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Evaluation of Antimicrobial Efficacy of Chitosan as Root Canal Irrigant on Enterococcus Faecalis

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ABSTRACT

Purpose: To evaluate the antimicrobial effect of chitosan as root canal irrigant on Enterococcus faecalis in comparison to 3%NaOCL in experimentally infected primary anterior root canals. **Materials and methods:** Access cavity and mechanical preparation of root canals of 30 extracted primary anterior teeth which were then sterilized at autoclave at 121^0 for 15 minutes. These teeth inoculated with Enterococcus *faecalis* with reference strain ATCC 19433.Then grouped into three groups which were irrigated with 3%NaOCl, 0.5% chitosan and saline respectively. Samples were cultured on bile auscline agar incubated at 37^0 for 24 hours. Counting of colony forming units formed before and after irrigation was performed. **Results:** Chitosan had higher antimicrobial effect than saline and lower than NaOCl. Statistical analysis between the groups revealed that there was a statistically significant difference between the three irrigants (*P*-value = 0.001). **Conclusion:** Chitosan solution can be used as a natural alternative to sodium hypochlorite in root canal irrigation, but may need to increase its concentration or the time of application inside the root canals and also should be supported by in vivo studies.

KEYWORDS

Enterococcus faecalis, sodium hypochlorite, chitosan.

INTRODUCTION

Complete debridement and effective disinfection of the endodontic space is an important goal for achieving long-term success of nonsurgical endodontics. Microorganisms' removal is important for pulpal disease treatment. Chemomechanical instrumentation decreases most of the invading bacteria and also reduces the debris of the necrotic pulp tissue. The medicament usage in endodontic treatment enables

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the elimination of bacteria that remain in spite of cleaning and shaping, so this makes the media suitable for healing [1–3].

Preservation of deciduous teeth is essential for the concord development of occlusion, maintenance of arch length, optimum function of speech and mastication and this allows the mouth tissues to be in a good state. Caries progresses rapidly in deciduous teeth leading to pulpal damage through contamination of the pulpal tissue by bacteria and their derived toxins, for this reason the root canal management is a mandatory process [4].

Many factors are required for effective root canal management such as a proper diagnosis, thorough cleaning, a powerful decontamination technique achieved with the use of various endodontic medications and irrigants and finally by obturation of the root canal and accurate permanent restoration. In endodontic infections of the deciduous teeth, while most microbes may be present in the main root canal, a considerable portion of infection is located in other parts of the root canal such as the root apex ramifications, the lateral branches and also the dentinal tubules. Clinically during the endodontic management, cleaning, shaping and irrigation of the root canal takes so much time and is considered the most important step in children's teeth management [5].

Enterococcus *faecalis* is the most common microorganism cultured from failed root canals that undergo retreatment and also from primary endodontic infected cases. It is a facultative, gram positive anaerobic organism that grows by the formation of a biofilm and can survive chemomechanical preparation and root canal medication and it can result in failure of the root canal treatement after obturation [6,7].

Irrigation has recently been considered the best way for lubrication, elimination of bacteria and removal of pulpal tissue and dentinal debris during mechanical preparation. The simple act of irrigation allows the loose, necrotic, infected substances to be flushed away before they are pushed deeper into the root canal and apical tissues, causing a harmful effect on the periapical tissue and subsequently the successor tooth germ. So the use of cleansers in the irrigation process is an important task [8].

Sodium hypochlorite is still considered one of the most common endodontic irrigants due to its antimicrobial efficacy and its ability to remove organic material. However, it is not only irritant to the periapical tissues, but also owns certain disadvantages such as staining of instruments, burning of nearby tissues, uncomfortable taste, high toxicity, erosion of instrument, inability to detach the smear layer and reduction in modulus of elasticity and flexural strength of dentin [9–11]5.25%.

Because of the disadvantages of the antimicrobial irrigants that are present, the search for new alternatives such as chitosan is essential. Chitosan is a non-synthetic polysaccharide, which consists of conjugated polymers of glucosamine and N-acetyl glucosamine. Partial deacetylation of chitin results in chitosan production. It is biocompatible, biodegradable, bioadhesive and has no documented toxicity. In addition it is a good disinfectant agent. Its decreased price has increased its usage for many applications in the fields of medicine and pharmaceuticals. Also due to the high capability of chitosan to chelate different metal ions in acidic environment, it can successfully remove smear layer from the root canals after mechanical preparation[12,13].

MATERIALS AND METHODS

Teeth selection:

A total of thirty extracted single rooted teeth were used in this study, the teeth were taken from children patients (between 2 to 8 years) who lost those teeth due to trauma which resulted in avulsion or intrusion or extraction of primary teeth or due to delayed exfoliation of primary teeth. These

teeth were collected from outpatient dental clinic, hospitals and Faculty of Dental Medicine for Girls Al-Azhar University over a period of six months.

Inclusion criteria: All teeth were deciduous anterior teeth, single-rooted, free of caries and had complete root formation.

Exclusion criteria: Permanent, posterior multi-rooted and carious teeth were discarded.

Enterococcus *faecalis* reference strain

Enterococcus *faecalis* reference strain (ATCC 19433) was supplied from Microbiological Resources Centre (Cairo MIRCEN) Faculty of Agriculture, Ain-Shams University to be used in this study. Its name is Enterococcus *faecalis*, its synonyms is Streptococcus *faecalis* and was supplied as actively growing culture on slope agar.

Teeth preparation:

The teeth were cleaned from outside to remove any soft or hard tissue debris by scaler, and then washed with water; the teeth were disinfected with 1% NaOCl and stored in thymol solution to preserve the teeth until their use.

Then gaining access in all teeth was performed and all pulp tissue debris were removed with H file then the root canals of all teeth were prepared mechanically with K files starting with size 15 up to size 40 to the full working length. 1ml of 1% NaOCl was used as irrigant to remove the organic debris of pulp tissue after each use with K file and 17% EDTA was used to remove smear layer (inorganic) of root dentin after each file size.

All teeth were then decoronated below the level of cementoenamel junction until having standardized root length with 8mm of all teeth using diamond disc in low speed straight hand piece (Allowable max. speed 40.000 rpm weight 48g.NSK, Japan) under coolant water spray. Then all teeth were sterilized in autoclave (at 121 ^{0}c, for 15 minutes).

Teeth grouping:

The samples were divided into three groups (10 for each):

Group I: samples irrigated with saline, the negative control.

Group II: samples irrigated with chitosan, the test material.

Group III: samples irrigated with 3% NaOCL, the positive control.

Preparation of chitosan acetate solution:

Chitosan was supplied in the form of powder to be mixed with 10% acetic acid. The mixing occured by using thermostatic magnetic heating stirrer for 2hours at room temperature until homogenous mix of chitosan acetate solution was obtained inside a sterile beaker (40ml) and this mix was freshly prepared just before use.

Sterility test:

Sterility test was performed to make sure that there was no bacterial or fungal contamination of the sterilized teeth samples. It was carried out by injection of sterile saline inside the root canals then three sterile absorbent paper points were inserted into canals for 1 minute for each paper point to be saturated with all fluids then transferred into Nutrient Agar, MacConkey's agar, Sabouraud dextrose agar, and then the plates were incubated at 37˚C for 24 hours, and observed if they were clear, which indicated that the sterilization was effective.

Selection and preparation of bacterial microorganisms:

The microorganisms were maintained at -70˚C in brain heart infusion broth with 15% glycerol. The bacteria was obtained by making freshly prepared subculture on bile auscline media to produce separate colonies and incubated for 24 hours at 37˚C before work.

Few separate colonies on the bile auscline media were inoculated inside the Brain Heart Infusion Broth to produce the bacterial suspension. The turbidity of this suspension was adjusted to a 0.5 Mc-Cfarland standard by adding more organism if the suspension is too clear or diluting it with the brain heart infusion broth if the suspension is too heavy. After adjustment, the suspension was ready for use.

1ml of bacterial suspension was injected inside each root canal by sterile plastic syringe under pressure to make sure that it reached to the full working length, and then these samples were placed individually inside eppendorf tubes and submerged with brain heart infusion broth, closed and inserted inside a rack, then incubated at 37^0C for 24 hours to allow bacteria to multiply and proliferate.

Estimation of bacterial count:

The bacterial sample was taken from each root canal by insertion of three sterile absorbent paper points size #30, #35 and #40 inside each root canal for 1 minute for each paper point to be saturated with the bacterial suspension and this was the first microbial sample (S1). The paper point specimens were removed from the canal using sterile tweezer and placed in a sterile falcon tube containing 1 ml saline

Serial 10 fold dilution of bacterial suspension in sterile saline (1/10, 1/100, 1/1000, 1/10000, 1/100000) was made then 0.1 ml from each dilution was plated on the bile auscline agar and aerobically incubated at 37^0C for 24 hours. The colony forming unit was counted by multiplication of number of colonies/plate by the dilution and volume factor (10x104x2) = 200000/organisms /ml.

Application of irrigant solution:

After the incubation of the teeth with the bacteria, they were irrigated with 1ml of irrigant solution according to the groups:

Group I: 0.9% sterile saline solution (the negative control)) and left in the root canals for 5 minutes then three sterile paper points were inserted inside the root canals and this was the second sample (S2a).

Group II: 0.5% chitosan solution (the test material) and left in the root canals for 5 minutes then three sterile paper points were inserted inside the root canals and this was the second sample (S2b).

Group III: 3% of NaOCl (the positive control) and left in the root canals for 5 minutes then three sterile paper points were inserted inside the root canals and this was the second sample (S2c).

Then the estimation of bacterial counting was performed as mentioned before.

STATISTICAL ANALYSIS

Data were represented as mean, standard deviation (SD), median, range and 95% Confidence interval (95% CI) values. Wilcoxon signed-rank test was used to study the changes in bacterial counts after irrigation within each group. Kruskal-Wallis test was used to compare between the three groups. Dunn's test was used for pair-wise comparisons.

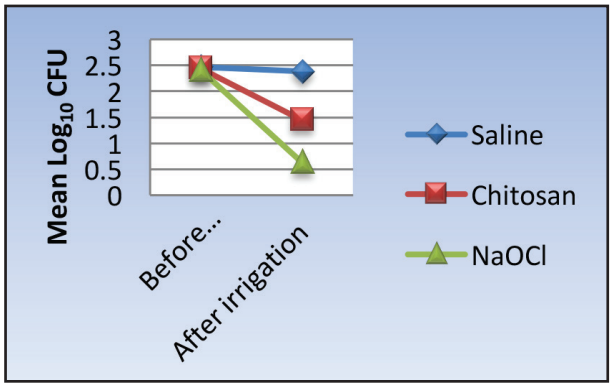
The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20.

RESULTS

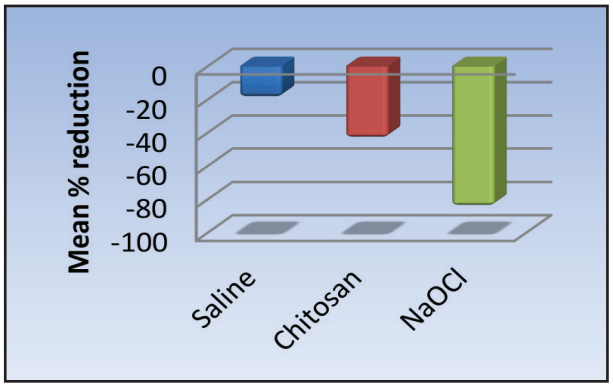
There was a statistically significant difference between percentage reduction in E. faecalis counts after using the three irrigants (P-value = 0.001). Pair-wise comparisons between the groups revealed that NaOCl group showed the statistically significantly highest mean percentage reduction in E. faecalis counts. Chitosan group showed statistically significantly lower mean value. Saline group showed the statistically significantly lowest mean percentage reduction in E. faecalis counts table1.

Table (1): *Descriptive statistics for Log_{10} CFU counts of E. faecalis in the three groups before and after irrigation and % reduction in E. faecalis counts*

Irrigant	Time	Mean	SD	Median	Minimum	Maximum	95% CI	
							Lower bound	Upper bound
Saline	Before irrigation	2.47	0.37	2.55	1.70	2.87	2.21	2.73
	After irrigation	2.38	0.38	2.48	1.57	2.79	2.11	2.65
	Reduction %	-18.92	4.83	-17.08	-26.98	-14.52	-22.37	-15.46
Chitosan	Before irrigation	2.44	0.31	2.46	1.85	2.79	2.22	2.66
	After irrigation	1.45	0.81	1.60	0	2.18	0.86	2.03
	Reduction %	-43.40	10.14	-83.37	-100	-70	-91.56	-77.05
NaOCl	Before irrigation	2.41	0.35	2.55	1.88	2.79	2.16	2.66
	After irrigation	0.63	1.33	0	0	3.30	-0.32	1.58
	Reduction %	-84.30	121.18	-100	-100	227.87	-130.09	43.28



Fig(1): Line chart representing mean Log_{10} CFU counts of E. *faecalis* before and after irrigation.



Fig(2): Bar chart representing mean percentage reduction in E. *faecalis* counts after using the three irrigants.

DISCUSSION

The success of endodontic therapy is dependent on chemomechanical preparation, irrigation, microbial disinfection and complete root canal obturation. Root canal treatment is required for microbial pulpal infection. Bacteria are the main causative factor in the development of pulp and periapical diseases[14,15].

In this study Enterococcus *faecalis* was chosen because it is the dominant species associated with secondary infections and appears more resistant to endodontic treatment. Also Enterococcus *faecalis* has some virulence factors like lytic enzymes, cytolysin, and aggregation substances. It is also capable of adhering to host cells and express protein that permits it to compete with other bacteria and change host response. Enterococcus *faecalis* is also able to inhibit the effect of lymphocytes, contributing in the failure of endodontic therapy [16,17].

In this study the teeth samples used were primary anterior teeth to simulate the primary teeth in children's mouth at our clinic. This was in agreement with another study but it was an in vivo one[18].

While another previous study [19] used posterior primary teeth. However because of the many root canals it would be difficult to obtain standardization of root canals for more accurate results.

In the present study chitosan was used as a natural material to be compared with sodium hypochlorite. While the later has high surface tension allowing direct contact of the irrigant with the dentinal walls of the root canals, the use of it has a risk of extrusion into periapical tissues that result in inflammation, hematoma and also necrosis and paresthesia [20,21].

So in this study chitosan was used to represent an alternative natural root canal irrigant with antimicrobial potency due to the interaction between chitosan which has a positive charge and the bacterial cell which has a negative charge. This causes increase in increase the bacterial cell permeability, leading to the leakage of intercellular components and cell death. Also chitosan attaches to DNA and inhibits mRNA synthesis by passing to the microorganisms nuclei and interfering with mRNA and proteins synthesis [22].

In the present study 0.5% concentration of chitosan was used to test its antibacterial efficacy and the results showed that there was a statistically significant difference between percentage reduction in Enterococcus faecalis counts after using the three irrigants (P-value = 0.001). Chitosan when compared with 3%NaOCl showed that NaOCl group showed statistically significantly highest mean percentage reduction in Enterococcus faecalis counts. Chitosan group showed statistically significantly lower mean value than 3%NaOCl. Saline group showed statistically significantly lowest mean percentage reduction in Enterococcus faecalis counts.

Another recent study is in accordance with the present study which considered that NaOCl had higher antibacterial effect than chitosan. In this recent study 3% NaOCl was compared with both 0.25% chitosan and 0.5% chitosan and showed that the colony forming units for them was as following, 3% NaOCl (CFU=32/ml) then 0.5% chitosan (CFU=33/ml) and finally 0.25% chitosan (CFU=35/ml). So the antibacterial effect of the test irrigants were ranked from strongest to weakest as following 3% NaOCl, 0.5% chitosan followed by 0.25% chitosan which had the least antibacterial effect [23].

Also another recent study using 0.2% chitosan did not show any inhibition zones when compared to 3% NaOCl, so this explained that 0.2% chitosan had no antibacterial effect in this recent study. This may be due to the lower concentration of the chitosan. As well as, this recent study displayed that when 1% chitosan was compared with 3% NaOCl, both of them had the same antibacterial effect with no statistically significant difference between them (p=0.352) [24].

CONCLUSION

Under the condition of this study we concluded that: 0.5% chitosan acetate solution has more antibacterial effect than 0.9%Saline and less than that of 3% NaOCl.

This method could be an effective line for bacterial eradication in primary root canals and needs to be confirmed clinically due to presence of other different microorganisms.

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