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Research Article

IN VITRO EVALUATION OF ANTIBACTERIAL EFFICACY OF SODIUM HYPOCHLORITE, CHLOROQUICK, OZONATED OIL AND CHITOSAN NANOPARTICLE AGAINST 6 WEEK ENTEROCOCCUS FAECALIS BIOFILMS

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A B S T R A C T

Aim / Objectives: To Compare The Antibacterial Efficacy Of Sodium Hypochlorite, Chloroquick (1-Hydroxyethane 1, 1-Diphosphonic Acid), Ozonated Oil and Chitosan nanoparticle Against 6 week Enterococcus Faecalis Biofilms.
Material And Method: Access opening and biomechanical preparation were performed on 40 freshly extracted mandibular second premolars. The specimens were sterilized; 15 µm of E. faecalis was inoculated into each canal and incubated at 36°C for 6 weeks. Later, specimens were randomly divided into 4 groups of 10 each and following procedures was carried out: (i) Conventional irrigation with 3% NaOCl (ii) Conventional irrigation with Chloroquick (iv) Conventional irrigation with Ozonated oil (iv) Conventional irrigation with Chitosan nanoparticles solution . Samples were collected from each canal using sterile paper points which were deposited in brain heart infusion
broth, and microbiological evaluation was carried out.
Statistical Analysis: One-way ANOVA, post hoc Tukey's honest significant difference test.
Results: Maximum disinfection was found in Ozonated oil group (group 3) .
Conclusion: Within the limitation of this study, it was found that Maximum disinfection was found in Ozoanted Oil group .

INTRODUCTION

Efficient disinfection of the root canal system and prevention of reinfectionis responsible for the successful outcome of root canal treatment. [1] The microbiota associated with the secondary root canal infection markedly differs from that of untreated teeth.[2]Free floating microorganisms in the root canal space can attach to each other and grow into biofilm as a microbial community on the dentin walls.[3] The maturity of the biofilm is known to influence its resistance to being killed by antibacterial agents. [4] *Enterococcus faecalis* is gram positive anaerobic facultative cocci that has been most commonly found in the root canals undergoing retreatment cases. It has inherent antimicrobial resistance, the ability to adapt to harsh environmental changes and the ability to invade into dentinal tubules and are difficult to eliminate.

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Effective control of pathogenic microorganisms and mechanical stabilization of root canal dentin can significantly improve the long term survival of endodontically treated teeth.[7] Bacterial biofilms are structured communities of cells adhered to an organic surface and stabilized in an extracellular matrix. [8] Bacterial biofilm is highly resistant to conventional irrigants because of the extracellular polymeric matrix formation.

Moreover, bacterial biofilm provides nutrition and protects the bacteria from the immune system thus; it increases the resistance of microorganisms. [6]

Endodontic biofilms can resist the action of antibacterial irrigants using three mechanisms: failure of irrigant penetration into the biofilm, especially in deep portions; bacterial cells that present slow growing due to nutritional limitations; and adoption of phenotypes resistant to antimicrobial agents .[9]

The high density of bacterial cells in biofilm communities and the inherent resistance of biofilm bacteria to antimicrobials/host defence systems are the main factors responsible for most persistent and chronic bacterial infections.[8] The combined use of mechanical instrumentation and chemical irrigation has been recommended to obtain effective elimination of biofilms from the root canal system. [10]

Several root canal irrigants and disinfection techniques have been introduced in dentitsry to further decrease the intra-radicular bacterial count from the root canal system . However, evidence of their complete elimination has not been recorded in literature. Insufficient eradication of intra-radicular bacteria could be attributed to the complex root canal morphology and the organization of intra-canal bacteria into biofilms. Moreover, the protective layer formed by dentine matrix and dentine powder inhibits the antimicrobial activity of the root canal irrigants.[11]

Sodium hypochlorite is used as potent Universal root canal irrigant since many years. Despite of its advantages, NaOCl has extensive drawbacks; it is cytotoxic if accidently injected into periapical tissues, has a foul taste and smell, bleaches clothes and has a potentiality to corrode metallic objects.[12]Over and above it doesn't eradicate all bacteria [13, 14, 15], or does it completely remove the smear layer. [16]NaOCl also changes dentin characteristics .[17, 18]Knowing this information, an ongoing search for a safe irrigant with good antibacterial effect is recommended.

Chloro Quick is a combination solution of stabilized Sodium Hypochlorite solution with buffer and HEDP (with detergent and system activator along with other excipients). The freshly mixed solution has advantages over using multiple solutions.

ONE STEP SOLUTION ACT AS: Antibacterial Agent, Proteolytic Agent, Chelating Agent, Lubricating Agent, Prevention and Removal of smear layer, Emulsifying Agent.

Ozonated oils are pure plant extracts, through which pure oxygen and ozone are passed. The plant extracts undergo a chemical reaction to form a thick, viscous oil which act as an efficient antimicrobial agent. It is now a days used as root canal irrigant for effective removal of bacterial biofilms.

Another chelating agent named as Chitosan is quite unique, natural polysaccharide, bio-based polymer with different characteristics that make it one of the promising exploitable material in medicine. Now a days, as an alternative to antimicrobial agent, chitosan was proposed in dentistry.[19, 20]Chitosan is a natural polysaccharide found in shells of crab or shrimp. It is also known to be biocompatible, biodegradable, shows bio-adhesion and nontoxic, and therefore, has been used in drug delivery systems and also in biomedical applications. It also shows a broad-spectrum antimicrobial property and is associated with high chelating characteristics; therefor its use in endodontics is of interest. [21]

Literature search reveals that very few studies have been carried out to study the antibacterial efficacy of Chitosan nanoparticles and Ozonated Oil against 6 weeks E. faecalis biofilms and hence the study was performed .

Hence, the purpose of this study was to compare the antibacterial efficacy of Sodium hypochlorite, Chloroquick, Ozonated oil and Chitosan nanoparticleswith conventional irrigation technique against 6 week *Enterococcus faecalis* biofilms.

Aim: To Compare The Antibacterial Efficacy Of Sodium Hypochlorite, Chloroquick , ozonated oil and chitosan nanoparticle with Conventional Irrigation Against *Enterococcus Faecalis* Biofilms.

Null Hypothesis: There will be no difference in the Antibacterial Efficacy Of *Sodium Hypochlorite, Chloroquick , ozonated oil and chitosan nanoparticle* With *Conventional Irrigat Iion* Against *Enterococcus Faecalis* Biofilms

MATERIAL AND METHODOLOGY

Sample size selection criteria: Assuming that the effect size of 1.0 for the proposed study, 40 extracted single rooted premolars per group (Total=40) will be collected and handled as per guidelines by OSHA & CDC. The power of the study is 80% and 95% confidence level.

Forty freshly extracted mandibular second premolars from patients for orthodontic or periodontal purposes were collected for the study.

Conventional access to the root canal system was performed. Patency of each canal was established by placing a size 10 K-file (Mani Inc., Tochigi, Japan) until it was visible in the apical foramen. Working length was established 1 mm short of the apex, and the canals were enlarged sequentially up to a size F2 protaper (Dentsply, Maillefer, Switzerland) as per the manufacturer's recommendation. EDTA (RC Help) was used as a lubricant, and canals were irrigated with 2.25% NaOCl (VIP Vensons, India) during the preparation. After root canal preparation, the enlarged apical foramina were sealed with glass Ionomer cement to prevent bacterial leakage, and the specimens were then sterilized in an autoclave at 121°C for 20 min at 20 psi pressure.

Pure culture of E. faecalis (ATCC 29212) grown in brain heart infusion (BHI), broth was used to contaminate the root canals. The root canals were inoculated with 15 µm of the turbid suspension of E. faecalis ATCC 29212 using a micropipette (Kasablanka, Digital Variable Micropipette, Mumbai, India). The turbidity was verified using the McFarland turbidity scale, and adjusted to 0.5, corresponding to 10[8] organisms per milliliter. The specimens were incubated at 36.5°C for 24 h. Autoclavable foam with punch holes was used to hold the prepared specimens. Asepsis was maintained throughout the procedures using standard precautions with two flames in a biosafety cabinet. Pre operative microbial count was carried out before treatment protocol was done on each tooth samples. The teeth were randomly divided into four groups of 10 teeth each:

(i) **GROUP I:** Conventional irrigation with 3% NaOCl (ii) **GROUP II** : Conventional irrigation with Chloroquick (iii) **GROUP III** : Conventional irrigation with Ozonated Oil (iv) **GROUP IV**: Conventional irrigation with Chitosan Nanoparticle solution.

Group I: (n = 10) Conventional irrigation with 3% NaOCl

After incubation, samples were retrieved from the incubator. The canals were subjected to copious irrigation with 2 ml of 3% NaOCl solution (VIP Vensons, India) for 2 min. Finally, canal was washed with 2 ml of saline to remove any residual

irrigant. A sterile paper point was used to obtain the sample from the canal which was deposited in a sterile Eppendorf tube containing 200 µl of BHI broth.

GroupII: (n=10) Conventional irrigation with Chloroquick: Same procedure was carried out as group I with 2ml of Chloroquick for 2 min .

Group III: (n=10) Conventional irrigation with Ozonated Oil: Same procedure was carried out as group I with 2ml of Ozonated Oil for 2 min.

Group IV: (n=10) Conventional irrigation with Chitosan Nanoparticlessolution: Same procedure was carried out as group I with 2ml of Chitosan nanoparticle for 2 min .

Microbiological Evaluation

Later, the specimens were incubated at 36.5°C for 24 h. Using sterile micropipettes, decimal series of dilutions were made up to 10−4 for each sample. The BHI agar plates were incubated at 36.5°C for 24 h. Colony count of E. faecalis was done using semi-quantitative method and expressed as CFU/mL.

The statistical analysis across groups was evaluated using appropriate non-parametric tests. The analysis was performed using SPSS ver20.0.

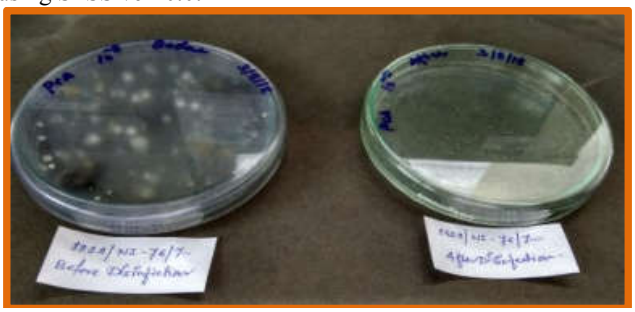

Fig 1 microbial Plating done before and after disinfection in all the 4 experimental groups.

RESULTS

Result showing the % reduction in bacterial count in all the four experimental group are given in table I.

Statistical analysis was done by using descriptive and inferential statistics using one way ANOVA and Multiple Comparison: Tukey Test and software used in the analysis were SPSS 24.0 version and Graph Pad Prism 7.0 version and $p<0.05$ is considered as level of significance.

Mean reduction in total plate count in group 1 was 82.37±1.44, in group 2 it was 95.36±0.54, in group 3 it was 95.70±1.23 and in group 4 it was 95.40±0.28. (Table II)

By using Multiple Comparison: Tukey Test statistically significant difference was found between group 1 and group 2(p=0.0001), group 1 and group 3(p=0.0001), group 1 and group 4(p=0.0001) and no significant difference was found between group 2 and group 3(p=0.872), group 2 and group 4(p=1.00) and between group 3 and group 4(p=0.913).

Table I Result showing the % reduction in bacterial count in all the four experimental group.

S.N.	Test Parameter	Measurement Unit	% reduction in Sodium hypochorite group	% reduction in Chitosan nanoparticle group	% Reduction in Ozonated oil group	% Reduction in Chloroquick group
1	Total Plate count	cfu/swab	84.7 %	96.2 %	97.7 %	95.83%
2	Total Plate count	cfu/swab	83 %	95.5 %	95.1 %	95.65 %
3	Total Plate count	cfu/swab	82 %	95.3 %	94.2%	95.32%
4	Total Plate count	cfu/swab	81 %	95.6 %	96.1%	95.51%
5	Total Plate count	cfu/swab	83 %	95.5 %	95.4%	94.79%
6	Total Plate count	cfu/swab	80 %	95.6 %	97.7 %	95.65%
7	Total Plate count	cfu/swab	82 %	95.6 %	95.1 %	95.32%
8	Total Plate count	cfu/swab	81 %	94.1%	94.2%	95.33%
9	Total Plate count	cfu/swab	84 %	95.2%	96.1%	95.32%
10	Total Plate count	cfu/swab	83 %	95%	95.4%	95.35%

Table II Descriptive Statistics of all thefour experimental groups

Descriptive Statistics								
Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	10	82.37	1.44	0.45	81.33	83.40	80.00	84.70
Group 2	10	95.36	0.54	0.17	94.97	95.74	94.10	96.20
Group 3	10	95.70	1.23	0.39	94.81	96.58	94.20	97.70
Group 4	10	95.40	0.28	0.08	95.20	95.60	94.79	95.83

DISCUSSION

Enterococci are usually isolated in root canals undergoing standard endodontic treatment because of low sensitivity to antimicrobial agents or their ability to inactivate antimicrobial agents.[22,23] *E. faecalis* has the ability to survive under various environmental stresses. It has also been speculated that *E. faecalis* can enter the canal, survive the antibacterial treatment, and then persist after obturation.[24,25]Hence, in the present study, the root canals were contaminated with *E. faecalis* (ATCC 29212) strain that was obtained by growing the cells in BHI broth. In this present study comparison of antibacterial efficacy of various newer irrigating solutions has been tested against *E. faecalis*.

NaOCl is commonly used in concentrations between 0.5% and 6%.[26] It is reported to kill the target microorganisms in seconds, even at low concentrations, although some reports have shown that considerable longer time for the elimination of the same microorganisms. Furthermore, there is evidence that hypochlorite is not effective against all pathogenic bacteria specifically *E. faecalis* which is associated with recalcitrant canals.[27] In this present study the mean reduction count for Group I was 82.37 % which is lowest along all experimental group and statistical significant with other groups. The above result are in agreement with the study conducted by Gomes *et al* (2001).[28]

In this present study Chloro Quick is used which is a combination solution of stabilized Sodium Hypochlorite with buffer and HEDP (1 hydroxyl ethylene 1,1 diphosphonic acid) .The mean reduction in the bacterial load in Chloroquick group was found to be 94.5% in the present study which is more than sodium hypochlorite group .This result was in accordance with the study conducted by Arias-Moliz MT (Dec 2014;40:199-2). This can be due to the fact that increase in antibacterial efficacy with addition of HEDP in NaOCl.[29]

In the present study Ozonated Oil group showed the mean bacterial reduction of 95.70% which was highest among all the experimental groups . It was found to be statistical significant with sodium hypochlorite group while it was statistical insignificant with all other groups. The highest antibacterial efficacy of Ozonated oil is due to its oxidant action on cells by damaging its cytoplasmic membrane due to ozonolysis of dual bonds and also Ozone-induced modification of intracellular contents because of secondary oxidant effects.[30] Ozone first damage to cytoplasmic membrane of cells, as a

consequence to ozonolysis of dual bonds and secondly modification of intracellular contents because of secondary oxidant effect that leads to oxidation of protein loss of organelle function. Using ozone gas and ozonated oils and water, the depth of penetration of this therapy can reach its intended targets. Additonally, the bone at the end of the roots is often shown to harbor pathogenic bacteria for many years after traditional root canal therapy has been completed. Ozone can eliminate those bacteria that infest this region and remove toxic waste products that effectively prevent complete healing of the osseous structures.[31]

Chitosan is a novel natural biocompatible polysaccharide obtained by the deacetylation of chitin, which is found in crab and shrimp shells. Chitosan has introduced in dental research and gained popularity because of its biodegradability, bioadhesion and lack of toxicity.[32,33] It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas.[34] The antibacterial nanoparticles having the dimensions in the range of 1-100nm have greater surface area and charge density, which enable them to achieve a higher degree of interaction with the negatively charged surface of bacterial cells and have the ability to disrupt the extracellular polymerase matrix.[35] In the present study mean reduction in bacterial count for chitosan nanoparticle was superior than sodium hypochlorite which was in accordance with the study done by Shretha *et al*.(2010) [35] and Carpio-Perochena *et al* (2015). [36] This can be attributed to the fact that CS-NPs are biocompatible and positively charged, so they interact with negatively charged bacterial cell wall causing disruption of the cell wall leading to leakage of intracellular components and cell death.

So the Null Hypothesis of this present study, that there would be no difference in the antibacterial efficacy of Sodium hypochlorite, Chloroquick, Ozonated Oil and Chitosan nanoparticle with conventional irrigation technique against 6 week *Enterococcus faecalis* biofilms was rejected.

CONCLUSION

Within the limitation of this study, it was found that

- Chitosan nanoparticle, Ozonated Oil and Chloroquick showed highest antibacterial efficacy against E faecalis as compared to sodium hypochlorite.
- Maximum disinfection was found in Ozonated Oil group.
- However, further in vivo studies are required to corroborate the present in vitro study to intra oral condition.

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