COMPARITIVE STUDY OF SMEAR LAYER REMOVAL AND ANTIBACTERIAL ACTIVITY OF SILVER, CHITOSAN NANOPARTICLES AND SODIUM HYPOCHLORITE AGAINST ENTEROFECALIS WHEN USED AS ROOT CANAL IRRIGANTS- IN VITRO STUDY

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Abstract

Background: As a very resistant microorganism in infected root canals, Enterococcus faecalis (E. faecalis) can stubbornly survive lethal challenges and invade dentinal tubules, making it the most persistent pathogen in root canal treatment. Enterococcus faecalis (E. faecalis) is the most commonly encountered microorganism detected in persistent root canal infections. These bacteria possess certain virulence factors, invade dentinal tubules and resist nutritional deprivation. Proper irrigation is an essential step for success in root canal therapies which is achieved by using excellent endodontic irrigants. Aim: To evaluate the antimicrobial efficacy of Sodium Hypochlorite (NaOCl), Silver Diamine Fluoride (SDF), Chitosan Nanoparticles (CNPs) root canal irrigants against the bacterial strain of Enterococcus Faecalis (E. faecalis) using agar well diffusion method. Materials and Methods: In this in-vitro study, the test materials were manipulated in accordance with the manufacturer’s instructions. The antimicrobial properties of root canal irrigants were evaluated by using agar diffusion method using bacterial strain of Enterococcus faecalis (ATCC 29212). 0.25 mL of each irrigant was placed on to 6.5 mm diameter blotting papers which were placed in 7 mm diameter wells on the Mueller Hinton agar plates. Later, E. faecalis strains were inoculated with sterile cotton swab on to the agar plates. Results: The p-value Sodium Hypochlorite showed the greatest zone of inhibition followed by SDF and Chitosan Nanoparticles respectively (p Sodium Hypochlorite showed the greatest zone of inhibition followed by SDF, Bioactive Glass Nanoparticles and Chitosan Nanoparticles respectively. Conclusion: Sodium Hypochlorite was the most effective root canal irrigant followed by SDF, whereas Chitosan Nanoparticles was the least efficacious compared to the rest against Enterococcus Faecalis.

Keywords: smear layer removal, antibacterial activity, silver, chitosan nanoparticles, sodium hypochlorite, enterofecalis, root canal irrigants, in vitro study, endodontics.

INTRODUCTION

Microorganisms predominantly bacteria are the primary etiological factor in the development of pulp and periapical lesions. Successful root canal therapy depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and microorganisms. Microorganisms predominantly bacteria are the primary etiological factor in the development of pulp and periapical lesions. Successful root canal therapy depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and microorganisms. Bacterial elimination in infected root canal systems is performed using mechanical debridement and chemical elimination of intraradicular microorganisms [1-5]. As a very resistant microorganism in infected root canals, Enterococcus faecalis (E. faecalis) can stubbornly survive lethal challenges and invade dentinal tubules, making it the most persistent pathogen in root canal treatment. Sodium Hypochlorite (NaOCl) is the medicament of choice due to its ability to
dissolve organic substances present in the root canal system, its affordability, its efficacy against pathogenic organisms like E. faecalis and pulp digesting property in endodontic therapy [6-9]. Sodium hypochlorite is used in non-surgical endodontic treatment as a powerful antimicrobial agent for its chemical dissolution properties and as a lubricant during instrumentation [10]. Silver Diamine Fluoride (SDF), is an anti-cariogenic agent, that is deemed to be very powerful as an antimicrobial root canal irrigant and for inter-appointment dressing specifically in pediatric dentistry [11,12]. Chitosan is a polymer with wide range of application in the medical field. It is either in part or completely de-acetylated chitin. Fungal cell walls and crustacean shells, for example, contain chitin naturally, it is completely biodegradable and biocompatible and may be used as an adhesive and wound dressing material [13]. Chitosan has been investigated as an antimicrobial material against target organisms like algae, micro-organism (gram positive and gram negative), Chitosan has been investigated as an antimicrobial material in various in-vitro and in-vivo experiments designed to study interactions with chitosan against target organisms like algae, gam positive and gram negative bacteria and fungi such as yeasts [14] Hence, this study was carried out to evaluate the antimicrobial efficacy of various root canal irrigants like Sodium hypochlorite, SDF, Chitosan Nanoparticles (CNPs) against E. faecalis bacteria in vitro using disc diffusion method and the relative efficacies were recorded after 24, 48 and 72 hours of incubation

MATERIALS AND METHODS

An in -vitro study conducted and the study was approved by the Institutional Ethics Committee. Sampling Technique Randomised sampling technique was carried out for sample size determination for minimum number of petri plates required using the following formula. The minimum sample size required was determined to be 10 after substituting the values in the above formula. Hence, the total number of petri dishes for the study equalled to 10. The antimicrobial property of root canal irrigants was evaluated by agar diffusion method [9] using strains of Enterococcus faecalis (ATCC 29212). Primary isolation was done on Mueller Hinton agar plates and magenta pink-coloured colonies were grown. In the present study, four endodontic irrigants were used i.e., 2.5% Sodium Hypochlorite, 38% SDF, and Chitosan Nanoparticles (1 mg/mL in 0.1% acetic acid). The test materials were manipulated in accordance with the manufacturer’s instructions. Solution of 2.5% Sodium hypochlorite and 38% SDF were used which were commercially available. Solution of Chitosan Nanoparticles was prepared by diluting 1 mg/mL in 0.1% Acetic acid [20]. Bacteria were diluted to obtain suspension of approximately 5x10^6 colony forming units in sterile Trypticase Soy Broth (TSB) obtained by spectrophotometer by forming two layers, one is the seed layer and next is the second layer. Microbial strains were confirmed by colony forming units and growth characteristics. A 10 mL of Mueller Hinton agar was poured in petri plates to form a base layer. When it was solidified, a second layer containing 10 mL of Mueller Hinton agar and 200 µL of microbial standardised suspensions were poured over it. Wells of 7 mm diameter were prepared on Mueller Hinton agar plates using sterile core borer. Followed by this 0.25 mL of each irrigant was placed on to the 6.5 mm diameter blotting paper. The soaked 6.5 mm blotting paper was placed into the wells which was created on the agar plates. Later E. faecalis strains were inoculated with sterile cotton swab on to this agar plate [22]. After pre-diffusion of test materials for two hours at room temperature, all the plates were incubated at 37°C and evaluated at 24, 48 and 72 hours to analyse whether there were any significant changes in zone of inhibition of individual irrigant after 24, 48 and 72 hours [Table/Fig -1-3], respectively. Generation time is the time duration for any bacteria to become double by binary fission which was 72 hours for E. faecalis [23]. In case of E. faecalis, the growth usually comes after 24 hours, but some studies conclude that growth gets completed after 72 hours [24]. So, to confirm the growth and for further confirmation of the results, bacteria were incubated for 72 hours and readings were recorded at 24, 48 and 72 hours. A study verified three-day-old biofilm showing E. faecalis colonising the dentin surface and starting to invade the patent dentinal tubules [24]. A 0.5 mm precision ruler was used to determine the microbial inhibition zones and the results were expressed as the mean and standard deviation. The test results were measured by the author, double blinding was ensured by two other authors to eliminate the bias. The results were analysed using one-way ANOVA with INSTAT software (GraphPad, San Diego, CA) to find out if there was a significant overall difference between the mean zones of inhibition of various irrigants and Post-hoc Tukey HSD test to find out where the differences occurred. The p-value was considered significant at (p-value was considered significant at (p<0.01).

RESULTS

Table 1 represents zones of inhibition (in mm) of root canal irrigants after 24, 48 and 72 hours of incubation of 10 Petri Plates. Mean zones of inhibition after 24 hours were observed. Significant difference was present in the overall test means (p<0.01).
Table 1: Zones of inhibition (in mm) of root canal irrigants after 24, 48 and 72 hours of incubation

<table>
<thead>
<tr>
<th>Sodium hypochlorite</th>
<th>Silver Diamine Fluoride</th>
<th>Chitosan Nanoparticles (CNPs)</th>
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<tr>
<td><strong>24 hours</strong></td>
<td>48 hours</td>
<td>72 hours</td>
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DISCUSSION

It is crucial to consider that most of the irrigation solutions used during root canal treatments modify the chemo-mechanical properties of the root canal dentin, thus affecting the performance and longevity of all materials used for root canal obturations and restorations. One of the most challenging tasks in endodontic treatments is the accomplishment of a comprehensive elimination of indwelling microorganisms, as well as a suitable sealing of the root canal system [1-10]. The fact that mechanical endodontic instrumentation allows clinicians to obtain a bacteria-free root canal system by itself has no evidence-based support in modern literature. In endodontics, the use of intracanal disinfectant solutions with adequate efficacy and acceptable biocompatibility will lead to the eradication of resistant microorganisms within the root canal and periapical areas and will consequently decrease the failure rate of root canal treatment. E. faecalis is the most common bacterial species found in association with endodontically treated teeth with chronic apical periodontitis. The development of calcified biofilm on root canal dentin by E. faecalis can be a potential factor that enables them to persist even after endodontic treatment. Sodium Hypochlorite is the most commonly used solution in root canal treatments, as it’s a low-cost approach that demonstrates a very powerful antimicrobial activity against microbiota of infected root canals [6]. NaOCl ionises in water into Na+ and the hypochlorite ion, OCl-, establishing equilibrium with hypochlorous acid (HOCl). Chlorine exists predominantly as HOCl at acidic and neutral pH, whereas OCl- predominates at pH of 9 and above. Hypochloric acid is responsible for the antibacterial activity [7]. In this study, NaOCl showed significant results in antimicrobial efficacy as compared to other root canal irrigants. It has been approved that 2.5% NaOCl is extremely useful in the removal of vital pulp tissue from dentinal walls. A 1.5-2.5% Sodium Hypochlorite solution as an endodontic irrigant is preferred as the gold standard for root canal cleansing and disinfection [9]. Jaiswal et al., found out that NaOCl was highly effective in eliminating E. faecalis grown in biofilm [3]. SDF is an anti-cariogenic material with a high fluoride release capacity. SDF is very effective as an antimicrobial endodontic irrigant and for dressings given in between the appointments [11-15]. Other root canal irrigants like Sodium Hypochlorite and Chitosan Nanoparticles considered in this study fail at releasing fluorides which adds to the antimicrobial efficacy. As fluoride has a direct inhibitory effect on the metabolic activity of bacteria (gram positive and gram negative). The Silver Nanoparticle solution has been endorsed as an alternative to root canal irrigating solutions not only for its effective bactericidal capacity but additionally for its biocompatibility, specifically at lower concentrations. SDF can enhance the hardness of enamel surface and re-mineralise it [12,15,16]. In an in -viro model studied by Prabhakar AR and Kumar SC, BAG was effective against E. faecalis as an intra canal medicament [16]. In this study, showed less antimicrobial effect when compared to NaOCl, SDF and showed higher antimicrobial efficacy than CNPs. Yadav P et al., proved the anti-bacterial efficacy of Chitosan to be almost equivalent to 3% NaOCl, suggesting that CNPs can be used as an endodontic irrigant to overcome the deleterious consequences, concentration and time dependent outcomes of the conventional irrigants like NaOCl and Chlorhexidine on dentine [15]. According to this study, CNP showed less antibacterial efficacy when compared to other irrigants. Disagreement may be due to different concentration of Chitosan solution, discrepancy in tested strains, variances in methodology, and may be because of different incubation conditions. In-vitro studies have the limitation of laboratory and clinical setup errors. Contamination of the irrigants with other biofilms and fluids in oral cavity may alter the results. The microorganisms of root canal other than E. faecalis may show unpredictable reactions to the studied irrigants. This study has exclusively used E. faecalis strain ATCC29212. In-vitro studies cannot re-multiply the microorganism which is the possibility in host in-vivo. Agar depth
can have an effect on the accuracy of plate-based assays and the probable reason for the same could be antimicrobial agent diffusion. Therefore, further clinical studies are indicated.

CONCLUSION

Our study concludes that Sodium Hypochlorite showed better results as compared to the other irrigants in the following order of decreasing antimicrobial efficacy against E. faecalis: SDF, and Chitosan Nanoparticles. Therefore, it is safe to say that Sodium Hypochlorite is still the most effective ‘gold standard irrigant’. No decrease in zone of inhibition after 72 hours proved that the efficacy of the root canal irrigants is maintained at least till three days. Further studies should be carried out for longer period to substantiate findings of this study.

REFERENCES