



Effect of Different Nano Irrigant Solutions as a Final Rinse on Depth of Penetration of Bioceramic Root Canal Sealer

(An in vitro study)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سببناك لا تعلم لنا
إلا ما علمتنا إنك أنت
العظيم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Dedication

To the soul of my father, who taught me to believe in God, myself.

To my mother who is my role model, she inspires and motivates me to grow without any barriers. To my sister, the pillar of my life for her endless support and help.

I dedicate this work.....

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List of Abbreviations

Abb.	Full term
°C	<i>Celsius</i>
<i>BCSHF</i>	<i>Bioceramic sealer hiflow</i>
<i>CBCT</i>	<i>Cone beam computed tomography</i>
<i>CHX</i>	<i>Chlorohexidine</i>
<i>CLC</i>	<i>Cold lateral condensation</i>
<i>CLSM</i>	<i>Confocal laser scanning microscopy</i>
<i>CNI</i>	<i>Conventional needle irrigation</i>
<i>CSBs</i>	<i>Calcium silicate-based sealers</i>
<i>DOM</i>	<i>Dental operating microscope</i>
<i>EA</i>	<i>Endo activator</i>
<i>EDTA</i>	<i>Ethylenediaminetetraacetic acid</i>
<i>ER:YAG</i>	<i>Erbium:yttrium–aluminum-garnet</i>
<i>kHz</i>	<i>kilohertz</i>
<i>MDA</i>	<i>Manual dynamic activation</i>
<i>MTA</i>	<i>Mineral trioxide aggregate</i>
<i>N/cm</i>	<i>Newton centimeter</i>
<i>NaOCl</i>	<i>Sodium hypochlorite</i>
<i>NiTi</i>	<i>Nickel Titanium</i>
<i>NPs</i>	<i>Nano particles</i>
<i>PBS</i>	<i>Phosphate buffer solution</i>
<i>PIPS</i>	<i>photon-initiated photoacoustic streaming</i>
<i>PUI</i>	<i>Passive ultrasonic irrigation</i>
<i>RCS</i>	<i>Root canal sealer</i>
<i>Rpm</i>	<i>Rotation per minute</i>
<i>SEM</i>	<i>Scanning electron microscope</i>
<i>SEM</i>	<i>Scanning electron microscopy</i>
<i>SMTSC</i>	<i>Single matched tapered sized cone</i>
<i>SWEEPS</i>	<i>Shock wave enhanced emission photoacoustic streaming</i>
<i>TEM</i>	<i>Transmission electron microscopy</i>
<i>WVC</i>	<i>Warm vertical compaction</i>
<i>XPF</i>	<i>XP finisher</i>

1. INTRODUCTION

The basic requirements of root canal treatment procedure are effective mechanical preparation, proper disinfection and three-dimensional sealing of complex root canal system therefore, many final rinse solutions have been developed in an attempt to disinfect it and remove the smear layer ⁽¹⁾.

Root canal irrigant solutions have been employed along with mechanical instrumentations to eliminate the pulp remnants and the smear layer formed after preparing the root canal. Irrigant solutions can also open the dentinal tubules to clean the root canal space adequately and therefore resulting in better conditions for subsequent canal obturation and adhesion. Moreover, smear layer removal from the root canal may improve the contact of root canal filling materials and root canal sealers with the canal walls and thus may improve the penetration of sealers into the dentinal tubules ⁽²⁾.

There are various irrigants used in root canal treatment but none of them meet all the requirements to be considered the ideal irrigant. The most commonly used irrigants are sodium hypochlorite (NaOCl), Ethylenediaminetetraacetic acid (EDTA), chlorhexidine (CHX) ⁽³⁾.

Nano-irrigants may help in improving the cleanliness of the root canal system by deeper penetration into the dentinal tubules as they have distinct advantages and unique physico-chemical properties in comparison to bulk counterparts due to their high surface to volume ratio. Several nanoparticle materials are available such as Silver nanoparticles (AgNPs) which introduced as an irrigant, intracanal medicament and incorporated in root canal filling due to strong antibacterial effect,

Chitosan nanoparticles is a natural biopolymer have been established for antimicrobial applications, Magnesium oxide nanoparticle (MgoNPs) is excellent antimicrobial irrigant with minimal cytotoxic effect on living cells, Titanium dioxide nanoparticles (TiO₂NPs) are highly stable particles with superior antibacterial property ⁽⁴⁾.

In order to agitate and improve the flow of irrigants to the intricacies of root canal system by mechanical or other energy forms. There are many irrigant activation systems available in the market. Some of them use ultrasonic activation by high frequency, low frequency by sonic devices, manual dynamic agitation or by rotary files like XP endo finisher that helps three-dimensional cleaning of unreachable areas ⁽⁵⁾.

The complete sealing and filling of the cleaned and shaped root canal system are important steps that can affect the long-term success of root canal treatment. Because of the complexity of root canal system, sealers need to be used to fill the irregularities and to penetrate deep into dentinal tubules to obtain a fluid tight seal of the root canal system. Meanwhile, root canal sealers should provide adherence between gutta-percha and dentinal walls to avoid gap occurrence at the sealer-dentin interface ⁽⁶⁾.

Traditional obturating sealers do not provide the best seal as they shrink after having been set, have little or no adhesion to dentin, and are not dimensionally stable when in contact with moisture, which will likely lead to leakage over time leading to recontamination and cytotoxic to periradicular tissues ⁽⁷⁾.

Recently bioceramic sealers have been developed to overcome the drawbacks of the other sealers. The potential advantages of bioceramic materials in endodontics are related to their physico-chemical and biological properties. Bioceramics are biocompatible, non-toxic, non-shrinking, and usually chemically stable within the biological environment. A further advantage of these materials is their ability to form hydroxyapatite and ultimately create a bond between dentin and the material ⁽⁸⁾.

The impact of nano final rinse solutions at various root levels on the penetration of bioceramic sealers has not been well studied. This study spots the light on the depth of penetration of bioceramic sealer into the dentinal tubules utilizing nano technology.

2. REVIEW OF LITERATURE

2.1 Irrigation of the root canals.

2.1.1 Different root canal irrigants.

2.1.2 Effect of root canal irrigants on depth of sealer penetration into dentinal tubules.

2.2 Activation of the root canal irrigants.

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2.4. Effect of different obturation techniques on depth of penetration of various root canal sealers.

2.5. Methods of evaluation of depth of penetration of root canal irrigants and sealers into dentinal tubules.

2.1 Irrigation of the root canals

Chemical debridement and disinfection of the root canal system has an important role in success in root canal treatment procedure. The bacterial biofilm and variable number of micro-organisms are obstacles to completely clean root canals. The irrigants used in chemical debridement should have the ability to penetrate dentinal tubules and maintain long term antibacterial effect on planktonic microbes and microbes in biofilm as well as remove the smear layer as it affects the penetration depth of variable sealers ⁽⁹⁾.

2.1.1 Different root canal irrigants.

Sodium hypochlorite (NaOCl) is the most used irrigation in endodontics due to the anti-microbial effect and proteolytic action on organic tissues NaOCl reacts with organic tissue resulting in saponification, amino acid neutralization, and chloramine reactions. NaOCl ionizes in water into Na^+ and the hypochlorite ion OCl^- establishing an equilibrium with hypochlorous acid (HOCl). At acidic and neutral PH chlorine exists predominantly whereas at high PH above (9) OCl^- predominates. Hypochlorous acid is responsible for the anti-bacterial effect. Whereas hypochloric acid disrupts vital functions of the microbial cell, Although, the hypochlorite alone does not remove smear layer, it affects the organic part of the smear layer making its complete removal is possible with subsequent irrigation with chelating agents ⁽¹⁰⁾.

Forough et al.⁽¹¹⁾ evaluated the effectiveness of different concentrations of sodium hypochlorite solution in reducing bacterial growth of *E.faecalis* in root canals. Chemo-mechanical preparation was done for 104 roots of maxillary central incisors.104. In order to remove

the smear layer, 5.25% sodium hypochlorite solution was used for 3 minutes in the root canals. Then, the samples were immersed in 1 mL of 17% EDTA for 3 minutes. Finally, the root canals were irrigated with phosphate-buffered saline (PBS) solution. Then *E. faecalis* biofilms formed within the root canals for 4, 6, and 10 weeks were evaluated. Each group was divided into 4 subgroups in terms of the antibacterial treatment: group 1: 1% NaOCl solution; group 2: 2.5% NaOCl solution; group 3: 5.25% NaOCl solution; and group 4: PBS solution. After preparation of root canal filings, the counts of live bacteria were calculated through the classic method of counting: colony forming units (CFU). In groups 2 and 3, there was no bacterial growth due to complete removal of *E. faecalis* biofilms, while the bacterial counts in group 1 at 4, 6 and 10 weeks decreased compared to the control group, so the recommended concentrations of sodium hypochlorite to obtain the most anti-bacterial effect are 2.5% and 5.25%.

Moreover, NaOCl is a potential irritant to periapical tissues associated with accidental extrusion ⁽¹²⁾. On contrary, sodium hypochlorite cannot dissolve inorganic dentin and smear layer ⁽¹³⁾.

Ethylenediaminetetraacetic acid (EDTA) is generally used after endodontic instrumentation for its chelating action by which it removes the inorganic part of smear layer. EDTA was introduced in 1957 by Ostby, in the form of aqueous solution at 15.5% and pH 7.3. It has the ability to demineralize dentin and remove the inorganic component of the smear layer. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentin to a depth of 20-30 μm in 5 min. The application of EDTA resulted

in effective opening of the dentinal tubules with very little superficial smear debris ⁽¹⁴⁾.

EDTA can be used as a final rinse to increase the amount of opened dentinal tubules. Also, the aggressive effect of EDTA was noted on root canal walls that appeared as erosion and degradation which interfere with the mechanical properties of dentin. Also, it may remain inside the canals after finishing the root canal preparation which could affect the adaptation of filling material. Demineralization takes a place after using the chelating agents depends on the of time application ⁽¹⁵⁾.

Citric acid (CA) is a weak organic tribasic acid occurring naturally in citrus fruit, used as chelating irrigant in concentration between 1-50% to remove smear layer after root canal instrumentation. Citric acid shouldn't be used with sodium hypochlorite as it reduces the available chlorine ⁽¹⁶⁾. **Machado et al.** ⁽¹⁷⁾ compared the effectiveness of smear layer removal and sealer penetration when either 17% EDTA or 10% CA was used. They found that sealer penetrates the dentinal tubules equally with both chelating solutions. pH and time of exposure are the main factors that affect the amount of smear layer removal.

Chlorhexidine (CHX) is used for disinfection of the root canals because of its good anti-microbial activity through the cationic ions that are negatively charged. They rapidly attract to the inner cell membrane of the bacteria and other microbes and exerts a bactericidal effect. Chlorhexidine lack of the tissue dissolving capacity exhibited by sodium hypochlorite, however the substantivity of chlorhexidine provides an advantage over sodium hypochlorite ⁽¹⁸⁾.

Hydrogen peroxide, one of the anti-microbial irrigant solutions, acts through the reaction of superoxide against lipid structure of the micro-organism. Hydrogen peroxide produces nascent oxygen which reduces the flushing property of the irrigants ⁽¹⁹⁾.

MTAD is a mixture of 3% doxycycline, 4.25% citric acid and polysorbate detergent. MTAD was introduced by Torabinejad and Johnson at Loma Linda University in 2003. The use of MTAD has been reported to be more efficient in removing smear layer as compared with the use of EDTA and NaOCl, especially from the apical third ⁽²⁰⁾.

Torabinejad et al. ⁽²¹⁾ showed that MTAD effectively removed smear layer and did not significantly change the structure of the dentinal tubules when used after NaOCl (5.25%) as compared with irrigation with EDTA and 5.25% NaOCl.

QMIX is a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX and detergent. **Stojicic et al.** ⁽²²⁾ reported that QMiX and NaOCl were superior to CHX and MTAD under laboratory conditions in killing *E. faecalis* in planktonic and biofilm culture. QMiX is comparable to EDTA in smear layer removal due to presence of polyaminocarboxylic acid ⁽²³⁾.

Maleic acid (MA) is a mild organic acid used as an acid conditioner in adhesive dentistry. It has been found to possess the smear layer removing quality when used as an acid etchant in restorative dentistry. It was found that final irrigation with 7% maleic acid is more efficient than 17% EDTA in the removal of smear layer from the apical third of the root canal system ⁽²⁴⁾.

Nanotechnology is a science dedicated to the manipulation of materials and structures, materials or solutions in nanometer scale range from 1 up to 100 nanometers. The term nano originated from the Greek meaning dwarf. Nano sized particles, such as clusters of small numbers of atoms or molecules in nanostructured biomaterials, used in biomedical research have demonstrated significantly superior properties from the properties of the same material at larger scales of measurement. Nanomaterials are available in various size and shape ⁽²⁵⁾. Nanomaterials have various properties like ultra-small sizes, large surface area to mass ratio and increased chemical reactivity. In endodontics it was introduced to improve antimicrobial efficacy, tissue regeneration and mechanical integrity of already affected dentin. Based on the composition, nanoparticles are categorized according to the origin into naturally occurring or synthetic, according to shape and structural composition. In comparison to bulk materials, nanoparticles are low solidicity modicum with low coordination and unsatisfied bond, so these particles are easy to interconnect with other molecules easily. These particles exhibit a high surface area to volume ratio. Likely to bulk materials, nanoparticles disrupt the cell wall synthesis, but it also inhibits various enzymes ⁽²⁶⁾.

Chitosan is a deacetylated derivative of chitin and one of the natural biopolymers. Chitosan have been established mainly to be antimicrobial and when used as an irrigant in endodontic treatment it enhances disinfection. Chitosan nanoparticles have the potential to be used as a final irrigant due to its ability to remove smear layer and reduce bacterial biofilm ⁽²⁷⁾.

Silva et al. ⁽²⁸⁾ studied the time-dependent effects of chitosan on dentin structures. The aim is to evaluate the effects of chitosan. At

different concentrations on the removal of the smear layer and on dentin structure after 3 and 5 min of application. They used 12 recently extracted maxillary canine teeth. Instrumentation done using the crown down technique and irrigated with 1% sodium hypochlorite. The specimens were distributed according to the time and concentration of the final irrigating solution: G1: 0.1% chitosan for 3 min; G2: 0.2% chitosan for 3 min; G3: 0.37% chitosan for 3 min; G4: 0.1% chitosan for 5 min; G5: 0.2% chitosan for 5 min; G6: 0.37% chitosan for 5 min. All samples were prepared for scanning electron microscope analysis. G1 exhibited removal of the smear layer, but not the smear plugs. G2 showed visible and open tubules with slight erosion of the peritubular dentin. Cleaning in G3 was similar to that in G2, however, the erosive effect was greater. There was expansion of the diameter of the tubules in G4; and in G5 and G6, there was severe erosion with deterioration of dentin surface. They conclude that 0.2% chitosan for 3 min appeared to be efficient for removing the smear layer, causing little erosion of dentin.

Silver nanoparticles (AgNPs) have been used in many health-care fields because of their broad-spectrum antibacterial and antiviral properties. AgNPs have a high surface area to volume ratio and unique chemical and physical properties, which result in increased reactivity. A previous study showed that 0.1% AgNPs solution has a strong antibacterial effect against *E. faecalis* biofilm formed on dentin after 24 hours of exposure. Despite this antibacterial effectiveness, the probable adverse effects of AgNPs such as cytotoxicity to the host cells ⁽²⁹⁾. silver nanoparticles have demonstrated the same bactericidal effect as 5.25% NaOCl against *E. faecalis* ⁽³⁰⁾. Silver nanoparticles (AgNPs) are utilized as final irrigant due to its supposed ability to remove smear layer ⁽³¹⁾.

However, silver in various application methods should be used with caution because of its toxicity ⁽³²⁾.

Titanium dioxide nanoparticles (TiO₂NPs) have also been used in dentistry as an antibacterial agent via lipid peroxidation causing disruption of microbial cell wall. TiO₂NPs have high antibacterial properties and have a high biocompatibility in tissue environment ⁽³³⁾.

2.1.2 Effect of root canal irrigations on depth of sealers penetration into dentinal tubules.

Tuncer et al. ⁽³⁴⁾ evaluated the effect of different irrigant solutions on sealer penetration into dentinal tubules using confocal laser scanning electron microscope. 32 human lower premolars were used. The samples were divided into 4 groups according to the final irrigation used: (1) 17% EDTA + 2.5% NaOCl, (2) 7% MA + 2.5% NaOCl, (3) 10% CA + 2.5% NaOCl, and (4) the control group: 2.5% NaOCl. All teeth were obturated using the cold lateral condensation technique with gutta-percha and AH26 sealer labeled with fluorescent dye. The teeth were sectioned at 2, 5, and 8 mm from the root apex. They concluded that the coronal sections in each group showed a significantly higher percentage and maximum depth of sealer penetration than did the apical and middle sections. Final irrigation with EDTA, MA, and CA after the use of NaOCl affected sealer penetration. However, there was no significant difference between these experimental groups (EDTA, MA, and CA) in all sections.

Aydin et al. ⁽³⁵⁾ compared the effect of chitosan nanoparticle, QMix, and 17% EDTA on the penetrability of a bioceramic sealer into dentinal tubules using a (CLSM). 60 lower premolars were selected and randomly divided into 3 groups (n=20) before root canal preparation.

according to the solution used in the final rinse protocol: chitosan, QMix, and EDTA groups. The teeth were filled with a TotalFill BC sealer single gutta-percha cone and with 0.1% Rhodamine B. The specimens were horizontally sectioned at 3 and 5 mm from the apex. Total percentage and maximum depth of sealer penetration were measured. They concluded that greater depth of sealer penetration was recorded at 5 mm as compared to 3 mm in all the groups. Within the limitation of the present study, it can be concluded that QMix and EDTA promoted sealer penetration superior to that achieved by chitosan nanoparticle.

Martinho et al. ⁽³⁶⁾ analyzed the penetration of bioceramic sealer into dentinal tubules after using different irrigations using confocal laser scanning microscopy (CLSM). 29 teeth with single roots were separated into 3 groups according to the final irrigation protocol: G1 (n= 10) EDTA + 3% (NaOCl), G2 (n= 10) 17% EDTA + 2% CHX and G3: Control group (n= 9) 17% EDIA + saline solution. Root canals were filled with MTA Fillpex sealer and gutta-percha. The sealer was labelled Rhodamine B. The teeth were segmented at the middle and apical sections. They concluded that final irrigation with NaOCl promoted similar sealer penetration in the apical and middle sections. On the other hand, a significant decrease in the sealer penetration of the middle section was observed for the CHX and saline groups. Compared to other irrigants, NaOCl promotes more uniform sealer penetration.

Shenoy et al. ⁽³⁷⁾ evaluated the effect of final rinsing solution on smear layer removal through penetrability of the root canal sealer. 30 teeth were selected and prepared till H-file no 30. 3% NaOCl was used during cleaning and shaping of the canals. The teeth were divided into 3 groups of 10 teeth each. G I was irrigated with 17% EDTA, G II with

TUBLICID plus, and G III with Biopure MTAD. ISO 30 size gutta-percha points were selected as master cones. The Acroseal sealer was mixed with 0.1% fluorescent Rhodamine B dye. Obturation was done and after 48 hours, the roots were sectioned and subjected to CLSM. They concluded that the maximum depth of sealer penetration was observed in G II.

Jardine et al. ⁽³⁸⁾ compared the effect of Mix, BioPure MTAD, 17% EDTA, and saline on the penetrability of a resin-based sealer into dentinal tubules using a CLSM and to describe the cleaning of root canal walls by SEM. 80 disto-buccal roots of upper molars were selected and divided into 4 groups (n=20) before root canal preparation according to the solution used in the final rinse protocol: QG (QMix), MG (BioPure MTAD), EG (17 % EDTA), and CG (control group: saline). 10 roots of each group were prepared for SEM. The remaining canals were filled with a single gutta-percha cone and AH Plus with 0.1 % Rhodamine B. The specimens were horizontally sectioned at 4 mm from the apex. They concluded that QG and EG presented similar amounts of sealer penetration. MG and CG presented the lowest penetrability values. 17% EDTA and QMix promoted sealer penetration superior to that achieved by BioPure MTAD and saline.

Abusteit et al. ⁽³⁹⁾ assessed the depth of sealer intra- tubular penetration following different final rinses using CLSM and indirectly evaluated precipitation of irrigations. 52 maxillary incisors were prepared to size #40/04 with 6% NAOCL irrigating solution. Teeth were randomly divided into 4 groups (n=13) according to final rinse. G 1: 17% EDTA, Saline and 2% CHX solution; G2: Smear OFF; G 3: Qmix; and G4: saline control. Obturation was performed with gutta percha and resin sealer

mixed with Rhodamine B dye. Teeth were sectioned into apical, middle and coronal thirds from apex. They concluded that G 3 had the highest depth of penetration. There were statistically significant differences between G2 and G3 and control for all sections, G1 and control in apical section only. Recently developed final rinses produced higher sealer penetration and more patent dentinal tubules than using EDTA and CHX.

Moon et al. ⁽⁴⁰⁾ evaluated the effect of different final irrigations on the sealer intra-tubular penetration of curved canals using CLSM. The mesio-buccal canals from 45 upper and lower molars were prepared. The samples were divided into 3 groups according to the final irrigation used into group N (control), 3.5% NaOCl; group E, 17% (EDTA); and group EN, 17% EDTA followed by 3.5% NaOCl. All teeth were obturated with gutta-percha and AH plus sealer labeled with fluorescent dye. Transverse sections at 2 mm and 5 mm from root apex were examined. They concluded that the apical sections in each group showed significantly lower penetration than the coronal sections. In apical levels, group E and EN resulted in a higher penetration than the control group. In curved canal, final rinse with NaOCl after the use of EDTA had no additional effect on sealer penetration. Complete debridement with EDTA remains a challenge in the apical area of curved canals.

Machado et al. ⁽¹⁷⁾ compared the efficacy of different chelating solutions (17% EDTA and 10% CA) on the smear layer removal, and their effect on tubular dentin sealer penetration. 60 root canals were prepared and distributed into four groups (n=15) according to the final irrigation protocol: G1, final irrigation with 2.5 mL of distilled water; G2, final irrigation with 2.5 mL of 2.5% NaOCl; G3, final irrigation with 2.5 mL of 17% EDTA; and G4, final irrigation with 2.5 ml of 10% CA. 5

specimens from each group were not filled to assess smear layer removal by SEM. 10 specimens from each group were filled for analysis of sealer penetration into dentinal tubules by CLSM. They concluded that G3 and G4 had greater smear layer removal in the cervical and middle thirds, in comparison with G1 and G2 and G4 had the highest percentages of sealer penetration in all thirds, in comparison with G1 and G2.

Thota et al. ⁽⁴¹⁾ evaluated the effect of different irrigating solutions used in final irrigation on depth of sealer penetration into dentinal tubules. 30 recently extracted, human mandibular premolar teeth with single canals were randomly divided into two groups, and one of the two irrigants was used in each group. Group A (Chitosan) and Group B (EDTA), all the teeth were obturated with gutta-percha and AH 26 sealer labeled with fluorescent dye. The teeth were sectioned at distances 2, 5, and 8 mm from the root apex. Maximum depth of sealer penetration was measured using CLSM. They concluded that at coronal third depth, the sealer penetration was greater in (EDTA) group, and the depth of sealer penetration was greater at apical third in chitosan group. Final irrigation with EDTA and chitosan after the use of NaOCl affected sealer penetration.

2.2 Activation of the root canal irrigations.

Conventional irrigations methods deliver the irrigant just 1mm beyond the needle tip and the irrigation is purely dependent on positive pressure injection to flow. This may help microbes to persist and thrive after root canal treatment in lateral, accessory canals, fins and anastomoses. Activation of the irrigation may be defined as using a method either in energy or mechanical form to agitate and improve the

flow of the irrigants to the intricacies of the complex root canal system. There are many methods for activation ⁽⁴²⁾.

2.2.1 Different activation methods

Conventional needle irrigation (CNI) has been advocated as an efficient method of irrigant delivery. This technique involves dispensing the irrigant into the canal through needles with variable gauges passively or with agitation. The latter is done by moving the needle up and down. Variable designs of the needles in order to dispense the irrigation either from the end or through the side. Side vented needles improve the hydrodynamic effect of the irrigant and reduce the chance of apical extrusion. To avoid the extrusion of the irrigant apically its crucial to remain the needle loose inside the canal ⁽⁴³⁾.

Manual dynamic activation (MDA) is achieved by gently moving a well-fitting gutta-percha master cone up and down in short 2- to 3-mm strokes while the cone is grasped 1mm set back from the working length within an instrumented canal can produce an effective hydrodynamic effect and significantly improve the irrigant flow. the push-pull motion of a well-fitting gutta-percha point in the canal might generate higher intracanal pressure changes during pushing movements, leading to more effective delivery of irrigant to the untouched canal surfaces the frequency of push-pull motion of the gutta-percha point 100 strokes per 30 seconds ⁽⁴⁴⁾.

Ultrasonic irrigation

The literature describes that there are two types of ultrasonic irrigation. The first one is the simultaneous of ultrasonic irrigation with instrumentation, while the second is without instrumentation which is

known as passive ultrasonic irrigation (PUI). The first one has been discarded in the daily practice because the difficulty of controlling the cut of dentin resulting in altering the canal morphology ⁽⁴⁵⁾. During PUI the energy is transmitted from the oscillating wire at frequencies between 25-30kHz to the irrigant by waves that induce two physical phenomena. The first one is acoustic streaming which is a rapid movement of the irrigant in a circular or a vortex shape, the second is cavitation by creation of bubbles inside the irrigation. Two flushing methods can be used during PUI, continuous method by uninterrupted supply of irrigant which is responsible for dissolving the organic pulpal tissue and have antibacterial property. The intermittent flush by injecting the irrigant with a syringe then activated by the oscillating wire and the canal is refilled several times after each activation cycle ⁽⁴⁶⁾.

Sonic irrigant activation

EndoActivator System (EA) (Dentsply Tulsa Dental Specialties, Tulsa, OK) is a sonically driven irrigant activation system designed to produce vigorous intracanal fluid agitation that has been shown to increase the efficacy of irrigation activation better than traditional needle irrigation ⁽⁴⁷⁾.

EDDY (VDW, Munich, Germany) is a new sonic device, which is driven by an air scaler at a rate of about 6000 Hz. EDDY (VDW) has a non-cutting disposable polyamide tip, which prevents cutting of the root canal dentin. EDDY (VDW) transmits a vibration to the moving polyamide tip in a high amplitude oscillating motion due to the high frequency vibration. The three-dimensional movement results in highly

effective release, which promotes the cleaning effect, such as cavitation and acoustic flow in the irrigation solution ⁽⁴⁸⁾.

Negative pressure irrigation

EndoVac system is a negative pressure system invented by John Schoeffel. The system generates negative pressure that draws the irrigation apically via high volume suction. The system is composed of a master delivery tip (MDT), macrocannula and microcannula. The MDT delivers copious amounts of irrigation inside the access. The macrocannula removes debris from inside the canal. The microcannula evacuates the debris and irrigation from the apical part down to the level of the working length ⁽⁴⁹⁾.

XP endo-finisher (XPF) has been introduced to improve the irrigant efficiency. XPF is an ISO 25 without tapering produced using a special alloy called martensitic, austenitic electropolished file -X (MaxWire) martensite-austenite electropolish-flex. The file is straight in M phase at room temperature and change into A phase when the file is exposed to body temperature. In the A phase the file has a unique spoon shape with a length of 10 mm from the tip. It is suggested to be used at 800 RPM and torque of 1 N/cm ⁽⁵⁰⁾.

Laser irrigant activation

It has been introduced in addition to the traditional procedures. Photon-induced photo acoustic streaming (PIPS) is a laser agitation technique, which uses an erbium:yttrium–aluminum-garnet (Er:YAG) laser. This technique is based on the high absorption of Er:YAG laser wavelength into water-based irrigants that fill the pulp chamber. When an Er:YAG laser is shot in aqueous medium, the irrigants are locally and

instantly heated beyond their boiling point and a vapor bubble starts to form at the fiber tip's end after each pulse. This vapor bubble collapses after reaching its maximum volume with a subsequent cavitation effect. This phenomenon produces turbulent photoacoustic agitation of irrigants streaming the fluids three-dimensionally throughout the root canal system and leads to effective removal of smear layer. SWEEPS (Shock Wave Enhanced Emission Photoacoustic Streaming) is a more recent Er:YAG laser modality launched to improve the cleaning and disinfecting efficacy by photon-initiated photoacoustic streaming (PIPS) technique. It is based on the emission of a couple of consecutive laser pulses, with the second subsequent laser pulse that shoots into the liquid at an optimal delay time from the first pulse, when the initial bubble is in the final phase of its collapse. PIPS and SWEEPS techniques only require the tip to be placed into the coronal reservoir of the pulp chamber ⁽⁵¹⁾.

2.2.2 Effect of activation methods on depth of sealers penetration into dentinal tubules.

Ismail et al. ⁽⁵²⁾ studied the comparison of sealer Penetration by using different irrigation techniques. They used 48 single rooted maxillary central incisors were taken. Access cavity was done then canal instrumentation was prepared by step back technique and the apical preparation was done till 40 k-file. The samples were randomly assigned into three experimental groups based on the final irrigation technique used. Group I: Apical negative pressure (Endovac); Group II: passive ultra sonic irrigation (PUI); Group III: combination of apical negative pressure and PUI. All the samples were obturated using AH plus sealer and the sections were observed under confocal laser scanning microscope to evaluate the percentage and maximum depth of sealer penetration at

1mm, 3mm and 5mm levels. They conclude that the combination group was the only group to achieve better sealer penetration at 1mm and 3mm levels from the working length.

Aydin et al. ⁽⁵³⁾ studied the effect of different irrigation activation techniques on sealer Penetration. They used 45 mandibular premolars that were instrumented up to size #30/ 09 taper and randomly divided into three groups (n = 15) depending on the final irrigation activation technique: EDDY, PUI or CNI. After the final irrigation procedures, the root canals were obturated with labelled sealer mixed with 0.1% Rhodamine B. Then transverse sections at 3, 5 and 7 mm from the root apex were examined using CLSM to measure the maximum depth and total area and percentage of sealer penetration. They found that the penetration depth in the EDDY group the was higher compared to the CNI group in the apical and middle sections and compared to the PUI group in the apical section ($P < 0.05$). The penetration area in the EDDY group was higher compared to the CNI group in all sections and compared to the PUI group in the coronal section. The percentage of penetration was higher in the EDDY group compared to the CNI group in all sections and compared to the PUI group in the coronal section. They conclude that sealer penetration was superior in the EDDY group than the CNI group in the apical section. In the middle and coronal sections, sealer penetration was similar for the EDDY and PUI groups.

Mancini et al. ⁽⁵⁴⁾ evaluated the effectiveness of different irrigating techniques in removing the smear layer at 1,3,5 and 8 mm from the apex of the root canals. They used 65 extracted single rooted human premolars and decoronated at 16 mm length to standardize the root length. Specimens were randomly divided into 2 control groups (n = 10) and 3

experimental groups (n = 15) according to the activation method (sonic by EndoActivator, passive ultrasonic and negative apical pressure by EndoVac. Specimens were shaped up to F4 protaper rotary file and irrigated with 5.25% NaOCl. Root canals were split longitudinally and observed by field emission scanning electron microscope (SEM) to evaluate the presence of debris and smear layer at all sections. They found that The EndoActivator system was more efficient than passive ultrasonic irrigation and control groups in removing smear layer at 3,5,8 sections. The EndoVac System removed more smear layer at all sections. At 5 and 8 mm from the apex the EndoVac and PUI didn't differ statistically significantly, but both are better than the control groups.

Yilmaz et al. ⁽⁵⁵⁾ studied the effectiveness of various final irrigation techniques on sealer penetration in curved roots. They used 65 freshly extracted maxillary first molar teeth with mesio-buccal roots having more than 20° of root curvature were used. The root canals were instrumented and randomly divided into four experimental groups and one control group. In the four experimental groups, 3 ml of EDTA followed by 3 ml of NaOCl was delivered with the use of the following protocols: Group 1: manual dynamic activation (MDA), Group 2: sonic irrigation (SI), Group 3: passive ultrasonic irrigation (PUI), Group 4: conventional needle irrigation (CI). All teeth were obturated with gutta-percha and AH plus and sealer labeled with fluorescent dye. Transverse sections at 2 mm and 4 mm distance from the root apex were examined with the aid of confocal laser scanning microscopy. Total percentage and maximum depth of sealer penetration were measured. They found that PUI, SI, and MDA did not significantly improve sealer penetration in the apical portion of curved root canals when compared to conventional needle irrigation.

2.3 Different calcium silicate-based sealers (CSBS)

calcium silicate-based sealers (CSBs) demonstrate favorable properties such as hydrophilic nature, high PH above 12, antimicrobial properties, expansion on setting, insolubility in the presence of tissue fluids and osteogenic potential ⁽⁵⁶⁾.

2.3.1 Portland cement

Derived from the calcination of mixture of the limestones from portland and silicon-argillaceous materials. Portland cement exhibits both antibacterial and antifungal that are similar to MTA ⁽⁵⁷⁾. Portland cement produces large amounts of lead and arsenic released from portland cement added to its high solubility when compared to MTA and it concerns about the safety with respect to the surrounding tissues. Uncontrolled setting expansion of Portland cement could lead to crack formation on the tooth ⁽⁵⁸⁾.

2.3.2 Mineral trioxide aggregate (MTA) sealers are calcium silicate cement, consisting of tricalcium silicate, dicalcium silicate and tricalcium aluminate. The radiopaque compound is bismuth oxide. The material comes in two forms, grey and white. In the first form grey color is given by iron ions, which were removed to obtain the white form. MTA's setting reaction is by hydration, obtaining hydrated calcium silicate and calcium hydroxide, which is released over time, therefore the MTA is strongly antimicrobial. MTA's biological integration is due to the calcium ions which form hydroxyapatite in contact with phosphate ions present in the body. MTA Fillapex is a sealer that is composed of MTA, salicylate resin, natural resin, bismuth oxide, and silica. A recent study showed that

this sealer has suitable physicochemical properties, such as good radiopacity, flow, and alkaline PH ⁽⁵⁹⁾.

2.3.3 Bioceramic sealer can be categorized as bioinert with biological systems and bioactive that can undergo interfacial interactions with surrounding tissues (bioactive glasses, bioactive glass ceramics, hydroxyapatite, calcium silicates, alumina, zirconia). The setting reaction of their main component calcium silicate results in precipitation of calcium phosphate. In addition, calcium phosphate enables to form the chemical composition and crystalline structure similar to teeth. The improved bond between a sealer and root dentine encourages bioactivity and tissue growth in comparison to other commercially available root canal sealers ⁽⁶⁰⁾. bioceramic sealers have been advocated for use in a single-cone technique since the slight expansion of the material negates minimizing the amount of sealer. sealing ability as assessed by fluid filtration, and push-out bond strength, have shown that bioceramic sealers maintain the ability to provide an adequate seal when used with a single cone obturation technique ⁽⁶¹⁾.

2.4. Different obturation techniques and their effect on depth of penetration of various types of sealers

Dasari et al. ⁽⁶²⁾ evaluated the impact of three different obturation techniques on the penetration depth and adaptation of a bioceramic root canal sealer (BioRoot RCS) to root dentin using scanning electron microscope and confocal laser scanning microscope. In this study a recently extracted ninety mandibular premolar teeth of similar dimensions with straight single root canals were grouped into three experimental groups (n=30 each group) based on the obturation technique: lateral

compaction technique, warm vertical compaction technique and injectable thermo-plastisiced technique. After obturation half of the root samples in each group were sectioned horizontally by hard tissue microtome at 3, 6 and 9 mm respectively from root apex to measure the depth of penetration of the sealer using CLSM, other halves were sectioned longitudinally to measure the adaptation of the sealer to radicular dentin with CLSM. The results showed that warm vertical condensation technique penetrated deeper root dentin penetration of the sealer. Better adaptation of the sealer to apical root dentin with minimal voids was achieved with warm vertical compaction in comparison to the injectable gutta-percha method and lateral condensation obturating techniques.

Alshehri et al. ⁽⁶³⁾ studied the quality of obturation and presence of voids in the apical third of the root with the warm vertical compaction (WVC) and single matching taper sized cone (SMTSC) techniques. Mesial roots of 16 freshly extracted mandibular first molar teeth (with a total of 32 canals) were used. Canals in each mesial root were shaped to a size F3 Protaper and were randomly assigned to either continuous wave vertical compaction or single match tapered size cone technique AH plus sealer. The volume of voids and gaps in the obturated roots were measured using micro-CT scanning at 1, 3, and 5 mm from the apex of the root. The measurements at 1, 3, and 5 mm from the apex were measured the area of voids and gaps in square micrometers then the ratio between voids, gaps and the total canal area in the section was calculated. Voids were more often found in canals obturated using the warm vertical compaction technique compared to the single matching taper sized cone technique. Voids at 1 and 5 mm from the apex measured 0.14 mm² and

0.07 mm² for both groups respectively. At 3 mm from the apex, voids were found to be 0.15 and 0.08 mm² for the WVC and SMTSC groups, respectively. However, these differences found were statistically insignificant.

Turkyilmaz and Erdemir ⁽⁶⁴⁾ investigated the tubular penetration depth of four different root canal sealers (AH26, AH plus, RealSeal and MTA Fillapex) using two different obturation techniques: cold lateral condensation and single cone obturation. Eighty-two single rooted teeth were collected and instrumented till F4 ProTaper universal rotary file. The root canals were irrigated with 5 ml 17% EDTA for removing the smear layer. Finally, the canals were rinsed with 5 ml distilled water to purify the remnant of previous solutions. Each solution used was applied for 3 min. All root canals were dried with sterile paper points with a size 40. At the end of these procedures, two roots were reserved as a negative control group to show the absence of smear layer on dentin walls. The remaining eighty root canals were randomly divided into four groups (n=20) according to root canal filling materials used. The root canal sealers were applied to root canals with a #25 lentulo spiral that rotated at low speed. In each group, half of the samples were filled using 2% tapered gutta-percha cones using cold lateral condensation technique (CLC) (n=10) and the other half were filled with ProTaper F4 wide tapered gutta-percha cones using SMTSC technique (n=10). All obturated root canals were stored at a 100% humidity environment at 37C for 2weekss for the complete set of root canal sealers. Two longitudinal slots were prepared along the buccal and lingual surfaces of each root with a diamond disc without perforation into the canal. Then the roots were split into two halves with a chisel, producing two specimens per teeth. One of

the two halves was used for examination. The notches were formed on each half of root at 2, 5, and 8 mm from root apex. There was no statistical difference between the obturation methods. When the sealers are evaluated within themselves AH26 was affected by obturation methods significantly. Also, the results revealed a significant difference between root regions. AH26 and RealSeal root canal sealers had the highest penetration values with CLC and SMTSC obturation techniques, respectively. The obturation method did not affect the penetration amount of root canal sealers. RealSeal root canal sealer performed better penetration ability than the other sealers. Apical third of the root in all groups showed lowest penetration depth.

Reynolds et al. ⁽⁶⁵⁾ compared the depth and percentage of dentinal tubule penetration for single cone (SC) and warm vertical compaction (WVC) obturation techniques with two different bioceramic sealers: BC Sealer (BCS), BC Sealer, HiFlow (BCSHF) and an epoxy resin-based sealer (2Seal easymix). 50 single canals teeth including incisors, canines and premolars were decoronized till the level of cemento- enamel junction and shaped up to rotary instruments to a final size of 40 6% EndoSequence (Brasseler, USA). Canals were irrigated between instruments with 1 mL of 6% NaOCl using a 30-G side-vented needle. For the final irrigation protocol canals were irrigated with 3 mL of 17% EDTA for 1 min followed by 3 mL of 6% NaOCl for 1 min. Canals were then irrigated for 1 min with 5 mL saline and dried with matching paper points prior to obturation. Teeth were then randomly divided into five groups: 1- resin-based Sealer with warm vertical obturation (RBWV), 2- bioceramic sealer with single cone obturation (BCSC), 3- bioceramic sealer with warm vertical obturation (BCWV), 4- bioceramic Sealer

HiFlow with single-cone obturation (HFSC), 5- bioceramic sealer HiFlow with warm vertical obturation (HFVV). The roots were sectioned at 3 mm and 6 mm levels from the apex. The roots were evaluated with a confocal laser scanning microscopy. There was significantly greater depth and percentage of sealer penetration at the 6 mm section compared to 3 mm. No statistically significant difference was found in sealer type or obturation technique at the examined levels. In conclusion, dentinal tubule penetration was similar comparing bioceramic sealer, bioceramic sealer HiFlow and resin-based sealer using single cone and warm vertical compaction techniques.

Jeong et al. ⁽⁶⁶⁾ investigated the depths of penetration of bioceramic sealer in dentinal tubules by using 3 different obturation methods. One hundred (47 canines and 53 incisors) extracted human permanent anterior teeth were endodontically prepared and divided equally into 3 experimental groups and 1 control group as follows: CPoint single cone (CPSC), gutta-percha single cone (GPSC), gutta-percha vertical compaction (GPVC), all with a calcium silicate-based sealer and calcium indicator Fluo-3, and CPoint single cone with a calcium indicator Fluo-3 (CPF3) without sealer as the control. Root canal chemo-mechanical preparation was done with rotary instruments using a crown-down technique to a master apical file #40/06. During instrumentation, the root canals were copiously irrigated with 10 mL 5.25% NaOCl. After instrumentation, the canals were irrigated with 10 mL 17% EDTA, followed by 3mL 5.25% NaOCl each for 3 minutes and then followed by a final flush with 10 mL deionized water. All root canals were irrigated by using 30-gauge irrigation needles and dried with 2 paper points for 3 seconds each. The teeth were then randomly divided into 4 groups. The

roots of the teeth in each group were axially cross-sectioned, and the surfaces were examined under confocal laser scanning microscopy at 10 and 20 magnifications. The sealer penetration depths were measured at their maximum depths and at 4 circumferential depths (12, 3, 6, and 9 o'clock). Statistical analysis by using one-way analysis and they concluded that the pressure derived from hygroscopic expansion of CPoint or warm vertical condensation did not enhance penetration depths of the calcium silicate-based sealer. Sealer penetration into the dentinal tubules occurred independent of the obturation technique.

2.5 Evaluation methods to determine depth of penetration into dentinal tubules.

Confocal laser scanning microscopy (CLSM):

Confocal microscopy or wide-field fluorescence microscopy is a particular version of conventional fluorescence microscopy that provides high-resolution images of material labeled with fluorescent dyes using certain optical components like Rhodamine B or Flouro-3 methyl. The ability to directly, noninvasively and serially optically segment thick, intact living specimens and an appreciable gain in lateral resolution is also provided. CLSM have several properties like shallow depth of field, the lack of out-of-focus glare, and the capacity to acquire serial optical slices from thick samples that give several advantages: higher resolution images can be obtained using a shorter wavelength, greater contrast can be achieved as compared to conventional microscopes and three-dimensional reconstruction of the images using the slices obtained in different focal planes ⁽⁶⁷⁾. CLSM has been used in the field of dentistry since the early 1990s including Analysis of the surface roughness of

dental materials, dental erosion, in studies involving the evaluation of micro tensile bond strength ⁽⁶⁸⁾.

Scanning microscopes (SEM):

SEM is mostly used in endodontics to assess root canal bacterial leakage. At atomic-scale resolution, the SEM delivers structural and chemical information about a material using traditional transmission electron microscopy methods. A broad spectrum of molecular and supramolecular structures may now be regularly weighed using elastically dispersed electrons. Scanning TEM is an effective method for determining the chemical composition of biological specimens because of recent advances in the collection and processing of electron energy-loss spectroscopy data. SEM is mostly used in endodontics to analyze bacterial leakage in the root canal, bacteria ⁽⁶⁹⁾.

Stereomicroscope:

The stereomicroscope is a valuable instrument in various fields of dentistry. Stereo and optical microscopy highlights aspects of external morphology, as well as root canal space of extracted teeth. studies have been conducted to evaluate apical microleakage around retrograde filling materials, studying the sealing abilities of root-end filling materials, for example, amalgam, mineral trioxide aggregate, and determination of working length during root canal procedures. Studying differences in canal configuration, lateral, foramina and auxiliary canals, and apical deltas at root apex is also very beneficial ⁽⁷⁰⁾.

3. AIM OF THE STUDY

This study aimed to evaluate the effect of different nano-particles final rinse solutions on depth of penetration of bioceramic root canal sealer into the dentinal tubules. The null hypothesis states that there is no difference among the tested groups.

4. MATERIALS AND METHODS

Section outline

- 4.1. Study design and ethical committee approval.
- 4.2. Sample size calculation.
- 4.3. Selection of teeth.
- 4.4. Decoronation of the samples.
- 4.5. Chemo-mechanical preparation of the samples.
- 4.6. Preparation of final rinse solutions and grouping of the samples.
 - Group (I) Silver nanoparticles 0.1%. (AgNPs).
 - Group (II) Chitosan nanoparticles 0.2%.
 - Group (III) Titanium dioxide nanoparticles 0.1%. (TiO₂NPs).
 - Group (IV) Ethylenediaminetetraacetic acid 17% (EDTA).
- 4.7. Obturation of the samples.
- 4.8. Sectioning of the root specimens.
- 4.9. Evaluation of the specimens using confocal laser scanning microscopy.
- 4.10. Statistical analysis of the data.

4.1. Study design and ethical committee approval:

This is an in vitro randomized, interventional prospective study. The study was accepted by the ethical committee of the Faculty of Dental Medicine, Al Azhar University Cairo, boys with a code number (803/211).

4.2. Sample size calculation:

Based on the results of Tuncer AK, and Tuncer S, ⁽³⁴⁾ the sealer penetration into dentinal tubules at apical and middle third sections were recorded mean \pm SD of (54.93 \pm 20.50) and (75.39 \pm 27.32) respectively. The sample size of 10 per group was obtained for the present study using unpaired two sample two tail t-test. The effect size (df=18.55) and the required sample size were calculated for alpha level of significant 5%, assuming a normal distribution with 95% confidence intervals. A total sample size of 40 samples used in this study.

4.3. Selection of the teeth:

A total 67 of Freshly extracted human mandibular premolars were collected from the clinic of oral and maxillofacial surgery department at the faculty of dental medicine Al-Azhar University Cairo, boys on patient range between 18 to 30 years old. The teeth were extracted due to medical reasons not related to this study. The teeth were cleaned to remove any hard deposits using hand periodontal curette and scalpel blade, then disinfected by immersion 2.5% Sodium hypochlorite (NaOCl) (Egyptian Company for household bleach, Cairo, Egypt) for 10 minutes then rinsed with distilled water. The teeth were evaluated under dental operating microscope (DOM) (S2350, Zumax Medical Company, Jiangsu, China) at 16x magnification to detect any external defects on

root surface. Periapical radiographs were taken from buccolingual and mesiodistal directions using a digital sensor size 2 (New IDA, Dabi Atlanta, Brazil) and hyperlight x-ray (Eighteenth Medical Technology Co., Changzhou, China). A pre-interventional Cone beam computed tomography was taken as follows:

Fabrication of 4 artelion plastic models were done as a modification of **Alkawas M. et al** ⁽⁷¹⁾ in (9X9 cm in diameter, 17 mm in thickness) and contains 10 circular holes 5mm in diameter. The roots of each sample were painted with two successive layers of colored nail polish (Yolo, Yolo Cosmetics, Cairo, Egypt). The circular holes filled with pink wax used for holding the teeth during imaging from tooth apex up to 17 mm coronally. CBCT was done using Planmeca Promax 3D machine (Planmeca Promax 3D Mid Machine, Helsinki, Finland) with exposure time 15 seconds with 90 KV, 12.5 mA and voxel size 70 microns. Teeth were examined in axial, coronal, and sagittal views. The values of image contrast and brightness were constantly adjusted using the software (Planmeca, Romexis dental software) image-processing tool to ensure optimal standardization and visualization (figure 1).

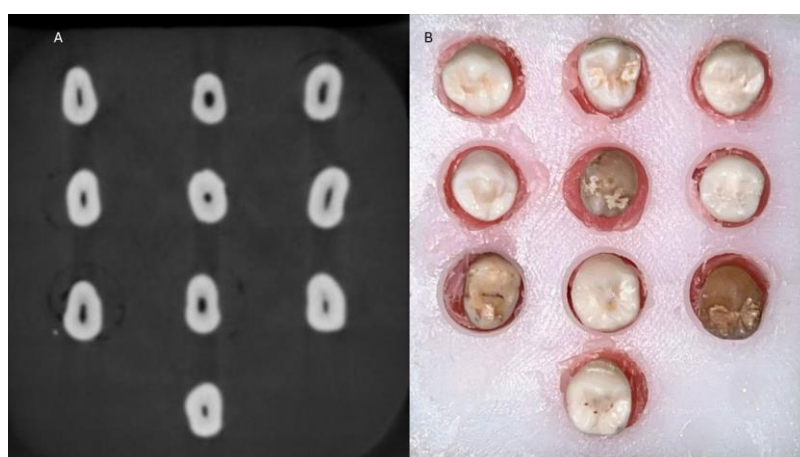


Figure (1): (A) CBCT showing an axial view for the teeth (B) A photograph showing the mold holding the teeth.

Teeth were selected according to the following inclusion criteria:

- Teeth with type I root canals anatomy according to Vertucci's classification ⁽⁷²⁾.
- Teeth with mature apices.
- Teeth with root curvature from 0°–10° according to Schneider's method ⁽⁷³⁾.
- Teeth with average length from 20 to 22mm from the buccal cusp to the root apex.
- Teeth with dentin thickness 2.23 +/- 0.3mm at the middle third.

Teeth that did not follow the inclusion criteria were excluded from the study as.

- Teeth with internal root resorption.
- Teeth with immature apices.
- Teeth with calcified root canals with or without pulp stones.
- Teeth with external root fractures or defects.
- Teeth with root caries.
- Teeth with previous root canal treatment.

4.4. Decoronation of the samples:

Forty mandibular premolars were selected based on the inclusion and exclusion criteria to be used in the study. Decoronation of the selected teeth was done at the coronal level of the plastic model using low speed diamond saw 0.3mm with water coolant to be 17 mm root length.

Each sample was stored in a numbered eppendorf containing normal saline solution at room temperature for one week for future randomization of the samples.

4.5. Chemo-mechanical preparation of the samples:

The samples were removed from the plastic models. Root canal patency was checked using #10-K file (Dentsply Sirona, Baillagues, Switzerland). The working length was measured as 16mm. Root canal preparation was done in crown down manner using Endostar E3 Azure system (Poldent, Warsaw, Poland) attached with a rotary file handpiece powered by a cordless torque-limited electric motor with a 16:1 reduction handpiece (E-connect pro, Eighteeth Medical Technology Co., Changzhou, China) at 300 RPM and 2 N/Cm by the following sequence, #30/08 orifice opener for the coronal 5mm, #20/04 ,#25/04, #30/04 and finally #40/04 to the full working length. The root canal irrigated between each file using 3 ml of 5.25% sodium hypochlorite with a side vented needle prorinse needle 30-gauge tip size in diameter (Dentsply Sirona, Baillagues, Switzerland) mounted on a Luer-lock 3 ml plastic syringe placed 2 mm short of the working length. The master cone was checked clinically and radiographically to confirm the cone fitting to the full working length. Finally, the root canals were irrigated by 3ml 5.25% sodium hypochlorite activated by passive ultrasonic irrigation using the silver tip attached to ultra-x (E-connect pro, Eighteeth Medical Technology Co., Changzhou, China) then, flushed by 3 ml of distilled water then, dryness obtained using sterile paper points.

4.6. Preparation of final rinse solutions and grouping of the samples:

According to the final rinse solution used, the numbered eppendorfs containing the samples were randomly divided using sample randomized software (<http://www.randomized.org>).

Group (I): (n=10)

Silver nanoparticles solution 0.1%. (AgNPs).

Preparation method: Silver nanoparticles have been prepared by chemical reduction method as reported by Turkevich⁽⁷⁴⁾. A solution of AgNO₃ has been used as Ag¹⁺ ions precursor and borohydride used as a reducing agent. The color of the solution slowly turned into grayish yellow, indicating the reduction of the Ag¹⁺ ions to Ag nanoparticles. The characterization test of the solution was done using TEM (figure 2). The PH of the solution is 7.

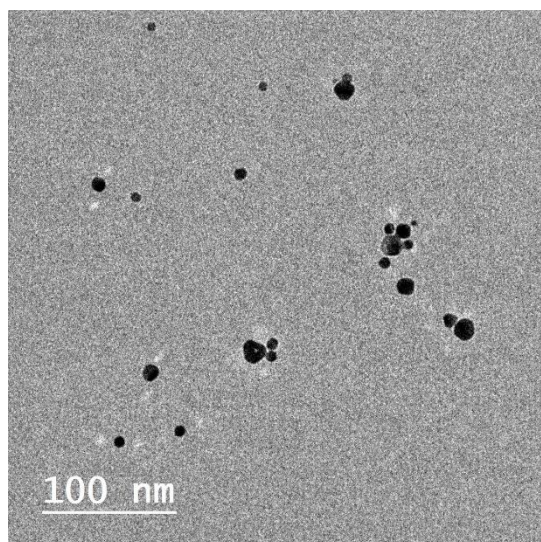


Figure (2): A microphotograph Showing the TEM image of AgNPs.

Group (II): (n=10)

Chitosan nanoparticles solution 0.2%.

Preparation method: Chitosan nanoparticles were prepared according to the ionotropic gelation process. Nanoparticles were obtained upon the addition of a tripolyphosphate aqueous solution to a Chitosan solution. Briefly, 0.2gm of Chitosan powder with a 90% degree of acetylation was dissolved in 200ml 1% acetic acid (pH = 4) and stirred for 6h to get homogenous solution then, add 150ml of tripolyphosphate 0.2% w/v dropwise. The clear solution turned to turbid indicating formation of chitosan NPs. The suspension was washed by centrifugation for 30min at 12000 rpm (Hermle Z32 HK, Germany) three times with water ⁽⁷⁵⁾.

Testing the size and shape: transmission electron microscopes were used by utilizing electrons that have short wavelengths to allow observation of matters with atomic resolution at an accelerating voltage of 200 kV. The samples were prepared by placing a droplet of colloidal suspension in respective solvent on a Formvar carbon-coated. Distribution and average size were determined using an image analysis software package (figure 3).

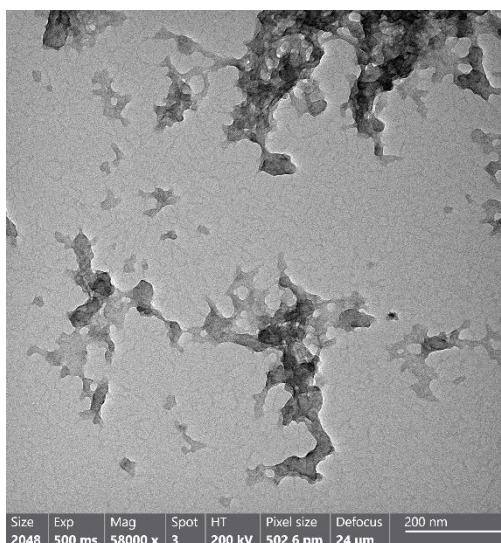


Figure (3): A microphotograph showing the TEM image of Chitosan NPs.

Group (III): (n=10)

Titanium dioxide nanoparticles solution 0.1%. (TiO_2 NPs).

Preparation method: anatase particles were prepared by precipitation from homogeneous solution using titanium (IV) isopropoxide (Sigma-Aldrich) as precursor in aqueous solution acidified with nitric acid to pH 2 using a water-to-titanium mole ratio of about 200 ⁽⁷⁶⁾.

Testing the size and shape: TEM were performed on 2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV (figure 4). The PH of the solution is between 4 and 5.

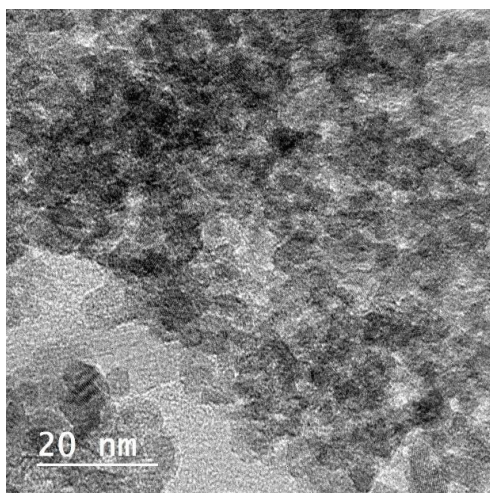


Figure (4): A microphotograph showing the TEM image of the prepared TiO_2 NPs.

XRD analysis: XRD pattern has been performed using XPERT-PRO Powder Diffractometer system (figure 5).

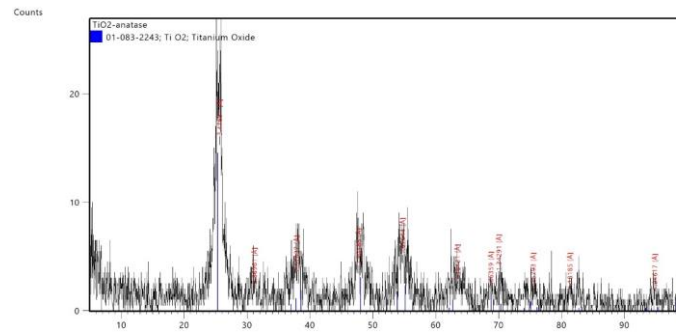


Figure (5): A microphotograph showing the XRD pattern of the prepared TiO₂ NPs.

Group (IV): (n=10)

Ethylenediaminetetraacetic acid (EDTA) 17% as control group.

In each group the samples were irrigated by 3ml of final rinse solution and activated by passive ultrasonic activation technique for 30 seconds using ultra x at 25 KHz.

4.7. Obturation of the samples:

The master cone was checked clinically and radiographically to confirm the cone fitting to the full working length. Matched sterile paper points (Dia-Proseal, Diadent, Cheongju, Korea) were used to dry the root canals. A bioceramic calcium silicate-based sealer composed of calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agent (Bio-C Sealer, Angelus, Londrina, PR, Brazil) was mixed with Rhodamine B fluorescent dye for further analysis using confocal laser scanning microscope (figure 6). Each 1gm of the sealer was mixed with 0.001gm of 0.1% Rhodamine B dye measured by micro pipette (figure 7). The sealer was placed into the canal using a master cone size #40/04. The cone was inserted slowly to the full working length. A single matched taper sized cone obturation technique was done. Sealing off the excess gutta-percha 2 mm below the level of canal orifice using heat carrier plugger (fastpack, Eighteeth-China) at

150°C and finally condensed by a cold plugger. Excess sealer was removed using a cotton pellet. The 2 mm coronal part was sealed using resin modified glass ionomer restoration (3 M ESPE; St Paul, MN, USA). The samples were kept at 37°C in 100% humidity in an incubator for two weeks to ensure complete setting.



Figure (6): A photograph the (bio c) bioceramic sealer.



Figure (7): A photograph showing the micro pipette.

4.8. Sectioning of the samples:

Each root was sectioned horizontally using low speed diamond saw with water coolant (IsoMet 4000 Precision Saw, Telangana, Secunderabad, India) at 3 mm, 7 mm and 13 mm from root apex. The sections were polished with silicone carbide abrasive papers. For each slice with a thickness of 1 mm was mounted onto glass coverslip 22X50 mm to be examined under confocal laser scanning microscopy (figure 8,9,10,11).



Figure (8): A photograph showing the wax holding the root sample.

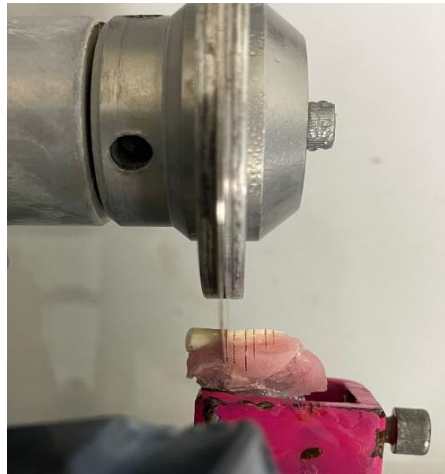


Figure (9): A photograph showing the isomet sectioning the root samples.

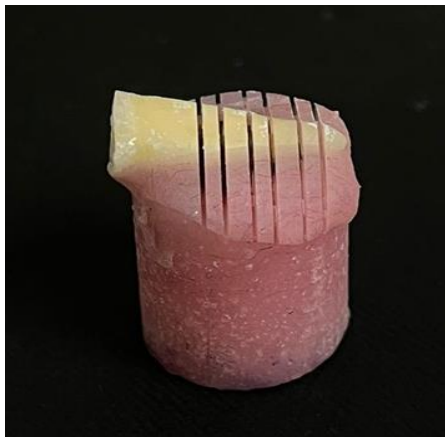


Figure (10): A photograph showing the horizontal sections of the root sample.

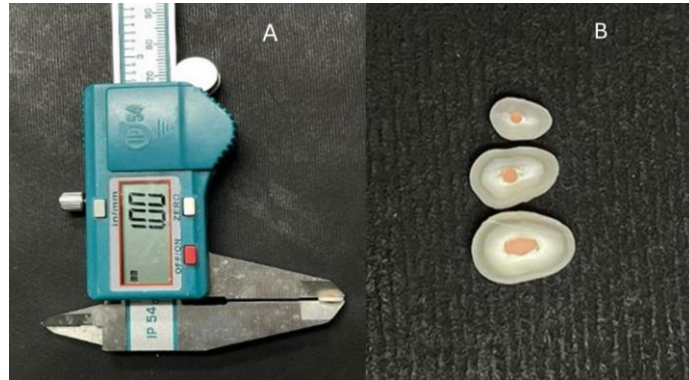


Figure (11): (A) photograph showing the thickness of each root specimen, (B) A photograph showing each root specimen.

4.9. Scanning of the samples:

The slices were investigated under a Zeiss confocal laser scanning microscopy (Carl Zeiss, Jena, Germany) was set at the excitation wavelength 543 nm and the emission wavelengths of 546 – 735 nm in low light condition as the Rhodamine B dye is sensitive to light at digital zoom. The full sample acquisition was imaged with a 10x objective lens in the format of $1,024 \times 1,024$ pixels (figure 12). While the 40x oil lenses confirm the content of the sealer inside the dentinal tubules, each sample was evaluated for a consistent fluorescent ring around the canal wall, indicating the sealer dye distribution. The penetration depth of the sealer into the dentinal tubules was illustrated by the fluorescence, which started from the outer surface of the canal until the maximum depth around the canal wall which serves as the starting point. The analysis of images acquired by CLSM were done by two equations:

The percentage of sealer penetrated into the dentinal tubules was calculated by:

Maximum area filled with sealer = Total cross section of the root – root canal cross sectional area (figure 13).

$$\text{Percentage of sealer penetration} = \frac{\text{maximum area filled by sealer} - \text{root canal cross sectional area}}{\text{Dentin area of the root canal}}$$



Figure (12): A photograph showing the CLSM machine.

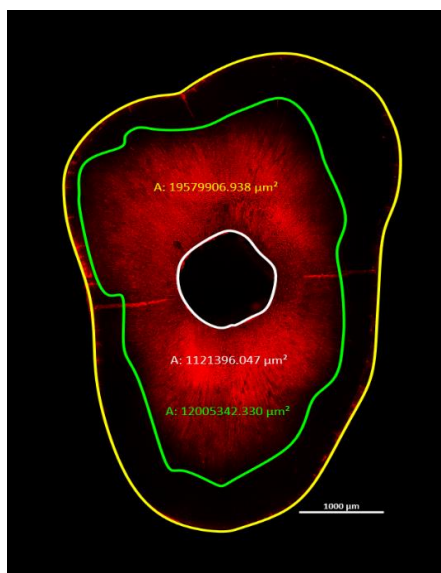
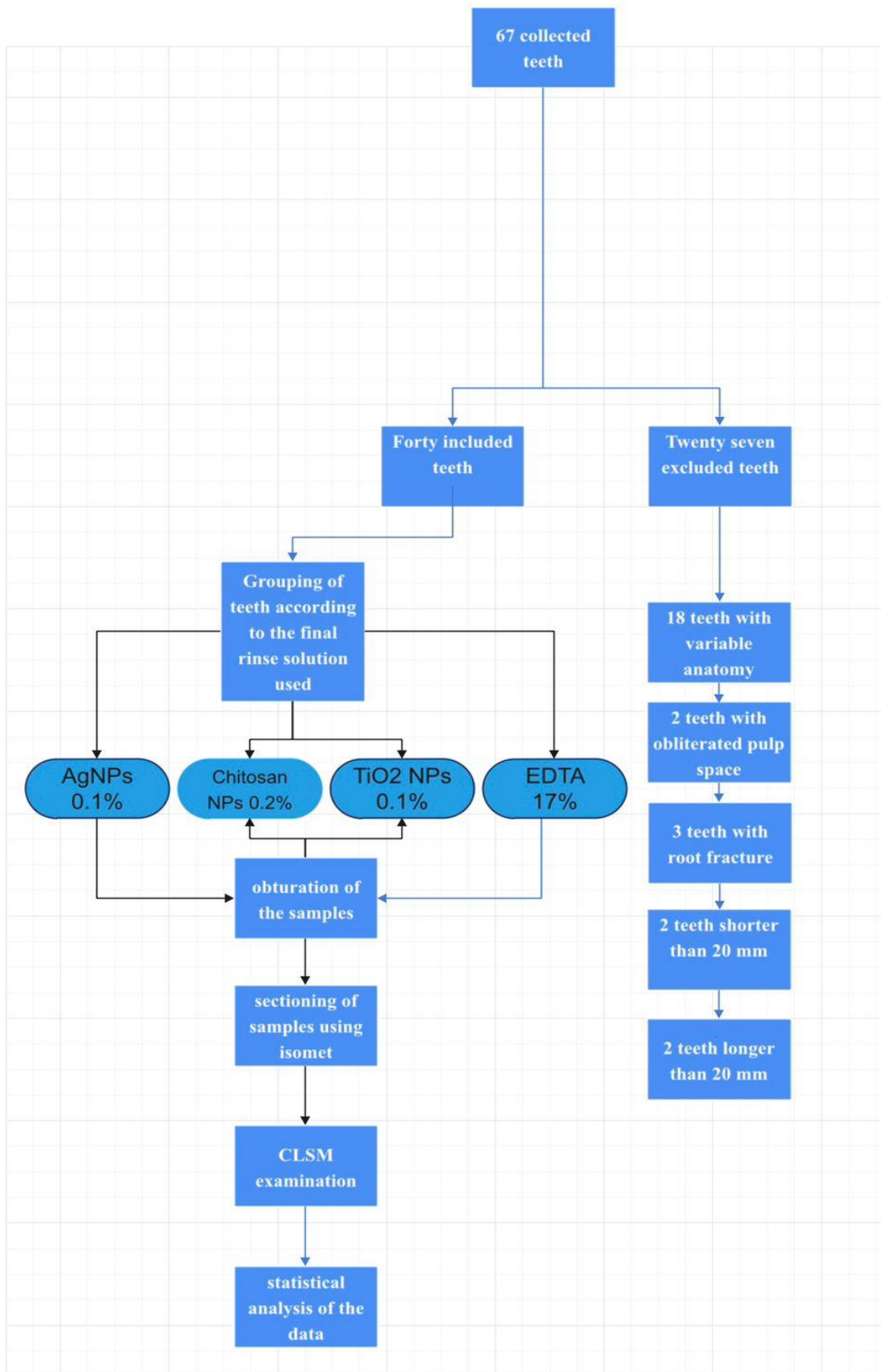


Figure (13): A microphotograph showing the analysis of the photos acquired from the CLSM software. The yellow zone: total cross section of the root, the green zone: maximum area filled by the sealer, the white zone: the root canal cross sectional area.

4.10. Statistical analysis of the data

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Sealer penetration % data showed normal (parametric) distribution while depth of penetration data showed non-normal (non-parametric) distribution. Data were presented as mean, standard deviation (SD), mean and standard deviation values. For parametric data, repeated measures ANOVA test was used to study the effect of irrigation technique, root level and their interactions on different variables. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. For non-parametric data, Kruskal-Wallis test was used to compare between final rinse irrigation techniques. Friedman's test was used to compare root levels. Dunn's test was used for pair-wise comparisons when Kruskal-Wallis test or Friedman's test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.



5. RESULTS

5.1 Effect of final rinse solutions and different root levels on percentage of sealer penetration.

5.2 Effect of type of final rinse used (regardless of the root level).

5.3 Effect of root level (regardless of final rinse type).

5.4 Effect of different interactions of variables on percentage of sealer penetration.

5.1. Effect of final rinse solutions and different root levels on percentage of sealer penetration.

Repeated measures ANOVA results:

The results showed that the type of final rinse used as a final rinse (regardless of root level) had a statistically significant effect on mean sealer penetration. The Root level regardless of final rinse technique had a statistically significant effect on mean sealer penetration. The interaction between variables had no statistically significant effect on mean sealer penetration. Since the interaction between the variables is non-statistically significant, the variables are independent from each other (table 1).

Table (1): Repeated measures ANOVA results for the effect of different variables on percentage of sealer penetration

Source of variation	Type III Sum of Squares	df	Mean Square	F-value	P-value	Effect size (<i>Partial eta squared</i>)
Final rinse type	13729.385	3	4576.462	36.279	<0.001*	0.983
Root level	5632.3	2	2816.15	38.379	<0.001*	0.578
Final rinse technique x Root level interaction	984.885	6	164.147	2.237	0.053	0.193

*df: degrees of freedom = (n-1), *: Significant at $P \leq 0.05$*

5.2. Effect of type of final rinse used (regardless of the root level).

The results showed that there was a statistically significant difference among the type of final rinse used. Pair-wise comparisons revealed that there was no statistically significant difference between Chitosan NPs (57.2 ± 12.1) and Titanium dioxide NPs (57.3 ± 10) which showed the significantly highest mean percentage of sealer penetration followed by Silver NPs (42.6 ± 13.9) while EDTA (28.9 ± 13.5) showed the lowest mean percentage of sealer penetration P-value < 0.001 (table 2), (figure 14).

Table (2): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between percentage of sealer penetration after different final rinse used.

Final rinse technique	Mean	SD	P-value	Effect size (Partial Eta squared)
Chitosan	57.2 ^A	12.1	<0.001*	0.983
EDTA	28.9 ^C	13.5		
Silver nanoparticle	42.6 ^B	13.9		
Titanium dioxide	57.3 ^A	10		

*: Significant at $P \leq 0.05$, Different superscripts indicate statistically significant difference between types

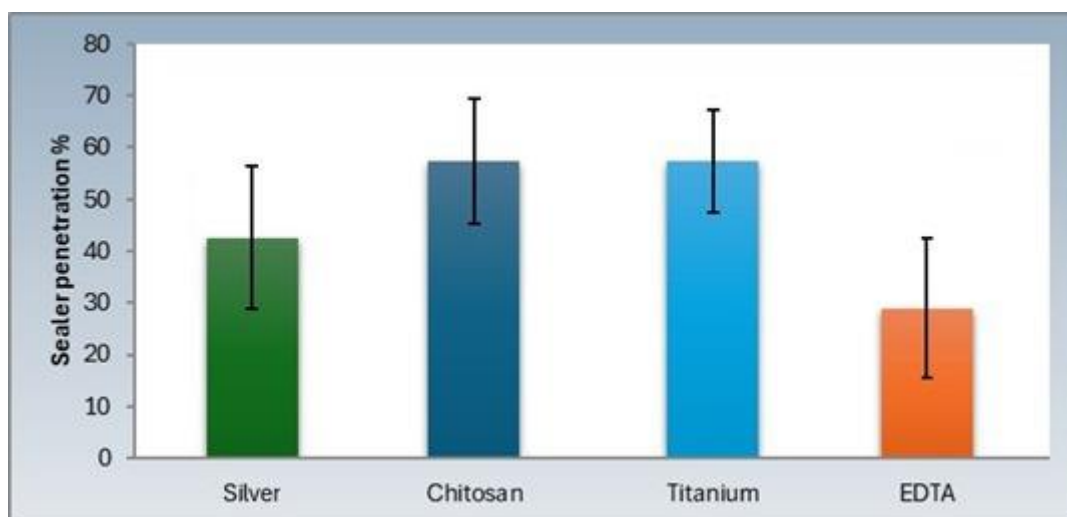


Figure (14): Bar chart representing mean and standard deviation values for percentage of sealer penetration of different final rinse types regardless of root level.

5.3 Effect of root level (regardless of final rinse type).

There was a statistically significant difference between root levels. Pair-wise comparisons revealed that coronal level showed the statistically significantly highest mean percentage of sealer penetration (56.1 ± 16.5) followed by middle root (44.8 ± 14.1). While apical root level showed the lowest mean percentage of sealer penetration (37.5 ± 15.9) P -value < 0.001 (table 3), (figure 15).

Table (3): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between percentage of sealer penetration at different root levels regardless of final rinse solution used.

Coronal		Middle		Apical		P -value	Effect size (Partial Eta squared)
Mean	SD	Mean	SD	Mean	SD		
56.1 ^A	16.5	44.8 ^B	14.1	37.5 ^C	15.9	$< 0.001^*$	0.578

*: Significant at $P \leq 0.05$, Different superscripts indicate statistically significant difference between root levels

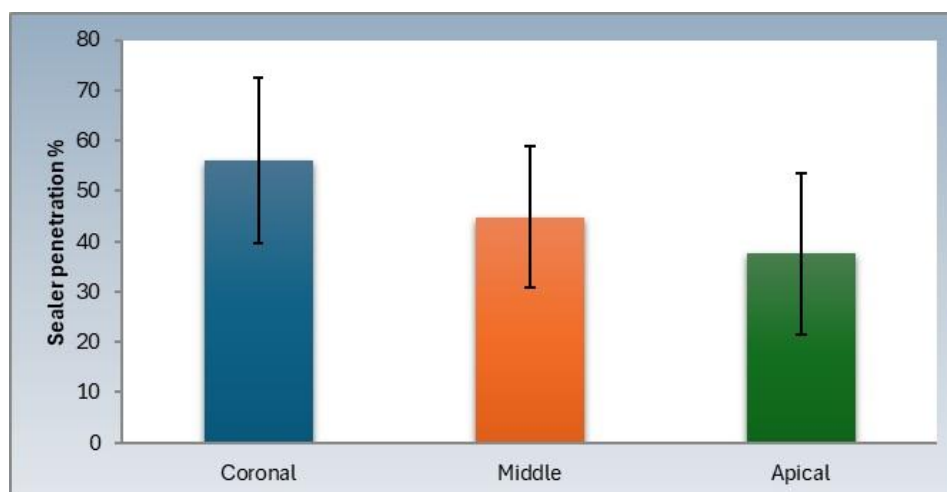


Figure (15): Bar chart representing mean and standard deviation values for percentage of sealer penetration at different root levels regardless of final rinse solution used.

5.4 Effect of different interactions of variables on percentage of sealer penetration.

At the coronal root level, there was a statistically significant difference between the final rinse types used. Pair-wise comparisons revealed that there was no statistically significant difference between Chitosan (66.8 ± 12.1), Silver nanoparticle (56.9 ± 11.3), and Titanium dioxide (62.8 ± 13.9) all showed statistically significantly higher percentage of sealer penetration than EDTA (38.1 ± 13.2) P -value < 0.001 .

At the middle root level, there was a statistically significant difference between the final rinse types used. Pair-wise comparisons revealed that there was no statistically significant difference between Chitosan NPs (59.4 ± 6.5) and Titanium dioxide NPs (52.2 ± 5.6) both showed the statistically significantly highest mean percentage of sealer penetration followed by silver which showed (38.2 ± 9) while EDTA showed the lowest mean percentage of sealer penetration (29.5 ± 10) P -value < 0.001 .

At the apical root level, there was a statistically significant difference between the final rinse types used. Pair-wise comparisons revealed that Titanium showed the statistically significantly highest mean percentage of sealer penetration (53.5 ± 5.4) followed by Chitosan which showed (45.8 ± 5.8) followed by silver nanoparticle (35 ± 10.2). EDTA showed the lowest mean percentage of sealer penetration (15.7 ± 5.5) P -value < 0.002 (table 4), (figure 16).

Table (4): The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between percentage of sealer penetration with different interactions of variables.

Final rinse type	Coronal		Middle		Apical		P -value	Effect size (<i>Partial eta squared</i>)
	Mean	SD	Mean	SD	Mean	SD		
Chitosan	66.8 ^{AE}	12.1	59.4 ^{AE}	6.5	45.8 ^{BF}	5.8	$< 0.001^*$	0.49
EDTA	38.1 ^{BE}	13.2	29.5 ^{CE}	10	15.7 ^{DF}	5.5	$< 0.001^*$	0.514
Silver	56.9 ^{AE}	11.3	38.2 ^{BF}	9	35 ^{CF}	10.2	$< 0.001^*$	0.46
Titanium	62.8 ^A	13.9	52.2 ^A	5.6	53.5 ^A	5.4	0.088	0.165
P -value	$< 0.001^*$		$< 0.001^*$		0.002*			
Effect size (<i>Partial eta squared</i>)	0.464		0.711		0.825			

*: Significant at $P \leq 0.05$, A, B, C, D superscripts in the same column indicate statistically significant difference between types, E,F superscripts in the same row indicate statistically significant difference between root levels

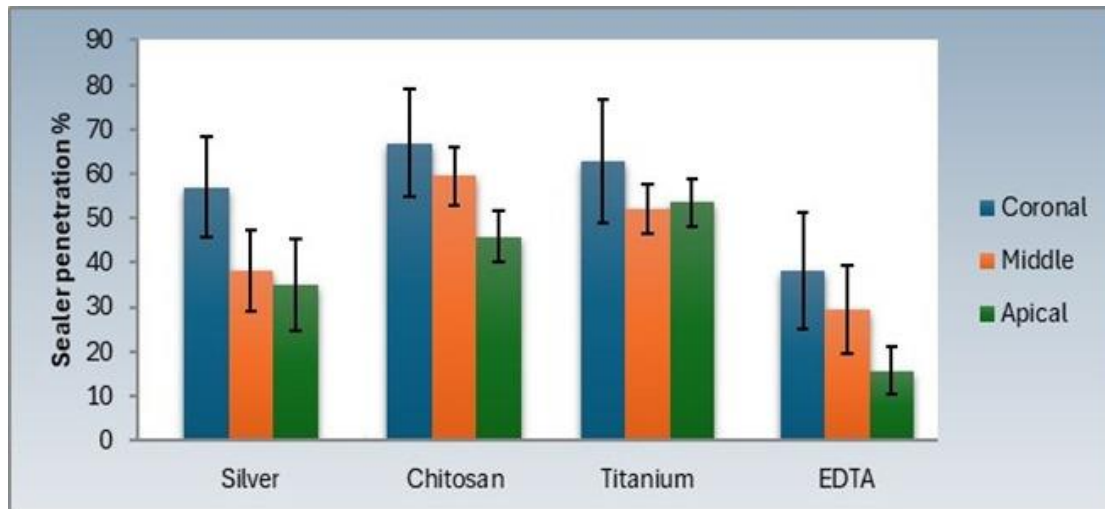


Figure (16): Bar chart representing mean and standard deviation values for percentage of sealer penetration with different interactions of variables.

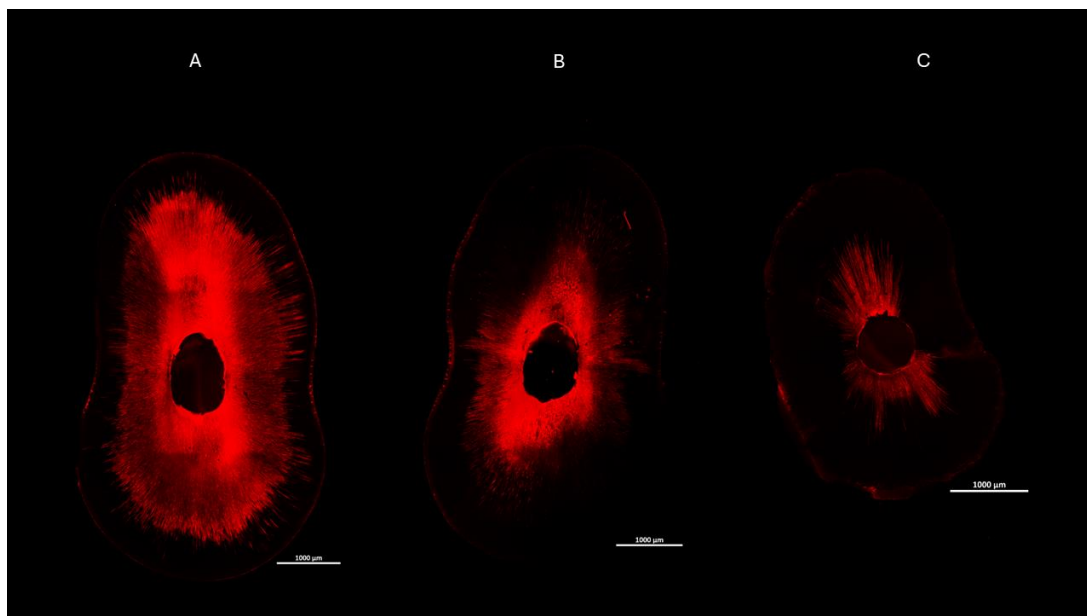


Figure (17): A microphotographs showing chitosan NPs at the coronal root level (A), Middle root level (B), Apical root level (C)

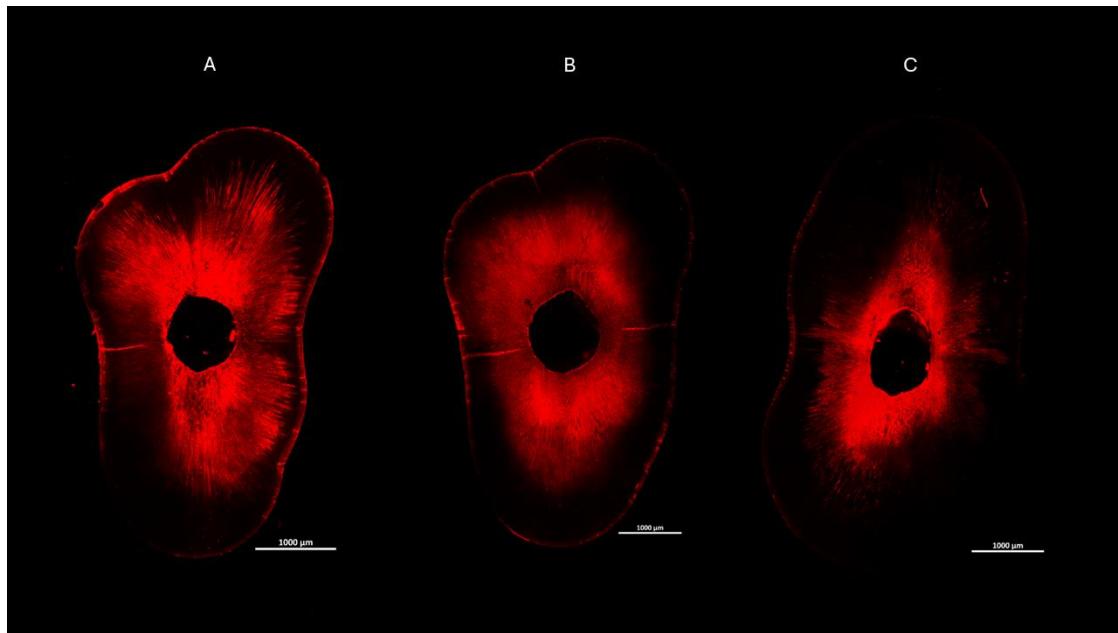


Figure (18): A microphotographs showing TiO₂ NPs at the coronal root level (A), Middle root level (B), Apical root level (C)

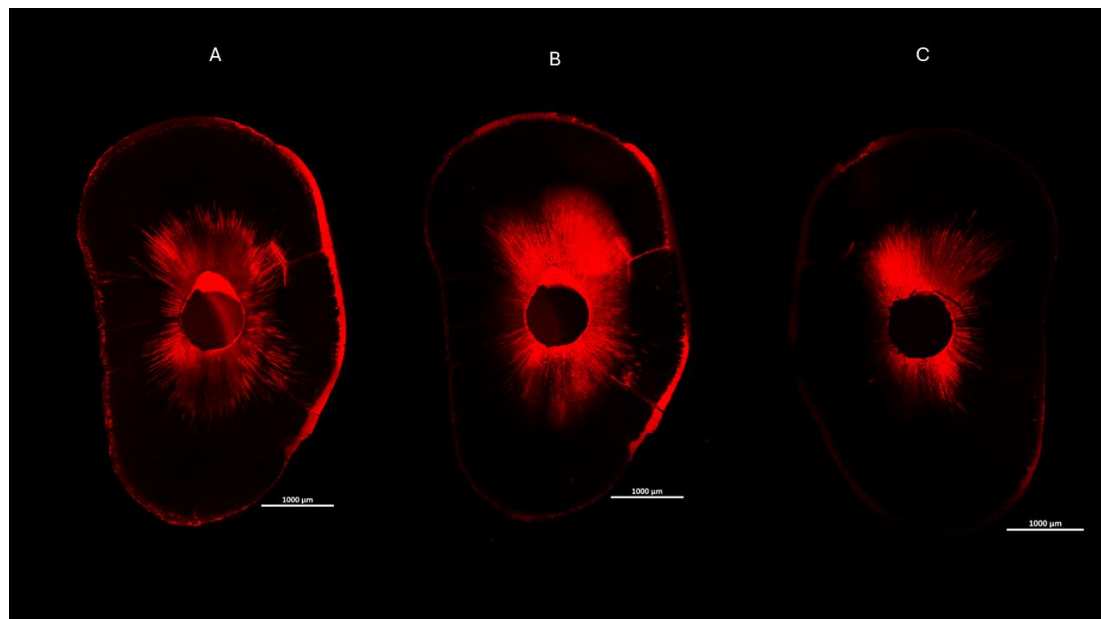


Figure (19): A microphotographs showing Ag NPs at the coronal root level (A), Middle root level (B), Apical root level (C)

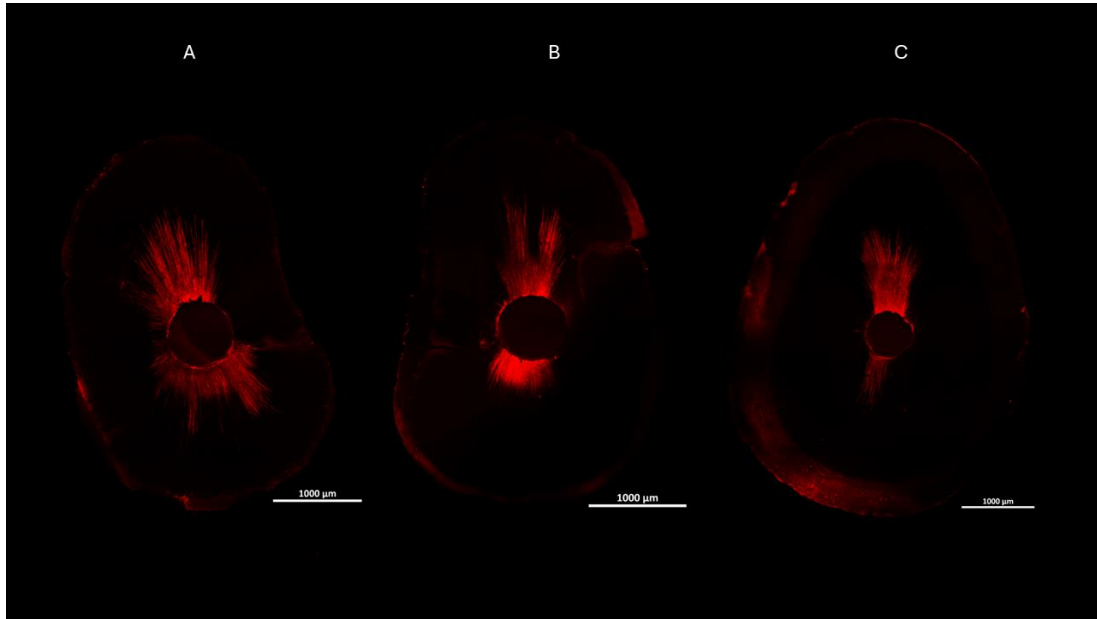


Figure (20): A microphotographs showing EDTA at the coronal root level (A), Middle root level (B), Apical root level (C)

6. DISCUSSION

Successful endodontic outcome depends mainly on effective disinfection and fluid tight seal of the root canal system. To obtain such goals, maximum depth of penetration of both irrigating solutions and the obturating materials are required. Multiple factors may affect the depth of penetration of both irrigating solution and obturating materials into the dentinal tubules such as presence of the smear layer, type of irrigant and its particle size, obturation techniques, type of root canal sealer ⁽⁷⁷⁾.

The smear layer represents one of the most obstacles that may limit the tubular penetration of the root canal sealer as it may penetrate to a depth up to 40 μm . It consists of organic matter trapped within translocated inorganic superficial layer with 1-5 μm thickness. Although, complete removal of the smear layer is impossible to obtain ⁽⁷⁸⁾, different materials and protocols have been suggested to remove it. Traditionally, a final rinse of a chelating agent has been recommended following sodium hypochlorite to remove the inorganic part of the smear layer resulting in more penetration depth of the sealers into the dentinal tubules and improving the fluid tight seal of the root canal system. On the other hand, the particle size of the irrigating solutions may affect its penetration into the dentinal tubules. Unlike the traditional irrigants ⁽⁷⁹⁾.

Nano technology is advocated to remove the smear layer and improve the depth of penetration of both the irrigating solution and root canal sealers due to their unique physico-chemical properties. It allows for reaching the complex root canal anatomy such as apical ramifications and canal anastomoses due to the unique particle size that represents tenth of the diameter of the tubular dentin for a depth of 300 to 1500 μm ⁽⁸⁰⁾.

Recently, different nano-irrigants have been suggested to improve the tubular penetration of the root canal sealer such as Silver nanoparticles, Magnesium oxide nanoparticles, Chitosan nanoparticles and Titanium oxide nanoparticles ⁽⁸¹⁾.

This interventional, randomized, prospective study aimed to evaluate the effect of different nano-irrigants as a final rinse solution on the depth of penetration of bioceramic sealer. Single rooted premolar teeth with single root canals were selected as they the most teeth extracted due to orthodontic reasons with type I vertucci canal anatomy for standardization purposes ⁽⁸²⁾. Teeth were collected from patients aged between 18 and 30 years old to minimize variations in dentin nature ⁽⁸³⁾.

Out of 67 extracted mandibular premolars, 40 teeth were included in this study. A 27 teeth were excluded due to the following reasons: 18 teeth with variable anatomy other than Vertucci type I, 2 teeth due to obliterated pulp chamber, 3 teeth with root fracture, 2 teeth shorter than 20 mm and 2 teeth longer than 22 mm. The roots of each tooth were painted with nail polish to prevent blockage of the root openings with future embedding in pink wax that was done to secure the teeth during CBCT. A pre-operative periapical x ray to explore the teeth anatomy and CBCT were done to confirm the root anatomy⁽⁸⁴⁾. All molds were fabricated at 17 mm for future decoronation of all teeth embedded in it for standardization using slow-speed diamond saw with water coolant to avoid heat generation.

Root canal instrumentation was done in crown down manner using Endostar E3 Azure system that is using heat treatment technology allowing the file to transform, at body temperature, from its martensite

phase at the time of manufacturing to the austenite phase. It offers extreme flexibility and great resistance to fracture and the austenite phase which increases the cutting ability ⁽⁸⁵⁾. The master apical preparation was done using 40/04 rotary file to shape the root canal allowing for deeper penetration both irrigating solution and the root canal sealer. Apical enlargement above size #35/0.04 is essential to enhance removal of smear layer apically, and there is no significance difference between #40/0.04 and 45/0.04 ⁽⁸⁶⁾. Irrigation between each file was done using 5,25% sodium hypochlorite due to its superior antibacterial effect, the capacity to dissolve organic matter and act as a lubricant for all shaping files ⁽⁸⁷⁾. Many chelating agents are supposed to be used in the final rinse protocol due to its ability to dissolve the inorganic part of the smear layer ⁽⁸⁸⁾. Activation of both irrigants was done using passive ultrasonic irrigation (PUI) because it improves the dispersion of root canal irrigants that enables deeper penetration to inaccessible areas via cavitation through bubble implosions and acoustic streaming. Besides, it improves smear layer and hard tissue debris removal ⁽⁸⁹⁾.

A single matched tapered sized cone obturation technique was selected utilizing hydraulic bioceramic sealer to fill the root canals as the sealer is chemically bonded to dentin providing the same quality of obturation of the warm vertical compaction without application of heat that may cause some changes in the physical properties of the sealer such decrease flowability and significant weight loss ⁽⁹⁰⁾. The bioceramic sealer was mixed with fluorescent Rhodamine B dye to allow differentiation of the sealer from dentinal tubules for further analysis using CLSM. The dye concentration to root canal sealer is 0.1% because higher concentrations may cause excessive fluorescence in the image. This dye does not cause

any changes to the physical properties of all root canal sealers ⁽⁹¹⁾. Confocal laser scanning microscopy is a noninvasive evaluation method that produces high resolution pictures that is easily distinguishable by adding fluorescent dyes to the tested material with no need for sample preparations such as gold plating as in SEM that may damage the sample. In addition, the advantage of its low magnification allows for better interpretation of the entire surface to be examined ⁽⁹²⁾.

Chitosan and Titanium dioxide nanoparticles have the highest positive effect on sealer penetration power compared to the other final rinse solutions used.

Chitosan is composed of amino acids (Chitin dimer with N-acetyl-D glucosamine chain) that protonate into negatively charged amines (-NH₃) which chelate with the calcium ions in dentin resulting in formation of a stable water-soluble complex that is easily flushed with irrigation. The chelating mechanism of Chitosan is either utilizing multiple amino acids (bridge model) or utilizing only one amino acid (free-arm model) depending on the number of the calcium ions that are bound to the amino acids ⁽⁹³⁾. The result of this study agrees with **Pedro et al.** ⁽⁹⁴⁾ that measured the amount of calcium ions released after using either Chitosan or EDTA with different activation techniques including manual, sonic, and ultrasonic activation. Chitosan with passive ultrasonic activation technique showed the highest amount of calcium ions released after spectrometric analysis compared to EDTA and distilled water with all activation techniques. Similarly, **Kamble et al.** ⁽⁹⁵⁾ concluded that Chitosan is more effective than EDTA in removing the smear layer. Adversely, final rinse using chitosan nanoparticles showed the least sealer penetration power compared to Qmix and EDTA, this discrepancy may

be because of lack of activation of the final rinse utilizing single cone technique with bioceramic sealer unlike the current study utilizing single matched tapered sized cone technique ⁽⁹⁶⁾.

Regarding the Titanium dioxide nanoparticles solution, its unique preparation using nitric acid that has a decalcifying action on the tooth structure gives a possible reason for increasing the depth of penetration of the sealer ⁽⁹⁷⁾. Also, a possible chelating action of the Titanium dioxide nanoparticles on dentin is supposed by **Pelozo et al.** ⁽⁹⁸⁾ Furthermore, Titanium dioxide Nanoparticles are highly stable particles with suitable photocatalytic properties that aid in lysis of the organic components of the smear layer. Although Silver nanoparticles final rinse has lesser sealer penetration into the dentinal tubules than Chitosan nano particles and titanium dioxide nanoparticles, it can also remove the smear layer that may be due to physical interaction between nanoparticles and debris when compared with EDTA ⁽⁹⁹⁾.

Regarding EDTA, it has the least effect on sealer penetration power compared to other final rinse solutions used. This may be related to its high surface tension that decreases wettability to the dentin ⁽¹⁰⁰⁾. Another possible reason is that the demineralization produced by EDTA leads to a relatively smooth dentine surface with reduced surface area, which could lead to reduced dentine wettability for adhesion ⁽¹⁰¹⁾. On the contrary, **Aydin et al.** ⁽³⁵⁾ stated that EDTA as a final rinse showed a high mean percentage of Total Fill bioceramic sealer penetration that Chitosan, this may be attributed to lack of activation for final rinse solutions.

Regarding the root level, the highest effect of the tested final rinse solutions on sealer penetration power is at the coronal level and

decreasing in an apical direction. This may be attributed to the technique of ultrasonic activation of the final rinse solutions as the tip of the device does not reach to the full working length and relatively small tip size (25/00) in a wide canal prepared up to size 40/04⁽¹⁰²⁾. Anatomically, there is increased number (57,400 per mm²) and diameter of dentinal tubules coronally while decreasing apically (14,400 per mm²) in the root canal. Furthermore, some areas in the apical third of the root canals are free of the dentinal tubules or it may be occluded by tissue-like cementum⁽¹⁰³⁾. The sealer penetration into the dentinal tubules was not homogenous as sealer penetration was found to be greater in the buccolingual direction compared with the mesiodistal direction, and this finding is in line with that of a previous study⁽¹⁰⁴⁾ because of increased sclerosis in the dentinal tubules located on the mesial and distal sides of the canal lumen⁽¹⁰⁵⁾. It is the time to reject the null hypothesis of the study as there is a difference among the tested groups.

7. SUMMARY

Nano-irrigants may help in improving the cleanliness of the root canal system that allows deeper penetration into the dentinal tubules as they have distinct advantages and unique physico-chemical properties in compared to bulk counterparts due to their high surface to volume ratio. Several nanoparticle materials are available such as silver nanoparticles (AgNPs) which introduced as an irrigant, intracanal medicament and incorporated in root canal filling due to strong antibacterial effect. Chitosan is a natural biopolymer that has been established for antimicrobial applications as well as a final rinse solution due to its action on smear layer. Titanium dioxide (TiO₂NPs) are highly stable particles with superior antibacterial property.

forty mandibular premolars were selected according to specific inclusion criteria and then prepared until file #40/04. According to the final rinse solution type the teeth were grouped into 1-AgNPs group 2-Chitosan NP group 3- TiO₂ NP group 4- EDTA group. All samples were obturated using bio c bioceramic sealer with single matched tapered sized cone technique. The sealer was labelled with Rhodamine B dye for further evaluation of the specimens under confocal laser scanning microscope. The samples were sectioned horizontally into three slices at 3, 7, and 13 mm (apical, middle, and coronal) sections from the apex. The results showed that Chitosan NPs and Titanium dioxide NPs solutions both showed the statistically significantly highest percentage of sealer penetration. Silver nanoparticle solution showed statistically significantly lower percentage of sealer penetration. EDTA showed the statistically significantly lowest percentage of sealer penetration. Coronal level

showed the statistically significantly highest percentage of sealer penetration. Middle root level showed statistically significantly lower mean value. Apical root level showed the statistically significantly lowest percentage of sealer penetration regardless of the final rinse solution used.

8. CONCLUSIONS

Within the parameters of this study, the following conclusions could be drawn:

- 1- Chitosan, titanium dioxide and silver nanoparticles final rinse solutions have superior effect on depth of penetration of bioceramic sealer into the dentinal tubules.
- 2- The depth of penetration of bioceramic sealer increases in an apico-coronal direction.
- 3- EDTA has lesser effect on depth of penetration of bioceramic sealer than nano-irrigant solutions used in this study.

9. RECOMMENDATIONS

- 1- Further research should be done to evaluate the depth of penetration of different bioceramic sealers.
- 2- Further research should be done to evaluate the effect of different activation methods of the nano-irrigants on the depth of penetration of bioceramic sealer.
- 3- Further research should be done to evaluate the effect of different activation methods of bioceramic sealers on its depth of penetration of bioceramic sealers into the dentinal tubules.
- 4- Further research should be done to evaluate the effect of different access cavity designs on depth of penetration of bioceramic sealers into the dentinal tubules.
- 5- Further research should be done to evaluate the effect of apical size preparation of root canal on depth of penetration of bioceramic sealers into the dentinal tubules.
- 6- Further research should be done to evaluate the effect of canal preparation with different taper on depth of penetration of bioceramic sealer into the dentinal tubules.
- 7- Further research should be done to evaluate the dentin micro-hardness for teeth irrigated by nano irrigants.
- 8- Further research should be done to measure the fracture resistance for teeth irrigated by nano irrigant solutions.

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المخلص العربي

قد تساعد محاليل الارواء متناهيه الصغر في تحسين نظافه قناة الجذر عن طريق اختراق أعمق في الأنابيب العاجية لأنها تتمتع بمزايا مميزة وخصائص فيزيائية كيميائية فريدة مقارنة بنظيراتها ذو الاحجام الطبيعيه نظرًا لارتفاع نسبة السطح إلى الحجم. تتوفر العديد من مواد السوائل النانوية مثل سوائل الفضة النانويه التي تم تقديمها كدواء للري داخل القناة وتم دمجها في حشو قناة الجذر بسبب تأثيرها القوي المضاد للبكتيريا، والكيوتوزان هو بوليمر حيوي طبيعي تم إنشاؤه كمضاد للميكروبات، وسائل أكسيد المغنيسيوم النانوي يعتبر مضاد للميكروبات مع الحد الأدنى من التأثير السام للخلايا على الخلايا الحية، سائل ثاني أكسيد التيتانيوم النانوي عبارة عن جزيئات مستقرة مع خاصية مضادة للجراثيم متفوقة.

تم اختيار أربعين من الضواحك السفلية وفقًا لمعايير الاشتمال ثم تم تحضيرها حتى المبرد رقم ٤٠ تحذب ٤٪. وفقا لنوع الغسول النهائي تم. تجميع الأسنان في ١-مجموعه سائل الفضة النانوي ، ٢-مجموعه سائل الكيوتوزان النانوي، ٣-مجموعه سائل ثاني اكسيد التيتانيوم النانوي ، ٤-مجموعه الاي دي تيه ايه. تم حشو العينات بماده لاصق علاج الجذور مخلوط بماده الرودامين بي مع وضع قمع وحيد من الجاتا بيركا للفحص تحت مجهر المسح بالليزر متحد البؤر بعد ان تم تقسيم العينات الي ثلاث شرائح بأطوال ٣-٧-١٣ (قمي و متوسط واكليلي) من ذروه السن. أظهرت النتائج أن كلاً من سائل الكيوتوزان النانوي وسائل ثاني أكسيد التيتانيوم النانوي أظهرت أعلى متوسط تغلغل للماده اللاصق بنسبة معنوية إحصائياً. أظهر سائل الفضة النانويه قيمة متوسطة أقل. و ان بشكل ملحوظ من الناحية الإحصائية. أظهرت بان ماده الاي دي تي ايه أقل متوسط تغلغل للماده اللاصق إحصائياً وكشفت أن المستوى الإكليلي أظهر أعلى متوسط تغلغل للماده لاصق القناة. أظهر مستوى الجذر الأوسط قيمة متوسطة أقل بكثير من الناحية الإحصائية. أظهر مستوى الجذر القمي أقل متوسط تغلغل لماده اللاصق إحصائياً بغض النظر عن نوع الري.

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أستاذ ورئيس قسم علاج الجذور

كلية طب الأسنان- جامعة الأزهر (بنين – القاهرة) - مشرفا ومناقشا

لجنة الاشراف

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**تأثير محاليل الإرواء متناهيه الصغر المختلفه كغسول نهائي علي عمق اختراق
لاصق حيوي لقتاه الجذر.**

(دراسه خارج الجسم الحي)

بحث مقدم كجزء من مقومات الحصول على درجة الماجستير في علاج الجذور

مقدم من

الطبيب/محمد مجدي بيومي محمود

بكالوريوس طب وجراحة الفم والأسنان - ٢٠١٧, كلية طب الأسنان - جامعة المستقبل
معيد بقسم علاج الجذور في الجامعه المصريه الروسيه

المشرفون

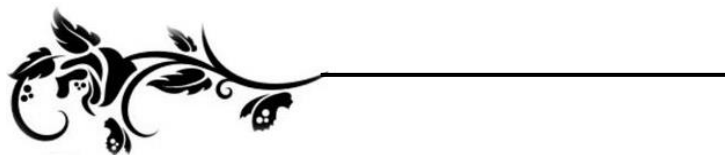
أ.د/ معتز بالله أحمد الخواص

أستاذ علاج الجذور - رئيس قسم علاج الجذور
جامعة الأزهر - كلية طب الأسنان بنين - القاهرة

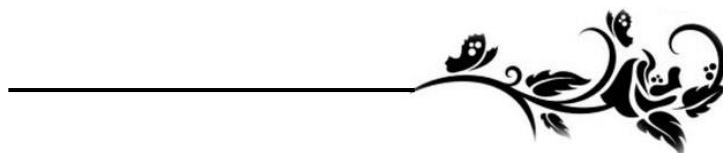
د/ عمرو عبد الوهاب بيومي

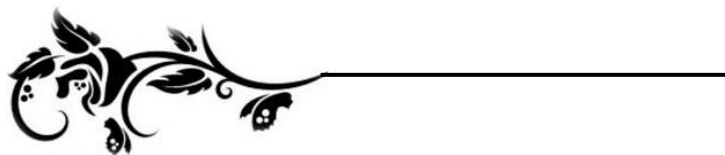
مدرس علاج الجذور
جامعة الأزهر - كلية طب الأسنان بنين - القاهرة

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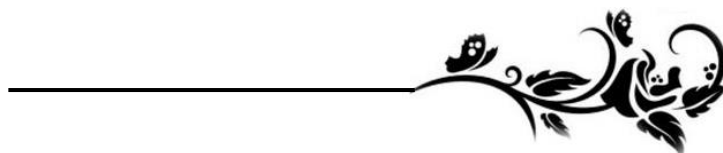


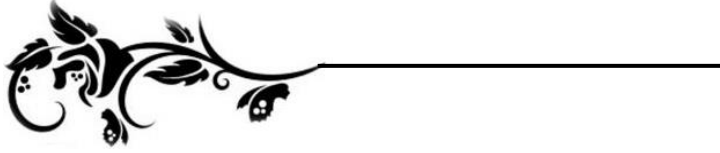
Introduction



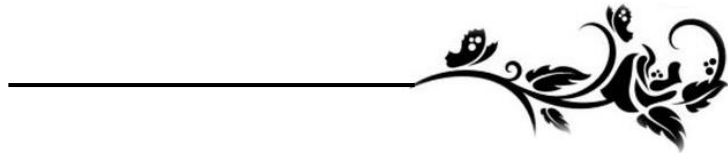


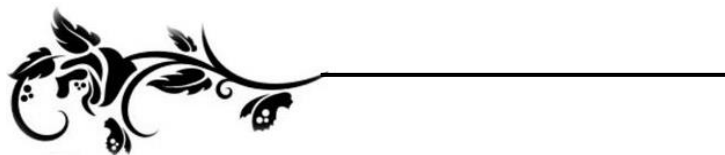
Aim of the study



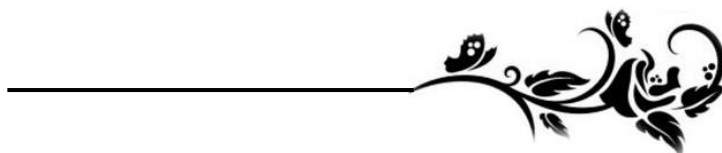


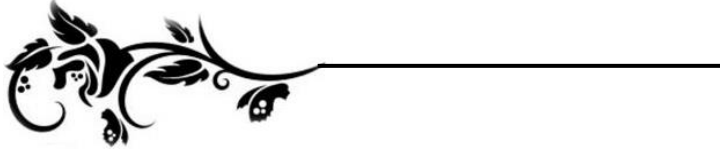
Review of Literature



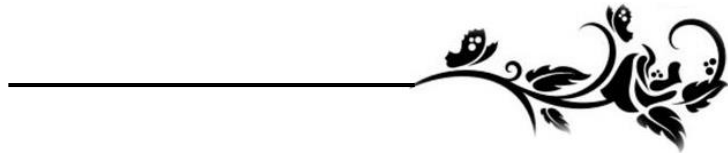


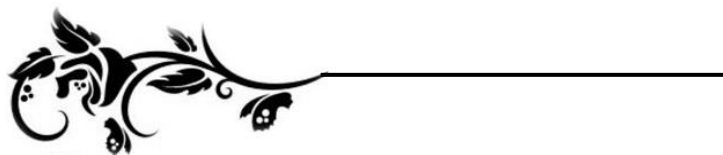
Materials and Methods



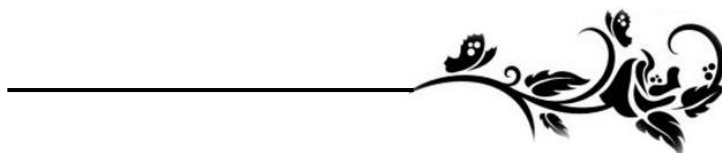


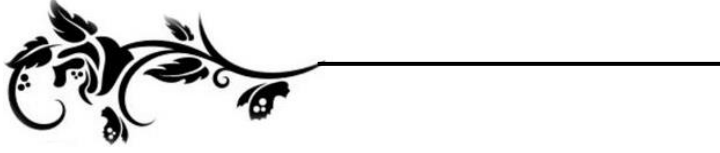
Results



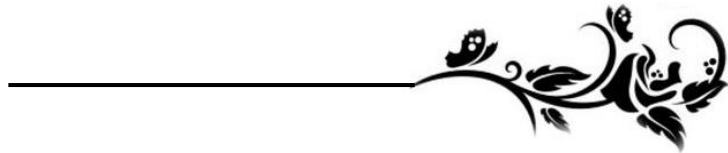


Discussion



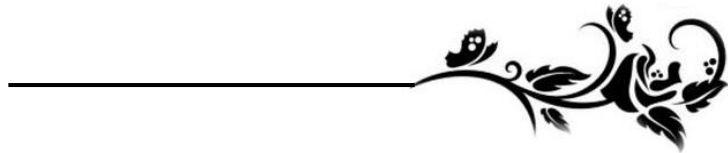


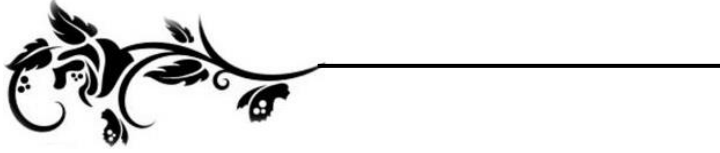
Summary



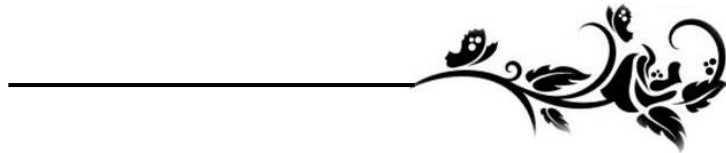


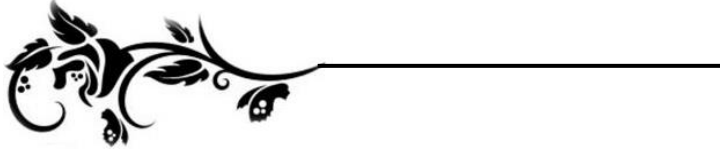
Conclusion



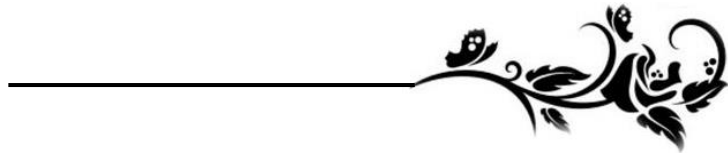


Recommendations





References





Arabic Summary

