NANO-ENGINEERED IRRIGATING SOLUTIONS AND LASERS – AN ANTIBACTERIAL STUDY AGAINST ENTEROCOCCUS FAECALIS

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Abstract

Background: Enterococcus faecalis (E. faecalis) is the most commonly detected micro-organism in asymptomatic and persistent endodontic infections. Thorough disinfection of the root canal is more important than proper shaping for a successful endodontic treatment. This study is an attempt to implement nanoparticles which have proven anti-bacterial efficacy as irrigating solutions against E. faecalis.

Aim: To compare and evaluate the anti-bacterial efficacy of 5% sodium hypochlorite (NaOCl), 0.2% chitosan nanoparticles solution (ChNP) and 0.01% silver nanoparticles solution (AgNP) against E. faecalis with and without diode laser activation.

Materials and Methods: 70 single rooted mandibular premolars were included in this study. Access opening, working length determination and biomechanical preparation were standardized. Samples were embedded in putty material inside an endodontic tube to simulate periodontal ligament and autoclaved followed by inoculation of E. faecalis (MTCC 439) and incubated at 37°C for 7 days. The samples were divided into 7 groups (10 samples in each group). Group 1- No treatment (Negative control), group 2 - 5% NaOCl without activation (Positive control), group 3 - 5% NaOCl with diode laser activation, group 4- 0.01% AgNP without activation, group 5- 0.01% AgNP with diode laser activation, group 6- 0.2% ChNP without activation and group 7- 0.2% ChNP with diode laser activation. A final rinse of the respective irrigants and activation were performed, followed by counting the colony forming units. Statistical analysis used were one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc tests. Results showed that diode laser in combination with 5% NaOCl and 0.2% ChNP had significant effects in the reduction of microbial colony counts of E. faecalis in comparison to other experimental groups.

Keywords: Nanoparticles, Irrigation, LASER.

Introduction

Biofilm is a microbial sessile community which contains cells attached to a substratum, within a matrix of extracellular polysaccharides and it shows alterations in phenotype, growth and gene expression. (1) E. faecalis present in the biofilms plays a significant role in the primary and persistent pathologies in the root canal system. (2) This was attributed to multiple factors, especially its capacity to survive in environments of nutritional deprivation and when commensality with other bacteria is meagre. (3) Ultimately, the success of root canal treatment relies on the elimination of the microbial film, which can be challenging due to the invasion of the microorganism into the dentinal tubules and the anatomic complexities of the root canal. E. faecalis, a gram positive facultative anaerobe, is reported to be present in approximately 20-30% of primary infections and 67-77% of secondary infections. (4)

Hence, thorough disinfection of the root canals is highly essential to overcome the ill-effects of these microbes. Root canal disinfection includes mechanical cleaning and irrigation using solutions with antimicrobial potential.

Sodium hypochlorite is considered as the gold standard root canal irrigant, because of its antimicrobial efficacy and tissue dissolution capacity. (5)

Antimicrobial delivery system through nanoparticles is one of the innovations to improve the characteristics of antibacterial agents used in root canal treatment. (6) Nanomaterials are particles with external dimensions of 1–100 nm, large surface/area mass ratio and increased chemical reactivity, which enable them to interact to a greater extent with the
negatively charged surface of bacterial cells, resulting in enhanced antimicrobial activity.(7,8) Nanoparticles have also been studied in the endodontic field in an attempt to reduce E. faecalis adherence to dentine, eliminate biofilms and enhance root canal disinfection of dentinal tubules.(6)

Silver nanoparticles are capable of attaching to and penetrating into the cell walls of both Gram-positive and Gram-negative bacteria, disturbing cell function by releasing silver ions; thus, they are used for the treatment and prevention of drug-resistant microorganisms and inhibition of the biofilm formation.(7)

Chitosan, a primary component of crustacean exoskeletons procured by alkaline deacetylation from chitin is a cationic biopolymer,(9) which is known to remineralize the demineralized structure with its functional phosphate groups and combines with calcium ions for crystal nucleation, leading to the formation of a calcium phosphate layer. It improves the resistance of the dentinal surface to degradation by collagenase.(10) Furthermore, chitosan presents with biocompatibility, chelating capacity and also antimicrobial effects against a broad range of gram-positive and gram-negative bacteria as well as fungi.(11)

Lasers being another phenomenal field, have excellent bactericidal effect.(12) Diode lasers emit radiation within the visible (mostly 660 nm) and infrared (810 to 980 nm) range of the electromagnetic spectrum. Because of the higher absorption coefficient in water (0.68 cm⁻¹), diode lasers have lower penetration depth into the dentine (up to 750 µm) compared to Nd:YAG laser.(13) There are only few data about the antimicrobial effectiveness of diode lasers in root canal treatment available in the literature so far.

Thus, the aim of this study is to compare and evaluate the anti-bacterial efficacy of 5% NaOCl, 0.01% silver nanoparticles (AgNP)(14) and 0.2% chitosan nanoparticles (ChNP)(15) solutions against E.faecalis, with and without laser activation.

Materials and Methods
Specimen preparation
Seventy non-curious, extracted single-rooted premolars were used. The teeth presented straight root canals, fully formed apices and root canal length was standardized to 12 mm by decoronating with a diamond disc. Apical patency was established with a #10-K file (Mani Inc., Japan). The orifice enlargement was done using #6, 5, 4 Gates Glidden drills (Dentsply Maillefer, USA), followed by biomechanical preparation up to F3 (Protaper gold, Dentsply Maillefer, USA), 1 mm short of the apex i.e., working length [WL]. Intermittent 5% NaOCl (Prime Dental Products Pvt., Ltd., India), 0.9% saline (Fresenius Kabi Private Limited, India), 17% EDTA (RC Help, Prime Dental Products Pvt. Ltd., India) irrigation were carried out between each instrumentatation. The apex of all specimens were made impermeable with two layers of epoxy adhesive (LOCTITE Pvt., Ltd., India).(16) To obtain a closed root canal system that mimicked clinical in vivo scenarios and to ensure easy handling, a customized model was fabricated for each tooth by mounting into a 1.5-mL polypropylene Eppendorf tube (Eppendorf Private Limited, India) filled with silicone rubber impression material.(17) These specimens were then autoclaved at 121 °C, 15 lbs for 30 minutes.

Biofilm formation
Pure standard strain of MTCC 439 Enterococcus faecalis (CSIR Institute of Microbial Technology, Chandigarh, India) was inoculated in 5ml of brain heart infusion (BHI) broth (HI Media, India) for 24 hrs at 37°C. The cell suspension was adjusted spectrophotometrically to match the turbidity of 3.0 x 10⁶ CFU/mL (equivalent to 1.0 McFarland standard). Under laminar air flow chamber ensuring asepsis, 0.1-mL aliquots of E. faecalis culture was inoculated into each canal with a sterile needle. The medium was replaced every 24 h, for seven days, with fresh BHI broth (100µl). The entire set-up was incubated at 37°C during the experimental period.

Groups
Seventy root segments were randomly divided in seven groups (n = 10).
Group 1- No treatment (Negative control )
Group 2- 5% NaOCl (Prime dental products Pvt. Ltd., India) without activation (Positive control)
Group 3- 5% NaOCl with LASER activation (IMDSL Automatic Dental Diode Laser - 10W, Macromed, India)
Group 4- 0.01% AgNP solution (Nano Research, Bihar) without activation
Group 5- 0.01% AgNP solution with LASER activation
Group 6- 0.2% ChNP solution (Everest Biotech, Bangalore) without activation

Group 7- 0.2% ChNP solution with LASER activation

For all the non-laser activation groups, the respective irrigating solutions were used. A sterile needle (26 gauge) was introduced into the root canal until 1 mm short of WL and 5 mL of the specific solution was irrigated, for 5 min. Thereafter, the root canals were washed with 2 mL of sterile saline solution.

For the laser activation groups, a sterile needle was introduced into the root canal until 1 mm short of WL and 2.5 mL of the specific solution was injected, for 2 min. LASER activation by diode laser, delivered by 400 μm fiber tip kept 1-2 mm away from the apex was activated at 25 µJ Energy; 1 W power; Pulse mode; 20 sec- 3 cycles in sweeping motion from apical to coronal third for 1 min. Then, irrigation was done with respective irrigating solutions (2.5 ml) again for 2 min. Finally the canals were flushed with 2 ml of sterile saline solution.

Colony counting

Immediately after each irrigation protocol, three #35 sterile absorbent paper points (META BIOMED INC., USA) were consecutively inserted into the root canals until the WL. After 1 min, they were transferred to sterile test tubes containing 1 mL of sterile BHI broth. All tubes were vortex mixed for 1 min to dislodge bacteria from paper points. The bacterial suspension was serially diluted. Aliquot portions (100 μL) of 1000-fold and 10,000-fold dilution were taken from each sample and pipetted in triplicate onto the surface of BHI agar plates, which were incubated at 37°C for 24 h. Then, the number of Colony Forming Units (CFU) per plate was determined.

Statistical Analysis

Data was tabulated and the results were presented in mean±SD. One way analysis of variance followed by Tukey’s post-hoc tests were used to compare CFU among the groups. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results

Figures 1- 7 show the CFU of various groups. There was significant (p=0.01) difference in CFU among the groups (p<0.05). Table 1 and graph 1 show the inter group comparison on the basis of colony forming units. The lowest CFU were noted in Groups 3 and 7 (NaOCl + Laser and ChNP + Laser) and highest CFU were noted in Group 4 (AgNP) among experimental groups. Intergroup comparison between Group 4 and 5 (AgNPs without and with laser) and other groups were also found to be significant. Although significant differences were noted among all the groups (p<0.05) but inter group comparison between group 3 (NaOCl + laser) and group 7 (ChNPs + laser) was found to be insignificant (p>0.05).
Table 1: CFU of various groups

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>COLONY FORMING UNITS (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – No treatment</td>
<td>12306</td>
</tr>
<tr>
<td>2- NaOCl</td>
<td>694</td>
</tr>
<tr>
<td>3- NaOCl + Laser</td>
<td>23</td>
</tr>
<tr>
<td>4- AgNP</td>
<td>7146</td>
</tr>
<tr>
<td>5- AgNP + Laser</td>
<td>836</td>
</tr>
<tr>
<td>6- ChNP</td>
<td>721</td>
</tr>
<tr>
<td>7- ChNP + Laser</td>
<td>17</td>
</tr>
</tbody>
</table>

Graph 1: CFU of *E. faecalis* after treatment with various irrigants

Discussion

The major challenges faced by endodontists in routine practice are attributed to the complexities of the root canal anatomy, polymicrobial nature of infection and biofilm formation by microbes. *E. faecalis*, a gram-positive facultative anaerobe can be encountered even in a perfectly obturated root canal and produce the most resistant biofilm which is substantiated in any study that had evaluated the antimicrobial efficacy of root canal irrigants.(18) Hence in the present study *E. faecalis* was used as the test microorganism against which the antimicrobial efficacy of irrigants was evaluated.

NaOCl, especially with high concentrations has a great amount of undissociated hypochlorous acid (HClO) and chloride ions in solution, which is directly related to its disinfecting efficiency.(19) 5% NaOCl was used because of its high efficacy.

Nanotechnology created a great revolution and impact on all aspects of health including the dentistry by producing functional materials which exert their antimicrobial effect by interacting with the negatively charged bacterial cell wall.(8)
AgNPs interact with the cell membrane, increase permeability and prevent DNA replication of bacteria. Though they have proven antibacterial properties, higher concentrations may be toxic to the host cells due to their small size, chemical composition, surface properties and nonspecific oxidative damages. In this study, there was inadequate interaction period between positively charged AgNPs and negatively charged bacterial cells and this might be the reason for their inability to reduce the microbes effectively. When AgNPs were used as medicament for 7 days, adequate interaction occurred between the positively charged nanoparticles and resident biofilm bacteria/structure, resulting in marked destruction of biofilm structure and killing of biofilm bacteria.

Chitosan nanoparticles are good antibacterial agents aiding in destruction of biofilms of root canal. The pronounced antibacterial efficacy of cationic nanoparticles might be due to the fact that positively charged nanoparticles electrostatically interact with the negatively charged bacterial cells, resulting in altered cell permeability, leakage of intracellular components and killing microbes. Cationic antibacterial nanocomposites such as Zinc oxide(ZnO)-NP, Ch/ZnO-NP, or Ch-layer-ZnO-NP significantly inhibited bacterial adherence to dentin, which, in turn, would prevent bacterial recolonization and biofilm formation.

Diode lasers have lower thermal risks and penetration depth into the dentine up to 750 µm. Looking back at the literature, there are only few data about the antimicrobial effectiveness of diode lasers in endodontics. A 97.56% reduction in the amount of bacteria using a 830-nm diode laser with output power of 1.5 W and claimed that diode laser can be considered as an alternative technique for root canal disinfection. Diode laser application after smear layer removal could help in occluding the dentinal tubules particularly in the apical third area which will help in decreasing the risk of reinfection. The results of a study by Mehrvarzfar et al is in accordance with this study stating that diode laser has the potential to significantly reduce the microbes when compared with the non-laser groups.

The limitations of this study were:

Live and dead microbes or penetration depth of microbes are unknown, since advanced technologies or instrumentations were not used. Further in-vivo studies are necessary to evaluate the efficacy of irrigants and diode lasers against polymicrobial scenario. Further studies are necessary to know the effect of nanoparticles on viable tissues.

**Conclusion**

Diode laser in combination with 5% NaOCl and 0.2% ChNP solution had significant effects in the reduction of microbial colonies of *E. faecalis* in comparison to other experimental groups. It was also evident that all the laser activated groups, irrespective of the solutions, had statistically significant results when compared to their non-laser counterparts.

**References**


