A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF SILVER NANOPARTICLES, NANO-CHITOSAN, GRAPHENE NANOPARTICLES AND CHLORHEXIDINE AGAINST ENTEROCOCCUS FAECALIS AS ROOT CANAL IRRIGANTS : AN IN-VITRO STUDY

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree

of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

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Under the guidance of

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

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I hereby declare that this dissertation entitled **"A comparative evaluation of antimicrobial efficacy of Silver Nanoparticles, Nano-Chitosan, Graphene Nanoparticles and Chlorhexidine against Enterococcus Faecalis as root canal irrigants : An in-vitro study"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Vishesh Gupta**, Reader, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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OM NAMAH SHIVAAY

Dr. Sankalp Nigam

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
E. faecalis	Enterococcus faecalis
RCT	Root Canal Treatment
RCS	Root Canal System
POE	Portal of Exit
NaOCl	Sodium Hypochlorite
CHX	Chlorhexidine
EDTA	Ethylene Diamine Tetra Acetic Acid
$Ca(OH)_2$	Calcium Hydroxide
AgNP	Silver Nanoparticles
CsNP	Chitosan Nanoparticles
GNP	Graphene Nanoparticles
W/v	Weight by Volume
%	Percentage
&	And
BHI	Brain Heart Infusion
mL	Millilitre
µL	Microlitre
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
GO	Graphene Oxide
GIC	Glass Ionomer Cement

ABSTRACT

The aim of endodontic treatment is to clean the root canal system all vital and necrotic tissues, microorganisms, and its byproducts. The root canal system anatomy is extremely complex and diverse, it's not always possible to clean and shape it effectively. So, irrigation and disinfection of Root Canals plays a very important role in the success of a Root Canal Treatment. The aim of this study is to evaluate & compare the antimicrobial efficacy of Silver Nanoparticles, Nano-Chitosan, Graphene Nanoparticles and Chlorhexidine as root canal irrigants against Enterococcus faecalis.

Sixty-five permanent extracted anterior human teeth were taken and decoronated to standardize the canal length. After biomechanical preparation, teeth were inoculated with E.faecalis & randomly divided into five groups and the final irrigation was carried out with tested irrigants. Group I(n = 15): 0.2% Silver Nanoparticles; Group II(n = 15): 0.2% Nano-chitosan; Group III(n = 15): 0.2% Graphene Nanoparticle; Group IV(n = 15): 2% Chlorhexidine; and Group V(n=5): normal saline (negative control).

The obtained constituent was cultured on agar plates & the number of CFUs (Colony forming units) per plate was determined using a digital colony counter, and statistically analysed using one-way ANOVA followed by post hoc Tukey test.

Statistically no significant difference was observed between the tested irrigants i.e. 2% Chlorhexidine, 0.2% Silver Nanoparticles, 0.2% Nano-chitosan, 0.2% Graphene Nanoparticles, when compared to the control group i.e. Normal Saline; with mean colony count being 2.34, 2.5, 3.35, 3.38 & 5.66 CFU/ml (10^6) respectively, against Enterococcus faecalis.

The present in-vitro study indicates that all the tested irrigants : Chlorhexidine, Silver Nanoparticles, Nano-chitosan & Graphene Nanoparticles, have exhibited effective antimicrobial efficacy against E. faecalis, with Chlorhexidine being the most effective, followed by Silver Nanoparticles, Nano-Chitosan and Graphene Nanoparticles respectively.

Further, more in-vivo studies are suggested to co-relate clinically and evaluate which nanoparticles are more appropriate as root canal irrigants, not just against E. faecalis, but also other persistent endodontic pathogens.

Keywords: Enterococcus faecalis, Graphene, Nano-chitosan, Nanoparticles, Root Canal Irrigant, Root Canal Irrigation, Silver Nanoparticles.

INTRODUCTION

Microbes have colonised every surface of the human body. Microorganisms were observed in samples from teeth by Leeuwenhoek soon after he invented the microscope in 1684. Since Babylonian times, it was believed that a 'tooth worm' lived in the hollow portion of the tooth and caused decay. Leeuwenhoek challenged the 'tooth worm' theory of decay by identifying worm-infested cheese that he thought may be the source of disease [1]. Leeuwenhoek also described microorganisms that he scraped from teeth as 'cavorting beasties'. However, it took over 200 years before his observation was confirmed and a cause and effect relationship was suggested by Miller. Since 1890, when Miller first observed microorganisms associated with pulp tissue, microorganisms have been implicated in infections of endodontic origin.[2]

Normal flora is the outcome of bacteria colonising the environment on a long-term basis in a favorable symbiotic relationship. Normal oral bacteria, on the other hand, may become opportunistic and cause disease if they get access to typically sterile regions of the body, such as the dental pulp or periradicular tissues, under favourable conditions.[3]

Microorganisms play an important role in the development of pulp & periapical diseases.[4] Almost 700 bacterial species can be found in the oral cavity, with any particular individual harbouring 100–200 of these species.[5] Microorganisms play an unequivocal role in infecting root canal system. Endodontic infections are different from the other oral infections with respect to the environment in which they occur, since the root canal system is an enclosed body, surrounded by hard tissues all around.[6,7] Endodontic infections initiate and progress when the root canal system gets exposed to the oral environment by one or more reasons and simultaneously compromised host's immune response [8].

Preservation of teeth by endodontic therapy has gained a lot of popularity because of increased and predictable success rate of endodontic procedures, the reason behind this is the complete understanding of root canal anatomy and endodontic pathology, with our ability to combat the same [9]. The rationale for endodontic treatment is to eradicate the

infection, to prevent microorganisms from infecting or re-infecting the root and/or periradicular tissues [10]. The micro-flora comprising the root canal is typically unique and specific. The endodontic infections constitute almost 40-50% of the overall oral diseases.[11] Pulpal and periapical pathology are the commonest debilitating form of oral diseases with systemic implications. Though success rate of endodontic therapy ranges from 30-90% of treated cases, failure rate is equally high accounting to millions[11].

Even after completion of proper cleaning and shaping procedure it has been observed that an average of 1 to 5 bacterial species have been found in the root canals and these counts were marked up to 102 to 105 cells per canal [12]. It is a well-established fact that despite following the standard protocol of endodontic treatment, failures do occur. This may be due to multiple reasons but among these microbiological factors have a significant role to play [6].

The commonest microbes which persist even after the chemo-mechanical preparation are most commonly anaerobic rods such as F. nucleatum, Prevotella species, and C. rectus or Gram-positive bacteria such as Streptococci (S. mitis, S. gordonii, S. anginosus, S. sanguinis, and S. oralis), P. micra, Actinomyces species (A. israelii and A. odontolyticus), Propionibacterium species (P. acnes and P. propionicum, P. alactolyticus), Lactobacilli (L. paracasei and L. acidophilus) and E. faecalis [12].

As far as the fungi are concerned, it is the Candida species that have been commonly seen in as many cases nearly around 18% [11]. To be more specific, it has been observed that C. albicans is the most commonly detected fungal species in retreatment cases [13]. From all reported failed cases with pain and infection after the completed endodontic therapy, it has been observed that E. faecalis microorganism is one of the most commonly found species with prevalence values reaching up to 90% [6].

E. faecalis are gram positive cocci and facultative anaerobes. They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; but if it is in large numbers, it is difficult to eradicate. E. faecalis has many distinct features which makes it an exceptional survivor in the root canal, such as:

- Live and persist in poor nutrient environment
- Survive in the presence of several medication (e.g., calcium hydroxide) and irrigants (e.g., sodium hypochlorite)
- Form biofilms in medicated canals
- Invade and metabolize fluids within the dentinal tubules and adhere to collagen
- Convert into a viable but non-cultivable state
- Acquire antibiotic resistance
- Survive in extreme environments with low pH, high salinity and high temperatures
- Endure prolonged periods of starvation and utilize tissue fluid that flows from the periodontal ligament [10]

As a consequence of these features of E. Faecalis, endodontic treatment can fail. This may be when root canal treatment has not been adequately performed to eliminate or reduce the intraradicular bioburden. [14-16] Root canal system anatomy plays a significant role in endodontic success and failure. These systems contain branches that communicate with the periodontal attachment apparatus furcally, laterally, and often terminate apically into multiple portals of exit [17]. Consequently, any opening from the root canal system (RCS) to the periodontal ligament space should be thought of as a portal of exit (POE) through which potential endodontic breakdown products may pass.[18,19] Failures do occur even when the highest standard and the most consistent procedures are done to because of root canal complexities that cannot be shaped or cleaned and obturated with current technologies. Under these circumstances, infection can persist or quickly re-establish by itself [14-16].

Endodontic failures can be attributed to inadequacies in cleaning and shaping. Such failures are most often caused by microorganisms that have survived the conventional root canal treatment procedures [20]. In order to combat the infection, the root canal has to be properly disinfected with irrigants.

Irrigation has an important role for successful endodontic treatment. During and after instrumentation, the irrigants facilitate removal of microorganisms, tissue remnants, and dentinal chips from the root canal through a flushing mechanism. Irrigants also prevent packing of the hard and soft tissue in the apical root canal and extrusion of the infected material into the periapical area. Some irrigating solutions dissolve either organic or inorganic tissue in the root canal. In addition, several other irrigating solutions have antimicrobial activity that actively kill bacteria and yeasts when introduced in direct contact with the microorganisms. However, few irrigating solutions also exhibit cytotoxic potential, and they may cause severe pain if they get extruded into the periapical tissues [21].

So, an optimal irrigant should possess all or most of the positive characteristics listed below: [22]

- Be an effective germicide and fungicide
- Be nonirritating to the periapical tissues
- Remain stable in solution
- Have a prolonged antimicrobial effect
- Be effective even in the presence of blood, serum, and tissue protein derivatives.
- Have low surface tension
- Not interfere with repair of periapical tissues
- Not stain tooth structure
- Be capable of inactivation in a culture medium
- Not induce a cell-mediated immune response
- Be able to completely remove the smear layer, and be able to disinfect the underlying dentin and its tubules
- Be nonantigenic, nontoxic, and noncarcinogenic to tissue cells surrounding the tooth
- Have no negative effects on the exposed dentin's physical properties
- Have no negative effects on the sealing ability of restorative materials

- Have a convenient application
- Be relatively inexpensive

None of the available irrigating solutions can be regarded as an universally accepted irrigant. A combination of two irrigants in the correct irrigation sequence can contribute to a successful endodontic treatment outcome.[22]

Several chemical irrigants are commonly used for root canal disinfection, few of them like Sodium hypochlorite (NaOCl), Disodium ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide (H_2O_2), Citric acid, Chlorhexidine (CHX), and Calcium hydroxide $Ca(OH)_2$. It has been shown by various in vitro and in vivo studies that NaOCl and CHX were recommended as ideal irrigant because of their effective antimicrobial activity and other properties such as pulp digestion [23]. However, few studies have demonstrated that both NaOCl and CHX have shown not only a low ability to eradicate pathogens like E. faecalis, but also caused a significant toxicity on periodontal ligament cells in relation with their concentration and exposure time [24-28].

In order to overcome the limitations of these conventional root canal irrigants and medicaments, use of nanoparticles to disinfect the canal system has been proposed. Nanotechnology is considered to be a breakthrough in the field of medicine. The word "nano" originated from the Greek word meaning "dwarf". Nanotechnology was first explaned by Richard P. Feynman in 1960. Since then Nanotechnology has been used in various fields of science & technology. Nanotechnology is defined as a science that deals with the development it of new materials with new properties and functions through controlling and restructuring of the materials on a nanometre scale of less than 100nm.[29]

Nanomaterials exist in different forms and shapes. Nanoparticles can be classified as:[30]

A) According to origin:

1) Natural: Silver, Copper

2) Artificial/Synthetic: Chitosan, Graphene

B) According to Dimension:

1) Zero-dimensional or nanoparticles

2) One-dimensional or nanorods

3) Two-dimensional or thin films

4) Three-dimensional or nanocones

C) According to Structural Configuration:

1) Carbon-based : Graphene

2) Metal: Iron oxide, Silver

3) Dendrimers.

It can be useful in producing advanced biomaterials with unique physical, chemical and biological properties [31,32]. This is mainly approached by enhancing surface-to-volume ratio. To control various biological processes, nanomaterials can be modified with predefined geometries, surface characteristics and mechanical strength [33]. Since its advent Nanomaterials have been used in a variety of scientific fields. In this context, the use of nanoparticles in root canal space disinfection is an emerging topic or area of research. [34]

Nanomaterial denotes a natural or manufactured material containing unbound particles in which half or more of the particles in number and size are in the size range of 1-100 nm [35]. These materials present unique physicochemical properties, such as large surface area/mass ratio and increased chemical reactivity [31,36]. The increased number of atoms and increased surface to volume ratio compared with micro/macro-structures are suggested to contribute to the distinctly different properties of these nanomaterials. These advantages may be

employed to design highly specific materials and devices to interact with at the subcellular and molecular level of the human body in order to achieve maximal therapeutic efficacy with minimal side effects [37,38]. The electrostatic interaction between negatively charged bacterial cells and positively charged nanoparticles, and also accumulation of increased number of nanoparticles on the cell membrane of the bacteria have been associated with the loss of membrane permeability and unsuitable membrane function [40]. Antibacterial nanoparticles exhibit a broad spectrum of antimicrobial activity[38]. Many studies show that there is reliability in the use of different type of nanoparticles as antimicrobial agents especially against persistent endodontic pathogen such as Enterococcus faecalis.[35-38]

Therefore the aim of this in-vitro study is to evaluate and compare the antimicrobial properties of three nanoparticles (Silver Nanoparticles, Nano-Chitosan, Graphene Nanoparticles) as new irrigating agents against endodontic pathogen and to find out which irrigant is more appropriate for use as a root canal irrigating solution against Enterococcus faecalis.

AIMS & OBJECTIVES

Aim of the study:

The aim of this study is to evaluate & compare antimicrobial efficacy of Silver Nanoparticles, Nano-Chitosan, Graphene Nanoparticles and Chlorhexidine against Enterococcus faecalis as root canal irrigant.

Objectives of the study:

1) To evaluate the antibacterial effect of Silver Nanoparticle Root canal irrigating solution against Enterococcus faecalis.
2) To evaluate the antibacterial effect of Chitosan Nanoparticle Root canal irrigating solution against Enterococcus faecalis.
3) To evaluate the antibacterial effect of Graphene Nanoparticle Root canal irrigating solution against Enterococcus faecalis.
4) To evaluate the antibacterial effect of Chlorhexidine Root canal irrigating solution against Enterococcus faecalis.
5) Inter-group comparison of experimental Root canal irrigants.
6) To conclude which is the best irrigant to provide highest antibacterial effect against E. faecalis in Root Canal.

REVIEW OF LITERATURE

1. **Sundqvist G., Figdor D., Persson S. et al. (1998) [41] -** determined which microbial flora were present in teeth after failed root canal therapy and established the outcome of conservative re-treatment. Fifty-four root-filled teeth with persisting periapical lesions were selected for re-treatment. After removal of the root filling, canals were sampled by means of advanced microbiologic techniques. The teeth were then re-treated and followed for up to 5 years. The microbial flora was mainly single species of predominantly gram-positive organisms. The isolates most commonly recovered were bacteria of the species Enterococcus faecalis. The overall success rate of re-treatment was 74%. The microbial flora in canals after failed endodontic therapy differed markedly from the flora in untreated teeth. Infection at the time of root filling and size of the periapical lesion were factors that had a negative influence on the prognosis. Three of four endodontic failures were successfully managed by re-treatment.

2. **Love RM (2001) [42]-** identified a possible mechanism that would explain how E. faecalis could survive and grow within dentinal tubules and reinfect an obturated root canal. Cells of Streptococcus gordonii, Streptococcus mutans, or E. faecalis were grown in brain heart infusion broth containing various amounts of human serum for 56 days. The ability of the three species to invade dentine and bind to immobilized type 1 collagen in the presence of human serum was assessed by dentine invasion and microtitre well experiments. All three species remained viable over the period of the experiment when grown in human serum. Cells of all three bacteria were able to invade dentine and bind to immobilized collagen. Both of these properties were inhibited by the presence of collagen in the cell solution. Human serum inhibited dentine invasion and collagen adhesion by S. gordonii and S. mutans, whilst dentine invasion by E. faecalis was reduced in the presence of serum, but not inhibited, and binding to collagen was enhanced. It was postulated that a virulence factor of E. faecalis in failed endodontically treated teeth is related to the ability of E. faecalis cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.

3. **Afrouzan H., Amirinia C., Mirhadi S.A. et al. (2012)** [43]**-** evaluated the antibacterial and antifungal activity of propolis and nanopropolis, against staphylocuccus aureus and candida albicans collected from Ferula ovina (Boiss.) Taleghan, Iran. Agar well diffusion method was employed to determine the antimicrobial activity of propolis and nanopropolis. The nanopropoils was prepared by milling media method. Most of the nanopropolis size was under the 100 nanometers. There were significant differences between propolis and nanopropolis in inhibition of S. aureus and C. albicans. Findings of this study indicated that natural nanoparticles have the potential to be used efficiently in the control of bacterial and fungal diseases.

4. **Moghadas L., Shahmoradi1 M., Narimani T. (2012)** [44]- in their study evaluated antimicrobial efficacy of nanosilver particle based endodontic irrigation solution in comparison to 5.25% Sodium Hypochlorite against Enterococcus faecalis and Staphylococcus aureus, two most commonly isolated species of root canal space. The two facultative strains were inoculated in 7 mL of brain heart infusion and were incubated at 37°C for 24 h at same incubation conditions. They were then studied in different time intervals of 3, 5 and 15 minutes. After each contact period, taken sample was plated on Brain Heart Infusion agar to determine the number of colony-forming unit (CFU) per plate. No growth of E. faecalis and S. aureus was observed in any of irrigant groups and any of different time intervals. These results showed that the new nano silver particle based irrigant is as effective as Sodium Hypochlorite in preventing the bacterial growth of common root canal bacteria.

5. **Bhalla V., Bhoiwala V., Rajkumar B. et al. (2015)** [45]**–** compared the antimicrobial efficacy amongst 2% Tea Tree Oil, 2% Chlorhexidine Digluconate, 3% Sodium Hypochlorite and the control (Distilled Water) using the Minimum Inhibitory Concentration(MIC) Test. The MIC test was performed using 10-fold dilution in ninety-six U-Well Micro Test plates. The results were tabulated and statistically analyzed using binary statistics. It was seen that Tea Tree Oil was the most effective in inhibiting E. fecalis, followed by sodium hypochlorite, and chlorhexidine

digluconate was the least successful. Distilled water showed no effect on the gram positive organisms.

6. **del Carpio-Perochena A., Bramantel C.M., Duarte1 M.A.H. et al. (2015)** [46]**-** investigated the ability of bioactive Chitosan Nanoparticles (CNP's) to remove the smear layer and inhibit bacterial recolonization on dentin. One hundred bovine dentin sections were divided into five groups (n = 20 per group) according to the treatment. The irrigating solutions used were 2.5% sodium hypochlorite (NaOCl) for 20 min, 17% ethylenediaminetetraacetic acid (EDTA) for 3 min and 1.29 mg/mL Chitosan Nanoparticles for 3 min. The samples were irrigated with either distilled water (control), NaOCl, NaOCl-EDTA, NaOCl-EDTA-CNPs or NaOCl-CNPs. After the treatment, half of the samples (n = 50) were used to assess the chelating effect of the solutions using portable scanning electronic microscopy, while the other half (n = 50) were infected intra-orally to examine the post-treatment bacterial biofilm forming capacity. The biovolume and cellular viability of the biofilms were analysed under confocal laser scanning microscopy. The Kappa test was performed for examiner calibration, and the non-parametric Kruskal-Wallis and Dunn tests were used for comparisons among the groups. The smear layer was significantly reduced in all of the groups except the control and NaOCl groups. The CNPs treated samples were able to resist biofilm formation significantly better than other treatment groups. It was concluded that CNPs could be used as a final irrigant during root canal treatment with the dual benefit of removing the smear layer and inhibiting bacterial recolonization on root dentin.

7. **Makkar S., Aggarwal A., Pasricha S. et al. (2015)** [47]**-** evaluate & compared the antibacterial and antifungal properties of octenidine hydrochloride (OCT) and chlorhexidine gluconate (CHX) as endodontic root canal irrigant. The irrigants were tested at various concentrations OCT (0.025%, 0.05% and 0.1%) and CHX (0.2%, 1% and 2%) with reference bacterial strains of Enterococcus faecalis (ATCC: 29212). Sodium hypochlorite was used as control. 1 ml of organism suspension made equivalent to Mc Farland 0.5 was contacted with 1 ml of each concentration of irrigation solution and each mixture was removed in 3, 5 and 10 minutes. After 72

hours of incubation colony counts were measured using a microscope. Inhibition zones measured with Presterilised Whatman paper discs soaked with 20 µl of each irrigant were plated on bacterial plates. Kruskal Wallis test and post hoc tests, that is, Mann Whitney U test and Duncan's test of multiple comparisons were done. The number of CFUs dropped to zero after 3 minutes contact time with OCT 0.1% and CHX 2.0% with highly significance (P = 0.000). All the concentrations of OCT showed large inhibition zones and zones of CHX 1% and NaOCl 3% groups was significantly less than octenidine. Octenidine hydrochloride was found as an effective endodontic irrigant.

8. **Sharma D.K., Bhat M., Kumar V. et al. (2015)** [48]- evaluated the antimicrobial effectiveness of Graphene Silver Composite Nanoparticles compared to Saline and Sodium hypochlorite (3%) used as an endodontic irrigation solution. Thirty caries-free, single-rooted, mandibular premolar human teeth were prepared. The root canals were inoculated with a suspension containing E. Faecalis bacteria. The teeth were then randomly divided into three groups. Each group was irrigated with one of the following solutions: Saline (control), Graphene Silver Composite Nanoparticles and Sodium hypochlorite (3%). Antimicrobial effectiveness was evaluated immediately after irrigation and again after 3 days, by counting colony forming units on blood agar plates. The percentage reduction of E. Faecalis in Saline was 21.64 %, with Sodium hypochlorite it was 80.40% and the maximum reduction was observed in Graphene Silver Composite Nanoparticles with 86.85%. The result showed that Graphene Silver Composite Nanoparticles demonstrated maximum antimicrobial effectiveness against E. Faecalis bacteria.

9. **González-Luna P., Martínez-Castañón G. A., Zavala-Alonso N.V. et al (2016)** [49]- determined the bactericidal effect of silver nanoparticles as a final irrigation agent in endodontics. One hundred and twenty uniradicular extracted dental organs were inoculated with Enterococcus faecalis (E. faecalis) and organized into 4 groups: (A) 30 teeth irrigated with a dispersion of silver nanoparticles (537 µg/mL); (B) 30 teeth irrigated with a sodium hypochlorite solution (2.25%); (C) 30 teeth irrigated with a dispersion of silver nanoparticles (537 µg/mL) + EDTA (17%); and (D) 30 teeth with a saline solution. After the irrigation protocol, the samples were analyzed through a

spectrophotometer to measure the bactericidal effect and scanning electron microscope and atomic force microscope in order to observe the presence of dental smear layer. The results showed that nanoparticles of 10 nm and the sodium hypochlorite at 2.25% were effective for eliminating E. faecalis, with no significant difference between them.

10. **Rodríguez-Chang S., Ramírez-Mora T., Valle-Bourrouet G. et al. (2016)** [50]- measured the antibacterial efficacy of a dispersion of silver nanoparticles (AgNP) in a citrate medium tested in two E. faecalis strains. AgNP were synthesized, and AgNP citrate medium (AgNP-CM) dispersion was prepared at a concentration of 100 μg/mL. The antibacterial efficacy of AgNP-CM dispersion was evaluated over two E. faecalis strains: ATCC29212 and a wild strain collected from human necrotic teeth. 5% sodium hypochlorite (NaOCl) and sterile saline solution were used as positive and negative controls. 5 and 30-minute contact tests were conducted and each experimental group were replicated 10 times. After 24 hours of incubation, the Log CFU/mL were calculated. The AgNP obtained showed spherical shapes and had 30-60nm size. 5% NaOCl was able to completely eliminate both E. faecalis strains in all groups, showing a significant statistical difference when compared to AgNP-CM dispersion and negative control groups. AgNP-CM dispersion showed a statistically significant decrease in Log CFU/mL averages when compared to the sterile saline solution for the ATCC29212 strain during the 30-minute time. Between the 5-minute and 30-minute groups, a significant bacterial count decrease was also observed. The antibacterial efficacy of the dispersion was found greater for the ATCC29212 strain than the wild strain. AgNP-CM dispersion showed a significantly lower antibacterial efficacy against E. faecalis than the 5% NaOCl at the tested times.

11. **Sambandam C., Divyarani S., Kirubasankar L. et al. (2016)** [51]- evaluated the antibacterial activity of AgNPs prepared by the aqueous extracts of Ocimum sanctum (Thulasi) and Piper betle (betel leaf) against E. faecalis. 9ml of 5mM Silver nitrate was added to 1ml of aqueous extract of plants and incubated at 37^{o}C for 24 hrs. The change in colour from yellow to brown indicated the production of Silver nano particles. UV–Vis spectral analysis was done by UV–visible spectrophotometer. UV–Visible absorption between 200 and 800 nm was used. The antibacterial study was

done by well diffusion method. The silver nano particles were successfully synthesised from the aqueous extracts of O. sanctum (Thulasi) and P. betle (betel) leaves). The formation of AgNPs was indicated by the peak absorption between 410-450nm in UV-Vis spectrophotometer. The results showed that the AgNPs prepared from the two plant extracts have anti-bacterial activity against E. faecalis. It was concluded that the green synthesized AgNPs has antibacterial activity against E. faecalis and can be used for endodontic treatment.

12. **Samiei M., Shahi S., Abdollahi A.A. et al. (2016)** [52]- compared the efficacy of light-activated low-power laser, 2% chlorhexidine (CHX) and 2.5% NaOCl in eliminating Enterococcus faecalis (E. faecalis) from the root canal system. The root canals of 60 maxillary central incisors were contaminated with E. faecalis and then the bacteria were incubated for 24 h. All the root canals were instrumented in a crown-down manner with #4 and 3 Gates-Glidden drills, followed by RaCe rotary files (40/0.10, 35/0.08, and 30/0.06). The samples were randomly assigned to three experimental groups and one control group (n=15). In the control group no intervention was made. In the photo-activated disinfection (PAD) group, laser therapy was undertaken with diode laser beams (with an output power of 100 mW/cm2) for 120 sec. For the other two experimental groups, root canals were irrigated either with 5 mL of 2% CHX or 2.5% NaOCl solutions, respectively. The inhibition of bacterial growth in all the experimental groups was significantly superior to the control group. There was no significant difference between the effect of PAD and 2% CHX. The effect of 2.5% NaOCl was significantly better than that of the PAD technique. In addition, 2.5% NaOCl was significantly better than 2% CHX. Photodynamic therapy was effective in reducing the E. faecalis counts in comparison with the control group, but 2.5% NaOCl solution was the most effective protocol.

13. **Sedigh-Shams M., Gholami A., Abbaszadegan A. et al. (2016)** [53]**-** compared the antimicrobial efficacy of sodium hypochlorite (SH) and calcium hypochlorite (CH) against Enterococcus faecalis (E. faecalis) using quantitative real-time polymerase chain reaction (qPCR) analysis and they also compared their cytocompatibility on L929 murine fibroblasts using Mossman's tetrazolium toxicity (MTT) assay. A broth micro-dilution susceptibility test was used to determine the minimum inhibitory

concentration (MIC) of each irrigant against E. faecalis. Then, the root canals of 50 mature extracted human mandibular premolars were contaminated with E. faecalis and were randomly divided into three groups according to the irrigant used (n=20). Canals were irrigated with SH in group I (n=20) and CH in group II (n=20) at their obtained MIC. In group III (n=10), sterile saline was used. Microbial sampling was performed before and after biomechanical preparation. Quantitative PCR was used to quantify E. faecalis in the root canal samples. For cytocompatibility assessment, L929 murine fibroblasts were exposed to various concentrations of the irrigants. CH at an MIC of 5% was effective in eliminating E. faecalis in planktonic state and also its biofilm and exhibited comparable cytocompatibility to that of 0.5% SH.

14. **Alabdulmohsen Z.A. & Saad A.Y. (2017)** [54]- investigated the bactericidal effect of silver nanoparticles (AgNPs) in reducing bacterial infection in root canal when used as intracanal medicament alone or in addition to the conventionally used calcium hydroxide. The root canals of 110 single-rooted teeth were cleaned, shaped, and sterilized. All groups, except for negative control, were inoculated with Enterococcus faecalis for 48 h. The teeth were then divided into five groups according to the intracanal medicaments to be used. Group I (n = 30): $Ca(OH)_2$. Group II (n = 30): AgNP. Group III (n = 30): AgNP + $Ca(OH)_2$. Group IV (n = 10) was used as a positive control where the root canals were inoculated with E. faecalis and left without treatment. Group V (n = 10) was used as a negative control where the root canals were checked for the absence of bacterial growth. Specimens were incubated for 1 and 2 weeks. Pre and post-medication samples were obtained by paper points, and the colony-forming units were counted. $Ca(OH)_2$ resulted in a higher percentage of bacterial reduction in both 1 and 2 weeks of application (81.5% and 98%, respectively). AgNP was ineffective against E. faecalis with 32.9% bacterial reduction in 1 week and 56.5% after 2 weeks. It was concluded that the antibacterial effect of AgNP was lower than $Ca(OH)_2$ or combination of both materials.

15. **Babu B., Nair R.S., Angelo J.M.C. et al. (2017)** [55]- evaluated the efficacy of chitosan-silver nanocomposite on candida albicans when compared to three different antifungal agents- fluconazole, clotrimazole and amphotericin B in combination with standard irrigation protocol-5.25% NaOCl + 17% EDTA. Fifty experimental teeth

were biomechanically prepared and inoculated with suspension of C. albicans. At the end of 96 hours, teeth were divided into five experimental groups. The groups were treated with respective irrigating solutions for 1 min. An inoculation loop was used to remove aliquots from the fluid and was plated on 4% Sabouraud Dextrose Agar and incubated for 48 h. After incubation, the growth of C. albicans was assessed with light microscopy at ×400. The data were statistically analyzed. Colony forming unit (CFU) was determined for all five groups. 1% clotrimazole and chitosan-silver nanocomposite showed complete inhibition in all the samples. Control group (5.25% sodium hypochlorite, 17% ethylenediaminetetraacetic acid, and 0.9% saline), 0.2% fluconazole and 0.2% amphotericin B showed complete inhibition in 8 samples and reduced CFU in two samples. 0.2% fluconazole showed better inhibition of C. albicans compared to control group and 0.2% amphotericin B. It was concluded that Chitosan-silver nanocomposite as an endodontic irrigant can inhibit the growth of C. albicans in combination with standard irrigation protocol.

16. **Farshad M., Abbaszadegan A., Ghahramaniet Y. et al. (2017)** [56]- evaluated the effect of a imidazolium-based silver nanoparticle (ImSNP) irrigant on dentin roughness in comparison with three commonly used root canal irrigation solutions, which are 5.25% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA) and 2% chlorhexidine (CHX). Distilled water was used as control. Roots of 25 human anterior teeth were sectioned longitudinally to obtain 50 dentin samples. Roughness values were evaluated by atomic force microscopy analysis on 5 groups (n=10) after each group was treated in one of the tested irrigant solutions for 10 min. Values were statistically analyzed by Oneway analysis of variance, followed by a post hoc Tukey's test for pair-wise comparison. Dentin roughness significantly increased from 95.82 nm (control) to 136.02 nm, 187.07 nm, 142.29 nm and 150.92 nm with NaOCl, CHX, ImSNP and EDTA, respectively. CHX demonstrated a significantly higher roughness value compared to the other tested irrigants while no significant differences were seen in NaOCl, ImSNP and EDTA groups. ImSNP affected the physicochemical properties of dentin and raised its surface roughness; thus, this irrigant could impact bacterial and restorative material adhesion to root canal dentin walls.

17. **Hayam YH, Zakeer S., Mahmoud N.F. (2017)** [34]– evaluated the anti-bacterial activity of four selected solutions (Biopure MTAD, 2% Nano-Chitosan, 2% Chitosan, 2.5% Sodium hypochlorite) against Enterococcus faecalis (E. faecalis). The antimicrobial efficacy of four tested solutions was primarily determined using the agar disk-diffusion method (Mueller-Hinton agar plates). Furthermore, a total of 50 standardized single-canaled human teeth were collected and sterilized. Five teeth were cultured directly as negative control. Others were infected by a trypticase soy broth inoculated with E. faecalis. Infected roots incubated at 37ºC for 48 hours. Then five teeth cultured without treatment as positive control. While, the remaining were randomly divided into four groups (n=10) for being tested using the four selected solutions. Two or three sterile paper points were inserted into every root canal then transferred aseptically into corresponding Wassermann tubes that contain sterile Trypticase soy broth. All Wassermann tubes were incubated at 37ºC for 48 hours then microbial growth of E. faecalis was verified by turbidity of the broth. Growth was further checked after 2 and 10 days then obtained data was statistically analysed. Biopure MTAD significantly inhibited the bacteria growth recording 0%. Significant difference was found between the results of 2% Nano-Chitosan, 2% chitosan and 2.5% sodium hypochlorite. At 10 days' time interval the antimicrobial activity of all the tested solution generally decreased against E. faecalis. Biopure MTAD was found to efficiently improve antimicrobial root canal disinfection than other tested solutions against E. faecalis.

18. **Miranda J.S., Marques E.A., Landa F.V. et al. (2017)** [57]- evaluated the sealer capacity of dentinal tubules of teeth treated endodontically using different chelating solutions with the help of scanning electron microscopy (SEM). The root canals were irrigated with 2.5% sodium hypochlorite (NaOCl), replaced every instrument and filled. The auxiliary solutions used with a passive ultrasonic irrigation (PUI) for 1 minute were: 70% ethanol (control); 10% citric acid; 17% EDTA; and 0.2% chitosan. The roots were split lengthwise into two parts and samples were taken for analysis in a scanning electron microscope to obtain photomicrographs for the qualitative assessment. 17% EDTA solution and 10% citric acid removed the smear layer similar to each other. 0.2% chitosan solution removed the smear layer partially, with lower efficacy than 17% EDTA solution and 10% citric acid. There were a greater removal

of smear layer observed with the use of 17% EDTA, followed by 10% citric acid, 0.2% chitosan and 70% alcohol.

19. **Nabavizadeh M., Abbaszadegan A., Gholami A. et al. (2017)** [58]- investigated the antibacterial efficacy of Positively Carged imidazolium-based silver nanoparticle (PC Im-based AgNPs) at $5.7x10^{-8}$ mol/L in comparison with 2.5% sodium hypochlorite (NaOCl) and 2% chlorhexidine as the two broadly used endodontic irrigation solutions against biofilm E. faecalis using quantitative real-time polymerase chain reaction. In total, 48 premolar teeth with a single root were infected with E. faecalis and then prepared with ProTaper rotary instruments. The samples were randomly allocated into 4 groups of 12 samples. Sterile saline, PC Im-based AgNPs, NaOCl, and chlorhexidine were used as irrigants. Sampling the root canals was implemented with paper points and Gates-Glidden drills. The reduction in E. faecalis counts was calculated and statistically analyzed by means of the Kruskal-Wallis and Mann-Whitney U tests. Irrigation with PC Im-based AgNPs or NaOCl was significantly more effective in bacterial count reduction compared to irrigation with chlorhexidine or sterile saline. There was no significant difference between PC Im-based AgNPs and NaOCl irrigants when either Gates-Glidden drills or paper points were employed. Chlorhexidine was significantly less efficient than PC Im-based AgNPs and NaOCl solutions; however, it was significantly better than sterile saline in both sampling approaches. The PC Im-based AgNP solution revealed promising results as a root canal irrigant. This solution at $5.7x10^{-8}$ mol/L was effectively able to eliminate biofilm E. faecalis and this was not significantly different from that of 2.5% NaOCl.

20. **Benbelaïd F., Khadira A., Bendahou M. et al. (2018) –** proposed an alternative irrigation solution based on Cinnamomum cassia essential oil was evaluated for antimicrobial activity and total eradication of E. faecalis and C. albicans biofilms. The most used irrigation solutions by dentists, namely chlorhexidine digluconate and sodium hypochlorite, were also evaluated for comparison. Obtained data were analyzed statistically using two-way analysis of variance (ANOVA) followed by Bonferroni's test. The obtained results revealed that essential oils prepared in ethanol, in general, can be used as irrigation solution in endodontic treatments because of their effective antimicrobial activity against biofilms of pathogens responsible for post-

treatment apical periodontitis. C. cassia essential oil is a good example due to its proved effective antibiofilm activity that exceeded the most used irrigation solutions namely sodium hypochlorite and chlorhexidine digluconate.

21. **Bukhari S., Kim D., Liu Y. et al (2018)** [60]**-** tested a new disinfection technology using biomimetic iron oxide nanoparticles (IO-NPs) with peroxidase-like activity to enhance antibacterial activity on root canal surfaces and in dentinal tubules. The canal surfaces and dentinal tubules of single-rooted intact extracted teeth were infected by growing Enterococcus faecalis biofilms for 3 weeks. The samples were divided into 6 treatment groups: (1) phosphate-buffered saline (PBS) (negative control), (2) 3% hydrogen peroxide (H_2O_2) (test control), (3) IO-NPs (0.5 mg/mL) (test control), (4) IO-NPs (0.5 mg/mL) + 3% H_2O_2, (5) 3% sodium hypochlorite (positive control), and (6) 2% chlorhexidine (positive control). Environmental scanning electron microscopy coupled with energy-dispersive spectroscopy was used to confirm IO-NPs binding to the canal surface after a single treatment. Specimens were labelled with fluorescent staining for live/dead cells, and confocal laser scanning microscopy was used for the quantification of dead bacteria relative to the negative control (PBS). Both biofilm formation and dentinal tubule infection were successfully recapitulated using the in vitro model. IO-NPs were capable of binding to the infected canal surfaces despite a single, short-term (5-minute) treatment. IO-NP activation of H_2O_2 killed significantly more E. faecalis present on the canal surfaces and at different depths of dentinal tubules when compared with all other experimental groups. The results revealed the potential to exploit nanocatalysts with enzyme-like activity as a potent alternative approach for the treatment of endodontic infections.

22. **Charannya S., Duraivel D., Padminee K. et al. (2018)** [61]**-** evaluated the efficacy of AgNPs, 2% CHX gluconate, and the combination of two solutions against endodontic pathogens such as Enterococcus faecalis, Klebsiella pneumoniae, and Candida albicans. These organisms are frequently found in the root canal space and their persistence may lead to endodontic failure. The synergistic effect of the two solutions has been evaluated in this study. The antibiotic gentamycin was taken as the control group. CHX-AgNP combined solution exhibited the highest efficacy in comparison to these solutions used alone. They showed the highest efficacy against C. albicans

among the three organisms tested. The present study demonstrates the antimicrobial efficacy of a novel mixture of CHX-AgNP solution, and it may be developed as a promising antimicrobial agent against endodontic flora.

23. **de Almeida J., Cechella B.C., Bernardi1 A.V. et al. (2018)** [62]- evaluated the effectiveness of experimental solutions- 1% silver nanoparticles (Ag Np) Solution and 26% ZnO Np solution and conventional endodontic irrigants- 0.85% saline; 2% chlorhexidine gluconate (CHX); 5% sodium hypochlorite (NaOCl); 1% NaOCl; against Enterococcus faecalis biofilm, in root canals. Seventy-six extracted human teeth were biomechanically prepared and sterilized. The root canal surface was exposed to E. faecalis suspension to form a 7-day-old biofilm. Four teeth were analyzed by scanning electron microscopy (SEM) to confirm the presence of biofilm. The remaining teeth were randomly divided into 6 groups (n = 12) and treated with passive ultrasonic irrigation and different solutions: Group I – 0.85% saline (control); Group II – 2% chlorhexidine gluconate (CHX); Group III – 5% sodium hypochlorite (NaOCl); Group IV – 1% NaOCl; Group V – 1% silver nanoparticles (Ag Np) solution; and Group VI – 26% ZnO Np solution. The susceptibility of E. faecalis biofilms to disinfecting solutions (n = 10) was determined by quantification of colony-forming units. SEM analysis was also carried out to examine the biofilm structure after treatments (n = 2). Data were analyzed by Kruskal–Wallis and Dunn post hoc tests. All tested solutions showed superior effectiveness compared to 0.85% saline. Overall, 2% CHX presented the most effective action against E. faecalis biofilm, followed by 5% NaOCl, 1% Ag Np, 26% ZnO Np, and 1% NaOCl. 1% Ag Np and 26% ZnO Np were effective against E. faecalis biofilm similarly to conventional endodontic irrigants.

24. **Halkai K.R., Mudda J.A., Shivanna V. et al. (2018)** - [63]evaluated the antibacterial efficacy of biosynthesized silver nanoparticles (AgNPs) produced using the fungi against Enterococcus faecalis biofilm model on root dentin. Minimum inhibitory concentration (MIC) of AgNPs was determined by microbroth dilution method using series of dilutions. MIC dose was standardized to evaluate the antibacterial efficacy. For biofilm model, thirty root dentin blocks prepared using human extracted single-rooted teeth were inoculated with E. faecalis in Trypticase soy agar broth for 2

weeks with alternate day replenishment and randomly divided into three groups (n = 10 each) and treated as: Group I: Sterile distilled water, Group II: AgNPs, and Group III: 2% chlorhexidine gluconate (CHX) and incubated at 37°C for 24 h. Each dentin block was rinsed in saline, vortex shaken for 60 s, and serial decimal dilutions were prepared and plated on trypticase soy agar plates and incubated for 24 h followed by CFU colony counting and statistically analyzed using one-way ANOVA followed by post hoc Tukey honestly significant difference test. MIC of AgNPs for E. faecalis was determined as 30 mg/ml. No significant difference was seen between AgNPs and 2% CHX when compared to the control group against E. faecalis biofilm. Biosynthesized AgNPs exhibit effective antimicrobial activity against E. faecalis biofilm on root dentin. Therefore, it can be employed as antimicrobial agent for root canal disinfection.

25. **Patil P.H., Gulve M.N., Kolhe S.J. et al**. **(2018)** [64]**-** evaluated and compared the smear layer removal efficacy of etidronic acid-based irrigating solution with others in the apical third of the root canal. Forty human single-rooted mandibular premolar teeth were taken and decoronated to standardize the canal length. After biomechanical preparation, teeth were randomly divided into four groups (n = 10) and the final irrigation was carried out with tested irrigants. Group I: normal saline (negative control); Group II: 5.25% sodium hypochlorite (NaOCl) with surfactant and 17% ethylenediaminetetraacetic acid (EDTA) with surfactant; Group III: freshly mixed BioPure MTAD; and Group IV: freshly mixed Chloroquick solution. The teeth were split into two halves and observed under a scanning electron microscope to analyze the amount of smear layer present. Data were analyzed using the Kruskal–Wallis test and Mann–Whitney test. Group II (5.25% NaOCl with surfactant followed by 17% EDTA with surfactant) showed least smear layer scores. This was followed by Group III (MTAD) and then Group IV (Chloroquick). Sequential use of 5.25% NaOCl with surfactant and 17% EDTA with surfactant was found to be the most efficient than MTAD and Chloroquick in the removal of smear layer in the apical third of root canal.

26. Quiram G., Montagner F., Palmer K.L. et al. (2018) [65]- explored the use of a novel trilayered nanoparticle (TNP) drug delivery system that encapsulated chlorhexidine

digluconate, which aimed at improving the disinfection of the root canal system. Chlorhexidine digluconate was encapsulated inside polymeric self-assembled TNPs. These were self-assembled through water-in-oil emulsion from poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA), a di-block copolymer, with one hydrophilic segment and another hydrophobic. The resulting TNPs were physicochemically characterized and their antimicrobial effectiveness was evaluated against Enterococcus faecalis using a broth inhibition method. The hydrophilic interior of the TNPs successfully entrapped chlorhexidine digluconate. The resulting TNPs had particle size ranging from 140–295 nm, with adequate encapsulation efficiency, and maintained inhibition of bacteria over 21 days. The delivery of antibacterial irrigants throughout the dentinal matrix by employing the TNP system described in this work may be an effective alternative to improve root canal disinfection.

27. **Chávez-Andrade G.M., Tanomaru-Filho M., Basso Bernardib M.I. et al. (2019)** [66]– found out the antimicrobial and biofilm anti-adhesion activities of poly(vinyl alcohol)-coated silver nanoparticles (AgNPs-PVA) and farnesol against Enterococcus faecalis, Candida albicans or Pseudomonas aeruginosa. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of the solutions, as well as the effect on the biofilm biomass were evaluated. The biofilm anti-adhesion activity was evaluated using bovine root dentine treated with the solutions after 3 min of contact and analyzed by scanning electron microscopy (SEM) and by colony-forming units per milliliter (CFU/mL) counting. Data were analysed using ANOVA and Tukey's, the paired Student's t-test or Kruskal-Wallis and Dunn's tests (α=0.05). The MIC and MMC values (MIC/MMC) of the AgNPs-PVA and farnesol against E. faecalis were 42.5/50 μM and 0.85/1.0%, respectively. For C. albicans, the values were 27.5/37.5 μM and 1.75/2.5%; and for P.aeruginosa, 32.5/32.5 μM and 2.5/2.75%, respectively. Both solutions showed reduced biofilm biomass. SEM analysis showed that dentine blocks treated with both solutions had lower biofilm formation than the control (saline), except for C. albicans. In the CFU/mL counting, biofilm cells were viable in the all groups in comparison with control. AgNPs-PVA and farnesol showed antimicrobial and biofilm anti-adhesion activities, as well as potential for use as coadjuvant in endodontic treatment, and may

be an option as auxiliary procedure for root canal disinfection or to inhibit biofilm formation.

28. **Sridevi A., Sindhu J., Naveen D.N. et al. (2019)** [67]**-** evaluated the antimicrobial efficacy of zinc oxide (ZnO)-based sealer incorporated with fluorinated graphene and ZnO nanoparticles against Enterococcus faecalis. Sixty human extracted mandibular premolars with single canal were selected. After biomechanical preparation of all teeth and inoculation with E. faecalis, teeth were divided into three groups according to the type of the tested sealer, Group 1: Teeth were obturated with ZnO-based sealer only, Group 2: Teeth were obturated using sealer incorporated with fluorinated graphene, and Group 3: Teeth were obturated using sealer incorporated with ZnO nanoparticles. Each group was subdivided into two subgroups according to the timing of filling removal and culture sample. Subgroup A: Obturation removal and culture sample were taken after 1week. Subgroup B: Obturation removal and culture sample were taken after 3weeks. The number of colonies forming units was counted to assess the effect against E. faecalis. Data were recorded, tabulated, and statistically analyzed using the Kruskal–Wallis test was applied to find significance among the groups(1, 2, and 3). The Mann–Whitney U-test was applied to find significance between the groups. The Wilcoxon signed-rank test was applied to find significance between two-time intervals (24 h to 1 week, 24 h to 2 weeks) in each group. $P < 0.05$ was considered as statistically significant. After 3 weeks from obturation, fluorinated graphene had a better antibacterial effect and there was a significant difference between the three tested groups. After 1- and 3-weeks samples after obturation fluorinated graphene had a better antibacterial effect and there was a significant difference between the three tested groups.

29. **Abdelgawad L.M., Asmail N., Latif S.A. et al. (2020)** [68]– assessed the efficacy of agitation of chlorohexidine (CHX) and Silver nanoparticles (AgNps) with 810nm diode laser or sonic endoactivator compared to side-vented needle on infected root canals with Enterococcus Faecalis biofilms. Sixty-five extracted human premolars with single oval canals were instrumented by protaper system up to F3. Biofilms of E. faecalis were generated based on a previously established protocol. Two teeth were used to check the biofilm formation, then the remaining Teeth were randomly divided

into three equal experimental groups according to agitation techniques used: group 1 (810 nm diode laser with 1 watt), group 2 (sonic endoactivator) and group 3 (Side vented needle). Each group was further divided into three equal subgroups according to the irrigant solution into; subgroup A: chlorohexidine, subgroup B: silver nanoparticles and subgroup C: distilled water: Confocal laser scanning microscopy "CLSM" was used to assess bacterial viability. Data were analyzed by appropriate statistical analyses with P=0.05. Regarding the activation method, all groups had a significantly high percentage of dead bacteria. Diode laser agitation of AgNps irrigant showed the highest reduction percentage of bacteria (78.1%) with a significant difference with both CHX and water irrigation. Under the condition of the present study; results reinforced that laser activation is a useful adjunct, 810 nm diode laser agitation of AgNps or chlorhexidine was found to be more effective in disinfection of oval root canals than endoactivator and side vented needle techniques.

30. **Rafid J. Al –Badr & Hussain F. Al-Huwaizi (2020)** [69]**-** evaluated the antibacterial activity of chitosan-coated iron oxide nanoparticles (Chi-IONP) by agar direct diffusion method and determine the minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) of this nanosolution against E.faecalis, S.mutans and Candida albicans the three oral microorganisms commonly isolated from endodontic infections. The nanoparticles solution was prepared by functionalizing the 8nm iron oxide nanoparticles with glycine and coating them by chitosan dissolved in 0.25% v/v acetic acid. E.faecalis, S.mutans and candida albicans were isolated and identified with Vitek 2 system. Agar diffusion method was used to test three groups of materials, NaOCl 5.25% serve as positive control, 0.25% Acetic acid as negative control and Chi-IONP as experimental material. Broth microdilution method used to determine the MIC/MBC of the experimental material. Statistical analysis was conducted by independent variables t-test performed by using IBM-SPSS program version 22. The used microbial isolates were confirmed with Vitek 2 system with excellent confidence level of diagnosis. Chi-IONP showed antifungal activity higher than NaOCl 5.25%, and comparable effect to NaOCl 5.25% against E.faecalis and S.mutans. The negative group showed no effect on the tested microorganisms. The MIC/MBC were determined as following 125/250 µg/ml for E.faecalis, 250/ 500 µg/ml for S.mutans and the MIC/MFC was 62.5/ 125 µg/ml for

C.albicans. Chi-IONP was a powerful antimicrobial agent against the tested microorganisms, and it was found that antifungal activity of Chi-IONP was higher than NaOCl 5.25% and had comparable antibacterial effect to NaOCl 5.25%.

MATERIALS & METHODS

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in Collaboration with, Nivaran Pathology & Diagnostic Centre, Lucknow.

A total of 65 human permanent single rooted teeth were collected. The collected teeth were cleaned using ultrasonic scaler and then stored in 0.9% normal saline until further used.

The following inclusion & exclusion criteria were set to select the teeth:

INCLUSION CRITERIA

1. Non carious, sound and intact human single rooted teeth.
2. Tooth with single canal (one orifice and one foramen) determined radiographically.

EXCLUSION CRITERIA

1. Teeth with any crack, caries or calcification.
2. Teeth with developmental anomaly.
3. Teeth with any restoration.

Following materials and armamentarium were used:

TABLE-1

MATERIAL AND ARMAMENTARIUM USED

1) For Sample preparation:

S. No.	Material & Armamentarium	Manufacturer
1.	Ultrasonic Scaler with tips	Coltene, Switzerland
2.	Straight hand piece	Marathon, Korea
3.	Micro motor (Slow Speed)	Unicorn Denmart, India
4.	Diamond disc & Mandrel	Horico, Germany
5.	K-files (ISO #6,8,10,15,20)	Dentsply, U.S.A.
6.	Rotary HyFlex CM files (4% 20 & 4% 25)	Coltene, Switzerland
7.	17% Ethylenedieamine tetra acetic acid (EDTA) solution	SDFCL, India
8.	3% Sodium hypochlorite (NaOCl)	Vishal, India
9.	Normal Saline (0.9%w/v NaCl)	Beryl Drugs Ltd., India
10.	Disposable syringe of 5ml	Dispo Van, India
11.	30 gauge needle	Oro, India
12.	Endo block	Dentsply, U.S.A.
13.	Autoclave	Confident, India
14.	Glass ionomer Cement	Prevest DenPro, India
15.	Curing Light	Woodpecker, China
16.	Composite Restoration Instrument	GDC, India
17.	Modelling Wax	Pyrex, India

2) For Irrigation:

S. No.	Material & Armamentarium	Manufacturer
1.	Silver Nanoparticle 0.2 Wt%, 25 ml	Nano wings, India
2.	Nano Chitosan 0.2 Wt%, 25 ml	Nano wings, India
3.	Graphene Nanoparticle 0.2 Wt%, 25 ml	Nano wings, India
4.	Chlorhexidine 2%	Vishal, India
5.	Endo Activator with tips	Dentsply,U.S.A.
6.	Distilled Water	D.R.Laboratories, India

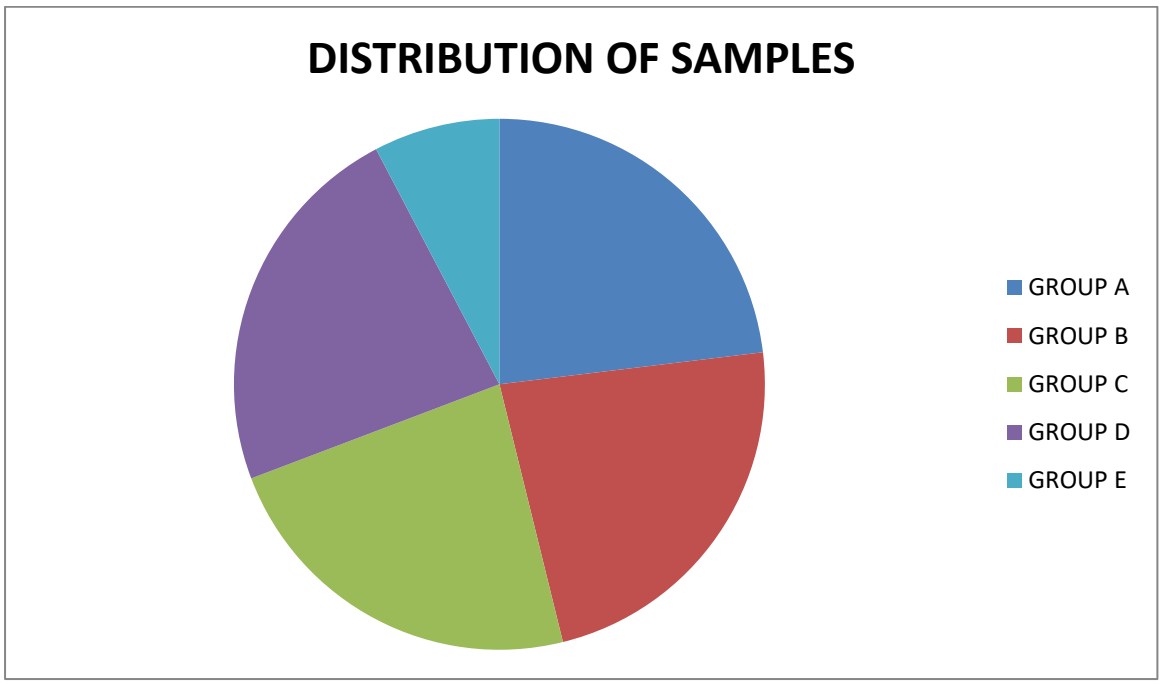
3) For Microbiology:

S. No.	Material & Armamentarium	Manufacturer
1.	Enterococcus Faecalis	ATCC 29212
2.	Absorbent Paper Points (4%, #20)	Meta-Biomed, Republic of Korea
3.	Sterile Tweezer	GDC, India
4.	Eppendorf tubes	Amanta, India
5.	Blood agar plates	Accumix, India
6.	Digital Colony Counter	ESICO, India

TABLE-2

DISTRIBUTION OF SAMPLES

GROUPS	NO. OF SAMPLES	TEST IRRIGANTS
GROUP A	15	0.2% Silver Nanoparticles
GROUP B	15	0.2% Nano Chitosan
GROUP C	15	0.2% Graphene Nanoparticles
GROUP D	15	2% Chlorhexidine
GROUP E	05	0.9% Normal Saline



Graph 1- Distribution of samples

METHODOLOGY

TEST ORGANISM

The microorganism used in this study was *Enterococcus faecalis* (ATCC 29212). (Fig.11)

TEST IRRIGANT

The irrigants tested were 0.2% Silver Nanoparticle (Nano wings, India), 0.2% Nano Chitosan (Nano wings, India), 0.2% Graphene Nanoparticle (Nano wings, India) (Fig.15) & 2% Chlorhexidine (Prevest Denpro, India) (Fig.17).

SAMPLE COLLECTION

Sixty five human mandibular premolars (Fig.1) extracted for orthodontic/Periodontal reasons were obtained from Department of Oral and Maxillofacial Surgery, BBDCODS, Lucknow after taking patient consent. Teeth collected were cleaned for any tissue remnants, plaque and calculus on the root with ultrasonic scaler. Out of the collected teeth the sample selection was done by following the inclusion and exclusion criteria. Digital Radiographs was taken to confirm the presence of single canal in collected sample teeth in order to fulfill for inclusion and exclusion criteria.

SPECIMEN PREPARATION

Standardization of teeth

All the selected teeth were marked and then sectioned 14mm from the apex (Fig.3), perpendicular to the long axis of the tooth, with a diamond disc fitted into mandrel held in straight handpiece of low speed micromotor hand piece with copious amount of water spray (Fig.2), to standardize root length for all selected teeth (Fig.4).

Root canal preparation

Working length was determined by passively inserting a size 10 K file into the canal & measuring on endoblock (Fig.5). Root canals were prepared using HyFlex CM files (4% 20 & 4% 25), using crown-down technique (Fig.6). During shaping, each canal was irrigated with 3% Sodium hypochlorite (Fig.19) and final irrigation was done with 17% EDTA solution (Fig.18).

Inoculation & Irrigation

The samples were autoclaved under steam at 121° C, 15 lbs pressure for 15 minutes (Fig.7,8). After sterilization, the samples root canal apex were sealed with light-cure GIC by instrument (Fig.9) and mounted on wax (Fig.10). Then the samples were inoculated with Enterococcus faecalis (Fig.12) and thereafter the teeth were randomly divided into 4 experimental groups of 15 samples each & 1 control group of 5 samples (Table 2,Graph 1). The following irrigation protocol was followed for all tested groups:

- To avoid contamination, all of the teeth were handled with sterile gloves and tweezers.
- With a sterile 5 mL syringe with a 30 gauge needle (Fig.13), 0.5 ml of irrigant was delivered into the canal for a period of three minutes (Fig.21), which was then agitated for 10 seconds with Endo-activator (Fig.14,22).
- To avoid irrigant carry-over, all experimental teeth were then flushed with distilled water (Fig.20,23).

Microbial Sampling

Using endoblock, an endodontic hand file was used in a filing motion to a level around 1 mm short of the root apex. A 4%, 20 paper point (Fig.13) was then placed into the canal till the working length for 30 seconds each to soak up the canal contents (Fig.24). Paper points were then transferred to the eppendorf tubes containing 1 mL saline and agitated in vortex for 1 minute (Fig.25,27). Aliquots of 500 µl dilutions were cultured into Blood agar plates (Fig.26,28,31). For 48 hours, all plates were cultured at 37°C in a microaerophilic environment with 5% CO2 (Fig.29,30). After that, a Digital Colony Counter (Fig.33) was used to determine the number of CFUs (Colony Forming Units) per plate (Fig.32).

Analysis of the sample

- The morphology of the colonies was used to identify them.

- The number of colony-forming units (CFU) per millilitre of sample was calculated as per the following formula :
- COLONY FORMING UNIT /ml= Number of colonies obtained X Dilution Factor
  Volume of sample inoculated

Statistical Analysis

After obtaining the mean colony forming unit (CFU) of each group, the following tests were performed using SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA):

- One-way analysis of variance (ANOVA)
- Post hoc Tukey's HSD analysis.

Fig.1- Samples

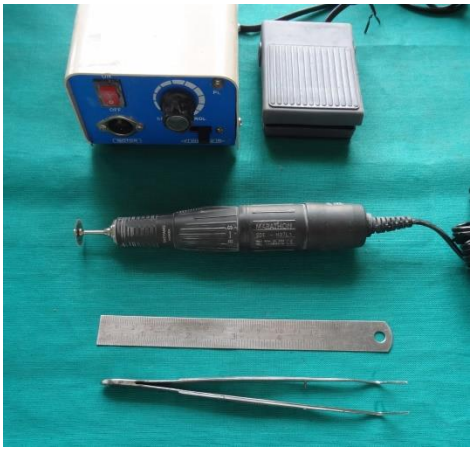


Fig.2- Armamentarium for Sample preparation

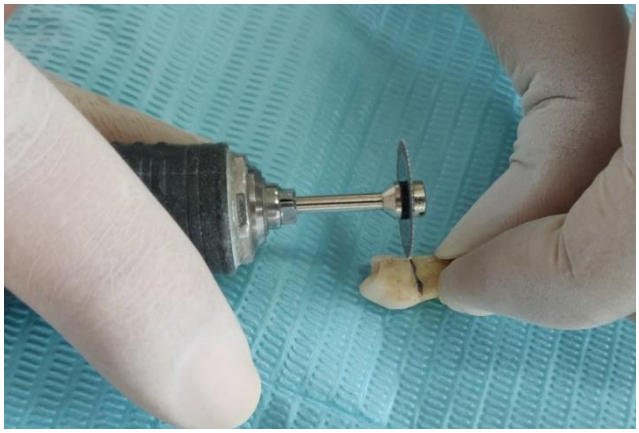


Fig.3- Decoronation of samples

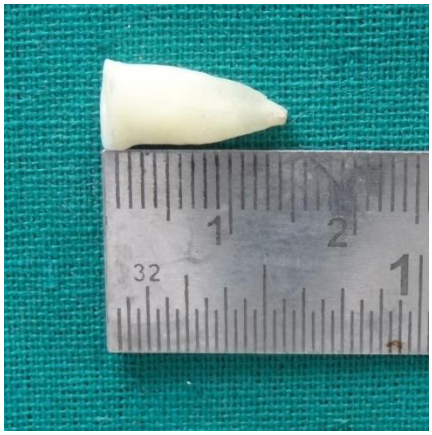


Fig.4- Standardization of length

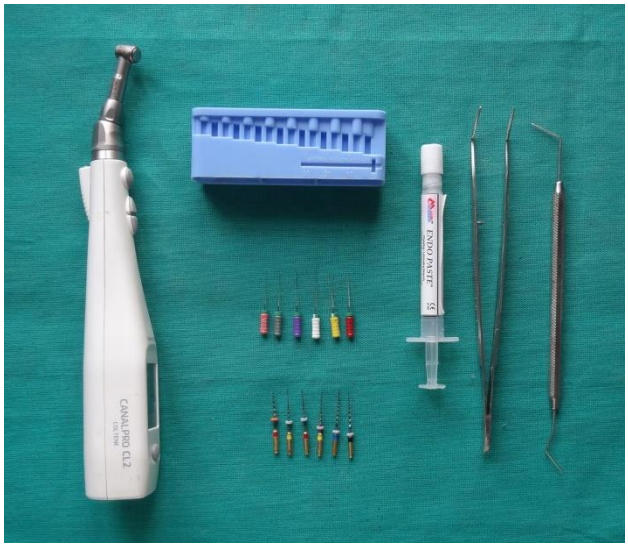


Fig.5- Armamentarium for Cleaning & Shaping

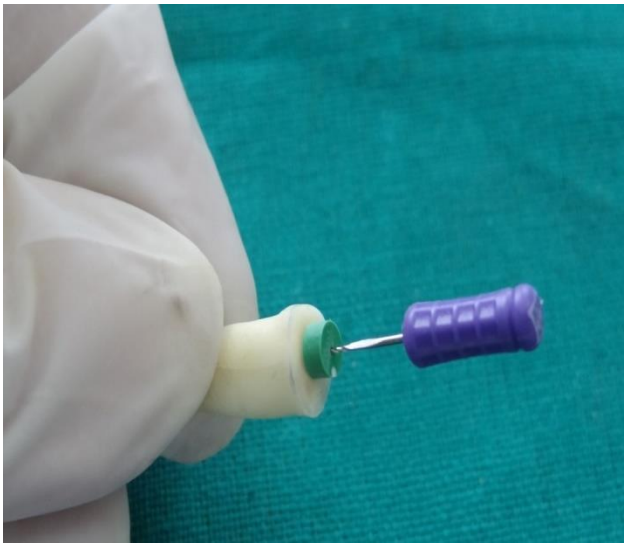


Fig.6- Cleaning & Shaping of Samples

Fig.7- Autoclave

Fig.8- Autoclaved samples stored in Saline

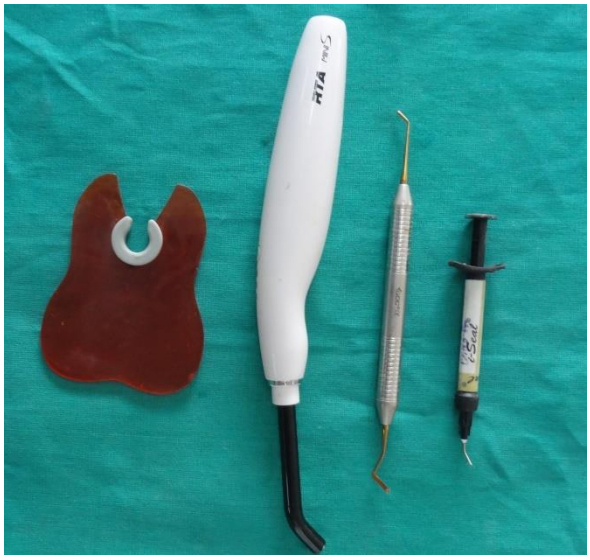


Fig.9- Armamentarium for sealing the apex

Fig.10- Wax mounted sample

Fig.11- Enterococcus faecalis (ATCC 29212)

Fig.12- Micropipette used to carry E. faecalis

Fig.13-Armamentarium for Irrigation

Fig.14-Endoactivator for agitation

Fig.15- Silver Nanoparticles,Nano-Chitosan,Graphene Nanoparticles Fig.16-NS Fig.17-CHX

Fig.18-EDTA Fig.19-Sodium Hypochlorite Fig.20-Deionized water

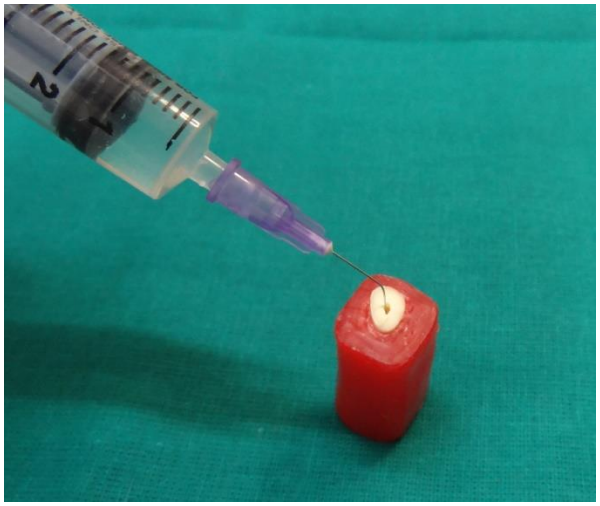


Fig.21- Irrigation with test irrigants

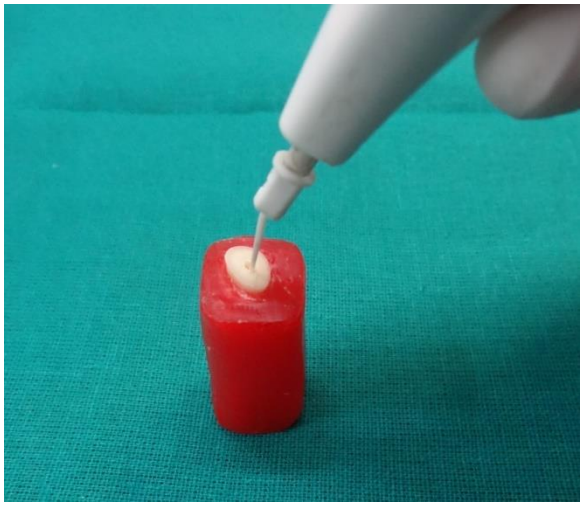


Fig.22-Endoactivator agitation

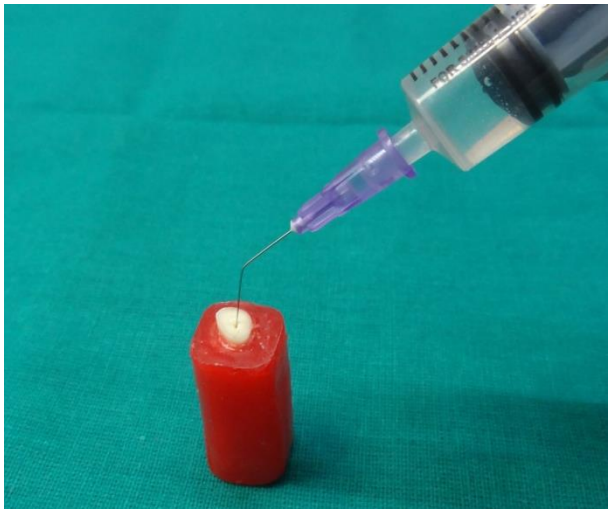


Fig.23-Flushing with Deionized water

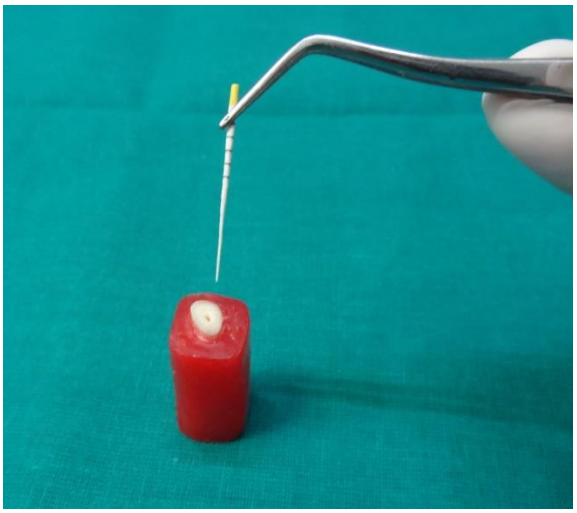


Fig.24-Paper point soaking canal content

Fig.25-Eppendorf tube

Fig.26- Inoculation loop

Fig 27- Eppendorf tube with paper point

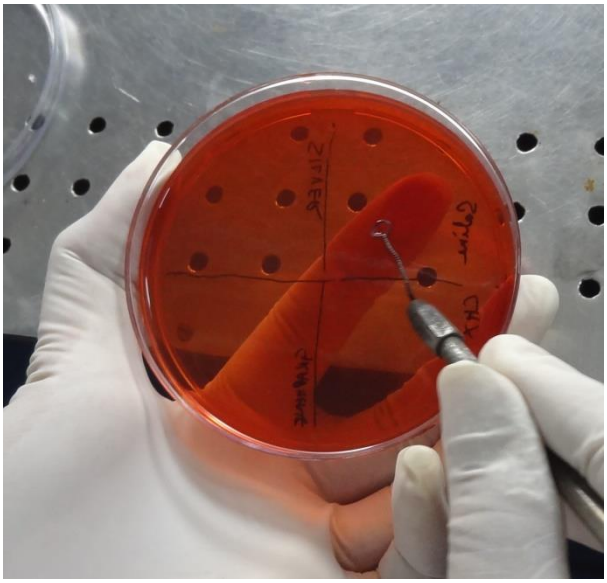


Fig.28-Culture of agar plate

Fig.29- Laminar flow chamber

Fig.30-Incubation Chamber

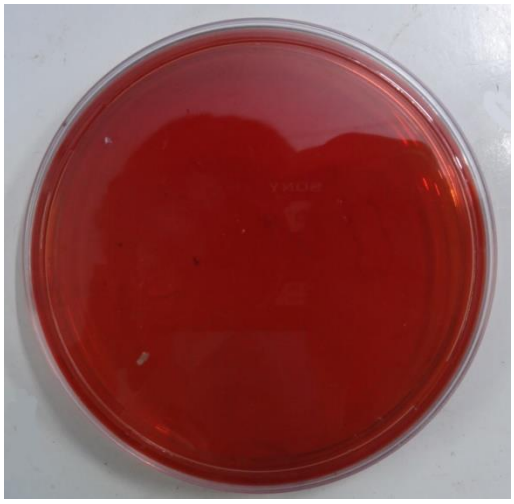


Fig.31-Blood agar culture plate

Fig.32-E. faecalis growth

Fig 33- Digital Colony Counter

OBSERVATIONS & RESULTS

The results obtained were tabulated (Annexure-3). Descriptive and analytical statistics were done. The normality of data was analyzed by the Shapiro-Wilk test. As the data followed normal distribution the parametric tests were used to analyze the data. The one-way analysis of variance (ANOVA) test was used to check mean differences among the groups. Post hoc analysis was done using Tukey's HSD test.

Software: SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA).

Output Tables:

Table 3: Comparison of mean CFU counts among the groups

Groups	N	Mean	S.D.	S.E.	Min.	Max	F-value	P-value[#]
Group A	15	2.50	0.11	0.03	2.20	2.70	387.288	<0.001[†]
Group B	15	3.35	0.15	0.03	3.10	3.60		
Group C	15	3.38	0.22	0.05	2.90	3.70		
Group D	15	2.34	0.19	0.04	1.90	2.70		
Group E	5	5.66	0.20	0.09	5.40	5.90		

[#]P-value derived from one-way ANOVA test; [†]significant at $p < 0.05$

The mean CFU counts were compared among the five groups. The analysis done by one-way ANOVA showed statistically significant differences (**p<0.001**) in mean CFUs counts. The Group D -2% Chlorhexidine had the least CFU count of 2.34 ± 0.19 followed by Group A - 0.2% Silver Nanoparticles (2.50 ± 0.11), Group B - 0.2% Nano Chitosan (3.35 ± 0.15) and Group C - 0.2% Graphene Nanoparticles (3.38 ± 0.22). The Control Group - 0.9% normal saline had the highest mean CFU count of 5.66 ± 0.20 (Table 3).

Table 4: Post hoc pair wise comparison of mean CFU counts among the groups

Groups	M.D.	95% C.I.	P-value*
Group A v/s Group B	-0.85	-1.03--0.67	<0.001†
Group A v/s Group C	-0.88	-1.06--0.69	<0.001†
Group A v/s Group D	0.15	-0.02-0.33	0.139
Group A v/s Group E	-3.16	-3.41--2.90	<0.001†
Group B v/s Group C	-0.02	-0.20-0.15	0.994
Group B v/s Group D	1.00	0.82-1.18	<0.001†
Group B v/s Group E	-2.30	-2.56--2.04	<0.001†
Group C v/s Group D	1.03	0.85-1.21	<0.001†
Group C v/s Group E	-2.28	-2.53--2.02	<0.001†
Group D v/s Group E	-3.31	-3.57--3.05	<0.001†

#P-value derived from Tukey's HSD post hoc test; †significant at $p < 0.05$

The post hoc pair wise comparative analysis was done. When group A was compared with group B, a mean difference of -0.85 (95% CI: -1.03--0.67) was seen which was statistically significant (p<0.001). When group A was compared with group C, a mean difference of -0.88 (95% CI: -1.06--0.69) was seen which was statistically significant (p<0.001). When group A was compared with group D, a mean difference of 0.15 (95% CI: -0.02-0.33) was seen which was NOT significant (p=0.139). When group A was compared with group E, a mean difference of -3.16 (95% CI: -3.41--2.90) was seen which was statistically significant (p<0.001).

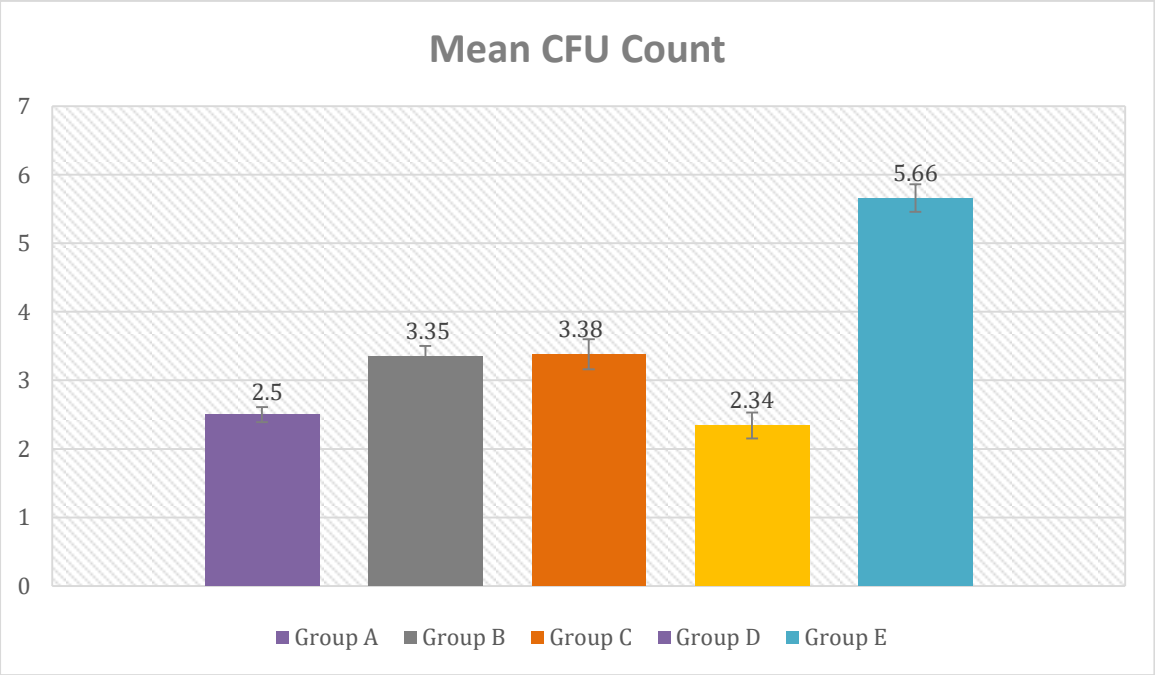
When group B was compared with group C, a mean difference of -0.02 (95% CI: -0.20-0.15) was seen which was NOT significant (p=0.994). When group B was compared with group D, a mean difference of 1.00 (95% CI: 0.82-1.18) was seen which was statistically

significant (p<0.001). When group B was compared with group E, a mean difference of -2.30 (95% CI: -2.56--2.04) was seen which was statistically significant (p<0.001).

When group C was compared with group D, a mean difference of 1.03 (95% CI: 0.85-1.21) was seen which was statistically significant (p<0.001). When group C was compared with group E, a mean difference of -2.28 (95% CI: -2.56--2.04) was seen which was statistically significant (p<0.001).

When group D was compared with group E, a mean difference of -3.31 (95% CI: -3.57--3.05) was seen which was statistically significant (p<0.001) (Table 4).

Graph 2: Comparison of mean CFU counts among the groups



Note: The error bar represents standard deviation
Standard Deviation is a measure of the amount of variation or dispersion of set of value.

The mean CFU counts were compared among the five groups. The Group D -2% Chlorhexidine had the least CFU count of 2.34 ± 0.19 followed by Group A - 0.2% Silver Nanoparticles (2.50 ± 0.11), Group B - 0.2% Nano Chitosan (3.35 ± 0.15) and Group C - 0.2% Graphene Nanoparticles (3.38 ± 0.22). The Control Group - 0.9% normal saline had the highest mean CFU count of 5.66 ± 0.20 (Graph 2).

DISCUSSION

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Nivaran Pathology and Diagnostic Centre, Lucknow.

The aim of this study was to evaluate & compare antimicrobial efficacy of Silver nanoparticles, nano-Chitosan, Graphene nanoparticles and Chlorhexidine against Enterococcus faecalis as root canal irrigant.

In the present study, 65 human mandibular premolars were taken into consideration after accomplishing the inclusion and exclusion criteria (Fig.1). Mandibular premolars were chosen because it was found by Slowey (1979) they are often called as "endodontist's enigma" and may present the greatest difficulty of all teeth to perform successful endodontic treatment and thus most prone to endodontic failure. Mandibular premolars have even shown high flare-up and highest failure rates because of extreme variations in root canal morphology.[70]

A major cause of endodontic failure is the inability to locate, debride and obturate the numerous lateral canals where endodontic instruments cannot reach during routine endodontic treatment.[71] To overcome this, root canal irrigants are being used to disinfect those areas.[72] Keeping this complexity of root canal system and the role of root canal irrigation in view, the present in-vitro study was undertaken.

Irrigation has a central role in endodontics. During & after instrumentation, the irrigant facilitates the removal of microorganisms, tissue remnants and dentin chips from root canals. Irrigants also help in preventing clogging of hard or soft tissue in the apical third of the root and extrusion of infected debris in the periapical area.[73] Commonly used irrigants not just possess tissue dissolving and antibacterial properties, but also have cytotoxic potential and may cause harm to the tissues[74]. The most popular irrigants being used are: Sodium

Hypochlorite, Chlorhexidine, EDTA, Hydrogen Peroxide and Normal Saline; but none can be regarded as optimal or ideal.[75]

Sodium Hypochlorite (NaOCl) is one of the most popular irrigating solution. Sodium Hypochlorite ionizes in water into Na^+ & hypochlorite ions, establishing equilibrium with hypochlorous acid (HOCl). This hypochlorous acid is responsible for the antibacterial activity.[76] The antibacterial & tissue dissolving property of Sodium hypochlorite was supported by Zehnder (2006). Sodium hypochlorite is a function of its concentration, and so is its toxicity.[77] The shortcomings of NaOCl include unpleasant taste, toxicity & its inability to remove smear layer by itself. The efficiency of Sodium Hypochlorite is counteracted by exudate from the periapical area, pulp tissue, dentin collagen, and microbial biomass.[78] Pashley *et al.*(1985) conducted a combination of in-vitro and in-vivo studies and concluded that sodium hypochlorite had caustic and deleterious effect on both hard and soft tissues of the oral cavity.[79] Therefore observing all the above harmful effects of Sodium Hypochlorite, it was not taken into the consideration in this in-vitro study.

Another common irrigant used is Chlorhexidine, appears to be the next most promising antibacterial root canal irrigant. Ribeiro et al. (2005) evaluated the genotoxicity of Chlorhexidine, Calcium Hydroxide, Formocresol and Paramonochlorophenol with concentration ranging from 0.01% to 1% and concluded that none of the tested irrigants contributed to DNA damage.[80] Jeansonne and White (1994) compared the antimicrobial efficacy of 2% Chlorhexidine with 5.25% Sodium Hypochlorite and concluded that 2% chlorhexidine was as effective as sodium hypochlorite as an antimicrobial endodontic irrigant.[81] Chlorhexidine's antibacterial activity is dependent on achieving an optimal pH of 5.5-7.[82] White et al.(1997) reported these effect of 2% Chlorhexidine persisted for 72 hours to 12 weeks and possesses substantivity because of its ability to bind to the hard tissue and maintain its antibacterial activityfor a long period of time.[83] These studies have assessed the antimicrobial efficacy of Chlorhexidine as a good irrigant, equivalent to Sodium Hypochlorite. It is relatively non-toxic and possesses a higher substantivity rate than other commonly used root canal irrigants, therefore 2% Chlorhexidine was taken into consideration in this study.

All the irrigating solutions have their share of limitations. Despite its usefulness as a final irrigant, still chlorhexidine cannot be advocated as the main irrigant because of its inability to dissolve tissue.[83] With the development of newer materials and technologies, the search for the optimal root canal irrigant continues.[77] The introduction of Nanoparticles in the field of endodontics has brought a new hope in the search of an ideal root canal irrigant.

The present study design is based on the studies conducted by Sharma DK. et al.[48], Hayam Y. Hassan, et al.[34] and Alabdulmohsen ZA, Saad AY [54]. This in-vitro study aimed to evaluate & compare antimicrobial efficacy of Silver nanoparticles, nano-Chitosan, Graphene nanoparticles and Chlorhexidine against Enterococcus Faecalis as root canal irrigant. It was decided to take sixty five samples as they showed statistical significance and reliability in results. All samples were inoculated with Enterococcus faecalis, as it has been reported that enterococci are one of the most resistant and most frequently isolated microorganism from obturated root canals that showed chronic periapical pathologies. [14,41] E. faecalis has been also responsible for 80–90% of human Enterococcal infections.[84] It has been found as the the most common enterococcus strains found in previously obturated root canals.[14,41,85] These findings suggest that E. faecalis plays a pathogenic role in chronic endodontic treatment failure and may survive in a harsh root canal environment without the help of other bacteria.[86]

To overcome the drawbacks of these conventional irrigants, antimicrobial nanoparticles offering numerous advantages like large surface-area:volume ratio, ultra-small sizes, and excellent chemical and physical properties have been introduced in the field of endodontics[87] Nanodentistry has various applications in dentistry like dental materials production, preventing diseases like dental caries and periodontal diseases; treatment of dentin hypersensitivity, oral cancer and diseases of endodontic origin. [88] Currently the application of Nanomaterials in the field of Endodontics is limited to:

1) Bone replacement material- Hydroxyapetite nanoparticles
2) Nanoneedles & tweezers
3) Endodontic Sealers – Bioceramic based nanomaterial (EndoSequence BC sealer)
4) Denbur Nanobrush
5) Nanoparticles as antimicrobial irrigants.[89]

In recent times, many materials have displayed good results as an endodontic irrigant. In this in-vitro study, nanoparticles based irrigants like silver nanoparticles, nano-chitosan and graphene nanoparticles were used as irrigants. Nanoparticles exhibit higher antimicrobial efficacy owing to its poly-cationic or poly-anionic nature. It also possess higher surface area and charge density, leading to better interaction with the bacterial cell.[90]

In the present study, Group A : Silver Nanoparticles irrigant was used as it is the most popular nanomaterial in endodontics. Jose Ruben *et al.* first described the antimicrobial properties of silver nanoparticles in 2005. Silver nanoparticles works by binding to the negatively charged parts of the cell membrane of bacteria, affecting its functions such as permeability, causing leakage of cytoplasm and its content and leading to rupturing of cell wall. As a result, the nanoparticles will infiltrate the cytoplasm and effect the DNA and RNA, causing more damage to the bacteria cell. [91]

Halkai et al. (2018) evaluated the antimicrobial effect of biosynthesized silver nanoparticles (using fungus- Fusarium semitectum) against E. Faecalis biofilm on root, and concluded that the biosynthesized silver nanoparticles were effective against E. Faecalis biofilm & thus can be used as root canal irrigants. [63]

In the present study, as Group B, Chitosan Nanoparticles (Cs-NPs) irrigant was used. It is one of the most researched polymeric nanoparticle material in endodontics. Chitosan is a natural polysaccharide, obtained by chitin deacerylation, which is one of the most abundant natural polysaccharide. Most-commonly found in exo-skeleton of shrimps & crabs. A modification in the chemical structure of chitin (1,4)-N-acetyl-D-glucos-2-amine, leads to the formation of (1,4)-2-Amino-2-desoxy- beta-D-glucan, known as Chitosan. [92]

Chitosan's antibacterial mechanism is still under research. One hypothesis states that chitosan has the ability to bind with the negatively charges cell membrane, which enhances its permeability, leading to cytoplasm and its content leakage and ultimately bacterial cell death. Other hypothesis state that chitosan has the property to chelate metal, which inturn reduces enzyme activity, inhibiting bacterial action.[93]

Kishen et al. (2008) & Shrestha et al. (2010) evaluated the efficacy of chitosan as an irrigant against E. Faecalis in a biofilm & planktonic state, and concluded that chitosan is effective in complete elimination in planktonic state & significant reduction in biofilm state.[94,95] Del

Caprio-Peeochena A et al. (2015) evaluated the efficacy of nano-chitosan in removing the smear layer and inhibit bacterial growth in dentin. They concluded that nano-chitosan has the capacity to eliminate the smear layer and prevent bacterial colonisation in dentin, thus can be used as a final irrigant & as an alternative to EDTA during root canal treatment. [46]

In the present study as Group C, Graphene Nanoparticle irrigant was used. It is an allotrope of carbon which forms an even crystal lattice excluding any structural dislocations. Graphene nanoparticles are being used for diagnosis of diseases and formation of antibacterial surfaces. Graphene Family Nanomaterials include Ultrathin graphite, few-layer graphene, graphene oxide, reduced graphene oxide and graphene nanosheets. Graphene oxide is one of the most popular graphene derivative. Graphene oxide is a chemically modified graphene with hydroxyl, carbonyl, and epoxy functional groups that is made by combining strong oxidising agents with graphite.[96]

Pulingam et al. were the first to report the antimicrobial activity of Graphene Oxide towards Gram-positive and Gram-negative bacteria. They found that Graphene Oxide antibacterial mechanism differed between Gram-positive and Gram-negative bacteria, where the majority of bacterial inactivation of Gram-positive bacteria occured through the bacterial wrapping mechanism. On the other hand, inactivation of Gram-negative bacteria mainly occured through physical contact, which led to membrane damage.[97] Martini *et al*. (2020) evaluate the antibacterial properties of graphene oxide (GO) against Enterococcus faecalis in vitro conditions. They concluded that Graphene Oxide possessed antimicrobial and antibiofilm properties against E. faecalis. Graphene Oxide film demonstrated a role in the inhibition of bacterial film proliferation and it showed acceptable adhesion properties to root dentin, without causing alteration of its structure. [98]

In the present study, a 30-gauge side-vented close-end irrigating needle was used to deliver irrigants to the root canal of the prepared tooth samples. It has been suggested that the side-vented needle is more efficient than the beveled and notched ones in removing bacteria[99]. The external diameters of 30 gauge needle is 0.32 mm which facilitates better penetration of irrigant into the canals.[22]

A keen interest was also there to find whether recent nanoparticles based irrigants were better against Enterococcus faecalis, than traditional irrigants or not. In this study, teeth samples inoculated with E. faecalis were used to determine the presence of bacteria in the canal. All the sample teeth irrigated with chlorhexidine and nanoparticles based irrigants showed a positive reduction in bacterial colony count. In the present study silver nanoparticle, graphene nanoparticles and nano-chitosan have shown antimicrobial potential as a root canal irrigants. There was no significant difference in bacterial colony count reduction between silver nanoparticle, graphene nanoparticle and nano-chitosan in this study.

In this study, 2% Chlorhexidine was found to be most effective against E. faecalis when compared to 0.2% Silver Nanoparticles, followed by 0.2% Nano-chitosan & 0.2% Graphene Nanoparticles respectively.

Similar results were obtained in the research done by Almeida et al. (2018) who evaluated the effectiveness of 1% silver nanoparticles (AgNP), 26% zinc oxide nanoparticles (ZnO Np), 2% Chlorhexidine (CHX), 1% Sodium Hypochlorite (NaOCl) and 5% Sodium Hypochlorite (NaOCl) against Enterococcus faecalis biofilm, and found out that overall 2% CHX presented the most effective action against E. faecalis biofilm.[62]

Also similar results were shown by study done by Hadil A. Sabry *et. al.* (2019) who compared the antimicrobial efficacy of Silver nano irrigant, Chlorohexidine and Sodium Hypochlorite against Enterococcus Faecalis and concluded that Silver Nanoparticles irrigant group showed the lowest antibacterial activity among the tested irrigants.[23]

Barreras *et al.*(2016) in their in-vitro study used Chitosan Nanoparticles and Chlorhexidine (CHX) alone and in combinations to remove Enterococcus faecalis from the infected root canals and concluded that Chitosan Nanoparticle root canal irrigant alone was least effective when compared with other test irrigants.[49]

In contrast to the results of the present in vitro study, the research done by Batool M AL-Fhham and Aseel Haidar MJ AL-Haidar (2019) found out that the antibacterial effect of Silver Nanoparticle was more than 2% Chlorhexidine, against E. faecalis biofilm.[101]

Also results of research done by Halkai *et al*. (2018) found no significant difference between Silver Nanoparticle and Chlorhexidine irrigants against E. Faecalis.[102]

In the present study, Graphene oxide (Group C) showed the least antimicrobial property against tested microflora. This result is in accordance to the research done by Wu et al (2018) who concluded that Graphene oxide is not a promising antimicrobial agent and serves as a general growth enhancer that can act as a biofilm to enhance bacterial attachment and proliferation.[103] The least antibacterial efficacy of Graphene Oxide can also be attributed to the impurity of the Graphene Oxide nanoparticle irrigating solution. Barbolina *et al.* (2016) stated in their research on Graphene Oxide Nanoparticle irrigant and its antimicrobial activity that the properties of Graphene Oxide are critically dependent on its manufacturing method, which includes purification and purification method. It was believed that the low antibacterial effect was due to impurities like boron and sulphur entrapped during its manufacturing process.[104]

A large number of nanoparticle materials are available today which provide a variety of options for better cleaning of the root canal with lesser adverse effects. Currently endodontic research is being focused on evaluating the antimicrobial efficacy of few nanoparticles as new irrigants against endodontic infection. Although previous studies also showed satisfactory results against persistent endodontic pathogen like Enterococcus faecalis and had overcomed the shortcomings of traditional irrigants but more in-vivo studies are still required to evaluate and to relate the findings of the present in-vitro study in terms of efficacy.

CONCLUSION

The present in-vitro study evaluated & compared antimicrobial efficacy of 0.2% Silver Nanoparticles, 0.2% Nano-Chitosan, 0.2% Graphene Nanoparticles and 2% Chlorhexidine against Enterococcus faecalis as root canal irrigants.

Within the limitations of this study the following conclusions were drawn:

1. All irrigants were effective against E. faecalis when compared to the control 0.9% Normal Saline, thus can be used as an effective root canal irrigant.
2. Statistically no significant difference was found between the antimicrobial efficacy of 2% Chlorhexidine and 0.2% Silver Nanoparticles.
3. 2% Chlorhexidine showed maximum antimicrobial activity against E. faecalis when compared to 0.2% Silver Nanoparticles, 0.2% Nano-Chitosan, 0.2% Graphene Nanoparticles.
4. Amongst the tested nanoparticles based irrigants, 0.2% Silver Nanoparticles was the most effective, followed by 0.2% Nano-chitosan & 0.2% Graphene Nanoparticles respectively.

However, further in-vivo studies are required to evaluate which nanoparticles are more appropriate as root canal irrigants, not just against E. faecalis, but also for other persistent endodontic pathogens.

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ANNEXURE-1

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 23 **BBDCODS/01/2019**

Title of the Project: A Comparative Evaluation of Antimicrobial Efficacy of Silver Nanoparticles, Nano-Chitosan, Graphene Nanoparticles and Chlorhexidine Against Enterococcus Faecalis as Root Canal Irrigants: An *In-Vitro* Study.

Principal Investigator: Dr. Sankalp Nigam **Department:** Conservative Dentistry & Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Sankalp Nigam,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10th January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 22/01/19

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
(Babu Banarasi Das College of Dental Sciences)
BBD City, Faizabad Road, Lucknow

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
BBDCODS

ANNEXURE-2

NIVARAN PATHOLOGY & DIAGNOSTIC CENTRE

HIG-91, Aliganj Ext. Sector-E, Lucknow. ☎ 0522-2329060, 2330983, 4109837, 3580925, 4319139, 9415067276, 9670129179 E-mail : nivaranpathology@gmail.com

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Clinical Laboratory

DIRECTOR

Dr. M. Mehrotra MBBS:MD (KGMU)
Ex. SR Immunologist Serologist & Microbiologist
Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow.
Professor & Head Dept. of Pathology & Microbiology SPIDMS, Lko.
Member Indian Assn. of Pathologist & Microbiologist

25 YEARS

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निवारण MEMBER

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FDA : 4/2015-DC-TRUENAT
PNDT : CMO : PNDIT/135/2002
GOVT. OF U.P : ADM/UC176534
ICMR : NIPDCLUP

This is to certify that Dr. Sankalp Nigam has conducted his dissertation study at Nivaran Pathology & Diagnostic Centre , Lucknow under the direct Supervision and guidance of Dr. Mridul Mehrotra (M.B.B.S. , M.D.) in patial fulfillment of the requirement for the degree of Master of Dental Surgery (M.D.S.).

Dr Mridul Mehrotra
M.B.B.S. , M.D. (KGMU)
Ex SR Sanjay Gandhi Post Graduate Institute of Medical Sciences
Demonstrator King Georges Medical College
Prof and Head Deparment of Pathology and Microbiology SPIDMS Lko

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ANNEXURE-3

DATA ENTRY

SAMPLE NO.	GROUP A AgNP	GROUP B NC	GROUP C GNP	GROUP D CHX	GROUP E Control	
1	2.6	3.3	3.2	2.5	5.9	
2	2.5	3.3	3.4	2.2	5.7	
3	2.6	3.3	3.1	2.3	5.5	
4	2.4	3.4	3.5	2.2	5.8	
5	2.5	3.6	3.3	1.9	5.4	
6	2.5	3.4	3.4	2.2	.	
7	2.5	3.5	2.9	2.4	.	
8	2.4	3.2	3.7	2.4	.	
9	2.7	3.1	3.4	2.5	.	
10	2.6	3.3	3.7	2.3	.	
11	2.4	3.6	3.5	2.7	.	
12	2.5	3.2	3.5	2.5	.	
13	2.2	3.4	3.6	2.2	.	
14	2.6	3.5	3.2	2.5	.	
15	2.5	3.2	3.3	2.4	.	
MEAN	2.5	3.35	3.38	2.34	5.66	

ANNEXURE-4

URKUND

Urkund Analysis Result

Analysed Document:	PLAGIARISM Sankalp.docx (D110162723)
Submitted:	7/5/2021 3:41:00 PM
Submitted By:	drvisheshgupta@bbdu.ac.in
Significance:	7 %

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Dr Sweta Choudhary.docx (D45168481)
Untitled.pdf (D34332733)
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Main thesis of Dr. nikita saini, Dept. of Pedontics & Prev. Dentistry, Ist submission.pdf (D91482912)
To evaluate the antimicrobial efficacy of 0.1% AgNP and conventional irrigants such as 2.5% NaOCl and combination of 2% CHX and 3% H2O2 with or without EndoActivator against E. faecalissis.docx (D81007879)
Antimicrobial efficacy of 0.1% AgNP and conventional irrigants with or without EndoActivator against E. faecalissis C Albicans -an Ex-Vivo Experimental Study.docx (D81009790)
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https://www.researchgate.net/publication/327136746_Evaluation_of_Antimicrobial_Efficacy_of_Four_Tested_Solutions_Against_Enterococcus_Faecalis_an_in_Vitro_Study

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