

The Efficacy of an Aqueous Solution of Magnesium Oxide Nanoparticles and Its Ultrasonic Activation on Root Canal *Enterococcus Faecalis* Biofilm (In Vitro Study)

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ABSTRACT

Background: Chemo-mechanical preparation is essential for the debridement of root canal, aiming to remove microorganisms, pulpal tissues and smear layer. Conventional techniques are not enough, incorporation of nanoparticles with ultrasonic activation is suggested to improve the antimicrobial properties of an endodontic irrigant. **Aim:** The present study compared the efficiency of magnesium oxide in the nano form with NaOCL when used as an endodontic irrigants against *E. Faecalis* biofilm. Furthermore, the influence of ultrasonic activation on the irrigant's action was evaluated. **Materials and methods:** Sixty intact human permanent single rooted teeth with mature apices were decoronated, instrumented to size #50, sterilized and incubated with culture suspension of *Enterococcus Faecalis*. Samples were divided into three groups equally according to the type of irrigant used. Group 1 were irrigated by MgO nanoparticles, group 2 were irrigated by NaOCL 5.25% , and group 3 were irrigated by saline. Each group were subdivided into two groups equally A and B. Subgroups A were irrigated passively, subgroups B the irrigant was ultrasonically activated 5 min, 25 HZ. Antimicrobial efficacy was evaluated by counting colony forming units. **Results:** Ultrasonic activation of nano MgO was the best in bacterial reduction followed by activated NaOCL. **Conclusions:** MgO in the nano form exhibit superior antibacterial action and its action was enhanced by ultrasonic activation.

Keywords: Root Canal Irrigants, Endodontic Biofilms, Magnesium Oxide, Nanoparticles.

INTRODUCTION

Enterococcus Faecalis (*E. faecalis*) is a commonly detected bacterial species in infected root canals. It rejoices virulence factors such as lytic enzymes and cytolysin that penetrate dentinal tubules and stick to

dentin surface of the root canal to establish a biofilm.^{1,2,3}

Complex anatomy of root canal system makes it difficult to achieve the optimal sterilization by the conventional

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instrumentation techniques. Many methods have been used to achieve sterilization of root canals. Management of endodontic biofilm can be done during biomechanical treatment, mechanically by cleaning and shaping and chemically by irrigation.⁴

Sodium hypochlorite (NaOCL) solution has been used as the conventional irrigant during the chemo-mechanical preparation of root canals. It is highly effective antimicrobial agent, dissolves necrotic tissues and aids in debridement of root canal.⁵

Radcliffe et al.⁶ investigated the antimicrobial action of different doses of sodium hypochlorite against three types of bacteria, one of them was enterococcus faecalis strains. Sodium hypochlorite concentrations were 0.5, 1.0, 2.5, and 5.25%. The results showed that the more concentration, the less time needed to disinfect the canal by NaOCL. On the other hand, **Gernhardt et al.**⁷ illustrated in their case report the toxicity of concentrated sodium hypochlorite on vital tissues and concluded that this report confirmed the known toxicity of NaOCL to soft tissues following inadvertent extrusion. Recommendation of using low concentration of NaOCL was presented by **Sermeno et al.**⁸ in their case report on tissue injury caused by accidental ejection of concentrated NaOCL

during root canal treatment. Chlorhexidine 2% have been suggested to be an alternative to NaOCL as an effective antibacterial irrigant.^{9,10}

Different agitation techniques have been introduced in endodontics as ultrasonic devices to improve the performance of different root canal Irrigants in comparison to conventional irrigation. Activation of NaOCL irrigant by ultrasonic energy helps in heating the irrigant, aids in the antibacterial and tissue dissolving properties, and removes debris as well as smear layer. Continuous ultrasonic irrigation for one minute after cleaning and shaping the root canal was shown to reduce the colony forming unit counts in infected teeth.^{11,12}

Recently, the incorporation of nanoparticles in different aspects of dental materials have been advocated. It has unique properties when compared to bulk or powder counter-parts. Nanoparticles exhibit higher antibacterial activity due to their polycationic/ polyanionic nature, higher surface area and charge density which provide greater interaction with bacterial cell. They have the ability to diffuse antimicrobial substances deep in dentin. The antimicrobial property of metals is considered a breakthrough in medicinal history. Particularly inorganic nano metal oxides has

gained more attention because of its biocompatibility and antimicrobial activity even in low concentration.^{1,13,14}

The incorporation of nanoparticles in irrigation has been discussed by many authors in literature. The antimicrobial properties of nano metal oxides have been proven. However, the antimicrobial efficacy of magnesium oxide nanoparticles as an irrigant using ultrasonic activation was not evaluated.

Therefore, the aim of the present study was to test the efficacy of an aqueous solution of magnesium oxide nanoparticles and its ultrasonic activation on root canal enterococcus faecalis biofilm in comparison with other irrigants.

MATERIALS AND METHODS

Sample size calculation

This power analysis for a 3 x 2 fixed effect analysis of variance. The first factor (Irrigant) included three levels while the second factor (Irrigation technique) included two levels. The effect sizes for the two factors (6.7 and 7) were calculated based upon the results of Seet AN.¹⁵ and an estimated percentage reduction in *E. faecalis* = 50% using Nano MG based upon expert opinion. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%, the minimum estimated sample size was 10

specimens per cell giving 60 specimens. Sample size calculation was performed using IBM® SPSS® Sample Power® Release 3.0.1

Selection of samples

Non-identified sixty intact human permanent, single-rooted teeth with mature apices were obtained from MIU dental bank. All selected teeth were free of caries, cracks or fractures. Teeth were radiographed buccolingual, and mesiodistal to detect canal calcifications and/or resorption to be excluded if present.

Preparation of the samples

All teeth were scaled to remove any calculus deposits. The teeth were decapitated with an IsoMet low speed cutting machine for precision sectioning below the cemento-enamel junction, perpendicular to the long axis and the length of the roots were standardized to 16 mm length. Root canals were enlarged to size #50 and irrigated with NaOCL 5.25% then were wrapped into gauze damped with saline solution and placed in sterilization pouches. The pouches were run into a sterilization cycle at 121⁰C, and 15 Psi for 20 min.¹⁶

Classification of the samples

The samples were divided into three groups according to the type of irrigant used. Group 1: (n=20) were irrigated by nano magnesium oxide (MgO), group 2: (n=20)

were irrigated by sodium hypochlorite (NaOCL) 5.25% and group 3: (n=20) were irrigated by physiologic saline. Each group was further subdivided into two equal subgroups A and B. Subgroup A was irrigated passively, while samples of subgroup B the irrigant were ultrasonically activated for 5 min, 25 HZ power.

Biofilm development

Sterilized samples had their root canals infected with *E. Faecalis*. A clinical isolate of *E. Faecalis* was obtained from the microbiology laboratory (Central Laboratories, Ministry of Health, Egypt), and the biofilm formation was done by the investigator. The bacterial strain was inoculated in Trypticase Soy Broth (TSB) and incubated at 37° C for 24 hours. The experimental suspensions were prepared by cultivating the biological markers on the surface of TSB agar following the same incubation conditions. The bacterial cells were resuspended in saline to reach a final concentration of about 3×10^8 cells/ML adjusted to No. 1 MacFarland turbidity standard. The bacterial suspension was introduced inside the sterile root canals and incubated. The teeth were filled with a 24-hour pure culture suspension of *E. Faecalis* grown in Trypticase Soy Broth (TSB). All teeth were incubated at 37° C in sealed vials

for 10 days replacing the intracanal fluids with freshly prepared 0.9% physiologic saline solution adjusted to No. 1 MacFarland turbidity standard every 72 hours.¹⁷

Preparation of MgO nanoparticles:

MgO nanoparticles were prepared as follows¹⁸: 2M magnesium nitrate ($MgNO_3 \cdot 6H_2O$) was prepared. 0.5M sodium hydroxide solution was added drop wise to the prepared magnesium nitrate solution while stirring it continuously. White precipitate of magnesium hydroxide appeared in beaker after few minutes. The stirring continued for 30 minutes. The pH of the solutions was 12.5. The precipitate was filtered and washed with methanol three to four times to remove ionic impurities and then centrifuged for 5 minutes at 5000 rpm/min and dried at room temperature.

The nanomaterial solution was diluted with water to prepare concentration of 5 g/L. The solution was sonicated for 20 min at 20 kHz, and stored at room temperature for one day before the experiment. The average nanoparticle size range from 30- 40 nm, spherical like sheath with purity more than 99%.¹⁶

Application of tested irrigants

The tested irrigants (nano-MgO and NaOCL 5.25%) were applied into the experimental groups. Control group received

saline solution irrigant. In experimental groups, the canals were irrigated by using 6 ml of the irrigants listed above for 5 min. Each irrigant was delivered by syringe with 27- gauge needle. All irrigation procedures were held at room temperature under aseptic conditions. For samples of subgroup A, the irrigant was allowed to stay in the canal passively for 5 min. For samples of subgroup B, irrigating solution was applied inside the canal by side vented needle then an ultrasonic tip was inserted inside the canal, reaching the midroot level and was activated for 30 seconds by short vertical strokes. This step was repeated 4-5 times. Irrigation lasted for 2 min and required 6 mL of each irrigating solution in the experiment.¹⁹

Method of evaluation

Microbiological test:

Colony forming units were counted before and after the application of irrigation. Petri dishes of try base medium were prepared for the culture. One sterile paper point was inserted into the depth of each canal sampled. Paper points were transferred to sterile 2 ml Eppendorf tubes containing sterile saline. Samples were vortexed for 5 min and shaken vigorously before culturing. Sterile 10th dilution to 10⁻³ dilution using 1% sterile peptone water as diluents. From the sterile dilution, 0.1 ml. were transferred,

plated on tryptone soy base plates and incubated anaerobically for 48 hrs. at 35 °C to 37 °C. After the incubation period, counting of the number of colony-forming units (CFUs) was performed on the plates.²⁰

RESULTS

Microbiological results

The bacterial count within each of the groups was different before and after application of the irrigant. Furthermore, there was change in the bacterial population when the irrigant was activated.

Comparing change in each group (before & after irrigation)

- **For group 1 (irrigation with MgO 5 mg) (Table 1, Figure 1)**

Without ultrasonic activation: The median bacterial count was 442 with range (8-939) before irrigation that decreased to median 22(0-89); recording median reduction =353 that represents 79.9% reduction (**Figure 2-A**). This difference was statistically significant (p=0.010).

With ultrasonic activation: The median bacterial count was 183 with range (16-290) before irrigation that decreased to median 0(0-5); recording median reduction =180 that represents 98.4% reduction (**Figure 2-B**). This difference was statistically significant (p=0.005).

Table (1): Results of irrigation with MgO 5 mg.

	Before	After	Mean reduction	Percentage reduction
Without ultrasonic activation (1A)				
Mean ±SD	338.6	31.5±29.5	307.2±315.1	90.7%
Median (Range)	442(8-939)	22(0-89)	-353(-878,10)	-79.9
With ultrasonic activation(1B)				
Mean ±SD	148.2±96.1	1.4±2.0	146.8±95.5	99%
Median (Range)	183(16-290)	0(0-5)	-180(-290,-16)	-98.4

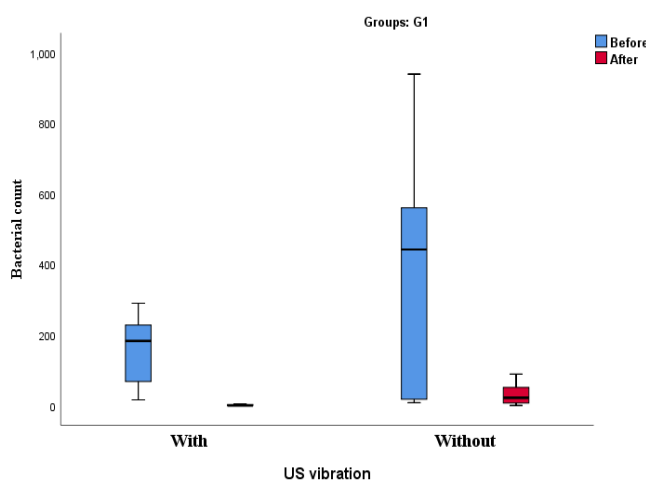


Figure (1): Boxplot representing bacterial count before and after irrigation by MgO 5 mg with and without ultrasonic activation.

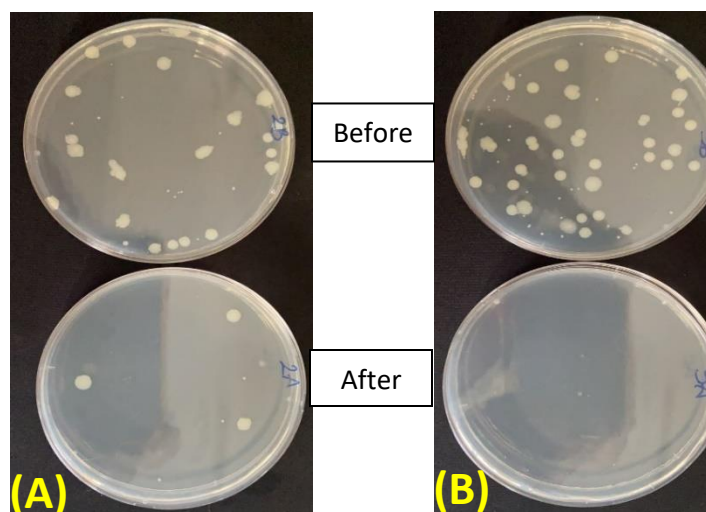


Figure (2): Tryptone soy base plates representing bacterial count before and after irrigation by MgO 5 mg; (A) without ultrasonic activation and (B) with ultrasonic activation.

- For group 2 (irrigation with NaOCL 5.25%.)(Table 2, Figure 3)

Without ultrasonic activation: The median bacterial count was 42 with range (7-122) before irrigation that decreased to median 4(0-15); recording median reduction =37 that represents 88.1% reduction (Figure 4-A). This was statistically significant (p=0.011).

that represents 100% reduction (Figure 4-B). This was statistically significant (p=0.008).

- For group 3 (irrigation with saline.) (Table 3, Figure 5)

Without ultrasonic activation: The median bacterial count was 12 with range (5-138) before irrigation that decreased to median 60(6-180); recording median increase = 42 that represents 350.0++%.

Table (2): Results of irrigation with NaOCL 5.25%.

	Before	After	Mean reduction	Percentage reduction
Without ultrasonic activation(2A)				
Mean ±SD	53.2±39.2	6.0±5.5	47±40.3	88.6%
Median (Range)	42(7-122)	4(0-15)	-37(-115,8)	-88.1
With ultrasonic activation(2B)				
Mean ±SD	56.9±52.6	3.7±5.3	53.3±49.0	93.6%
Median (Range)	36(8-140)	2(0-17)	-36(-123,-5)	-100.0

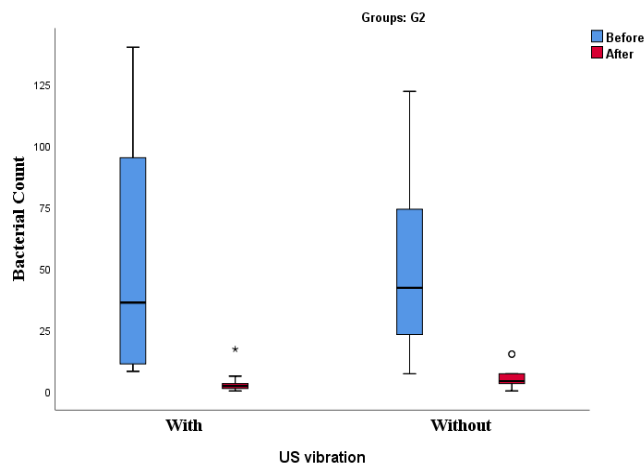


Figure (3): Boxplot representing bacterial count before and after irrigation by NaOCL 5.25%. with and without ultrasonic activation.

With ultrasonic activation: The median bacterial count was 36 with range (8-140) before irrigation that decreased to median 2(0-17); recording median reduction = 36

increased (Figure 6-A). This was statistically significant (p=0.012).

With ultrasonic activation: The median bacterial count was 56 with range (12-214).

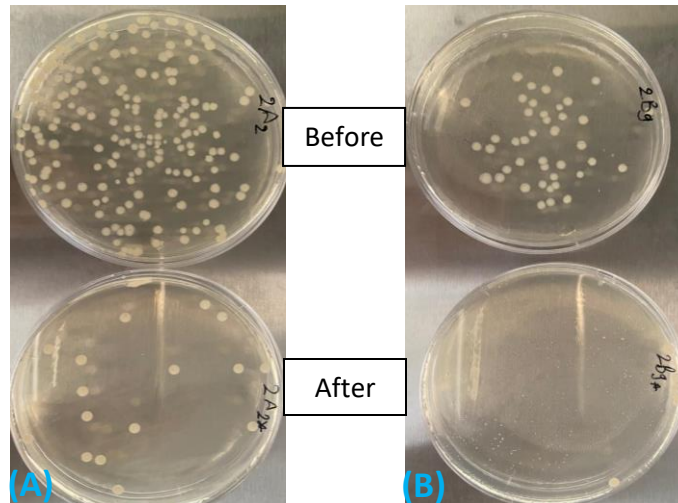


Figure (4): Tryptone soy base plates representing bacterial count before and after irrigation by NaOCL 5.25%; (A) without ultrasonic activation and (B) with ultrasonic activation.

Table (3): Results of irrigation with saline.

	Before	After	Mean reduction	Percentage reduction
Without ultrasonic activation(3A)				
Mean ±SD	26.8±42.9	71.4±56.0	+ 44.3±34.1	169.2%++
Median (Range)	12(5-138)	60(6-180)	42(0,99)	350.0++
With ultrasonic activation(3B)				
Mean ±SD	86.0±73.6	72.9±79.4	21.2±81.2	15%
Median (Range)	56(12-214)	35(3-224)	-27(-174,73)	48.0

before irrigation that decreased to median 35(3-224); recording median reduction =27

that represents 48% reduction (Figure 6-B). This was statistically insignificant (p=0.799).

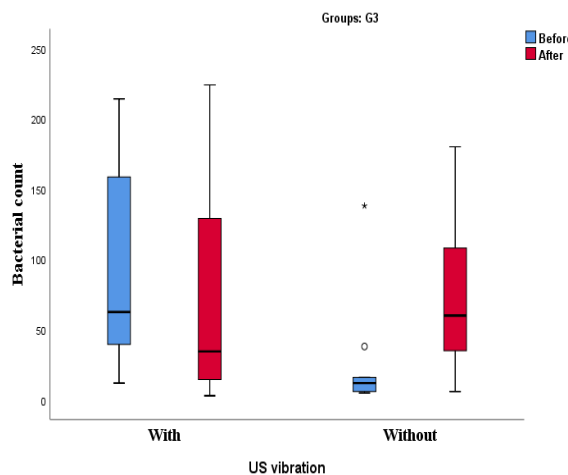


Figure (5): Boxplot representing bacterial count before and after irrigation by saline with and without ultrasonic activation.

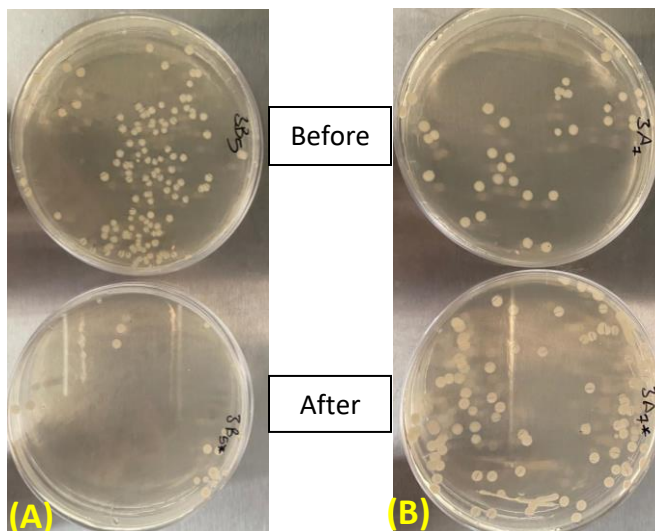


Figure (6): tryptone soy base plates representing bacterial count before and after irrigation by saline; **(A)** without ultrasonic activation and **(B)** with ultrasonic activation.

Irrigant type effects on bacterial count (Table 4, Figure 7 & 8)

Without ultrasonic activation: The median bacterial reduction was 353 with range (-878,10) in group 1, while it was 37(-115,8) for group 2, and on the contrary it increased for group 3 to reach 42(0,99) (Figure 7). There was a statistical significance difference between median bacterial reduction in the three groups.

With ultrasonic activation: The median bacterial reduction was 180 with range (-290, -16) in group1 while it was 36(-123, -5) for group 2 and 27(-174,73) for group 3. The differences between groups were statistically significant (p=0.016). (Figure 8)

Effect of ultrasonic activated irrigants on bacterial count reduction (Table 5)

Comparison of the bacterial count reduction with or without ultrasonic

Table (4): Statistical comparison between bacterial reduction in the three groups, after irrigation with and without ultrasonic activation, by Kruskal Wallis test followed by Dunn’s test.

	GP (1) irrigation with MgO 5 mg.	GP (2) irrigation with NaOCL 5.25%.	GP (3) irrigation with saline.	p value
	Median (Range)	Median (Range)	Median (Range)	
Without ultrasonic activation	-353(-878,10) ^a	-37(-115,8) ^b	42(0,99) ^a	<0.001
With ultrasonic activation	-180(-290, -16) ^a	-36(-123, -5) ^a	-27(-174,73) ^b	0.016

P<0.05 is statistically significant.

a: statistically significant from each other’s, b: statistically insignificant from other groups.

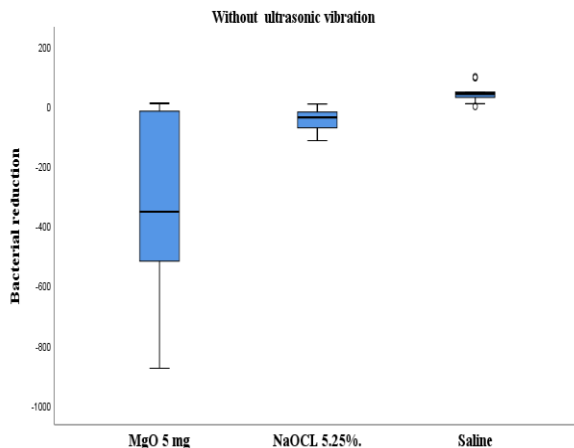


Figure (7): Boxplot representing bacterial reduction in the different groups without ultrasonic activation.

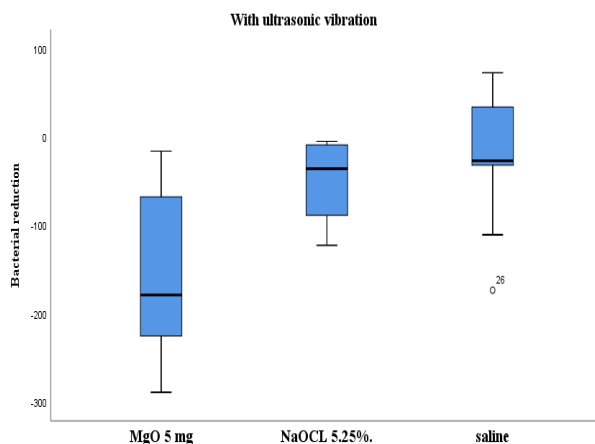


Figure (8): Boxplot representing bacterial reduction in the different groups with ultrasonic activation.

Table (5): Statistical comparison for bacterial reduction between irrigant with ultrasonic activation and irrigant without ultrasonic activation in the three groups, using Mann Whitney test.

	Without ultrasonic activation	With ultrasonic activation	P value
	Median (Range)	Median (Range)	
irrigation with MgO 5 mg.	-353(-878,10)	-180(-290, -16)	0.557
irrigation with NaOCL 5.25%.	-37(-115,8)	-36(-123, -5)	0.931
irrigation with saline.	42(0,99)	-27(-174,73)	0.063

P<0.05 is statistically significant.

activation among the three groups revealed that activation of the irrigant

improved its action. The bacterial count reduction among the three groups was

decreased after ultrasonic activation of the irrigant.

DISCUSSION

Limitations in current root canal irrigating solutions such as inactivation by dentin, limited long-term antibacterial effectiveness and the ability of bacteria to infiltrate deep inside dentinal tubules, reaching depths occupying the whole dentine thickness, has encouraged researchers to seek novel alternatives.²¹

Biofilm model of *Enterococcus Faecalis* has been chosen to test the antibacterial

efficacy of the tested irrigants for being the most resistable species among different endodontic microbial flora. It is the most common isolated species in failed endodontically treated teeth. It can penetrate deeply inside the dentinal tubules and can stick easily to the wall forming biofilm. It can remain alive inside dentinal tubules for up to 60 days if not entirely eradicated after single or multiple visits. Furthermore, *E. faecalis* can create biofilms after 24 hours.^{3, 22}

MgO nanoparticles were utilized in the present study because they are one of the functional nano metal oxide particles. Nano MgO function as a bactericidal agent. The positively charged particles and their large surface area interacts strongly with the negatively charged bacterial cell walls and

spores.²³ They also shows antibacterial effect against grams positive, gram negative bacteria and viruses.²⁴ The bacterial cell wall is the primary target of metal oxide nanoparticles, because it is composed of surface proteins for attachment and colonization of components such as teichoic acid and polysaccharides that defend against host defense and environmental conditions.²⁵

Nano MgO has low toxicity and can easily be prepared from readily available and inexpensive precursors in comparison to nano-silver, nanoTiO₂, nano-copper, and other types of nanomaterials and bactericides.^{26,27} Furthermore, no cytotoxicity effect of different concentration of Nano magnesium oxide was demonstrated after 24 hr.²⁸ Thus, it has significant potential as an acceptable bactericidal agent.

NaOCL was used in the present study because it has been the most effective and potent endodontic irrigant. In comparison to some other popular and potential root canal irrigants such as 10% chlorhexidine, 3% and 30% hydrogen peroxide, 10% peracetic acid, 5% dichloroisocyanurate (NaDCC), and 10% citric acid, 1% NaOCL was the only irrigant that has the capacity to dissolve necrotic tissues.²⁹

Saline was used because it has no antibacterial action, so testing it with and

without ultrasonic activation helps in revealing if the ultrasonic activation has an effect in eradication of *E. Faecalis* biofilm.

Because mode of irrigant application has an impact on its efficacy, passive ultrasonic activation (PUI) was used in the study in comparison to the traditional method of irrigation by syringe.³⁰ The hydrodynamic rinsing improves the penetration depth.³¹ It is critical for dissolving residual organic and inorganic material in places where the endodontic file cannot reach, and removes debris formed during instrumentation.³² Ultrasonic agitation improves the efficacy of the final rinse and extrudes much less irrigant solution with less apical debris collection.¹³

The antibacterial efficacy of the investigated root canal irrigants was assessed through the use of colony forming units (CFU). CFU is the most practical and accepted method for correctly identifying and comparing the antibacterial efficacies of various materials.³³

The results of the present study showed that there was a difference between groups. The MgO irrigation with ultrasonic activation was shown to be the best followed by NaOCL irrigation with ultrasonic activation, while irrigation with saline without ultrasonic activation showed increase in bacterial population.

This might be attributed to the fact that ultrasonic activation results in better penetration depth of the irrigant. This is in agreement with **Hauser et al.**³⁴ **Paragliola et al.**³⁵ as they documented that hydrodynamic rinsing improved the penetration depth of rinsing solution into root canal wall dentine. Also the PUI with a nickel titanium tip was found to have better tissue dissolving effects. Moreover, the bactericidal capability of the nano-sized MgO irrigating solution has a major role in these results. This is in agreement with **Jin et al.**³⁶ who demonstrated that MgO NP treatments deform and destroy the cell membrane, causing intracellular contents to seep out and bacterial cells to die. MgO nanoparticles were found to have antibacterial action against both bacteria and fungi. Moreover, **Golabek et al.**³⁷ stated that NaOCl solution's chemical activation was increased by ultrasonic agitation.

The results are not in agreement with **Wu. et al.**¹ who reported that the anti-biofilm efficacy of nanomaterials depends on their mode of application whether irrigation or medicament, and that the gel form was more efficient on biofilm disruption than solution. This difference in results may be due to the using of different material; silver nanoparticles as one of the nanometals in the gel form. Also, **Rödig et al.**³⁸ stated that

activating NaOCl with either sonic or ultrasonic devices did not improve its antimicrobial efficacy against *E. faecalis*. This difference in their study might be attributed to the low concentration of NaOCl (1%) used.

The overall results of the present study demonstrated that the activation of the irrigant was more effective despite the type of irrigant used. This was clearly demonstrated when reviewing the results of the saline solution. Without activation, saline did not exhibit any effect on the bacterial count, on the contrary, it increased the bacterial population. Being an inert material, this action of saline could be attributed solely on the activation process.

The overall results also demonstrated that the MgO in the nano form had a strong antibacterial action, it was even superior than NaOCl. This confirms the study hypothesis that nano MgO can be considered an acceptable root canal irrigant from the bacteriological point of view. Further studies on the other mechanical properties of nano MgO should be considered before recommending its addition to the endodontic armamentarium.

CONCLUSION

Based on the results of the present study, the following could be concluded:

1. MgO in the nano form exhibits superior antibacterial action.

2. Regardless of the chemical nature of the irrigant, ultrasonic activation plays a major role in its antimicrobial activity.

FUNDING RESOURCES

Non-funded research.

CONFLICT OF INTEREST

The authors deny any conflict of interest related to this study.

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