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The Effect of Silver Nano-Irrigant and Erbium Chromium: YSGG Laser on the Smear Layer Removal and Microhardness of Root Canal Dentine "In Vitro Study"

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ABSTRACT

Background: Root canal treatment involves irrigation protocols that affect both smear layer removal and microhardness of root canal dentine. ***Aim:*** Evaluate and compare the effect of using Silver Nanoparticles irrigant (AgNP), Erbium Chromium: YSGG Laser (Er;Cr: YSGG) and their combination on the removal of smear layer and microhardness of root canal dentine compared to 5.25% NaOCl and 17% EDTA. ***Materials and methods:*** Forty dentin samples from mandibular single-canaled premolars were selected, and twenty samples were allocated for each assessment. For microhardness, a baseline score was gained before irrigation using a Vickers microhardness tester. Samples for each assessment were randomly allocated in 4 groups (n=5): Group 1 (5.25% NaOCl + 17% EDTA), Group 2 (Er;Cr: YSGG), Group 3 (AgNP) and Group 4 (Er;Cr: YSGG + AgNP). Smear layer removal samples were examined under Environmental Scanning Electron Microscope (ESEM) at magnification 1000X to obtain a total average score out of 5 for each sample. Similarly, microhardness samples were assessed for after irrigation scores to obtain a percent change in microhardness. ***Results:*** Group 4 showed statistically significant higher score in the amount of smear layer left behind after irrigation than 3 and 2. Groups 1 and 4 reported statistically significant less percent decrease in microhardness than groups 2 and 3. ***Conclusion:*** Neither AgNP and Er;Cr: YSGG per se nor their combination was effective in removing smear layer. AgNP and Er;Cr: YSGG per se have decreased the microhardness of root canal dentine while their combination was similar to 5.25% NaOCl and 17%EDTA.

Keywords: Smear Layer Removal, Microhardness, Silver Nanoparticles, Erbium Chromium: YSGG Lasers.

INTRODUCTION

It is evident that the various instrumentation techniques used during root canal treatment result in the formation of a thick adhesive layer known as “smear layer”.[1] This layer is usually seen covering the dentinal walls of the root canal and blocking the dentinal tubules and hence preventing various irrigating solutions to

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penetrate the dentinal tubules to eradicate any bacteria harboring the tubules.[1]

Simultaneously, the various irrigating protocols known to us that help to remove such smear layer have their own impact on the microhardness of the root canal dentine once they expose the collagen.[2] Such an effect can predict the longevity and prognosis of the tooth being treated and, hence, the possibility of vertical root fracture.[2]

Among the most common irrigating protocols that we still consider as the gold standard protocol is the combination of both 5.25% Sodium Hypochlorite (NaOCl) and 17% Ethylenediaminetetraacetic acid (EDTA).[1] In such a combination, we benefit from the high disinfecting power of NaOCl and the chelating power of EDTA to remove the smear layer.[1]

Nevertheless, with the introduction of Laser Technology and Nanotechnology, thoughts of new irrigating protocols came to the surface. Silver nanoparticles (AgNP) and Erbium Chromium: YSGG Laser (Er;Cr: YSGG) have drawn considerable attention lately when it comes to disinfection and antibacterial action.[3,4] However, their ability to remove the smear layer and their effect on the microhardness of root canal dentine is still not clear. Hence, we conducted this study to consider the effect of AgNP and Er;Cr: YSGG on the smear layer removal and microhardness of root canal dentine when used alone or in combination.

MATERIALS AND METHODS

Materials

Materials used in the study are shown in **Table (1)**.

Methodology

Samples preparation:

A total of forty extracted human single-canaled mandibular premolars with an average length of 22 ± 1 mm were collected, where twenty premolars were allocated randomly for each assessment. Each tooth was examined radiographically to ensure the presence of a single mature canal without any anomaly. Moreover, they were examined by a Dental Operating Microscope (DOM) for any cracks or fractures. Then, each sample was decoronated using a low-speed diamond disc under copious irrigation to obtain a standardized root length of 15 mm measured by an endometer.[5]

For smear layer removal assessment, the root canal was then prepared by calculating the working length, subtracting 1 mm from the standardized root length.[5] Then with the help of the Protaper Next Rotary system, the canals were prepared until file X4 corresponding to #40/0.06 as a Master file.

During instrumentation, each canal was

Table (1): The materials, instruments and devices used in the study.

Material / Instrument / Device	Source / Company
27- gauge side vented needle	Hubei Guarddent Medical Technology,Co., Ltd, China
Acrylic Resin	Acrostone, Co., Ltd, Cairo, Egypt
Chisel	Dentsply-Sirona, Germany
Dental Operating Microscope	Leica, M320, Germany
Diamond disc	D&Z, Darmstadt, Germany
Digital Sensor	Nanopix, Eighteeth, China
Disposable plastic syringe	Global Med DEXI, China
Endomotor	E-cube, Saeshin America
Er;Cr: YSGG Laser	Waterlase MD, Biolase Technology, Inc., Irvine, CA
EDTA	EDTA PLUS +, Dental Plus, India
Fiber optics spectrophotometer	Ocean Optics USB2000+VIS-NIR, Oceans Insight, Orlando, Florida, USA
Isomet Linear saw	ISOMET 4000, Linear Precision Saw, Buehler, USA
K-files	MANI, China
Protaper Next	Dentsply-Sirona, Germany
Sodium Hypochlorite	Clorox, USA
Saline (Sodium Chloride solution 0.9%)	Al- Mottahidoon Pharma for Pharmaceutical Medical Production and Cosmetics, 10th of Ramadan City, Egypt
AgNP Solution (NT-SNP)	Nanotech Egypt for Photo-Electronics, City of 6th of October, Egypt
Stereomicroscope	Leica MC 190 HD, Germany
SEM	FEI Quanta 3D 200i, USA
Timer	Samsung A71 Mobile, Japan
Transmission Electron Microscope (TEM)	JEOL JEM-2100, Tokyo, Japan
Vickers Microhardness Tester	Wilson TUKON -1102 Microhardness tester, Buehler, USA
Wax	Cavex Set up Regular, Modelling Wax, Netherlands
Xray Machine	Fona XDG, Germany

irrigated with 3 ml of distilled water between each file size, and at the end of the preparation, a disposable plastic syringe with a 27-gauge side was used.[6]

For microhardness assessment, each tooth was split longitudinally using the Isomet. Each root was divided buccolingually into two halves.[7] A plastic ring with a diameter of 2.5 cm was filled with freshly mixed auto-polymerizing acrylic resin, into which a selected half of each tooth was embedded horizontally with the dentine surface exposed.[7] After curing the acrylic resin, the rings were removed, and each selected half was ground by silicon carbide abrasive papers (500, 800, 1000, 1200 grit) under distilled water to remove any surface scratches.[2] The samples were then tested for microhardness to obtain baseline measurements.[7] Each root was reassembled by placing the two halves on each other, and the root was completely and tightly surrounded by wax to mimic the clinical situation and to ensure the halves were in place during canal preparation. Cleaning and shaping of each canal were then performed as previously described.

AgNP Preparation

AgNP solution was prepared at Nanotech Egypt for Photo-Electronics, City of 6th of October, Egypt, using the Terkuvic method.[8] It involves the reduction of silver ions (Ag+) to metallic silver (Ag) using glucose as a reducing agent. The reaction is carried out under alkaline conditions to maintain the stability of the nanoparticles.

Characterization of AgNPs

The size and shape of the nanoparticles were characterized using a Transmission Electron Microscope (TEM) at an accelerating voltage of 200kV. As for the optical properties, Ultraviolet-Visible spectroscopy was obtained by a Fiber optics spectrophotometer. The properties of the material were as follows:

- **Appearance (Color):** Yellow
- **Appearance (Form):** Colloidal solution
- **Solubility:** Water soluble
- **Concentration:**100 ppm
- **Avg. Size (TEM):** 45 ± 5 nm
- **Shape (TEM):** Spherical like-shape
- **Expiration Date:** 3 months

Grouping of samples:

Samples were randomly allocated in 4 groups (n=5) according to final irrigating protocol: Group 1 (5.25% NaOCl + 17% EDTA), Group 2 (Er;Cr: YSGG), Group 3 (AgNP) and Group 4 (Er;Cr: YSGG + AgNP). A 5 ml disposable syringe with a 27-gauge side vented needle was used to reach 1-2 mm away from full working length. A standardized timing of one minute was used

in all groups.[6]

Application of Final Irrigating Protocol

This was done as follows in the corresponding groups:[6]

Group 1: NaOCl + EDTA

A syringe of 2.5 ml of 5.25 % NaOCl was applied for 30 seconds. The canal was then dried with paper points size #40 and washed with 5 ml saline. Then the canal was dried again using paper points size #40 and followed by 2.5 ml of 17 % EDTA that was applied for another 30 seconds.[6]

Group 2: Er;Cr: YSGG

In one minute and a total of 10 cycles, Er;Cr: YSGG Laser was applied into the canal at a wavelength of 2780 nm, power 1.5 W, Pulse Repetition rate of 50 Hz, Pulse duration 60 µs and water-air spray ratio of 80% - 30% (water used was distilled water). The laser beam was applied through Radial Firing tip size 2 (RFT2) with a length of 21 mm, where the tip was applied 1 mm shorter of the working length and moved in a coronal direction in a circular motion at a rate of 2 mm/sec.[4,9]

Group 3: AgNP

A syringe of 5 ml of AgNP with a concentration of 100 ppm was introduced within the canal of each sample for a duration of one minute.[10,11]

Group 4: AgNP + Er;Cr: YSGG

A syringe of 2.5 ml of AgNP with a concentration of 100 ppm was applied for 30 seconds and then activated using Er;Cr: YSGG Laser for another 30 seconds in with the same manner as in Group 2.[6,9,11]

Smear Layer Removal Assessment

After the final irrigation protocol, each tooth was then stored in a coded tube filled with saline solution. Then, each root was grooved buccally and lingually by a diamond disc without reaching the canal lumen and then longitudinally split into two halves using a chisel and mallet, resulting in two halves. One representative half was selected for scanning under ESEM.[6]

The selected half of each root was then marked at 4 mm, 8 mm, and 12 mm from the apex to define apical, middle, and coronal thirds. Each third was examined under the stereo microscope at a magnification of 10X to obtain a general idea about the number of debris remaining in each third after irrigation[1]. The sample was then examined using the ESEM., where each sample was fixed on aluminum stubs with standard diameter using a carbon double sticky tape at a magnification of 1000X at 4 mm, 8 mm, and 12 mm representing apical, middle, and coronal thirds.[5,6] The micrographs of each third in each root were coded and evaluated by two blinded observers using the five-level

scoring system to evaluate smear layer removal, as mentioned by Wang *et al.*:[6]

- Score 1: no smear layer and dentinal tubules open.
- Score 2: small amounts of scattered smear layer and dentinal tubules open.
- Score 3: thin smear layer and dentinal tubules partially open.
- Score 4: partial covering with a thick smear layer.
- Score 5: total covering with a thick smear layer.[6]

Each examiner evaluated the micrographs independently and blindly of each sample. Whenever there was conflict over the scoring, the micrograph of concern was discussed until an agreement on a definite score was reached. Finally, micrographs were decoded and scores were tabulated.[5,6]

Microhardness Assessment

The microhardness testing was performed twice. It was measured before shaping the canals, where the readings were considered baseline measurements and another time after the final irrigation protocol.[7] The test was performed in the middle third of the root half. A Vickers diamond indenter and a 20X objective lens were used to help apply a load of 100g oriented perpendicular to the dentine surface for a dwell duration of 20 seconds.[7] All measurements were taken by the same examiner using the same calibrated machine.

For baseline measurement, three indentations were made in the middle third of the selected root half. The first indentation was applied at the center of the middle third, just 0.5 mm away from the canal lumen. The second and third indentations were applied 0.5mm above and below the first indentation.[7,12] Baseline measurements were then converted into Vickers microhardness numbers using the following equation:[13]

$$\mathbf{VHN = 1.854\ P/d^2}$$

where,

VHN = Vickers hardness number in (Kgf/mm^2)
P = Load in (Kgf)
d = Length of diagonals (mm).

An average of all three values was calculated and considered the baseline microhardness value of the sample (B).[13]

After the final irrigation protocol had been applied, new sets of readings were obtained for each sample on the other side of the canal lumen as described before and an average value was calculated (A).[7] Both baseline and after final irrigation protocol values for each root half were used to calculate the percent change in microhardne-

ss for each sample according to the following equation:[14]

((B – A)/ B) x 100

RESULTS

Comparison between groups (at each third)

At the coronal root level, Er;Cr: YSGG Laser + AgNP showed the statistically significantly highest score. There was no statistically significant difference between the Er;Cr: YSGG Laser and AgNP groups; both showed statistically significantly lower scores. NaOCl + EDTA showed the statistically significantly lowest smear layer scores.

At the middle as well as apical root levels, there was no statistically significant difference between smear layer scores of different groups.

Comparison between root thirds within each group

As regards to NaOCl + EDTA, Er;Cr: YSGG Laser and Er;Cr: YSGG Laser + AgNP groups, there was a statistically significant difference between smear layer scores at different root thirds, where the apical root third showed the statistically significantly highest score. Middle root third showed a statistically significantly lower score. The statistically significantly lowest score was observed at the coronal third.

While in AgNP group, the apical root thirds showed the statistically significantly highest score. There was no statistically significant difference between the middle and coronal root thirds; both showed the statistically significantly lowest scores. (**Figures 1-5 and Table 2**)

Comparison between percent reduction in microhardness among groups:

Percent reduction in microhardness of root canal dentine was measured according to the following equation:

$$\textit{Percent reduction} = \frac{\textit{(MH before irrigation – MH after irrigation)}}{\textit{MH before irrigation}} \times 100$$

where, MH = Microhardness

A pair-wise comparison between irrigants revealed no statistically significant difference between Er; Cr: YSGG Laser and AgNP groups, where both showed the statistically significantly highest mean percentage reduction in microhardness. On the other hand, there was no statistically significant difference between NaOCl + EDTA and Er; Cr: YSGG Laser + AgNP groups, where both showed the statistically significantly lowest mean percent reduction in microhardness. **(Figure 6 and Table 3)**

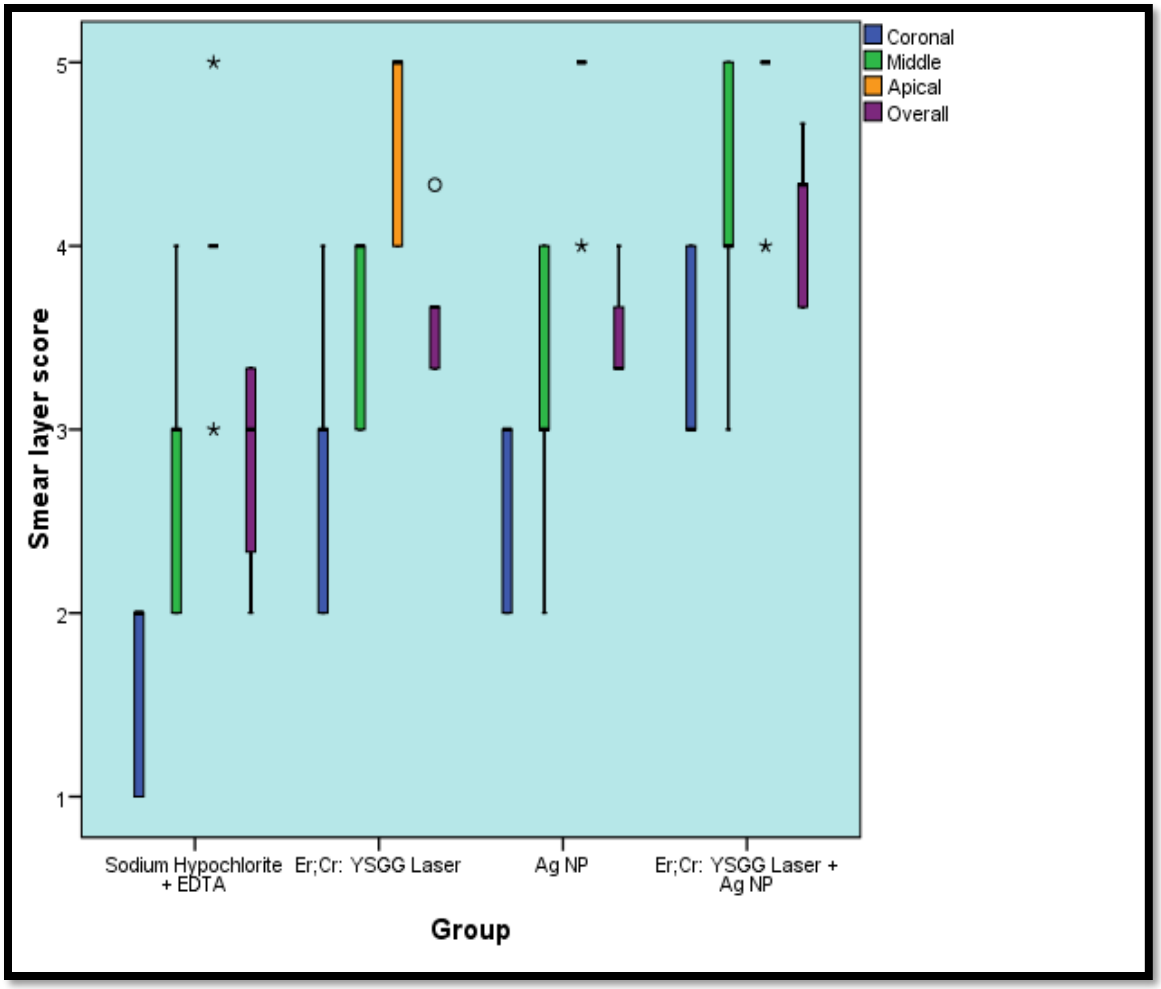
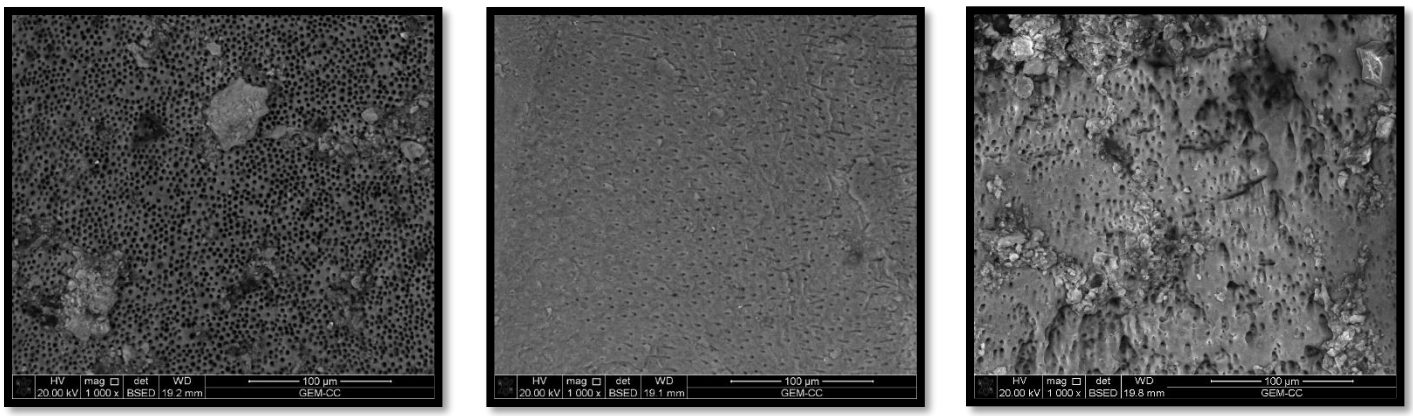
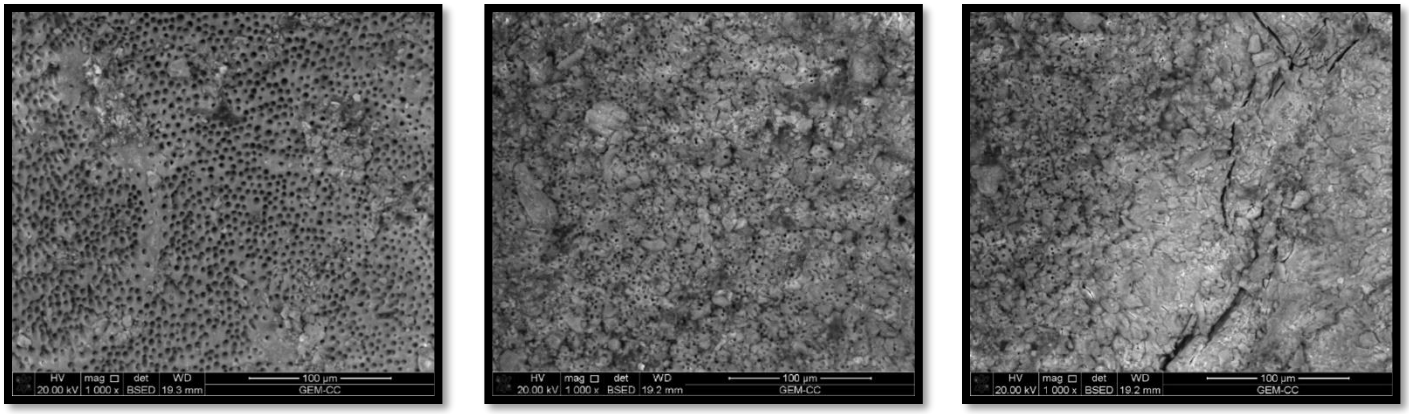


Figure (1): Box plot representing median and range values for smear layer scores of different groups (Circle and stars represent outliers).



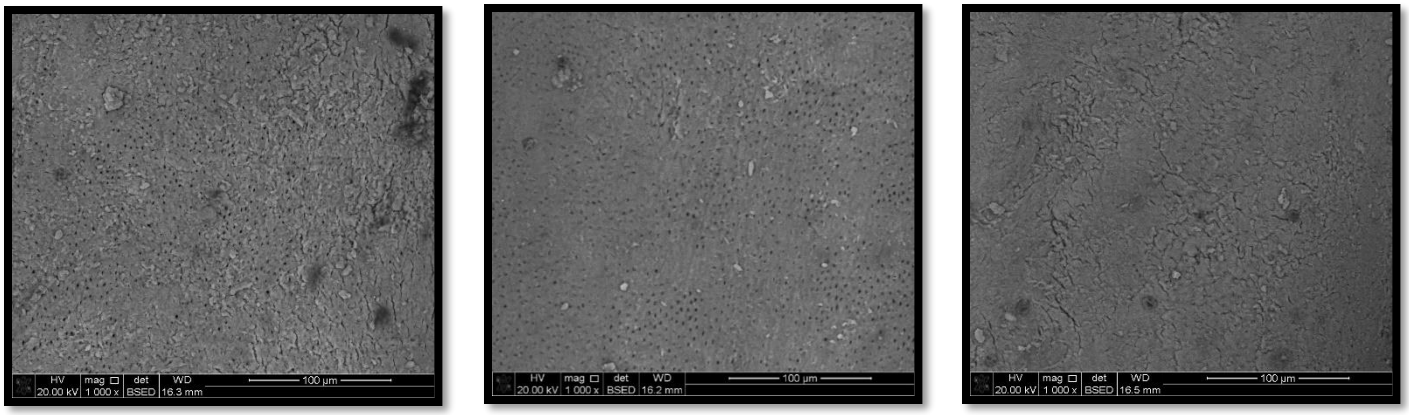
(a) **(b)** **(c)**

Figure (2): SEM Micrographs showing smear layer removal in samples of group 1 (NaOCl + EDTA) at 1000 x, (a) Coronal third, (b) Middle third, (c) Apical third.



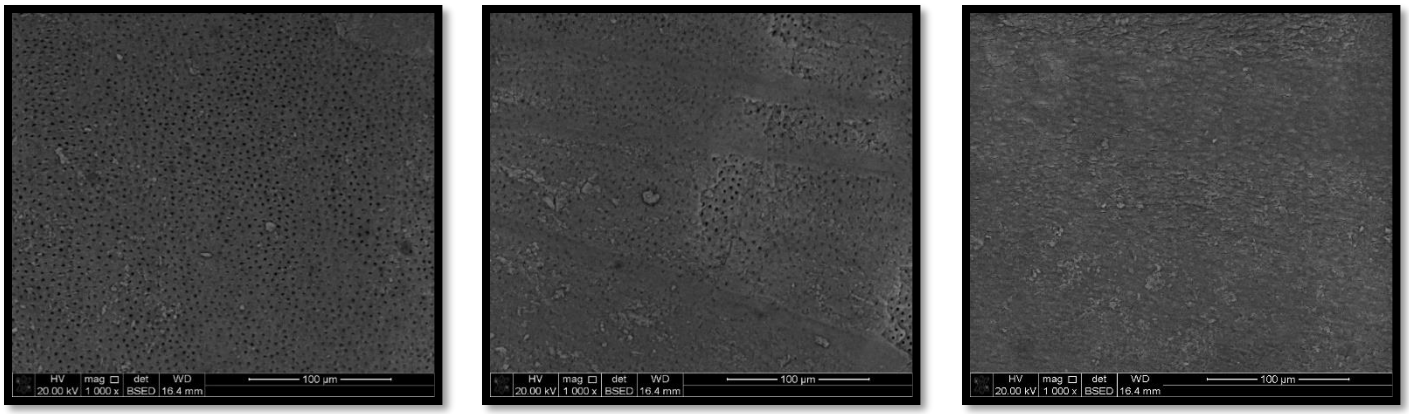
(a) **(b)** **(c)**

Figure (3): SEM Micrographs showing smear layer removal in samples of group 2 (Er;Cr: YSGG) at 1000 x, (a) Coronal third, (b) Middle third, (c) Apical third.



(a) **(b)** **(c)**

Figure (4): SEM Micrographs showing smear layer removal in samples of group 3 (AgNP) at 1000 x, (a) Coronal third, (b) Middle third, (c) Apical third.



(a) **(b)** **(c)**

Figure (5): SEM Micrographs showing smear layer removal in samples of group4 (Er;Cr: YSGG + AgNP) at 1000 x, (a) Coronal third, (b) Middle third, (c) Apical third.

Table (2): Descriptive statistics and results of Kruskal-Wallis test for comparison between smear layer scores in the four groups and Friedman's test for comparison between root levels within each group.

Root level	NaOCl + EDTA		Er;Cr: YSGG Laser		AgNP		Er;Cr: YSGG Laser + AgNP		*P*-value	*Effect size (Eta squared)*
	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)		
Coronal	2 (1-2) ^{CF}	1.6 (0.55)	3 (2-4) ^{BF}	2.8 (0.84)	3 (2-3) ^{BE}	2.6 (0.55)	3 (3-4) ^{AF}	3.4 (0.55)	0.012*	0.568
Middle	3 (2-4) ^{E}	2.8 (0.84)	4 (3-4) ^{E}	3.6 (0.55)	3 (2-4) ^{E}	3.2 (0.84)	4 (3-5) ^{E}	4.2 (0.84)	0.096	0.358
Apical	4 (3-5) ^{D}	4 (0.71)	5 (4-5) ^{D}	4.6 (0.55)	5 (4-5) ^{D}	4.8 (0.45)	5 (4-5) ^{D}	4.8 (0.45)	0.143	0.309
Overall	3 (2-3.33) ^{C}	2.8 (0.61)	3.67 (3.33-4.33) ^{B}	3.67 (0.41)	3.33 (3.33-4)^{B}	3.53 (0.3)	4.33 (3.67-4.67) ^{A}	4.13 (0.45)	0.008*	0.582
P-value	0.008*		0.014*		0.021*		0.022*			
Effect size (w)	0.958		0.859		0.768		0.760			

**: Significant at P ≤ 0.05,*
A,B,C superscripts in the same row indicate statistically significant difference between group.
D,E,F superscripts in the same column indicate statistically significant difference between root levels.

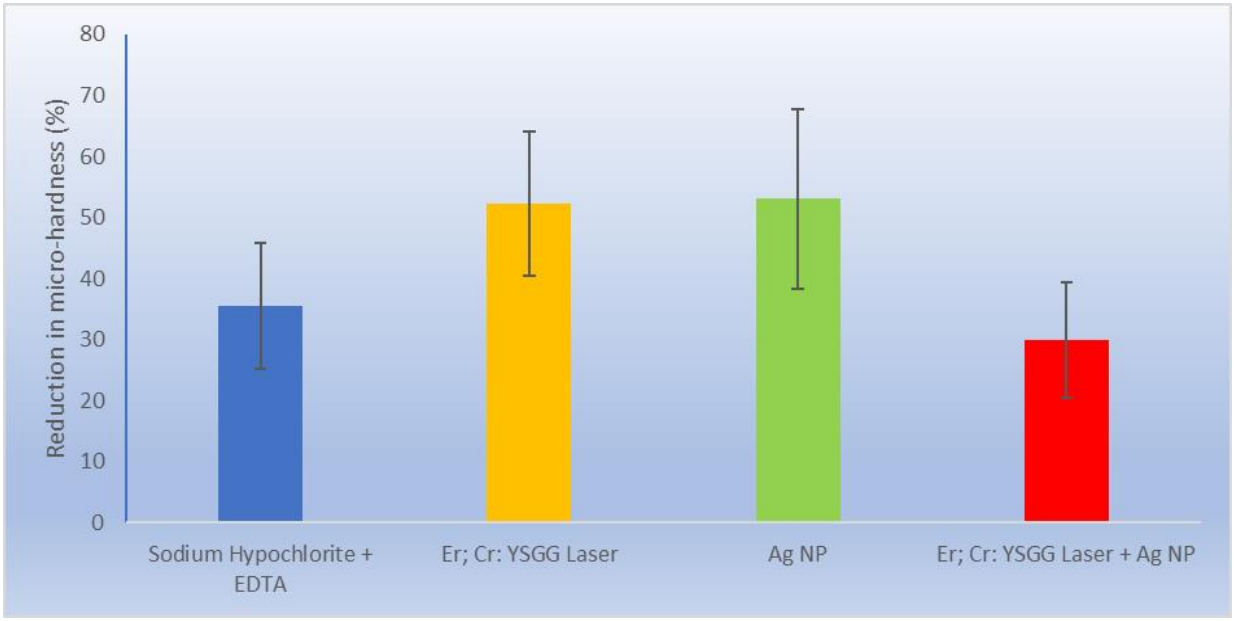


Figure (6): Bar chart representing percent reduction mean values of microhardness for the studied groups.

Table (3): The mean, standard deviation (SD) values and results of one-way ANOVA test for comparison between percent reduction in microhardness (%) of irrigant types.

NaOCl + EDTA		Er;Cr: YSGG Laser		AgNP		Er;Cr: YSGG Laser + AgNP		*P*-value	*Effect size (Eta squared)*
Mean	SD	Mean	SD	Mean	SD	Mean	SD		
35.5 ^{B}	14.2	52.2 ^{A}	10.2	53.1 ^{A}	14.8	30 ^{B}	11.9	0.025*	0.434

**: Significant at P ≤ 0.05.*
Different superscripts in the same row indicate statistically significant difference between irrigants.

DISCUSSION

Results of smear layer removal revealed that none of the studied irrigating protocols was capable of removing the smear layer completely, which coincides with previous studies.[3,6,15-17] Er;Cr: YSGG revealed a significantly lower smear layer removal score in the coronal third and along the entire canal length than in Group 1. This result is in agreement with that achieved by Bolhari *et al.*,[3] Alhadi *et al.*[18] and Aksoy *et al.*[19] According to their studies, the effectiveness of removing the smear layer via Er;Cr: YSGG depends on physical factors such as power level, duration of exposure, absorption of light into the dentinal tissues, the geometry of the canal and tip-to-target distance, rather than chemical factors.[18]

On the other hand, Shaheed *et al.*[20] reported that Er;Cr: YSGG significantly removed the smear layer more efficiently than the combination of Group 1 at the coronal and middle thirds.[20] This result could be attributed to the different study designs. Furthermore, Nasher *et al.*[21] recorded that Er;Cr: YSGG used at 2 W for one cycle in curved canals along with Diode 940 nm Laser at 2 W can significantly remove smear layer more efficiently than the combination of 5.25% NaOCl and 17% EDTA from the apical third.[21] This also could be attributed to their use of a higher power and dual wavelength of lasers.

When AgNP was used per se in Group 3, it recorded significantly less smear layer removal at the coronal third and along the entire canal length than in Group 1. This result coincides with that reported by Gozalez-Luna *et al.*[22] and Rajasekhar *et al.*[4] According to their study, the removal of the smear layer by AgNP was due to the physical interaction between AgNP and dentinal debris.[4,22] However, 5.25% NaOCl and 17% EDTA remove the smear layer by chemically interacting with dentinal debris.[2,22]

On the other hand, Rajasekhar *et al.*[4] and Martinez-Andrade *et al.*[10] reported that when AgNP was used along with 17% EDTA, it

resulted in a comparable efficacy in smear layer removal to that of the combination of Group 1.[4,10] This could be due to the 10 nm sized AgNP particles used in Rajasekhar *et al.*[4] study along with the chelating action of 17% EDTA in both studies. Furthermore, Tonini *et al.*[23] revealed that a combination of AgNP with citric acid, known as BioAkt, significantly removed smear layer as similar to 17% EDTA per se at coronal and middle thirds of root canals.[23] This could be attributed to the citric acid incorporated with AgNP.

The results in Group 4 were reported to be significantly less effective in the removal of the smear layer among all groups. This could be due to the application of Er;Cr:YSGG for a smaller number of cycles and hence less time as compared to Group 2. In addition, their combined use could have caused them to affect each other negatively.

The microhardness results reported a significant decrease in the mean root canal microhardness scores after irrigation within each group. These results coincide with several previous studies.[7,24–31]

Er;Cr: YSGG (Group 2) results coincide with those reported by Al-Omari and Palamara.[28] They explained that Er;Cr: YSGG laser beam is absorbed by hydroxyapatite crystals and water rich collagen fibrils leading to ablation[28]. Simultaneously, rapid heating of the mineral contents present in the root canal dentin occurs which leads to explosive breakdown of root canal dentine and hence decreasing the microhardness of the root canal dentine.[28]

Similarly, AgNP (Group 3) showed a significantly higher mean of percent reduction in microhardness than in Groups 1 and 4. This coincides with the findings of Sahebi *et al.*[32] They attributed this to the exhibition of a negative surface charge on AgNP particles as a result of the preparation procedure of AgNP irrigant solution employed in their study, which promoted the leaching out of Calcium (Ca^{+2}) ions from dentine. This would reduce the Ca/P ratio and hence decrease the root canal microhardness.[32]

On the other hand, Hassan and Khallaf[33] revealed an increase in root canal dentine microhardness, but when AgNP was used as an intracanal medicament.[33] This was rationalized due to the deposition of Ag on the root canal dentine by time and the use of propylene glycol that is capable of delivering AgNP deep into the dentine due to its low surface tension.[33] Similarly, Sahebi *et al.*[32] recorded an increase in root canal dentine microhardness when AgNP was incorporated with imidazole forming Im-AgNP.[32] This

was attributed to the ionic nature of imidazole liquid or the uneven charge distribution on the cationic part of imidazole and dentine surface, which eventually led to the deposition of IM-AgNP on the dentine surface and increased the root canal microhardness.[32]

The results in Group 4 revealed a significantly lower mean percent reduction in microhardness than Groups 2 and 3 and non-significant from that of Group 1. The results reported by Hamoudi *et al.*[34] could explain the results in the current study, where the use of Diode (808 nm) and Nd:YAG laser (1064 nm) resulted in the melting of the enamel surface. On re-solidification, a rougher enamel surface is formed, which might have attracted AgNP to nest on the surface and eventually become part of the enamel structure, altering the Ca/P ratio.[34] In addition, the combined use of Er;Cr; YSGG, and AgNP could have caused them to affect each other, leading to a mean percent reduction in microhardness that is significantly less than in Groups 2 and 3.

To date, the combination of NaOCl + EDTA remains the gold standard. Nevertheless, AgNP and Er;Cr: YSGG proved to be beneficial to a certain degree; hence, further research regarding their use in the Endodontic field is strongly required.

CONCLUSION

Under the circumstances of the current study, a combination of 5.25% NaOCl and 17% EDTA remains the gold standard protocol for removing the smear layer and having minimal effect on microhardness of root canal dentine, while the use of AgNP or Er;Cr: YSGG per se or their combination was not effective to remove smear layer. Though the combined use of AgNP and Er;Cr:YSGG minimally affected the microhardness of root canal dentine, their per se use negatively decreased the microhardness of root canal dentine Therefore, a chelating agent is recommended to be used along with AgNP or Er;Cr:YSGG but for a lesser period of time than in the current study to ensure smear layer removal with minimal effect on the microhardness of root canal dentin.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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