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Efficiency of Newly Introduced Root Canal Irrigants Based on Nano Particles (In Vitro Study)

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Aim of the study: This study aims to test the efficacy of newly introduced nano particles irrigations along with the golden standard irrigation regarding their antibacterial effect and the altering of dentin composition within the root canal dentin.
Materials and methods: A total of sixty-five single rooted human teeth were collected after being freshly extracted. Each tooth was accessed and prepared using one shape rotary files and then manual step back technique was performed at the apical portion to a size of 50#. The teeth were then divided into: antibacterial test group (45 teeth) and dentin composition test group (20 teeth).
Results: Regarding the antibacterial effect, among the study groups: Chitosan (nano particles) recorded the highest mean value followed by Silver (nano particles) the least mean value was recorded in sodium hypochlorite with no significant difference between these 3 groups. Regarding the dentin composition effect: Sodium hypochlorite showed the high mean calcium weight at middle and apical root levels followed by silver nano particles and chitosan nano particles. Sodium hypochlorite showed the highest mean phosphorous weight at middle and apical root levels followed by silver nano particles and chitosan nano particles. The calcium to phosphorous ratio among root levels apical and middle showed no significant difference among the study groups; But they all exhibited a higher Ca:P ratio to the control group which was statistically significant.
Conclusion: Silver Nano particles irrigation and Chitosan Nano particles irrigation showed lower antibacterial effects against *E. faecalis* biofilm than sodium hypochlorite irrigation. Similar effect on dentin composition was noticed among the three irrigants used silver Nano particles irrigation, Chitosan Nano particles irrigation and Sodium Hypochlorite irrigation. Chitosan Nano Particles irrigation and silver Nano particles irrigation showed no advantage to Sodium hypochlorite regarding; antibacterial effect nor the effect on dentin composition.

Keywords: Root Canal Irrigants, Nano Particles

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INTRODUCTION

There are numerous important treatments involved in the control of endodontic infection, but irrigation is critical in the removal of germs from the root canal. Irrigation of the root canal is a vital step in assuring root canal cleansing and eradicating bacterial toxins and biofilm, in addition to equipment deficiencies and anatomical problems.

Irrigation has numerous functions, the most important of which are flushing away loose debris, lubricating the dentinal walls, dissolving organic waste in the canal, and acting as an antibacterial agent (). According to research, if no irrigant is used during instrumentation, there will be around 70% more debris in the root canals than in irrigated canals (2). The mechanical and chemical effects of the irrigant have a significant impact on the intracanal cleansing and disinfection process.

According to research, the primary cause of pulpal and periapical disease is microbial infection within the root canal system, and bacteria in mature biofilms are difficult to eradicate due to their natural resistance to antimicrobial therapies. Meanwhile, current cleaning and shaping techniques cannot provide a clean, non-bacterial root canal, necessitating the use of an effective chemical irrigant to aid in the elimination of bacteria and associated toxins.

The Ca/P ratio of hydroxyapatite in dentin is regarded to be the fundamental component of tooth hard tissue surfaces. Certain chemical compounds have been shown in investigations to alter the chemical structure of human dentin and influence the Ca/P ratio of the dentin surface.

This change in the Ca/P ratio may have an impact on the initial ratio of organic and inorganic components. As a result, dentin permeability and solubility are altered, as is the adherence of dental materials to hard tissues.

For many years, sodium hypochlorite at a concentration of 0.5-5.25 percent has been the gold standard and one of the most regularly used irrigation solutions in endodontics. Sodium hypochlorite has various features, such as antibacterial activity due to its proteolytic potential and tissue-dissolving ability, but there are also drawbacks, such as toxicity and the removal of only organic debris from the smear layer, as well as an unpleasant odor and taste. Furthermore, NaOCl reduces dentin micro-hardness, alters its flexural strength and modulus of elasticity, and causes irreversible dentin microstructure erosion.

Nanoparticles have paved the door for new research opportunities in the fight against tooth illness and bacterial biofilm. Nanoparticles have recently been discovered to be useful in irrigation solutions, medications, and as an additive in sealers and restorative materials. Nanoparticles have polycationic or polyanionic properties, a wide surface area, and a high positive charge density, all of which contribute to their antibacterial action. The design, development, and use of structures, devices, and systems at the nanoscale scale by managing shape and size is referred to as nanotechnology (1 nm to 100 nm). It is a developing field with several applications in science and technology, particularly in the production of new materials. In recent decades, silver nanoparticles have been one of the most appealing research topics. Silver nanoparticles have exceptional antibacterial activity even at low concentrations due to their high surface-to-volume ratio. They are also cheap and have low cytotoxicity and immunological response.

They can also be used for disinfection due to their antibacterial properties. They are effective against a wide range of bacteria, including E. faecalis.

Similarly, chitosan, a cationic biopolymer, has aroused researchers' interest

in recent years due to its low toxicity and bio-adhesive properties. Chitosan is a naturally occurring polysaccharide that has aroused the interest of dental researchers because of its biocompatibility, biodegradability, bioadhesiveness, and lack of toxicity. Chitosan possesses a wide spectrum of antibacterial actions as well as a strong ability to chelate various metal ions in acidic conditions.

As a result, the effect of silver and chitosan nanoparticles in dispersion in conjunction with sodium hypochlorite irrigation, as well as the effect of these irritants on calcium to phosphorous ratios in root dentin, should be investigated.

AIM OF THE STUDY

The aim of this study was to evaluate the efficiency of two newly introduced root canal irrigants regarding antibacterial effect and effect on dentin composition.

MATERIALS & METHODS

Materials:

Table 1: Materials and instruments used.

Material Used	Composition	Manufacturer
Sodium hypochlorite	(5.25 % Sodium hypochlorite)	Clorox, Egyptian co, for household, Cairo, Egypt
Chitosan nano particles	2% chitosan nanoparticles in 80 ml suspension	Nano Gate company Nasr city, Cairo, Egypt
Silver nano particles	2% silver nanoparticles in 80 ml suspension 23 ppm	Nano Gate company Nasr city, Cairo, Egypt
K files	Manual K files Sizes 15 to 45	Maillefer DENTSPLY, Tulsa, USA
Rotary files	One Shape file M Wire Niti file	MicroMega Besançon Cedex - France
Rotary device	Endomotor System Speed from 350 to 500 rpm and torque 2.6N/CM	NAKANISHI INC. Tochigi, Japan
Saline	Sterile Non pyrogenic* Each 100 ml contains: Sodium Chloride 0.9 G Water for injections Q.S Sodium 150 mEq/L Chloride 150 mEq/L	Otsuka Pharmacutical Co., S.A.E. - A.R.E. Cairo, Egypt
Paper points	Sizes 35 _40_45	Maillefer DENTSPLY, Tulsa,USA
Contra	High speed Hand piece	DENTSPLY Sirona Charlotte, NC 28277, USA
Stones	Diamond round bur Diamond Tapered stone	Pro Medica (Cairo, Egypt)
Diamond disc	Thin flex diamond disc Abrasive technology	The KERR Manufacturing Company (USA, California)
Irrigation needle	Side vented needle Gauge size 27	Bibo dent 10th of Ramadan, Cairo, Egypt
E-faecalis Biofilm	Clinical isolate of *E-faecalis* from the microbiology laboratories	Central Laboratories, Ministry of Health, Cairo, Egypt
Brain Heart Infusion broth and agar	Cultivating medium for *E-faecalis* bacteria	BHI, Difco Labratories, Detroit, MI, USA
0.9% physiologic saline solution	Intracanal fluids replacement	Al Mottahedoon Pharma, 10th Ramadan City, Cairo-Egypt

I. Sample Selection:

A total of 65 single-rooted completely erupted human upper anterior teeth were selected from the surgical department of the Faculty of Dentistry based on the following inclusion criteria:

- The teeth were preserved in pure distilled water and were caries-free, single-rooted, with no fissures and no internal or external root resorption. Teeth that showed signs of anatomic abnormalities were removed. A stereomicroscope was used to check the external root surfaces of teeth to rule out cracks.

II. Sample Preparation:

- Access cavities were done in all teeth by diamond round stone until dentin drop then walls smoothened into convenience form by tapered cylindrical diamond stone.
- Pulp extirpation was done using k files size 10 and 15. Distilled water was used to irrigate all root canals. K-file 15 and 10 negotiated all canals to full working length until patency was established. The working length was confirmed until the tip of the file 15 was visible at the foramen.
- Each single root was then prepared with Rotary file: One Shape in crown down technique to the full working length of each tooth using NSK Endo motor (Speed from 350 to 500 rpm and torque 2.6N/CM) with irrigation of distilled water in between files. Manual k files size 35 _40_45_50 were gradually used in turn and pull motion manually until apical one third enlarged to size 50.
- All teeth were stored in distilled water after preparation and autoclaved for 20 minutes at 121ºC.

III. Preparation of irrigating Solution:

A- Chitosan nano particles:

Preparation Method:

- Ionotropic gelation was used to generate chitosan nanoparticles. When a

tripolyphosphate (TPP) aqueous solution was added to a Chitosan solution, blank nanoparticles were formed.

1. Exams: Dimensions and form: TEM was performed on a JEOL JEM-2100 high resolution transmission electron microscope at a voltage of 200 kV. [19]

2. Characterization:

- Appearance (Color): White.
- Appearance (Form): Suspension Avg.
- Size (TEM): Less than 50 nm.
- Shape (TEM): Spherical shape Expiration date: 3 months.

B- Silver Nano Particles:

1. Preparation Method:

Turkevich, Lee, and Meisel published a paper on the chemical reduction technique used to create silver nanoparticles. An AgNO3 solution was used as a precursor for Ag1+ ions (20). The stabilizer PVP was utilized, and the moderate reducing agent borohydrate was used. The solution turned grayish yellow, suggesting that the Ag1+ ions had been transformed to Ag nanoparticles.

2. Characterization:

UV-Vis absorption spectra were obtained using an Ocean Optics USB2000+VIS-NIR Fiber optics spectrophotometer.

Size & Shape: A JEOL JEM-2100 high resolution transmission electron microscope was used to perform TEM at a voltage of 200 kV. [21]

3. Properties:

Appearance (Color): Yellow.
Appearance (Form): Suspension.
Concentration: 100 ppm.
Optical Prop. (Abs.): λ_{max} ~410 nm.
Avg. Size (TEM): less than 20nm.
Shape (TEM): Spherical shape.

C- Sodium hypochlorite:

Standard house bleach agent is 5.25% Sodium hypochlorite.

IV. Sample Classification:

Samples of 65 prepared teeth will be classified according to the evaluation parameter:

45 teeth for antibacterial test and 20 teeth for dentin composition test.

1- Antibacterial test:

45 teeth divided into 5 groups:

- Group (1) (10 teeth) for Sodium Hypochlorite (in 2ml volume and 5.25% conc.) red color code
- Group (2) (10 teeth) for Chitosan Nano particles suspension (in 80 ml of chitosan particles 2%) black color code
- Group (3) (10 teeth) for Silver Nano particles suspension (in 80 ml of silver nano particles 2%) blue color code
- Group (4) Positive control group (10 teeth) yellow color code
- Group (5) Negative control group (10 teeth) grey color code

2- Dentin composition test:

20 Teeth divided into 4 groups:

- Group (A): 5 Teeth for Silver Nano Particles Suspension (In 80 ml of silver particles 2%)
- Group (B): 5 teeth for Sodium Hypochlorite (In 2ml volume and 5.25% conc.)
- Group (C): 5 teeth for Chitosan Nano particles suspension (In 80 ml of chitosan particles 2%)
- Group (D): 5 teeth Negative Control Group

V. Evaluation of Antibacterial effect:

1-Bacterial Sample preparation:

- **Biofilm Development**

For biofilm formation, a clinical strain of E. faecalis from the microbiology laboratory was employed.

The bacterial strain was grown in Brain Heart Infusion broth for 24 hours at 37°C. Growing the biological marker on the surface of Brain Heart Infusion agar and incubating them under identical conditions produced the experimental suspensions. The bacteria were resuspended in saline and adjusted to a final concentration of roughly 3 x 108 cells/mL using the No. 1 MacFarland turbidity standard.

- **Biofilm formation:**
- The teeth were filled with a 24-hour pure culture suspension of E. faecalis cultivated in Brain Heart Infusion (BHI) broth in the experimental groups. All teeth were incubated in sealed vials at 37°C for 21 days to ensure biofilm growth, which was later confirmed by SEM. Intra-canal fluids were replenished every 72 hours with freshly prepared 0.9 percent physiologic saline solution adjusted to the No. 1 Mac-Farland turbidity standard.
- To test the sterility of the sampling methods, the samples were infected with sterile BHI broth and replaced with sterile saline every 72 hours in the negative control group.
- At the end of the incubation period:

The infected teeth was brought and Irrigation protocol took place in the following order:

Irrigation Protocol:

- **Group 1: (10 teeth) Sodium Hypochlorite:**

Step 1:

A plastic syringe with a needle of gauge (27) filled with the Sodium Hypochlorite Irrigant. Irrigation performed with 2ml of sodium hypochlorite in 5.25% conc.

Step 2:

Activation done using a K file (08-10) and each single rooted tooth consumed a time of 2 minutes of manual agitation after the canal was filled from apex to pulp chamber with sodium hypochlorite irrigant in the accepted volume and conc.

Step 3:

After the consumption of the 2 minutes protocol, irrigant was cleaned from the canal with saline solution using a gauge 27 needle and a plastic syringe for 2 minutes, then dried to the full working length with paper points size 40.

- **Group 2: (10 teeth) Chitosan Nano Particles:**

80 ml (2%) of chitosan nano particles suspension solution irrigated to each group through a plastic syringe gauge #27 for 2 minutes each tooth. Manual agitation with file 15 for 2 minutes and then dried with paper points size 15, 20 and 30 to the full working length of each tooth.

- **Group 3: (10 teeth) Silver Nano Particles:**

80 ml (2%) of silver nano particles 23 ppm suspension solution irrigated to each group through a plastic syringe gauge #27 and manual agitated for 2 minutes each tooth with file 15 and then dried with paper points size 15, 20, 30 to the full working length of each tooth.

- **Group 4: (10 teeth) Positive control:**

Biofilm production was assured by filling the teeth with a 24-hour pure culture solution of E. faecalis cultivated in Brain Heart Infusion (BHI) broth for 21 days.

Group 5 (5 teeth) Negative control:

To ensure the sterility of the sample approach, the teeth were inoculated with sterile BHI broth, which was replaced with sterile saline every 72 hours.

3- Bacterial sampling

The transport fluid was absorbed with a sterile paper tip and then transferred to a test tube containing 1.0 mL of saline. Each sample was vortexed for 30 seconds to ensure complete homogeneity. In saline, serial 10-fold dilutions (1:10, 1:100, and 1:1000) were performed. The colony-forming units (CFU) per 1 ml were counted after 0.1 mm of each dilution was put to the plate media (BHI agar plates) and incubated for 48 hours at 37°C.

4- Bacterial count:

The total colony forming units (CFU) per ml of sample were calculated by multiplying the number of visible E. faecalis colonies per plate by the matching dilution factor and by 10.

VI. Evaluation of Dentin composition

Irrigation Protocol:

- A Total of 20 teeth chosen according to criteria of Sample selection and Sample preparation mentioned above. Each tooth after being prepared to the full working length and apical size 40.
- Irrigation protocol regarding each group was achieved as mentioned before.

Group A: 5 prepared teeth irrigated with 5.25% sodium hypochlorite, agitation with file 15 done for 2 minutes and then washed out with 2% saline using a plastic syringe with a #27-gauge needle and then dried using paper points #40.

Group B: 5 prepared teeth irrigated with 2% silver suspension in 80 ml, agitation with file 15 done for 2 minutes and then washed out with 2% saline using a plastic syringe with a #27-gauge needle and then dried using paper points #40.

Group C: 5 prepared teeth irrigated with 2% chitosan suspension in 80 ml, agitation with file 15 done for 2 minutes and then washed out with 2% saline using a plastic syringe with a #27-gauge needle and then dried using paper points #10 and #15.

Group D: 5 prepared teeth as negative group irrigated with saline only.

Sample Preparation:

- Each tooth was then sectioned in half vertically using safe sided diamond disk to be tested where each half is of 2 mm thickness to be appropriate for EDX analysis.
- Samples was then stored in cool dry atmosphere and prepared for EDX analysis.

Testing Under SEM/EDX:

- • Each tooth was tested twice, once in the middle third and once in the apical third of the canal lumen at random locations.
- The atomic percentage in dentin is calculated using the line scanning function, which starts at the main canal wall and extends 300 meters into the dentin to the outside surface of the root canal lumen of each specimen. Two parts of each sample, the apical and middle third surfaces, were chosen and line scanned. The mean value of the atomic percentage of each of the two locations was obtained during the 300-m line scanning.
- Dentin composition was evaluated using EDX analysis regarding the Ratio between calcium and phosphorus at each area mentioned above in addition to any findings.

Statistical Analysis:

Antibacterial test:

For data administration and statistical analysis, the Statistical Package for Social Sciences (SPSS) version 18 was utilized. To describe numerical data, the mean, standard deviations, standard error, minimum, maximum, and confidence intervals were employed. The data's normality was investigated by analyzing the distribution and applying the Kolmogorov-Smirnov and Shapiro-Wilk tests. Data were normalized

and examined using a one-way analysis of variance (ANOVA) test, followed by a pairwise comparison using Tukey's post hoc test, if ANOVA revealed a significant difference between groups. The p-values are all two-sided. Significant P-values were defined as those less than 0.05.

RESULTS

I. Bacterial growth

Data were collected, tabulated, and statistically analyzed and shown in table (2).

A significantly higher mean value was recorded in positive control (2050±291.55), (p=0.00). Among the study groups, chitosan (nano) recorded the highest mean value (10.8±1.87, followed by silver (nano) (5.88±1.25), with the least mean value recorded in sodium hypochlorite (2.14±1.07), with no significant difference between these 3 groups (Table 2).

Table (2): Descriptive statistics and comparison of bacterial count (x10^2) in different groups.

	Mean	Std. Dev	Min	Max	
Silver (nano)	5.88^b	1.25	4.00	8.00	P 0.001*
Chitosan (nano)	10.80^b	1.87	8.00	15.00	
Sodium hypochloride	2.14^b	1.07	1.00	4.00	
Positive control	2050^a	291.55	1600.00	2500.00	
Negative control	0.00				

Significance level p≤0.05, * significant

II. Dentin composition:

I. Calcium (Ca) weight %

1. Effect of different irrigation solutions:

There was a statistically significant difference in mean Ca weight percent between the four groups at the middle root level (P-value = 0.003). Sodium Hypochlorite group had the greatest mean Ca weight percent. No statistically significant difference between the silver nanoparticles and control groups while Chitosan nanoparticles had the lowest mean Ca weight percent by a statistically significant margin. At apical root level; There was a statistically significant variation in mean Ca weight percent between the four groups (P-value = 0.023). Sodium Hypochlorite and control groups; both had the greatest mean Ca weight percent values. Silver nanoparticles had a statistically substantially reduced mean Ca weight percent followed by Chitosan nanoparticles.

Table (3). Calcium weight percentage in the four groups.

Root level	Sodium Hypochlorite		Silver nano-particles		Chitosan nano-particles		Control		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Middle	43.9 ^{A}	5.9	27.5 ^{B}	8.1	21.1 ^{C}	6.3	33.2 ^{B}	4.7	0.003*
Apical	41.7 ^{A}	6.3	24.1 ^{B}	15.5	17.6 ^{C}	6.6	39.8 ^{A}	6.3	0.023*
P-value	0.715		0.686		0.465		0.080		

***: Significant at P ≤ 0.05, Different superscripts capital letters in the same row are statistically significantly different and different superscripts small letters in the same column are statistically significantly different.**

2. Effect of root levels on calcium weight:

In all groups, there was no statistically significant difference between mean Ca weight % in middle and apical root levels (*P*-value = 0.715), (*P*-value = 0.686), (*P*-value = 0.465) and (*P*-value = 0.080), respectively.

II. Phosphorus (P) weight %

1. Effect of different irrigation solutions:

There was a statistically significant difference in mean P weight percent between the four groups at the middle and apical root levels (P-value = 0.010) and (P-value = 0.043), respectively. There was no statistically significant difference between the Sodium Hypochlorite and control groups; both had the statistically significant greatest mean P weight percent values. The group of silver nanoparticles had a statistically significantly reduced mean P weight percent. Chitosan nanoparticles had the statistically lowest mean P weight percent.

Table (4). Comparison between Phosphorus weight percentage in the four groups.

Root level	Sodium Hypochlorite		Silver nano-particles		Chitosan nano-particles		Control		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Middle	16.5 ^{A}	1.9	12.3 ^{B}	4.2	9.3 ^{C}	2.3	17.7 ^{A}	2.4	0.010*
Apical	16.6 ^{A}	2.4	10.6 ^{B}	7.3	7.5 ^{C}	2.9	17.5 ^{A}	2.4	0.043*
P-value	0.686		0.686		0.273		0.893		

***: Significant at P ≤ 0.05, Different superscripts in capital letters in the same row are statistically significantly different and different superscripts in small letters in the same column are statistically significantly different.**

2. *Effect of root levels on phosphorous weight:*

In all groups, there was no statistically significant difference between mean P weight % at middle and apical root levels (P-value = 0.686), (P-value = 0.686), (P-value = 0.273) and (P-value = 0.893), respectively.

III. Calcium to Phosphorus weight %

1. *Effect of irrigation solutions:*

There was a statistically significant difference in mean Ca:P ratio between the four groups at the middle root level (P-value = 0.014). There was no statistically significant difference between the Sodium Hypochlorite, Silver nanoparticles, and Chitosan nanoparticles groups; all had statistically significantly higher mean Ca:P ratios than the control group. At apical root level; there was no statistically significant difference between mean Ca:P ratio in the four groups (P-value = 0.822)

2. *Effect of root levels on calcium to phosphorous weight ratio*

In Sodium Hypochlorite, Silver nanoparticles and Chitosan nanoparticles groups; there was no statistically significant difference between mean Ca:P ratio at middle and apical root levels (*P*-value = 0.174), (*P*-value = 0.414) and (*P*-value = 0.468), respectively. While for control group; middle root level showed statistically significantly lower mean Ca:P ration than apical level (*P*-value = 0.004).

Table (5). Comparison between Ca:P ratio in the four groups.

Root level	Sodium Hypochlorite		Silver nano-particles		Chitosan nano-particles		Control		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Middle	2.65 ^{A}	0.04	2.29 ^{A}	0.32	2.26 ^{A}	0.48	1.89 ^{b}	0.16	0.014*
Apical	2.46	0.23	2.38	0.26	2.35	0.32	2.28	0.32	0.822
P-value	0.174		0.414		0.468		0.004*		

***: Significant at P ≤ 0.05, Different superscripts in capital letters in the same row are statistically significantly different and different superscripts in small letters in the same column are statistically significantly different.**

DISCUSSION

One of the great challenges of endodontic treatment is the efficacy of proper cleaning and shaping the root canal system to eliminate any bacterial content and to preserve dentinal root structure. Irrigation is a pivotal step in the elimination of bacteria inside the root canal system, the lubrication of the files against the dentinal wall, dissolving of the organic tissues and removal of antibacterial biofilm.

According to (Haapasalo et al., 2014) the ideal irrigant should contain the following properties: dissolution of organic and inorganic matter, good root canal system penetration, killing of planktonic and biofilm microbes, biofilm detachment, nontoxic to tissue, nonallergenic, does not react with other dental materials, and does not weaken dentin. [22]

Endodontic treatment must achieve the best possible decrease of bacterial infection in the root canal system to a healing level (Siqueira & Rôças, 2008[23] and elimination of intra-tubular disinfection must be effective for long-term clinical success for the reasons stated. Also, after using chemical irrigants in the root canal, residual chemical irrigants and their byproducts have been shown to diffuse along the dentin tubules, potentially affecting resin penetration into the dentin structure or monomer polymerization in demineralized dentin, both of which can reduce bond strength to dentin (Erdemir, 2004[10] Mineral

ratio changes, especially the Ca/P ratio, can affect dentin permeability, solubility, and demineralization. (Yassen et al., 2013[25]; Rotstein et al., 1996[24]).

The application of nanomaterials in endodontics is primarily focused on improving antibacterial activity, tissue regeneration and mechanical integrity of previously damaged dentin matrix. (Del Pozo-Rodríguez et al., 2007) [26].

In our study, For standardization, single-rooted teeth with roughly equal apical diameters and root lengths were gathered in our study. In the current work, human teeth were extracted and used to imitate clinical settings (Hashem et al., 2009) [27].

Roots were cleaned and shaped with One Shape file as it has less morphological changes on root dentin and canal anatomy (Santhosh A. et al., 2019) [28]. For the dentin composition test, each tooth was sectioned in half vertically using safe sided diamond disk to be tested where a half will be of 2 mm thickness to be appropriate for EDX analysis once at the middle third and once at the apical third at random spots inside canal lumen, Sectioning of the teeth also allows fast and quantitative estimation of mineral in a given tooth sample in a non-destructive and accurate manner. (Suzuki et al., 2019) [29]

Preparation of irrigants took place, the silver nano particle irrigation was done in suspension at 23 ppm as used by (Suzuki et al., 2019)[29] *E. faecalis* was utilized since it is the most resistant bacteria and has been detected in significant numbers at areas of persistent endodontic infections as well as most primary endodontic infections (Lins et al., 2013) [30] Hypochlorite is utilized in a variety of concentrations, but we used 5.25 percent to maximize its efficiency (Clegg et al., 2006) [31]

Because it is relatively straightforward to manage the depth of needle penetration and the volume of irrigant released via the canal, a side vented needle was chosen for irrigation inside the root canal. (Van der, et al., 2006) [32] Then a swab was taken from each root by paper point into a saline test tube to assess bacterial growth.

To evaluate antibacterial effect on specimens, colony counting unit test was performed as used by (Pereira et al., 2020) [33] who assessed the presence of microorganisms from root canal using colony counting unit to measure antibacterial effect of irrigation . In addition, the effect on dentin composition was evaluated using energy-dispersive X-ray spectrometer. Single spot analysis was obtained for an easier more common approach to dentin element changes caused by irrigation (Wang et al 2016) [12]. Each spot was 300um deep respectively in different areas (apical and middle) The mean value of the atomic percentage of each of the 2 elements in the 300-um spot scanning was calculated. Calcium phosphorus levels were measured at random spots apically and at the middle part of each specimen According to the recent study by (Suzuki at al., 2019) [29] and (Teixeira et al., 2009) [34]

Our antibacterial test found that Sodium Hypochlorite had the most antimicrobial action, followed by Silver Nanoparticles, which were more effective than Chitosan Nanoparticles in lowering bacterial counts. There were no significant differences between these three groups.

The antibacterial activities of AgNPs against *E. faecalis* biofilms were investigated by Wu et al., (2014) [3]. After 2 minutes of contact with the AgNPs solution, the biofilm structure was not disrupted, but Chávez-Andrade (2019) [5] discovered anti-biofilm and anti-adhesion capabilities with poly (vinyl alcohol)-coated silver nanoparticles (AgNPs-PVA). On a 2018 study by Rodrigues CT [7], however, NaOCl was found to have the best antibacterial activity and biofilm dissolving capacity when compared to AgNp solution, which had a lower

antimicrobial effect in infected dentinal tubules.

Contact-mediated death is the mode of action of chitosan nanoparticles This causes increased cell wall permeability and, eventually, cell rupture, as well as protein-aceous and other intracellular component leaks (Qi L et al., 2004) [35] Previous studies as (Silva et al., 2012)[36] have shown that solutions with significantly higher concentrations and longer application times than the 2 minutes used in this study are required to remove this layer, whereas (Spano et al., 2009) [37] concluded that 0.2 percent chitosan for 3 minutes was sufficient to remove the smear layer and caused less erosion than EDTA. Nonetheless the desired results were not obtained in our study at 2 minutes of contact during manual agitation and concentration of 2%.

For the dentin composition evaluation, EDX analysis for specimens were assessed: Statistically significant differences between the Sodium Hypochlorite, Silver nanoparticles, and Chitosan nanoparticles groups were not found in pair-wise comparisons between the groups without the control group; however, all had statistically significantly higher mean Ca:P ratios than the control group. There was no statistically significant difference in the mean Ca:P ratio between the four groups at the apical root level. Suzuki et al 2019 [29] assessed the effect of silver nanoparticles on interradicular dentin corresponds with our findings; there was no statistically significant difference in different thirds of intraradicular dentin according to the different solutions utilized, as we used a 23 ppm Ag solution in suspension. According to their findings, silver nano particle dispersion at the same concentration (23 ppm) had no effect on the mechanical properties of dentin

Dentinal tubules in the apical region are smaller and less numerous than those in the middle and coronal regions (Wang et al 2016) [12] ; similar to our findings, no significant differences in element distribution within each group were found in their study in the apical or middle regions; one explanation is that irrigant penetration into dentinal tubules in the apical region was as good as that in the other areas. Despite its acidic pH, Ururahy et al. (2016) [38] observed that chitosan has a remarkable ability to chelate calcium ions in dentin, resulting in the depletion of inorganic material from the smeear layer. Silva et al. (2013) [29] found that using 0.3 percent chitosan for 3 minutes effectively eliminated the stain, implying that the effect is time dependent.

Within the constraints of the current investigation, it is possible to conclude that 5.25 percent NaOCl caused a time-dependent decline in the calcium/phosphorous ratio, similar to the effect of Ag nanoparticles, with chitosan nanoparticles having the least influence. According to the findings of this study, co-siding with Ghisi et al., (2015) [40] 5 percent NaOCl acts aggressively in the organic component of dentin, not only superficially but also deeply; thus, the advantages and disadvantages of using it as a root canal irrigant should be carefully considered. While Silver Nanoparticles and chitosan should be investigated as alternate irrigants, more research on the duration of each irrigant's exposure to root dentin is needed.

CONCLUSIONS

Based on the results of the present study:

1- Silver Nano particles irrigation and Chitosan Nano particles irrigation showed lower antibacterial effects against *E. faecalis* biofilm than sodium hypochlorite irrigation

2- Similar effect on dentin composition was noticed among the three irrigants used silver Nano particles irrigation, Chitosan Nano particles irrigation and Sodium Hypochlorite irrigation.

3- Chitosan Nano Particles irrigation and silver Nano particles irrigation showed no advantage to Sodium hypochlorite regarding; antibacterial effect nor the effect on dentin composition.

REFERENCES

1. Siqueira Jr, Rôças, Favieri, Lima. Chemomechanical Reduction of the Bacterial Population in the Root Canal after Instrumentation and Irrigation with 1%, 2.5%, and 5.25% Sodium Hypochlorite. Journal of Endodontics; 2000;26:331–334.
2. Baker NA, Eleazer PD, Averbach RE. Scanning electron microscopic study of the efficacy of various irrigating solutions. Journal of Endodontics, 1975;1:127–35.
3. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. Journal of Endodontics. 2014;40(2):285-90.
4. Del Carpio-Perochena A, Bramante CM, Aouada FA, Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restorative Dentistry & Endodontics. 2015;40(3):195-201.
5. Chavez-Andrade GM, Tanomaru-Filho M, Rodrigues EM. Cytotoxicity, genotoxicity and antibacterial activity of poly(vinyl alcohol)-coated silver nanoparticles and farnesol as irrigating solutions. Archives of Oral Biology. 2017;84:89-93.
6. Plutzer B, Zilm P, Ratnayake J, Cathro P. Comparative efficacy of endodontic medicaments and sodium hypochlorite against Enterococcus faecalis biofilms. Australian Dental Journal. 2018;63(2):208-216.
7. Rodrigues CT, de Andrade FB, de Vasconcelos L. Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. International Endodontic Journal. 2018;51(8):901-11.
8. L. M. Jonck, hristopher Eriksson and N. R. Comins. An EDX Analysis of the Root Dentin in Teeth Treated Endodontically with Zinc Oxide and Eugenol. Journal of Endodontics _1979;5 (1) : 489-3.
9. Dogan H, Qalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. Journal of Endodontics. 2001;27(9):578-80.
10. Erdemir A, Eldeniz AU, Belli S. Effect of gutta-percha solvents on mineral contents of human root dentin using ICP-AES technique. Journal of Endodontics 2004;30(1):54-6.
11. Pascon FM, Kantovitz KR, Soares LE. Morphological and chemical changes in dentin after using endodontic agents: fourier transform Raman spectroscopy, energy-dispersive x-ray fluorescence spectrometry, and scanning electron microscopy study. Journal of Biomedical Optics. 2012;17(7):075008.
12. Wang Z, Maezono H, Shen Y. Evaluation of Root Canal Dentin Erosion after Different Irrigation Methods Using Energy-dispersive X-ray Spectroscopy. Journal of Endodontics. 2016;42(12):1834-9.
13. Wang TF, Feng XW, Gao YXl. Effects of different concentrations and exposure time of sodium hypochlorite on the structural, compositional and mechanical properties of human dentin. Journal of Huazhong University of Science and Technology Medical sciences 2017;37(4):568-76.
14. Saha SG, Sharma V, Bharadwaj A. Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. Journal of Clinical and Diagnostic research : JCDR. 2017;11(4):ZC01-ZC4.
15. Kucukkaya Eren S, Uzunoglu E, Sezer B, Yilmaz Z, Boyaci IH. Mineral content analysis of root canal dentin using laser-induced breakdown spectroscopy. Restorative Dentistry & Endodontics. 2018;43(1):e11.
16. Souza MA, Lago BLT, Pletsch A. Association of calcium hypochlorite, reciprocating instrumentation and photodynamic therapy: Antimicrobial analysis and effects on root dentin structure. Photodiagnosis and photodynamic therapy. 2020;29:101625.
17. Yin IX, Zhang J, Zhao IS. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. International Journal of Nanomedicine. 2020;15:2555-2562.
18. Parolia A, Kumar H, Ramamurthy S, Davamani F, Pau A. Effectiveness of chitosan-propolis nanoparticle against Enterococcus faecalis biofilms in the root canal. BMC Oral Health. 2020;20(1):339.
19. Hasanin, Mohamed T., et al. Production of Well-Dispersed Aqueous CrossLinked Chitosan-Based Nanomaterials as Alternative Antimicrobial Approach. Journal of Inorganic and Organometallic Polymers and Materials, 2018; 1502-1510.

20. Hwang, M.G., Katayama, H. & Ohgaki, S. Inactivation of Legionella pneumophila and Pseudomonas aeruginosa: evaluation of the bactericidal ability of silver cations. Water Research, 2007; 41, 4097-4104.

21. Durán, N.; Marcarto, P.D.; De Souza, G.I.H. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of Biomedical Nanotechnology, 2007; 3(2): 203-208.

22. Haapasalo M, Shen Y, Wang Z. Irrigation in endodontics. British Dental Journal . 2014;216(6):299-303.

23. Siqueira JF Jr, Rôças IN, Ricucci D. Causes and management of post-treatment apical periodontitis. British Dental Journal. 2014;216(6):305-312.

24. Rotstein I, Dankner E, Goldman A, Heling I. Histochemical analysis of dental hard tissues following bleaching. Journal of Endodontics. 1996; 22(1):23-25

25. Yassen GH. The orthograde application of mineral trioxide aggregate apical plug may be an effective treatment approach in teeth with open apices. Dental Materials Journal. 2018;37(2):192-196.

26. Del Pozo-Rodríguez A, Delgado D, Solinís MA. Solid lipid nanoparticles: formulation factors affecting cell transfection capacity. International Journal of Pharmaceutical sciences and research. 2007;339(1-2):261-268.

27. Hashem AA, Ghoneim AG, Lutfy RA. The effect of different irrigating solutions on bond strength of two root canal-filling systems. Journal of Endodontics. 2009;35(4):537-540.

28. Santosh A, Kakade A, Mali S, et al. Scanning electron microscopic observation of primary tooth dentin following final irrigation with 95% ethanol: An in vitro study. Journal of Indian Society of Pedodontics and preventive Dentistry. 2019;37(3):271-274.

29. Suzuki TYU, Gallego J, Assunção WG. Influence of silver nanoparticle solution on the mechanical properties of resin cements and intrarradicular dentin. PLoS One journal. 2019; 14(6):e0217750.

30. Lins RX, de Oliveira Andrade A, Hirata Junior R. Antimicrobial resistance and virulence traits of Enterococcus faecalis from primary endodontic infections. Journal of Dentistry. 2013;41(9):779-786.

31. Clegg MS, Vertucci FJ, Walker C. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. Journal of Endodontics. 2006;32(5):434-437.

32. Van der Sluis LW, Gambarini G, Wu MK. The influence of volume, type of irrigant and flushing method on removing artificially placed dentine debris from the apical root canal during passive ultrasonic irrigation. International Endodontic Journal. 2006;39(6):472-476.

33. Pereira TC, Dijkstra RJB, Petridis X. The influence of time and irrigant refreshment on biofilm removal from lateral morphological features of simulated root canals. International Endodontic journal. 2020; 53(12): 1705-1714.

34. Vilela Teixeira AB, de Carvalho Honorato Silva C, Alves OL. Endodontic Sealers Modified with Silver Vanadate: Antibacterial, Compositional, and Setting Time Evaluation. Biomed Research International journal. 2019;4676354.

35. Qi L, Xu Z, Jiang X. Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate Research journal. 2004;339(16):2693-2700.

36. Silva PV, Guedes DF, Pécora JD. Time-dependent effects of chitosan on dentin structures. Brazilian Dental Journal. 2012;23(4):357-361.

37. Spanó JC, Silva RG, Guedes DF. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. Journal of Endodontics. 2009;35(5):727-730.

38. Ururahy MS, Curylofo-Zotti FA, Galo R. Wettability and surface morphology of eroded dentin treated with chitosan. Archives of Oral Biology. 2017;75:68-73.

39. Silva PV, Guedes DF, Nakadi FV. Chitosan: a new solution for removal of smear layer after root canal instrumentation. International Endodontic Journal. 2013;46(4):332-338.

40. Ghisi AC, Kopper PM, Baldasso FE, et al. Effect of superoxidized water and sodium hypochlorite, associated or not with EDTA, on organic and inorganic components of bovine root dentin. Journal of Endodontics. 2015;41(6):925-930.