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COMPARATIVE STUDY OF THE ANTIBACTERIAL EFFECT OF NANO-SILVER IRRIGANT, SODIUM HYPOCHLORITE AND CHLORHEXIDINE AGAINST ENTEROCOCCUS FAECALIS BIOFILM

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ABSTRACT

This study aimed to compare the antimicrobial effect of Nano-Silver Irrigant, Sodium Hypochlorite and Chlorohexidine against Enterococcus Faecalis bifilm. 60 Sixty, recently extracted, sound maxillary anterior teeth with completely formed apices and straight roots were selected in this study. Canals were instrumented using the Protaper Next system (Dentsply Sirona) 1 mm shorter from the apical foramen up to size X5 (size# 50/06). Sterility of the teeth after complete root canal cleaning and shaping were achieved by autoclaving for 15 min at 121ºC with the teeth immersed in distilled water. Enterococcus Faecalis Biofilm was prepared by mixing 5 mL of the bacterial inoculum with sterilized BHI, then inoculated in teeth samples for 60 days. Teeth were then divided after the incubation period into three groups; 20 teeth each, and were treated by delivering the each of the tested irrigating solution using sterile plastic syringes. The first group was treated with Nano-Silver 0.02mol/L (2000ppm) while the second was treated using with Sodium Hypochlorite (NaOCl) 5.25% and the third was treated usinwith Chlorohexidine (CHX) 2.0%. Serial rinses ensured 5 minutes contact period between the aseptic solution and the bacteria. Sterile paper points size 50 were introduced into the canals and maintained for 3 min for sample collection, after which the paper points were placed in test tubes containing 2ml of sterile saline and several dilutions were prepared. 100 μL of each dilution was applied to the blood agar culture plates and incubated at 37ºC for 24 hours. A classical bacterial counting technique was used for each group after the treatment application for the recovery of viable E. faecalis on agar plates The results showed that the NaOCl had the highest antibacterial activity with high statistically significant difference with the other groups followed by the chlorhexidine group which also show statistically significant difference than the other two groups, then came the nano silver irrigant group which showed the lowest antibacterial activity among all the groups with statistically significant difference with them.

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INTRODUCTION

Bacteria are the main etiology for the development of the dental caries and consequently to the progress of pulp and periapical diseases. [1]

The most common bacterial species recovered from the root filled teeth are *Enterococcus faecalis*.

Enterococci are gram positive cocci that can occur singly, in pairs or as small chains. They are facultative anaerobes, having the ability to grow in the absence or presence of oxygen also can cause different periradicular diseases and more likely in persistent lesions. [2]

This type of bacteria can survive different intracanal medications such as CaOH that leads to alkaline enviroment. [3]

E. faecalis possesses certain virulence factors that allows it to alter host response by adhering to host cells. These virulence factors include cytolysin, lytic enzymes, lipoteichoic acid and pheromones.

Studies showed the resistance of *E. faecalis* against intracanal medications is due to their ability to form a biofilm. Biofilm is composed of extracellular polymeric matrix which protect bacteria from nutrient deprivation, high alkaline media and salt concentrations caused by intracanal medicaments.[4,5]

The available endodontic methods for bacterial reduction include mechanical instrumentation and chemical disinfection.

The mechanical instrumentation includes cleaning and widening of root canal while the chemical disinfection includes the use of intracanal irrigation and medication.[6]

Therefore, widening of the apical third of the root canal will help eliminating microorganism allowing the instrument to reach inaccessible areas. This facilitates bacterial removal residing inside the dentinal tubules and allowing antimicrobials to penetrate effectively. [2, 7]

Sodium hypochorite (NaOCl), chlorhexidine gluconate (CHX), ethylenediaminetetacetic acid (EDTA), mixture of Tetracycline isomer acid and detergent (MTAD) and ozonated water are types of antimicrobial irrigating solutions. [8]

Celaletin and Ozkan in 2017 studied the various effect of irrigants and concluded that the future studies should concentrate on production of a single irrigant that is able to solubilize tissues, remove smear layer, biocompatible and has antibacterial effects. [9,10]

Sodium hypochorite (NaOCl) is the most commonly used root canal irrigant. It dissolves organic tissues inside the root canals and has antibacterial action. Several disadvantages for NaOCl includes inability to completely remove the smear layer, unpleasant taste, toxicity, minimizing the dentin modulus of elasticity and affects adhesion of resin to root dentin. [11, 12, 13]

Chlorohexidine (CHX) is widely used as mouth rinse for periodontal diseases and has been used as irrigating solution and intra-canal dressing. [14]

Chlorhexidine has an antibacterial effect against Gram-positive and Gram-negative bacteria and acceptable biocompatibility. Nevertheless, it has a significantly lower ability to dissolve biofilms compared with sodium hypochlorite. [15, 16]

The capability of disinfecting of difficult-to-reach areas such as lateral canals, fins and apical delta was also questionable. [12]

Due to this, researcher have focused on developing new medicaments based on nanomaterial to improve the properties of antibacterial agents used in root canal treatment. [17]

Nanomaterials has dimensions of 1–100nm, presenting small sizes, large surface/area mass ratio and increased chemical reactivity. [18] Their big surface area and charge density enable them to interact with the negatively charged surface of

bacterial cells, resulting in enhanced antimicrobial activity; thus, they have been applied in many health care fields. [13]

Silver nanoparticles have been used in dentistry in several forms. They have been incorporated in restorative materials and bonding agents to reduce caries and preventing biofilm formation into implant materials and into orthodontic adhesives [19, 20, 21]

Silver nanoparticles has direct effect on gram positive and negative bacteria. They release silver ions by penetrating their cell walls disturbing their functions leading to biofilm inhibition. [22]

Silver nanoparticles is being used in different forms; root canal irrigant, intracanal medicament, incorporated in root canal filling materials and bioceramic cements with decreased cytotoxic effects. [23, 24, 25, 26, 27]

This study aimed to evaluate the antibacterial effect of three different irrigating solutions against *E. faecalis*.

MATERIALS AND METHODS

Selection and preparation of teeth

Sixty, recently extracted, sound maxillary anterior teeth with completely formed apices and straight roots were selected to be used in this study from the surgery clinic October 6 University. The teeth were scaled and cleaned of debris and periodontal remnants. Samples were stored in distilled water until used.

Initial radiographs were taken, standard access cavities were prepared and working lengths were detected. Canals were instrumented using the Protaper Next system (Dentsply Sirona) 1 mm shorter from the apical foramen up to size X5 (size# 50/06).

During instrumentation saline was used as root canal irrigant. 17% EDTA followed by 5.25% NaOCl were used in a sequential manner to remove smear layer for 3 min each. The inactivation of NaOCl was then accomplished by the addition of 10 µl of 5% of sterile sodium thiosulphate pipetted into each tooth. Sterility of the teeth after complete root canal cleaning and shaping were achieved by autoclaving for 15 min at 121ºC with the teeth immersed in distilled water.

Biofilm formation

Enterococcus faecalis (#29212, ATCC Manassas, VI) was inoculated in 7 mL of brain heart infusion (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37˚C for 24 hours. Then, under the same incubation conditions suspensions were prepared on the surface of BHI plates; bacterial cells were resuspended in saline and adjusted to the# 1 McFarland turbidity standard (3×10^8 cells/ mL).

Five millimeters of the sterilized BHI were mixed with 5 ML of the bacterial inoculum, and then injected inside the root canals using sterile syringes for 60 days. At 72 hours intervals this process was repeated, always using 24-hours pure cultures prepared and adjusted to the #1McFarland standard. The teeth were kept in humid environment at 37˚C. All experimental procedures were carried out under septic conditions.

Tested materials:

1) Nano-Silver irrigant 0.02mol/L = 2000ppm.

2) Sodium Hypochlorite (NaOCl) irrigant 5.25%.

3) Chlorohexidine (CHX) 2.0%.

Canal treatment

After the incubation period for the biofilm formation the teeth were then divided into three groups; 20 teeth each, and were treated as follows:

1) Group A

The teeth were treated with Nano-Silver 0.02mol/L (2000ppm) by delivering the solution using sterile plastic syringes. Serial rinses ensured 5 minutes contact period between the aseptic solution and the bacteria.

2) Group B:

The teeth were treated with Sodium Hypochlorite (NaOCl) 5.25% by delivering the solution using sterile plastic syringes. Serial rinses ensured 5 minutes contact period between the aseptic solution and the bacteria.

3) Group C:

The teeth were treated with of Chlorohexidine (CHX) 2.0% by delivering the solution using sterile plastic syringes. Serial rinses ensured 5 minutes contact period between the aseptic solution and the bacteria.

Root canals in all groups were dried with sterile paper points and refilled with sterile distilled water using a sterile syringe. Thereafter, sterile paper points size 50 were introduced into the canals and maintained for 3 min for sample collection.

The points of each group were individually transported to test tubes containing 2 mL of sterile physiologic saline, and serial dilutions were prepared. 100 μL of each dilution was applied to the blood agar culture plates and incubated at 37ºC for 24 hours. All procedures were conducted inside a laminar flow chamber using sterile instruments to achieve strict asepsis.

A classical bacterial counting technique was used for each group after the treatment application for the recovery of viable E. faecalis on agar plates. The purity of the positive cultures was confirmed by Gram staining, by colony morphology on BHI agar $^{+}$ blood. The mean value of CFU for the plates of each group was then calculated

RESULTS

Statistical analysis

Kruskal Wallis ANOVA test followed by Mann-Whitney U test were used to compare between different parameter in the three studied groups. Data were revealed as mean ± SD. Statistical Package for Social Sciences (SPSS) computer program (version 19 windows) was used for data analysis. P value ≤ 0.05 was considered significant.

The lowest bacterial count was recorded by NaOCl (0.13±0.04) followed by Chlorhexidine group (1870.0±296.09), while the highest was recorded by Nano-silver (19500.00±4203.17) irrigant group.

The results showed that the NaOCl had the highest antibacterial activity with high statistically significant difference with the other groups. The Nano silver irrigant group showed the lowest antibacterial activity among all the groups with statistically significant difference with the other groups.

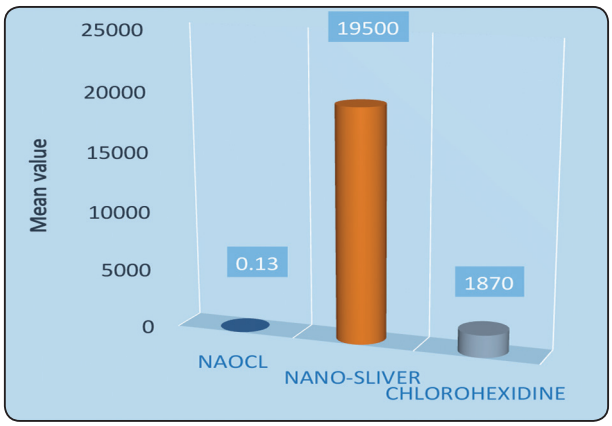

Fig. (1) Mean values of the bacterial count of the three studied groups.

TABLE (1): Comparison between mean values of the bacterial count of the three studied groups.

	NaOCl (n= 4)	**Nano-sliver (n= 4)**	**Chlorohexidine (n= 4)**	**P value**
Mean ± SD	0.13 ± 0.04	19500.00 ± 4203.17	1870.0 ± 296.09	0.007*
P value vs NaOCl	----	0.021*	0.029*	
P value vs Nano-Sliver	----	----	0.021*	

p≤ 0.05= Significant

DISCUSSION

Biofilms are made up of an extracellular polysaccharide matrix which make it difficult to eradicate the microorganisms that lies inside. Biofilms developed in vitro for short periods of time may not have the same resistance as a mature biofilm. [13]

In root canal treatment, biofilm dissolution is required due to the inability of the endodontic files to reach all areas inside the root canal. [16]

In the present investigation NaOCl as well as CHX irrigants exert their antimicrobial effect immediately after contact with bacterial suspension. This was in agreement with Vianna et al., 2004 who stated CHX and NaOCl liquids show an antimicrobial effect faster than gel formulation, which is more difficult to mix, prevented direct contact with the bacterial cells, thus requiring longer time to act against the microorganisms.[14]

Sodium hypochlorite had been the most efficient irrigating solution against *E. faecalis* this came in agreement with different studies that used NaOCl in deifferent concentrations. [13, 16, 28]

CHX is recommended as an alternative irrigant to NaOCl, due to its biocompatibility. The antimicrobial effect of CHX is related to its cationic molecule binding to negatively charged bacterial cell walls, consequently altering the bacterial osmotic equilibrium.

In the present study, the 2% of Chlorohexidine irrigant showed significantly better results in comparison to silver nanoparticles irrigant which is in agreement with previous study by Yadav et, 2017 who found that 2% of chlorohexidine had significantly better results than silver nanoparticles combination, although the combinations of silver nano particles with CHX and CaOH had better antimicrobial efficacy over time periods against *E. faecalis*. [29]

On the other hand, the inability of 2% chlorhexidine to eliminate biofilm has been demonstrated in previous studies. [15, 16, 31]

Studies showed that CHX and NaOCl does not inactivate lipopolysaccharide (LPS), which is a structural component of the Gram-negative bacteria's outer cell envelope and can be either secreted in vesicles by growing organisms or released after the organism's death. Despite this fact, if there is no symptoms and canal dryness is achieved a single visit root canal treatment could be successfully achieved. [14]

Silver nanoparticles irrigant have been suggested to be used against *E. faecalis* due to its ability to detach biofilm from root dentin. [28, 30, 32]

Silver nanoparticles increase cell membrane permeability and prevent DNA replication.[17,18,22] despite their antibacterial effectiveness, they also have adverse effects on human health. Their small size, chemical composition and non specific oxidative damages may lead to environmental toxicity. [22, 33]

The 2000 ppm silver nanoparticle solution tested was not effective in disrupting *E. faecalis* biofilm when compared with NaOCl and CHX. A previous study also demonstrated that AgNp as an irrigant had no capacity for disrupting biofilm. [28]

On the other hand, when Rodrigues et al, at 2018 used AgNp irrigant for 5 and 15 min, the total biovolume of biofilm was significantly lower compared with chlorhexidine.

It is important to clarify that silver nanoparticle used in this study was made with an aqueous vehicle with no additions in order not to provide any additional antibacterial effects.

The characteristics of nanoparticles, such as contact time, concentration, particle size and surface charge, influence their antimicrobial action against bacterial cells and mature biofilm. [23, 28, 30]

In this study, 24 hours of contact with *E. faecalis* biofilm was less effective in killing bacteria compared with chlorhexidine and NaOCl with the same time. These results were in agreement with Rodrigues et al studies when declared that, NaOCl had a significantly greater antimicrobial activity and more biofilm elimination ability with time intervals from 5 to 30 minutes compared with AgNp and CHX solutions. This differing from the findings of another study by Afkhami et al. that reported irrigation with 100 ppm AgNp had similar antimicrobial efficacy as that of 2.5% NaOCl. [34]

The resistance offered by the biofilm matrix and the insufficient time for interaction between positively charged AgNps and negatively charged bacterial cells are possible explanations for these results. A previous study, used an irrigant containing silver nanoparticles for 2 min and AgNp gel as a medicament for 7 days, and found that only the AgNp gel was able to disrupt *E. faecalis* biofilm. [28] Therefore, it was suggested that silver nanoparticles when used as an intracanal medication and not as an irrigant eliminate bacterial biofilms from inside root canals. [17, 28, 35]

CONCLUSIONS

- Sodium hypochlorite is the most effective antibacterial irrigant against *E. faecalis*.
- The silver nanoparticle solution was not effective in dissolving *E. faecalis* biofilm when used as root canal irrigant.

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