International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 702-710 www.ijdmsrjournal.com ISSN: 2582-6018

# Comparative Evaluation of Effectiveness of Nanoparticle Solutions and Conventional Endodontic Irrigants against Enterococcus Faecalis Biofilm

Dr.Sneha Mariam Lal [1], Dr.Shravan Kini [2], Dr Prathap MS [3]
*Postgraduate Student, Yenepoya Dental College, Mangalore, Karnataka*
*Reader, Yenepoya Dental College, Mangalore, Karnataka*
*Professor and HOD, Yenepoya Dental College, Mangalore, Karnataka*

Submitted: 05-02-2023 Accepted: 20-02-2023

**ABSTRACT**
**AIM:** To compare the effectiveness of NaOCl, EDTA and Nanoparticle Solution (Silver and Chitosan) against E. faecalis biofilm in the root canal
**METHODOLOGY:** An anonymous sample of twenty extracted human teeth was cleaned and shaped. Biofilm was grown on the root canal surface over a period of 1 week. SEM analysis of four teeth revealed the presence of biofilm. The remaining teeth were randomly split into 4 groups (n=4): G1- 1% NaOCl; G2- 17% EDTA; G3- 2mg/ml Silver nanoparticle solution; G4- 2mg/ml Chitosan nanoparticles solution. The susceptibility of E.facalis biofilm was determined by quantification of CFUs. Data was analysed using one way ANOVA and Tukey's post hoc analysis for multiple comparison ($p<0.05$)
**RESULTS:** NaOCl showed greatest reduction in CFUs followed by Chitosan nanoparticles, Silver nanoparticles and EDTA.
**CONCLUSION:** During the treatment of primary and recurring endodontic infections, it is essential to eliminate biofilms. Nanoparticles give new prospects for endodontic disinfection, thus they can be considered as alternative irrigants. It is, however, sodium hypochlorite which remains the gold standard among irrigants for effective bacterial elimination.
**KEYWORDS:** Biofilm, E.faecalis, Nanoparticles, Silver, Chitosan

## I. INTRODUCTION

Endodontic treatment success depends on the efficient removal of bacterial biofilm from the root canal system because endodontic infection is a biofilm-mediated infection. The intricate structure of the root canal system and innate microbiologic variables are the causes of the bacterial biofilm's resistance to current disinfectants. [1]

The capacity of E. faecalis to penetrate dentinal tubules and cling to collagen in the presence of human serum may be connected to its virulence factor in teeth that failed endodontic treatment. Its virulence may also be related to its adherence to host cells, resistance to intracanal medications, expression of proteins to ensure cell survival due to altered nutrient supplies, capacity to compete with other bacterial cells, ability to alter host response and environment, and expression of proteins to ensure cell survival. [2]

It has been established that 2.5% NaOCl is incredibly effective at removing pulp tissue. Yet, when driven into periapical tissues during irrigation, significant irritations have been described. The elastic modulus and flexural strength of dentin are markedly reduced by 5.25% NaOCl solution. This has prompted researchers to test other alternatives. [3]

Due of its capacity to chelate with calcium ions in dentin and dissolve the inorganic component from smear layers, ethylenediaminetetraacetic acid (EDTA) is frequently utilised in clinics as the gold standard for final irrigation solutions. However, the continuous administration of EDTA to the dentinal wall may result in dentin erosion and reduce microhardness. As a result, the quest for a substance with greater antibacterial capabilities and biocompatibility than EDTA has been noted more frequently. [4]

Nanomaterials are defined as particles with diameter of 1-100nm, exhibiting large surface/ area mass ratio and exponential chemical reactivity [5]. Nanoparticles have increased levels of contact with the bacterial cell as a result of their polycationic/polyanionic nature, which has higher surface area and charge density. The antibacterial activity of nanoparticles has been found to be strongly influenced by their size, with smaller particles exhibiting stronger antibacterial activity than macroscaled ones .[1]

Silver nanoparticles (AgNp) and chitosan nanoparticles (CNP) produced favourable antibacterial and antibiofilm outcomes. This stimulated the use of nanoparticles, as an irrigant or

International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 702-710 www.ijdmsrjournal.com ISSN: 2582-6018

intracanal dressing to clean root canals during endodontic therapy .[1, 6]

Due to their broad-spectrum bactericidal and virucidal characteristics, silver nanoparticles have been used extensively in numerous medical sectors. Due to its distunctive chemical and physical characteristics and high surface-to-volume ratio, silver nanoparticles are more reactive than larger particles. Silver nanoparticles exhibit a variety of antibacterial actions, including adhesion and penetration into the bacterial cell wall, which causes bacterial cell membrane integrity to be lost and cell wall permeability. [6]

According to earlier research, silver nanoparticles between 10 and 100 nm in size exhibited strong bactericidal ability against multidrug-resistant bacteria as well as gram-positive and gram-negative bacteria. Root canal irrigating solution has been advocated as an alternative to silver nanoparticle solution due to its significant bactericidal capability as well as biocompatibility, particularly at lower concentrations. [7,8]

Chitin, which makes up the majority of crab exoskeletons, can be converted into chitosan, a non-toxic cationic biopolymer, by alkaline deacetylation. In addition to having biocompatibility and chelating properties, chitosan also exhibits antibacterial properties against a variety of gram-positive and gram-negative bacteria as well as fungi. Chitosan polycations' interactions with the negatively charged surfaces of microbes are suggested as the mechanism of action. These interactions affect cellular permeability and result in cellular component leakage. [9]

Thus the purpose of this study is to evaluate the effectiveness of nanoparticle solutions and conventional endodontic irrigants against E. faecalis biofilm.

## II. METHODOLOGY

The research was carried out in the department of Conservative Dentistry and Endodontics, Yenepoya Dental College and Yenepoya Research Center

### SAMPLE SELECTION

Orthodontically extracted, anonymised, single rooted human premolar teeth (n=20) were used. The teeth were acquired from Department of Oral and Maxillofacial Surgery, Yenepoya Dental College. Sample size was calculated using G* power software. At 1% level of significance and power at 99% , the minimum sample required to test the above difference and with effect size 6.5 in each group is 4.(Total sample size=20)

The inclusion criteria were single rooted premolar teeth. Grossly decayed teeth, teeth with open apex, fracture lines, internal and external resorption, calcification, severely curved roots, more than one canal were excluded.

Teeth were scaled and cleaned of debris and periodontal remnants. Samples were stored in distilled water until use. Radiographs were taken, standard access cavities were prepared and working length was determined. Teeth were decoronated at a distance of 12 mm from the root end in order to standardise the length of the root canal.

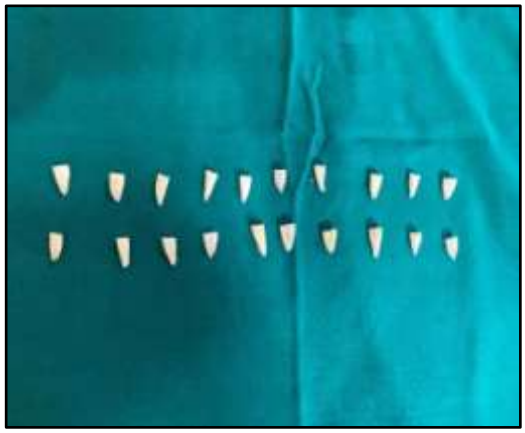


**FIGURE 1 DECORONATED TEETH**

Canals were instrumented using Protaper Next System 1mm shorter from apical foramen upto size X5. During instrumentation, 1% NaOCl was used as root canal irrigant. Final rinse of the canal was performed with 17% EDTA followed by 1% NaOCl to remove debris.

The inactivation of NaOCl was accomplished by addition of 10 µL of 5% sodium thiosulphate 703ipette into each tooth. Sterility of teeth after complete root canal cleaning and shaping was achieved by autoclaving for 15 minutes at 121°C with teeth immersed in distilled water.

### BIOFILM DEVELOPMENT

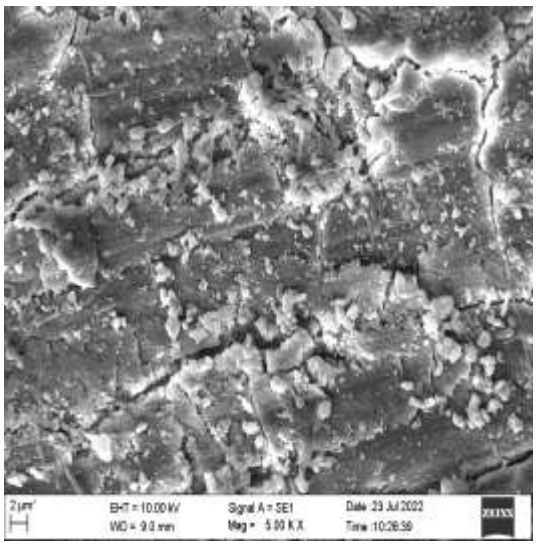
E. faecalis (ATCC 29212), a common strain was utilized. The E. faecalis counts in brain heart infusion broth (BHI) were established before testing. Aliquot portions were plated on the surface of BHI agar and incubated at 37°C for 48 h. The number of colony-forming units CFU/mL were calculated after incubation.

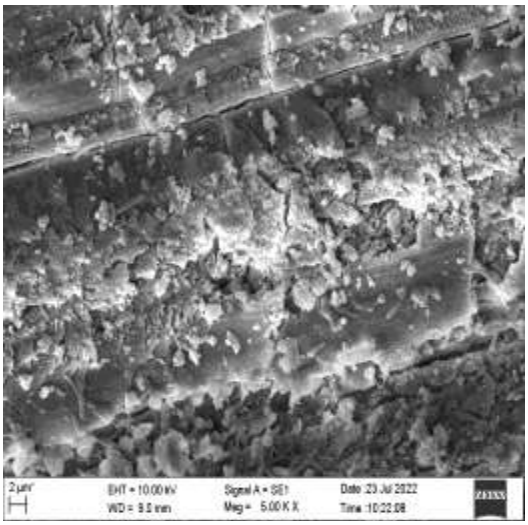
0.1-mL aliquots of E. faecalis culture enriched with 0.4% sucrose was introduced into the canals. Every 24 hours for seven days, the medium was changed by adding a fresh 100 L aliquot of sterile BHI in place of the BHI that had been

International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 702-710 www.ijdmsrjournal.com ISSN: 2582-6018

previously infused with E. faecalis. Throughout the experiment, the equipment was incubated at a temperature of 37 °C. Four roots were analysed by SEM to verify the presence of biofilm. Roots were longitudinally sectioned, fixed in 2.5% glutaraldehyde and 0.1M cacodylate solution, sputter-coated with a gold layer, and evaluated by Scanning Electron Microscope.

**FIGURE 2 TOOTH SAMPLES INNOCULATED WITH E. FAECALIS**





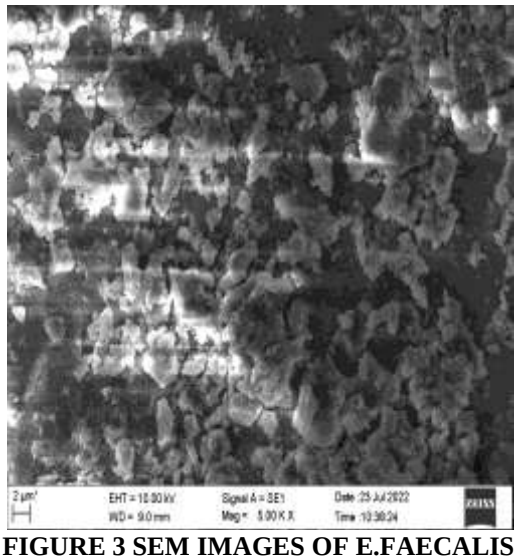


**FIGURE 3 SEM IMAGES OF E.FAECALIS WITHIN ROOT SAMPLES**

International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 702-710 www.ijdmsrjournal.com ISSN: 2582-6018

**Groups and disinfecting solutions**

The remaining roots were split into 4 groups (n = 4) : G1 – 1% NaOCl ; G2 – 17% EDTA; G3- 2 mg/ml Silver Nanoparticle solution; G4 – 2 mg/ml Chitosan Nanoparticle solution. The root canal was accessed with a sterile needle until it was 2 mm short of the working length and 3mL of the irrigant was administered for five minutes. Three millilitres of sterile saline solution was used to flush the canals.

**Colony counting**

Root canals in all groups were dried with sterile paper points and refilled with sterile distilled water using sterile syringe. Thereafter, sterile paper points, size #35 were introduced into the canals and maintained for 3 minutes for sample collection. One minute later, they were transferred into sterile Eppendorf tubes containing one milliliter of fresh saline. For the purpose of dislodging bacteria from the paper points, all tubes were vortex mixed for one minute.

Serial dilutions of all bacterial samples were made A 100 microliter aliquot of each sample was pipetted onto BHI agar plates and incubated at 37°C for 48 h.

A classical colony counting technique was used for each group, after treatment application for recovery of viable E. faecalis on agar plates. The mean value of CFU for plates of each group were calculated.

**FIGURE 4 COLONY FORMING UNITS IN GROUP 1**

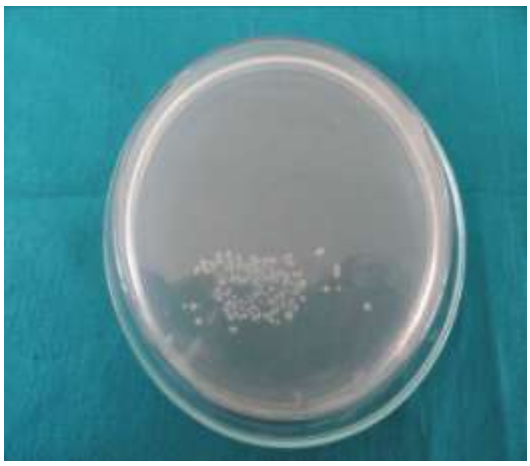


**FIGURE 5 COLONY FORMING UNITS IN GROUP 2**

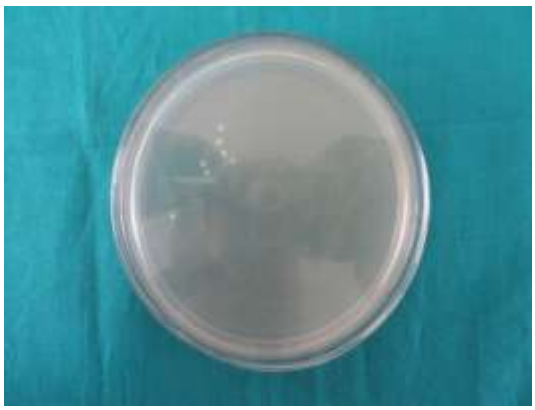


**FIGURE 6 COLONY FOMING UNITS IN GROUP 3**

**FIGURE 7 COLONY FORMING UNITS IN GROUP 4**

## III. STATISTICAL ANALYSIS

Descriptive statistics and Analysis of variance (One Way-ANOVA) technique/ Kruskal-Wallis test will be used to test the bacterial colony forming units among the groups Post-hoc analysis will be done using Tukey's test to assess the difference (pairs) in colony forming units

International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 702-710 www.ijdmsrjournal.com ISSN: 2582-6018

## IV. RESULTS

**Comparison between group 1 (NaOCL),group 2 (EDTA),group 3 (AgNP),group 4 ( Chitosan NP).**
We have used analysis of variance (ANOVA) technique to compare the groups.

| CFU/ml | Mean(Standard deviation) | F value | p value |
|---|---|---|---|
| group 1 (NaOCL), | 1.25(1.5) | 65.667 | 0.000 |
| group 2 (EDTA), | 19.25(2.986) | | |
| group 3 (AgNP) | 5.75(1.5) | | |
| group 4 ( Chitosan NP) | 5.25(1.258) | | |

p value<0.05 considered as significant.

The least number of CFUs were found in Group 1, thus proving NaOCl is effective compared to other groups.
Multiple Comparison

| | | p value | 95% Confidence Interval | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| group 1 (NaOCL) | group 2 (EDTA | 0.000 | -22.07 | -13.93 |
| | group 3 (AgNP) | 0.029 | -8.57 | -0.43 |
| | group 4 ( Chitosan NP) | 0.054 | -8.07 | 0.07 |
| group 2 (EDTA) | group 3 (AgNP) | 0.000 | 9.43 | 17.57 |
| | group 4 ( Chitosan NP) | 0.000 | 9.93 | 18.07 |
| group 3 (AgNP) | group 4 ( Chitosan NP) | 0.983 | -3.57 | 4.57 |

Intergroup comparison shows:
There is a significant difference between NaOCl and EDTA
There is a significant difference between NaOCl and AgNP
There is a significant difference between EDTA and AgNp
There is a significant difference between EDTA and CNP

## V. DISCUSSION

Over the years, extensive research has been done on the microbiota of root canals [10]. It appears that in 60% to 90% of teeth with apical periodontitis, bacteria colonise the root canals, canal ramifications, isthmuses, and other morphological abnormalities of the root before penetrating the dentinal tubules [11].

Constant infection in the peri-apical region could be the result of challenges encountered during or in the beginning stages of treatment. The primary causes of post-endodontic treatment diseases include the ongoing presence of microorganisms in the apical region of teeth with root canal fillings, insufficient aseptic control, poor cavity access design, missed canals, insufficient instrumentation, and improper temporary or permanent restorations [10]. Under the severe, nutrient-limited conditions of the root-filled teeth, resistant species have been found to survive, including enterococci and streptococci, followed by lactobacilli, Actinomyces species, peptostreptococci, Candida, Eubacterium alactolyticus, and Propionibacterium propionicum [12]

Enterococci, a gram-positive facultative anaerobe, can endure even the most adverse

environmental circumstances. They can propagate in the presence or absence of oxygen and at temperatures ranging from 10 to 45 degree Celcius at a pH of 9.6 in 6.5% NaCl broth. They have demonstrated their ability to survive root canal infections with fewer nutrients and fewer opportunities to elude root canal medications, even at 60 degree Celcius for 30 minutes. [13]

The ability to spontaneously mutate and the proton pump associated with the cell wall allow Enterococcus faecalis to survive as pathogenic bacteria [14]. This organism exists either in single, pairs or as short chains. This microbe appears to be the most effective in inducing gross infection through experimental penetration into dentinal tubules by forming chains. It was employed in earlier investigations on the effectiveness of irrigant solutions and intracanal medicines, but it is one of the few facultative organisms connected to chronic apical periodontitis. [15]

Proper instrumentation, irrigation, and obturation of the root canal are essential components of a successful root canal procedure. The root canal's irrigation, one of the three crucial procedures in root canal therapy, has the greatest impact on how quickly the periapical tissues heal. Optimizing root canal sanitation must be the primary endodontic therapy objective. [16] Literature lacks data demonstrating that mechanical instrumentation alone produces a root canal system free of germs. [17]

The success of the root canal procedure will be determined by the elimination of microorganisms and prevention of re-infection in the root canal system. To remove inflamed necrotic tissues, bacteria or biofilms, and debris from the root canal system, cleaning and shaping the root canal is done using hand and rotary devices along with irrigants. Instrumentation's main objectives are efficient irrigation, filling, and disinfection. Chemo-mechanical preparation using antibacterial irrigants and mechanical tools, which significantly reduces the amount of bacteria in the diseased root canal, is the key step in canal disinfection. According to a biological perspective, the goals of chemo-mechanical preparation are to eliminate microorganisms from the root canal system, remove pulp tissue that could support microbial growth, and avoid driving debris past the apical foramen to avoid inflaming the area. [18]

Mechanical instrumentation attempts at debriding the infected canal walls, but it cannot eliminate contamination from intricate areas. In teeth with intricate internal architecture, fins, or other anomalies that might have been missed during instrumentation, chemical debridement is crucial. By adding irrigants to mechanical debridement methods, these issues may be resolved. The optimal irrigant should have long-lasting antibacterial effects, eliminate smear layers, have low surface tension, and be capable of sterilising dentinal tubules. It should also be an effective fungicide or germicide. [16]

Given that it satisfies the majority of the requirements for an endodontic irrigant, sodium hypochlorite now appears to be the most optimal option. [16] In World War I, chemist Henry Drysdale Dakin and surgeon Alexis Carrel utilised a buffered 0.5% sodium hypochlorite solution to irrigate the infected wounds based on their research on the effectiveness of various solutions on infected necrotic tissues. The formulations of hypochlorite were sporicidal, virucidal, with wide range, nonspecific killing efficacy on all microorganisms and stronger tissue dissolving effects on necrotic than on live tissues. Due to these qualities, aqueous sodium hypochlorite has been used as a primary irrigant in endodontics since 1920.

Sodium hypochlorite solution is also affordable, accessible, and has a long shelf life. In essence, it performs as an organic and fat solvent that breaks down fatty acids to form soap and alcohol, thereby lowering the surface tension of the residual solution and is known as the saponification reaction. The neutralisation reaction that results when sodium hypochlorite neutralises amino acids produces water and salt. Hydroxyl ions leave the system, lowering the pH and Chlroamination reaction occurs when hypochlorous acid contacts organic tissue and releases chlorine.

Both amino acid hydrolysis and degradation are caused by hypochlorous acid (HOCl-) and hypochlorite ions (OCl-). Additionally, the sodium hypochlorite acts as a solvent, releasing chlorine that reacts with protein amino groups (NH) to create chloramines, known as the chloramination process, which disrupts cellular metabolism. The antibacterial effect of chlorine (a strong oxidant) inhibits bacterial enzymes, which causes an irreversible oxidation of the SH groups (sulfhydryl groups) of crucial bacterial enzymes. [16]

By changing pH, NaOCl's effectiveness can be improved. When 5.25% NaOCl is diluted, its capacity to dissolve tissue and fight bacteria is reduced. The following reaction occurs when NaOCl is introduced to water: NaOH + HOCl (hypochlorous acid) = NaOCl + H2O Hypochlorous acid partially dissociates into the anion hypochlorite (OCl) in aqueous solution. From 1% to 5.25 percent, sodium hypochlorite can be utilised in different concentrations. There will be

dangerous repercussions as the concentration rises. According to a study by Siquerra, using them at different doses had no effect on their antibacterial effects. The use of 1% NaOCl solution in root canals and dentinal tubules resulted in a sizable reduction in germs in the current investigation.[16,19]

EDTA is a white, water soluble substance that is a polyaminocarboxylic acid. Its capacity to chelate, or sequester, metal ions like Ca2+ and Fe3+, as well as its function as a hexadentate ligand, make it useful. Metal ions are still in solution after being bound by EDTA, although they are less reactive. Ferdinand Munz, who made the chemical from ethylene diamine and chloroacetic acid, originally characterised it in 1935. The major ingredients used nowadays to make EDTA are sodium cyanide, formaldehyde, and ethylene diamine. When EDTA interacts with the calcium ions in dentine, soluble calcium chelates are created. Dentin has reportedly been decalcified by EDTA to a depth of 20 to 30 m in about five minutes.

The chelation of cations from the bacterial outer membrane appears to be the cause of EDTA's antibacterial effect. According to Russell, 10% EDTA creates a zone that inhibits bacterial development in a manner akin to creosote. Lower EDTA concentrations, on the other hand, resulted in little to no inhibitory zone. According to Kotula and Bordácová, Na EDTA's antibacterial action is still there even after the chelators have broken their binding with metal ions. Yoshida et al. evaluated the clinical bactericidal efficacy of EDTA in conjunction with ultrasonic activation. Most patients were bacteria-free after 7 days without the use of any intracanal medications. [20]

Heling and Chandler found that Gram negative bacteria were more resistant to RC Prep than Gram positive ones. The effectiveness of RC Prep against Staphylococcus aureus was found to increase when the temperature was raised from 10°C to 45°C, according to Heling et al. The impact of RC Prep's ingredients on Streptococcus sobrinus was examined in a study. Findings showed that a bactericidal action required a minimum concentration of 0.25% for EDTA and 50% for glycol. Orstavik and Haapasalo, however, questioned the bactericidal efficacy of 17% EDTA. According to Ordinola Zapata et al., EDTA had no discernible impact on the structure and survival of the biofilm. According to Ballal et al., maleic acid and EDTA are equally effective against Enterococcus faecalis. Even after 60 minutes of contact, Arias Moliz et al. demonstrated that EDTA was ineffective against E. faecalis. Bystrom and Sundqvist demonstrated that the antibacterial activity of 5% NaOCl with EDTA was superior to that of NaOCl alone. [20]

However, none of the irrigants currently on the market meet the ideal criteria for an irrigant. As a result, ongoing research is being done to find the perfect irrigant. The Greek word "Nano" means "dwarf small old man." One billionth of a metre, or 10-9, is a nanometer. Physicist Richard P. Feynman initially proposed the idea of using nanotechnology in medicine in 1960. Nanomedicine is currently employed for illness diagnosis, treatment, and prevention.

Nanotechnology-based research tools, such as protein chips, are utilised to better understand the molecular causes of disease and to find novel molecular targets for treatment. Dental materials such ceramics, coatings for implants, bioceramics, fluoride-containing mouthwashes, fissure sealing materials, and light polymerization composite resins and bonding systems are all produced using nanotechnology. These nanoparticles are more active because to their larger surface area, which allows them to interact more strongly and enter dentinal tubules to increase "nano retention."

Different microorganisms in the root canal space are directly targeted by irrigant solutions based on nano silver. The biologically active silver ions that are released when silver and nano silver are dissolved in water really mediate the bactericidal effect. [8] Silver's toxicity is its most critical feature in biomedical applications. By creating smaller silver particles with lesser toxicity to human cells and more efficacy against microbes, nanotechnology is able to circumvent the toxicity effects in human cells that are only observed after prolonged exposures to silver. [21] The peptidoglycan cell wall and plasma membrane, cytoplasmic DNA, and bacterial proteins are the three primary components of the bacterial cell that interact with silver ions to create the bactericidal action. [22]

The CS-NPs' antibacterial activity is most likely caused by interactions with the bacterial cell membrane or cell wall. Various hypotheses have been put out in an effort to elucidate this mechanism. The electrostatic interaction between the positively charged amino groups of glucosamine and the negatively charged bacterial cell membranes is the most well-known CS-NPs paradigm of antibacterial activity. This interaction causes the cell's surface to change often, altering the membrane's permeability in the process. This causes an osmotic imbalance and the outflow of intracellular chemicals that kills the cell [23].

The antibacterial effect of the irrigants utilised in the current investigation had a noticeable impact on E. faecalis. All four of the experimental irrigants showed statistically significant reductions in E. faecalis when measured by colony forming units. This could be explained by the mode of action of specific irrigants.

In the present study on analyzing the CFUs for NaOCl group the mean CFU was least comapared to other experimental groups. This is consistent with research by CT. Rodrigues and et al (2018) At 5, 15, and 30 minutes, 2.5% NaOCl exhibited the greatest antibacterial activity and biofilm dissolving capacity. Compared to NaOCl, 94ppm AgNp solution demonstrated reduced antibacterial activity in infected dentinal tubules. After 5 minutes, the AgNp solution was more successful in removing planktonic bacteria. [5] According to Daming Wu et al (2014) the structure of the biofilm was not altered by treatment with a 0.1% AgNP solution. With a 2-minute irrigation, the 2% sodium hypochlorite group eradicated the E. faecalis biofilms.The structural integrity of the biofilms that were treated with 0.02% AgNP gel as a medication was considerably compromised. The results of this investigation revealed that the mode of application affects AgNPs' antibiofilm efficacy.AgNPs demonstrated the ability to remove persistent bacterial biofilms during root canal disinfection when used as a medication rather than an irrigant.[8]

Asmaa Faiek et al (2019) demonstrated that 0.5% chitosan had a stronger antimicrobial impact than saline but less potent than 3% NaOCl over a period of 5 minutes, indicating that it might be utilised as a natural substitute. [24]

However, the subsequent studies are incongruous. According to Sameer Makkar et al (2014) study, following contact with 0.1% AgNP solution for three minutes, the number of CFUs decreased and remained zero after 5 minutes.. 3% NaOCl showed bacterial growth in 3 minutes, 5 minutes and dropped to zero in 10 minutes. T his indicated a good antimicrobial effect for Silver nano particle in agar diffusion test. [25]

According to Leila Moghadas et al. (2012), who compared the effectiveness of 5.25% NaOCl with silver nanoparticle irrigant, the latter is just as effective .[22]

Abidin and et al (2022) stated that with the addition of PUI activation, irrigation of root canal therapy with nano-chitosan high molecular 0.2% demonstrated substantial antibacterial activity against E. faecalis. As a result, this research reveals a potential use for nano-chitosan in next biomaterial applications. [26]

From the results of the present study it can be inferred that sodium hypochlorite is an excellent antimicrobial and gold standard among irrigants.. However, in the present study various concentrations of silver and chitosan nanoparticle irrigant were not taken into consideration and different time period of application at different concentrations.

## VI. CONCLUSION

Recurrent infections and growing antimicrobial drug resistance could result in treatment failure in the field of endodontics. The pursuit of the optimal disinfectant irrigannt continues. Within the limitations of the current in vitro study, NaOCl, then chitosan NP, AgNp, and EDTA, showed efficiency in reducing the E. faecalis count in root canals.

Given the study's limitations, it may be said that using silver and chitosan nanoparticles to irrigate root canals has an antibacterial impact. It is possible to employ them as irrigants. However, the effectiveness of these nanoparticles depends on variables including particle size and the length of contact, which needs more research to fully comprehend their use as an irrigant.

## REFERENCES

[1]. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod. 2010;36(6):1030-1035.

[2]. Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405.

[3]. Moradi F, Haghgoo R. Evaluation of antimicrobial efficacy of nanosilver solution, sodium hypochlorite and normal saline in root canal irrigation of primary teeth. Contemp Clin Dent. 2018;9(2):S227- S232.

[4]. Ratih DN, Sari NI, Santosa P, Kaswati NMN. Time-Dependent Effect of Chitosan Nanoparticles as Final Irrigation on the Apical Sealing Ability and Push-Out Bond Strength of Root Canal Obturation. Int J Dent.;2020:8887593

[5]. Rodrigues CT, de Andrade FB, de Vasconcelos LR, Midena RZ, Pereira TC. Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. Int Endod J. 2018;51(8):901-911.

[6]. Del Carpio-Perochena A, Bramante CM, Duarte MA, de Moura MR, Aouada FA,

Kishen A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015;40(3):195-201.

[7]. de Almeida J, Cechella BC, Bernardi AV, de Lima Pimenta A, Felippe WT. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res. 2018;29(3):347-351.

[8]. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod. 2014;40(2):285-290.

[9]. Supotngarmkul A, Panichuttra A, Ratisoontorn C, Nawachinda M, Matangkasombut O. Antibacterial property of chitosan against E. faecalis standard strain and clinical isolates. Dent Mater J. 2020;39(3):456-463.

[10]. Vytaute Peciuliene, Rasmute Maneliene, Estera Balcikonyte, Saulius Drukteinis, Vygandas Rutkunas Microorganisms in root canal infections: a review Stomatologica , Baltic Dental and Maxillofacial Journal 2008;10:1

[11]. Sundqvist G, Figdor D. Life as an endodontic pathogen. Etiological differences between untreated and root-filled root canals. Endod Top 2003

[12]. Siqueira JFJr, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004

[13]. Sonia Bhonchal Bhardwaj Role of Enterococcus faecalis in failure of endodontic treatment Int.J.Curr.Microbiol.App.Sci(2013)2(8): 272-277

[14]. Tanriverdi, F., T. Erener, O. Erganis and Belli, S. An in vitro test model for investigation of dentinal tubules infected with Enterococcus faecalis- The principles of techniques for cleaning root canals Braz .Dent. J. 1997 8:67-72.

[15]. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001;27:76-81.

[16]. Deivanayagam Kandaswamy, Nagendrababu Venkateshbabu Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide International endodontic journal 2010,419-423

[17]. Mohammadi Z ,Jafarzadeh H Antimicrobial efficacy of chlorhexidine as a root canal irrigant Journal of oral science 2014; 56(2

[18]. Young GR, P Parashos, HH Messer The principles of techniques for cleaning root canals Australian Dental Journal Supplement 2007;52:(1 Suppl):S52-S63

[19]. Jefferson JCM, Frederico CM Efficiency of different concentrations of sodium hypochlorite during endodontic treatment: literature review Dental press endodontics 2012 2(4)

[20]. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid in endodontics. Eur J Dent 2013;7:135-42.

[21]. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli

[22]. Moghadas, L., Shahmoradi, M. and Narimani, T., 2012. Antimicrobial activity of a new nanobased endodontic irrigation solution: In vitro study. Dental Hypotheses, 3(4), p.142.

[23]. Chandrasekaran, M., Kim, K.D. and Chun, S.C., 2020. Antibacterial activity of chitosan nanoparticles: A review. Processes, 8(9), p.1173.

[24]. Faiek AM, EL-Malt MA, Ali HA, Mohamed EA. Evaluation of antimicrobial efficacy of chitosan as root canal irrigant on Enterococcus Faecalis. Al-Azhar Dental Journal for Girls. 2019 Oct 1;6(4):437-43.

[25]. Makkar S, Aggarwal A, Pasricha S, Kapur I. To evaluate the antibacterial properties of silver nano particle based irrigant as endodontic root canal irrigant. Int J Dent Health Sci. 2014;1(4):485-92.

[26]. Abidin T, Susilo D, Gani BA. The effectiveness of nano-chitosan high molecular 0.2% as irrigant agent against Enterococcus faecalis with passive ultrasonic irrigant. Journal of Conservative Dentistry. 2022 Jan 1;25(1):37.