ORIGINAL RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL EFFICACY OF CHITOSAN AGAINST ENTEROCOCCUS FAECALIS : AN INVITRO STUDY

ABSTRACT

Background: Enterococcus faecalis are considered the most resistant species of bacteria which are responsible for root canal treatment failures. Chitosan is a natural polysaccharide Research reveal that 0.2% chitosan has effectively removed smear layer from the root canals after instrumentation and has good antibacterial action. An ideal irrigant should have antimicrobial property comparable to NaOCl. This study was conducted to evaluate the antimicrobial efficacy of a higher concentration of chitosan (0.6%) against Enterococcus faecalis.

Materials and Methods: For preparation of the test solution (0.6% chitosan), 0.6g of chitosan was diluted in 100 ml of 1% acetic acid and the mixture was stirred for 2h using a magnetic stirrer until obtaining crystalline homogeneous solution. A sterile 96 micro titer well plate was labeled: Group I (control group) - 5.25% NaOCl, Group II (experimental group) - 0.6% Chitosan. A volume of 1 µl, 10 µl, 50 µl and 100µl of test material was pipetted into 10 wells each. Following which nutrient broth (100µl) was added and finally, microbial suspension (100µl) of E. faecalis was added to each well. After well -mixing, the plates were incubated at 370 C for 24 hours and Optical density (OD) reading was taken after incubation.

Results: Data was analyzed using one way ANOVA (analysis of variance) at a significance level of 0.05, using SPSS version 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). The statistical analysis of the result revealed that 5.25% NaOCl had significantly better antibacterial action than 0.6% chitosan.

Conclusion: Within the limitations of the study it was concluded that 0.6% chitosan shows antimicrobial properties comparable to that of 5.25% NaOCl.

Key words: Sodium hypochlorite, chitosan, Enterococcus faecalis, antimicrobial.

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INTRODUCTION

The success of endodontic treatment depends on the elimination of microbes from the root-canal system and prevention of reinfection. The root canal is shaped with hand and rotary instruments with continuous irrigation to remove the inflamed and necrotic tissue, microbes/biofilms, and other debris from the root-canal space.[1] Studies have demonstrated that large areas of the root-canal wall remain untouched by the instruments, emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.[2] Hence irrigation plays a central role in endodontic treatment. During and after instrumentation, the irrigants facilitate removal of microorganisms, tissue remnants; dissolve organic and inorganic matter and dentin chips from the root canal through a flushing and chelatingmechanism.[3]

Enterococcus faecalis are considered the most resistant species of bacteria which are responsible for root canal treatment failures. E. faecalis possesses certain virulence factors which include lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses.[4] In addition it is able to suppress the action of lymphocytes, potentially contributing to endodontic failure.[5]

Sodium hypochlorite has been used widely because of its ability to dissolve organic matter and its high antimicrobial potential. However it's toxic to the periapical tissues, weakens dentine by reducing its flexural strength and resilience thus making it more susceptible to deformation and possibly fracture.[6]

The ideal requirement of an irrigant includes the removal of both organic and inorganic material. In addition it is active only against the organic material; therefore other irrigants must be used to for the removal of the smear layer and dentin debris.[1]

Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine which is biocompatible, biodegradable, shows bioadhesion and lacks toxi-city. Chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells.[7] Previous research revealed that 0.2% chitosan has effectively removed smear layer from the root canals after instrumentation and has good antibacterial action.[8]An ideal irrigant should have antimicrobial property comparable to NaOCl. So the aim of this study was to compare the antimicrobial efficacy of a higher concentration of chitosan (0.6%) to 5.25% NaOCl against E. faecalis.

METHODOLOGY

Test solution preparation:

0.6% chitosan solution was prepared by diluting 0.6g of chitosan in 100 ml of 1% acetic acid and the mixture was stirred for 2h using a magnetic stirrer until obtaining crystalline homogeneous solutions.

Antimicrobial assay using micro titer plate method:

Broth dilution method:

One of the most basic anti-microbial susceptibility testing method is the Broth micro-or macro-dilution. The procedure involves preparing two-fold dilutions of the antimicrobial agent (8, 16 and 32 mg/ml) in a liquid growth medium dispensed in tubes containing a minimum volume of 2m - macro dilution method or with smaller volumes (1 µl, 10 µl, 50 µl and 100µl) using 96-well micro titration plate-micro dilution method. This method helps to find the minimum inhibitory concentration (MIC) which is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in tubes or micro-dilution wells as detected by the unaided eye. In the present study we used Broth micro dilution method.

A sterile 96 micro titer well plate was labeled:Group I (control group) - 5.25% NaOCl, Group II (experimental group) - 0.6% Chitosan. A volume of 1 µl, 10 µl, 50 µl and 100µl of test material was pipetted into 10 wells each (Fig 1). Following which nutrient broth (100µl) was added and finally, microbial suspension (100µl) of E. faecalis (ATCC29212) was added to each well (Fig 2). Control dilutions (drug free wells) were also kept. After well -mixing, the plates were incubated at 370 C for 24 hours and Optical density (OD) reading was taken after incubation (Fig 3).

Optical density was obtained from subtracting the control OD from the sample OD.

% of inhibition= (Control OD- Test OD)/Control ×100

RESULTS

TABLE 1

MEAN VALUE				
Concentration (µl)	1 µL	10 µL	50 µL	100 µL
% of inhibition	44.01	80.51	82.30	94.77

Sample 1:The percentage of inhibition of E. faecalis at different concentration of 5.25% NaOCl

TABLE 2

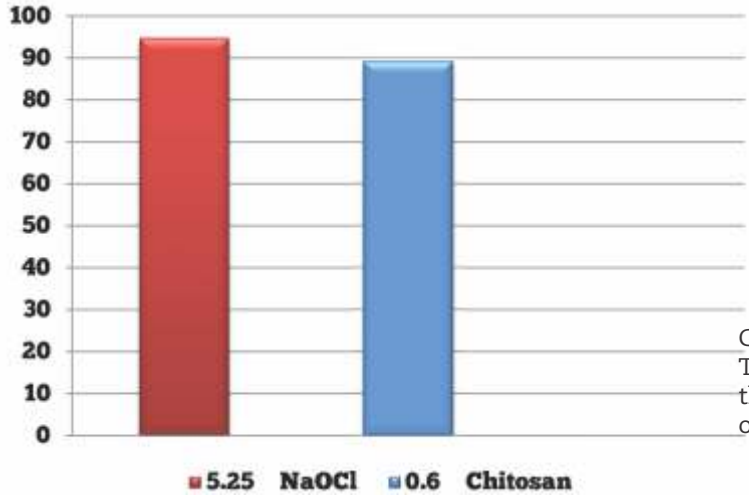
MEAN VALUE				
Concentration (µl)	1 µL	10 µL	50 µL	100 µL
% of inhibition	57.9	73.8	77.21	89.28

Sample 2: The percentage of inhibition of E. faecalis at different concentration of 0.6% Chitosan

TABLE 3

		F	Sig.
1 µL	Between Groups	295.25	.000*
10 µL	Between Groups	55.65	.000*
50 µL	Between Groups	13.527	.003*
100 µL	Between Groups	48.365	.000*

TABLE 3: Inter group comparison between 2 groups using ANOVA
*(P ≤ 0.05) – statistically significant.



Graph 1:
The graph representing the percentage of inhibition of E. faecalis at 100µl.

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The results were statistically evaluated using one way ANOVA. The $p \leq 0.05$ showed that the results were statistically significant. The statistical analysis was performed using SPSS version 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). The results revealed that 5.25% NaOCl had better antibacterial action followed by 0.6% chitosan solution.

DISCUSSION

Enterococcus faecalis is the most commonly implicated microorganism in asymptomatic persistent infections.[9] The highly complex nature of the organism poses a great challenge for endodontists. E.faecalispossess different virulence factors that avail their adhesion to host cells and extracellular matrix, which in turn facilitates tissue incursion, causes immune modulation and engenders toxin mediated damage.[10] It exhibits strong adhesion to collagen[11] and display resistance to chemomechanical preparation.[12] It can also survive in a quiescent phase with low metabolic activity for a long period of time.[13]

NaOCl has high antimicrobial property. Giardinoet al demonstrated that 5.25% NaOCl eliminated E. faecalisbiofilm in 30 seconds.[14] Dunavantet al, have shown that only NaOCl is able to kill the whole bacteria population organized in a biofilm.[15] Though sodium hypochlorite has been found to be the most potent endodontic irrigant but it has certain disadvantages like toxicity and to overcome this more biocompatible solution can be used. 0.2% chitosan is biocompatible, has good smear layer removal property and antibacterial action. But the search of an irrigant with better antibacterial action has lead to this study which compares a higher concentration of chitosan (0.6%) to NaOCl.

In this study NaOCl showed better results than 0.6% chitosan solution. In chitosan the cationically charged amino group may combine with anionic components such as N-acetyl muramic acid, sialic acid, and neuramic acid on the cell surface and suppresses growth of bacteria by impairing the exchanges with medium, chelating transition metal ions, and inhibiting enzymes.[16]

It binds to DNA and inhibits mRNA synthesis by penetrating toward the nuclei acid of microorganisms and interfering with the synthesis of mRNA and proteins.[16]

The results of this study was in concordant to a study done by Pankaj et al who checked the antimicrobial activity of chitosan and the data revealed that 0.25% chitosan and 0.5% chitosan has antimicrobial activity against E. faecalis and Candida albicans and showed no cytotoxicity.[17]

Several bioassays such as disk diffusion and well diffusion methods are commonly used to compare the anti microbial properties. But the dilution methods are the most appropriate ones for determination of the minimum inhibitory concentration (MIC) values.

One of the most basic anti-microbial susceptibility testing method is the Broth micro- or macro-dilution.[18] Unlike micro-dilution method, the main disadvantages of the macrodilution method are the tedious, manual undertaking, risk of errors in the preparation of antimicrobial solutions for each test, and the comparatively large amount of reagents and space required. Thus, the reproducibility and the economy of reagents and space that occurs due to the miniaturization of the test are the major advantages of the microdilution method.[19]

The results clearly demonstrated that the action of test irrigants could reduce the number of bacterial cells from the root canal. Bacterial reduction was significantly superior when NaOCl was used as irrigant. The present study demonstrates that the antibacterial efficacy of 0.6% chitosan is comparable to NaOCl. As 0.6 % chitosan is biocompatible, can effectively remove smear layer and has antimicrobial properties it can be used as an endodontic irrigant to overcome the deleterious effects of the conventional irrigants (NaOCl and EDTA) on dentine.[17]

CONCLUSION

Within the limitations of the study it was concluded that 0.6% chitosan shows antimicrobial properties comparable to that of 5.25% NaOCl.

Figure 1:
Pipetting the test materials into the wells

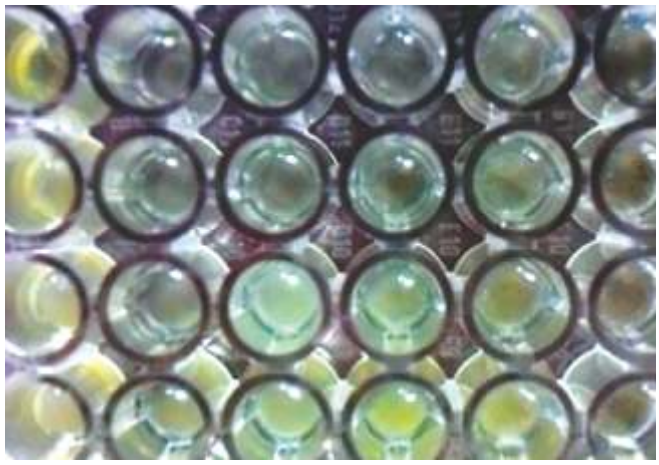

Figure 2:
Micro titer plates with the test materials

Figure 3:
Micro titer plate reader

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