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IMPACT OF TWO NANO IRRIGATING SOLUTIONS ON MICROHARDNESS OF ROOT CANAL DENTIN IN VITRO STUDY

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ABSTRACT

Background: Increased Dentin Brittleness means it is weaker and more liable to fracture under occlusal forces which adversely affects the strength of the tooth, the prognosis of the endodontic treatment, and the life time of the tooth under the occlusion forces. Irrigation solutions have different effects on the micro hardness and the micromorphology of the root canal dentin.

Aim: The current study was done to evaluate the effect of two different nano irrigating solutions on the dentin microhardness.

Materials and methods: Sixteen intact human maxillary incisors with mature apices were decoronated, after cleaning and shaping all the roots were longitudinally split by using Isomet. To get total of 32 root halves. Each root half was horizontally embedded in auto polymerized acrylic resin exposing the most dentin surface. Each root half was given a number and distributed into four equal groups. Group I Nano Magnesium oxide, Group II Chlorhexidine loaded chitosan nano particles, Group III 5.2% Sodium hypochlorite followed by 17% EDTA and Group IV Saline. Each half was immersed in the tested solution for 5 min. The microhardness of the root dentin was assessed by vicker's microhardness indenter

Results: Group III shoed statistically significant reduction in dentin microhardness compared to other groups.

Conclusion: CHX+CSNPs and MgO are suitable endodontic solutions because of their lower toxic effect on microhardness in comparison to 5.25NaOCL + 17% EDTA.

KEY WORDS: Root canal irrigation - Dentin hardness - nano irrigation – Magnesium oxide nano particles

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INTRODUCTION

Root canal treatment consist of endodontic instrumentation and irrigation solutions aiming to eliminate organic, inorganic elements and debris resulting from the endodontic procedures as well as the reduction of microbial content and its by products [1].

Irregularities in the root canal system, narrow canals, isthmus and apical deltas prevent complete cleaning and disinfection of the root canal system. That is why chemical disinfection by irrigation materials is a very important step in root can treatment. For clinical usage an ideal irrigation material should be capable of dissolve the organic and inorganic material, flush out the debris, lubricating the canal, has antimicrobial activity, remove smear layer and maintain the dentin hardness. Different irrigations solutions were introduced in endodontic field like sodium hypochlorite (NaOCL), (EDTA), Chlorhexidine (CHX) and iodine potassium iodide (IKI), till now no ideal irrigation solution in the market [2].

"Microhardness is defined as the resistance to local deformation". Its test is based on the intentional permanent surface deformation that remains permanently or temporarily after load removal. Hardness determination can be correlated with to the mechanical properties like fracture resistance and modulus of elasticity. There is a direct relationship between the micro hardness of dentin and the bond strength, so micro hardness could be the first indicator of the dentin / restoration interface. Differences in micro hardness can affect the equal distribution of stress along the interface and may lead to catastrophic failures [3].

Chitosan is anatural polysaccharide comprising od co-polymer =s of glucosamine and N-acetyl glucosamine. Partial deacetylationof chitin results in production of chitosan. It's a biodegradable, biocompatible, Bio adhesive, anti-bacterial agent, low cost product and nontoxic product [4].

In Dentistry Chitosan has been used as a barrier membrane for periodontal treatment and as oral mucosal delivery agent for Chlorhexidine. In a study conducted by Silva et al [5]. Chitosan has effectively removed smear layer from the root canal system after instrumentation but is role as antimicrobial or anti-fungal agent not yet proven. Literature has shown that the antimicrobial effect of CHX increase when combined with Chitosan. Combining the Chitosan and CHX to remove the smear layer and obtain high anti-microbial effect without harmful effect on radicular dentin would be very helpful in endodontic field [4, 5].

Metal oxide nano particles are promising dental irrigation solutions, because of their highly antimicrobial activity and their biocompatibility with human cells. Nanometer metal oxides particles have been investigated to be used as antimicrobial agents. It has been reported that nano-magnesium oxide (MgO) showed antibacterial activity against Gram-positive bacteria. In literature there was no study evaluating the mechanical properties of nano-MgO against root canal dentin and as a root canal irrigating solution. Nano-MgO is reported to be harmless to the human cells and the environment [6].

MATERIALS AND METHODS

Sample size calculation

MedCalc® version 12.3.0.0 program "Ostend, Belgium" was used for calculations of sample size, statistical calculator based on 95% confidence interval and power of the study 80% with α error 5%, According to a previous study [7], showed that the mean reduction values microhardness of canal dentin surface for irrigant solutions, at mean reduction Saline (0.47) compared to mean reduction NaOCl (5.15), with p-value <0.001 highly significant. So it can be relied upon in this study, based on this assumption, sample size was calculated according to these values produced a minimal samples size of 32 teeth, 8 teeth in each group enough to find such a difference.

Preparation of the nano irrigation materials

MgO nanoparticles

MgO Nanoparticles was prepared follows [6]. 0.2M magnesium nitrate (MgNO3.6H2O) was prepared. 0.5M sodium hydroxide solution was added drop wise to the prepared magnesium nitrate (MgNO3.6H2O) solution while stirring it continuously. White precipitate of magnesium hydroxide appeared in beaker after few minutes. The stirring was 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 dried white powder samples were annealed in air for two hours at 300 and 500ºC

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 % [8].

Preparation of Chlorohexidine loaded chitosan nano particles (CHX loaded CSNPs).

CHX loaded CSNPs

To get chlorhexidine 2% in final concentration, we use appropriate amount 100ml and evaporated to 10ml added to previously prepared CSNPs (10mg/ml) suspension with stirring and sonication for 1h. To get homogenous suspension of CHX loaded CSNPs [9, 10].

Selection of samples

Maxillary anterior teeth freshly extracted with mature apex and each tooth was radiographed to confirm the presence of single canal. Teeth with severe decay resorption (internal or external), root caries and vertical fracture were excluded.

Preparation of the samples

Crowns were sectioned transversally at the cemento- enamel junction (CEJ) with an Isomet cutting machine. The working length was obtained by using K-file #10. The file was inserted into the root canal and advanced until it was visible coming out of the apex. Up until it flushed with the apex, the file was withdrawn. The working length of the file was calculated by measuring it and deducting 1 mm. To lessen the strain on manual files, the pro taper rotary files' first orifice opener was utilized. Once the root canal had reached its documented operating length, file #15 was utilized to ensure its patency. File #20 and distilled water irrigation were then employed to flush out the debris. Following up on the preparation, F1, F2 and F3 pro taper rotary files were used. By using hand K files in sizes (30, 35, 40, etc.) up to 45, canal enlargement was completed. Canals in all groups were irrigated with a standardized volume of 3ml of distilled water using 30-gauge needle between each file [11].

All the roots were longitudinally split by using Isomet. To get total of 32 root halves. Each root half was horizontally embedded in auto polymerized acrylic resin exposing the most dentin surface.

Classification of the samples

The samples were divided into four groups according to the irrigation used. Group I: (n= 8) nano magnesium oxide, group II: (n = 8) chlorhexidine loaded chitosan nano particles, group III: (n= 8) 5.25% sodium hypochlorite followed by 17% EDTA, group IV (n=8) saline solution. Each half was immersed in the specific irrigating solution for five minute except group 3(Immersion of the sample in 5.25 NaOCL was only 3 min then cleaned by distilled water after that immersion in EDTA for 2 min was done).

Method of Evaluation

Microhardness test:

The microhardness of the root dentin was assessed using Vicker's tester for microhardness. The indentations were made at three separate locations on the cervical, middle, and apical dentin at approximately less than 0.5mm from the root canal space using a vicker microhardness tester with a vicker's diamond indenter and 20x objective lens. Each measurement were carried out by using a 200-g load for 15 second oriented perpendicular to the root surface. By using a built-in scaled micromerg, the diagonal lengths of the indentations were measured, and the measurements acquired for each depth were converted into Vickers hardness values (VHIN). One hardness value was generated for each sample by averaging the data.

RESULTS

Percent of change of microhardness was calculated for each sample. The mean and SD for each group was calculated

1- Impact of irrigation solution on dentin microhardness Taple (1) and Figures (1)

Coronal

There was a significant difference between different groups ($p<0.001$). The highest value was found in 5.25% NaOCl+17%EDTA (19.47±2.67), followed by CSNPs+CHX (13.74±5.29), then MgO (7.89±0.74), while the lowest value was found in saline (0.56±0.40). Post hoc pairwise comparisons were all statistically significant ($p<0.001$).

Middle

There was a significant difference between different groups ($p<0.001$). The highest value was found in 5.25% NaOCl+17%EDTA (21.93±0.49), followed by CSNPs+CHX (13.38±2.39), then MgO (8.88±2.24), while the lowest value was found in saline (0.69±0.40). Post hoc pairwise comparisons were all statistically significant ($p<0.001$).

Apical

There was a significant difference between different groups ($p<0.001$). The highest value was found in 5.25% NaOCl+17%EDTA (19.96±2.35), followed by CSNPs+CHX (13.28±2.31), then MgO (7.69±2.28), while the lowest value was found in saline (0.43±0.26). Post hoc pairwise comparisons were all statistically significant ($p<0.001$).

TABLE (1): Intragroup comparisons and mean ± standard deviation (SD) values of micro-hardness for different root sections

Irrigant	Micro-hardness (mean±SD)			p-value
	Coronal	Middle	Apical	
MgO	$7.89±0.74^{A}$	$8.88±2.24^{A}$	$7.69±2.28^{A}$	**0.475ns**
CSNPs+CHX	$13.74±5.29^{A}$	$13.38±2.39^{A}$	$13.28±2.31^{A}$	**0.942ns**
5.25% NaOCl+17%EDTA	$19.47±2.67^{A}$	$21.93±0.49^{A}$	$19.96±2.35^{A}$	**0.094ns**
Saline	$0.56±0.40^{A}$	$0.69±0.40^{A}$	$0.43±0.26^{A}$	**0.389ns**

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

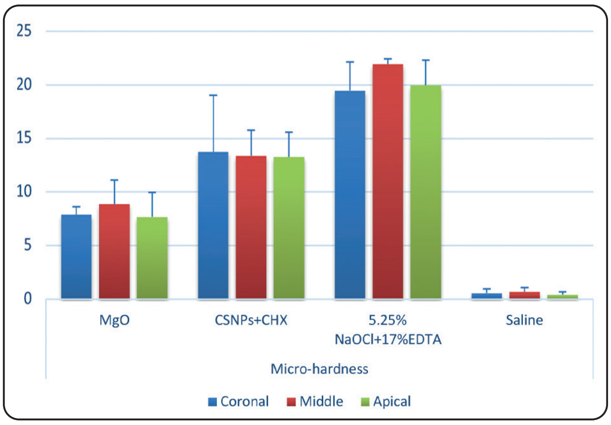

Fig. (1): Bar chart showing average micro-hardness for different root sections

2- Impact of irrigation solutions on different root levels Table (2) and Figure (2):

Group I MgO

There was no significant difference between values measured at different sections (p=0.475). The highest value was measured at the middle section (8.88±2.24), followed by the coronal section (7.89±0.74), while the lowest value was found at the apical section (7.69±2.28).

Group II CSNPs+CHX

There was no significant difference between values measured at different sections (p=0.942). The highest value was measured at the coronal section (13.74±5.29), followed by the middle section (13.38±2.39), while the lowest value was found at the apical section (13.28±2.31).

Group III 5.25% NaOCl+17%EDTA

There was no significant difference between values measured at different sections (p=0.094). The highest value was measured at the middle section (21.93±0.49), followed by the apical section (19.96±2.35), while the lowest value was found at the coronal section (19.47±2.67).

Group IV Saline

There was no significant difference between values measured at different sections (p=0.389). The highest value was measured at the middle section (0.69±0.40), followed by the coronal section (0.56±0.40), while the lowest value was found at the apical section (0.43±0.26).

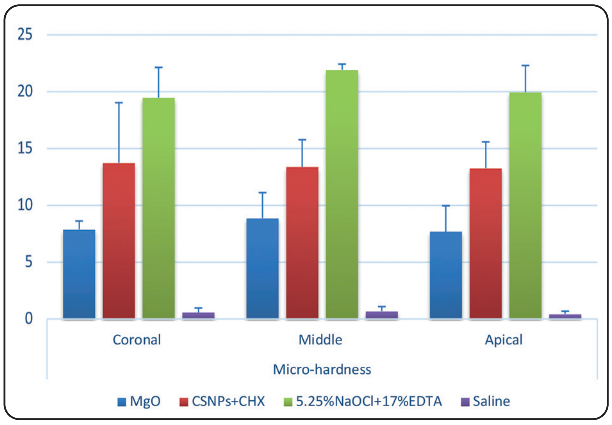

Fig. (2): Bar chart showing average micro-hardness for different irrigation materials

TABLE (2): Intergroup comparisons and mean ± standard deviation (SD) values of micro-hardness for irrigation materials

Root section	Micro-hardness (mean±SD)				p-value
	MgO	CSNPs+CHX	5.25% NaOCl+17%EDTA	Saline	
Coronal	7.89±0.74^{C}	13.74±5.29^{B}	19.47±2.67^{A}	0.56±0.40^{D}	**<0.001***
Middle	8.88±2.24^{C}	13.38±2.39^{B}	21.93±0.49^{A}	0.69±0.40^{D}	**<0.001***
Apical	7.69±2.28^{C}	13.28±2.31^{B}	19.96±2.35^{A}	0.43±0.26^{D}	**<0.001***

Means with different superscript letters within the same horizontal row are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

DISCUSSION

Endodontic instruments and irrigation solutions are used to remove organic, inorganic, and waste from the mouth during root canal treatment. It also seeks to reduce microbial content and byproducts.

The root canal system cannot be completely cleaned and disinfected because to irregularities, thin canals, isthmuses, and apical deltas. Because of this, a crucial stage in root irrigation is chemical disinfection of irrigation materials.

The aim of this study is the assessment of two nano irrigation materials on root canal microhardness by using vicker's microhardness indenter.

Metal oxide nano particles are promising dental irrigation solutions, because of their highly antimicrobial activity and their biocompatibility with human cells. Nanometer metal oxides particles have been investigated to be used as antimicrobial agents. It has been reported that nano-magnesium oxide (MgO) showed antibacterial activity against Gram-positive bacteria. In literature there was no study evaluating the mechanical properties of nano-MgO against root canal dentin and as a root canal irrigating solution. Nano-MgO is reported to be harmless to the human cells and the environment [6].

In Dentistry Chitosan has been used as a barrier membrane for periodontal treatment and as oral mucosal delivery agent for CHX. Literature has shown that the antimicrobial effect of CHX increase when combined with Chitosan. Combining the Chitosan and CHX to remove the smear layer and obtain high anti-microbial effect without harmful effect on radicular dentin would be very helpful in endodontic field [9].

Differences in micro hardness can affect the equal distribution of stress along the interface and may lead to catastrophic failures, although a decrease in microhardness makes it easier to instrument the root canal, it may also damage the root structure, making teeth that have had root canal therapy more likely to break. Additionally, it may worsen dental materials' capacity to seal against bacterial intrusion and adhere to dentin, increasing the permeability and solubility of the root canal dentin. This allows coronal leakage [12].

Instead of using the knoop test, the Vicker's microhardness test was used because it produces an indentation with a square shape, making it suitable for thick specimens, whereas the knoop test produces an indentation with a shallower depth and a longer elongation parallel to the specimen surface, making it suitable for thin specimens. In contrast to the knoop test, which relies only on one diagonal, Vicker's hardness number is based on the mean of two diagonals, providing more reliable results. Additionally, the little indenter tip of the Vicker is ideal for small specimens [13].

Cruz-Filho et al [14] noted that cutting the roots longitudinally rather than transversally into discs can produce more accurate representations of clinical scenarios, hence this method was chosen for the current investigation. The present study assessed the microhardness of the most superficial layer of root canal dentin since it is this layer that is initially in contact with irrigants in the root canal lumen. On the other hand, earlier research [7], measured the hardness of the area between the main canal and the cementum layer by transversely dividing the root into discs.

At various points on the same tooth, the radicular dentin's microhardness was different. Thus, indentations were created in the cervical, middle, and apical thirds of the radicular dentin in the current investigation to assess the Vickers hardness values for the dentin at a standard distance of less than 0.5 mm from the root canal walls.

In the current investigation, each irrigating solution was submerged for five minutes to approximate the period the irrigant solution would be applied clinically. According to the present study Cruz-Filho et al [12], Sayin et al. [15], Ulusoy et al [16] and Aslantas et al [17] employed root canal irrigants for five minutes in their microhardness tests, claiming that this duration is more practical in terms of clinical practise. Furthermore, De-Deus et al [18] assessed the impact of 17% EDTA for one, three, and five minutes on the microhardness of radicular dentin. They discovered that EDTA caused the largest reduction in microhardness from the reference state to 3 minutes, after which it remained unchanged. The effects of 2.5% and 6% salt were examined by Goldberg et al. Hypochlorite solutions were applied to root dentin for a variety of irrigation times, and it was discovered that irrigation for 5 minutes did not significantly affect dentin microhardness.

The results of this part showed that (17% EDTA + 5.25% NaOCL) recorded the highest microhardness reduction value followed by (CSNPs+CHX) Followed by (MgO) Followed by saline.

The results were full agreement with **Zaparolli et al** [19] who demonstrated that microhardness was reduced the most with EDTA/NaOCl against distilled water or NaOCL alone. **Ghisi et al** [20] found that EDTA with or without NaOCL have the most powerful effect in microhardness reduction against super-oxidized water and distilled water. **Kottor et al** [21] found that EDTA/NaOCL have greater effect in microhardness reduction than Qmix/NaOCL. **Nikhil et al** [22] found that Dentin microhardness was decreased more by EDTA than chitosan. **Bahgwat et al** [23] found that microhardness of dentin was most affected by EDTA in comparison to 4% propolis and 18% etidronic acid (HEBP). **UnniKrishnan et al** [24] found that EDTA+NaOCL group decreased microhardness much more in comparison to 10% citric acid, 17% Ethylene glycol tetraacetic aacid (EGTA) and MTAD solution.

The results were not in agreement with **Elgendy** [25] where they reported that 0.2% chitosan and 4% propolis were significally reduce dentin microhardness more 2.6% NaOCL or 17% EDTA. The disagreement may be related to the usage of pure chitosan rather than chx loaded chitosan nano particles. **Antunes et al** [26] reported that 15% EDTA did not show much difference against 0.2% chitosan on dentin microhardness by conventional irrigation, and both materials have the similar effect on dentin microhardness when activated by endovac. This difference in results may be due to the usage of different material concentration and activation of the irrigation materials by endovac.

CONCLUSION

Under the condition of the present study, it can be concluded that:

1- 5.25% NaOCL and 17% EDTA showed the highest percentage decrease in microhardness value.

2- CHX+CSNPs and MgO are suitable endodontic solutions because of their lower impact on microhardness in comparison to 5.25NaOCL + 17% EDTA.

3- The coronal third of the root canal is the most affected third by irrigation solutions, so it needs conservative approach.

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Non-Funded research.

Conflict of interest

The author deny conflict of interest related to this study.

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