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Efficacy of QMix ™ 2 in 1, Sodium Hypochlorite and Chitosan in Disinfection of Gutta Percha Cones

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ABSTRACT

Aim: The purpose of this study was to compare the efficacy of QMix ™ 2 in 1, Sodium Hypochlorite and Chitosan in disinfecting gutta-percha cones.

Materials and Methods: Gutta-percha cones were immersed in suspensions of *Enterococcus faecalis* and *Staphylococcus aureus* separately. The cones were then immersed in QMix ™ 2 in 1, Sodium Hypochlorite and Chitosan for 1 minute separately. The disinfected cones were then incubated in thioglycollate media for 7 days. The thioglycolate media was sub-cultured and colony forming units were counted. Data was statistically analyzed using one way anova.

Results: Qmix ™ 2 in 1 was found to be the most effective disinfecting solution followed by Sodium hypochlorite and Chitosan was least effective in disinfecting the cones.

Conclusion: QMix ™ 2 in 1 possesses superior bactericidal activity when compared with Sodium hypochlorite and Chitosan.

Keywords: Gutta Percha cones, Disinfection, QMix ™ 2 in 1, Sodium hypochlorite and Chitosan.

INTRODUCTION

A primary goal of endodontic treatment is the prevention and treatment of pulpal and periapical inflammation which is a body's response to endodontic infection. This is characterized by the destruction of the bone and periodontium around the apex of the tooth. Pulpal and periapical disease develops as a consequence of caries, trauma, periodontal disease and iatrogenic restorative procedures. In endodontics major efforts are made during instrumentation and debridement of the root canals to eliminate bacteria from the infected pulp space, because of this maintaining the chain of asepsis is extremely important to prevent bacterial contamination of the root canal system. Based on modern-day infection control concepts, the instruments and materials used during endodontic treatment, need to be free of contaminating microorganisms.

Occasionally re infection occur, in spite of utmost care during endodontic procedure.[1] One of the possible explanation is the contamination of gutta percha cones during its handling, though gutta percha points manufactured in aseptic conditions but some studies revealed the presence of micro organisms in freshly open boxes.[2, 3] According to

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various studies, *Staphylococcus* genus is found to be the most common microorganism contaminating gutta-percha cones in its boxes and after handling with gloves.[4] According to Guimaraes *et al*, the recovery rate of *Staphylococcus* genus from infected root canal is about 15.7%; this result justifies the need of gutta-percha disinfection.[5] Due to superior virulence of *Enterococcus faecalis*, it was selected in this study to represent the other possible organisms that may get contaminated with gutta-percha cones, more ever it is serves as a gold standard bacterium in endodontic research. [6]

Gutta percha cones (GP) have been regarded as best endodontic filling material.[7, 8] Gutta percha is polymer obtained by coagulation of latex produced by trees of the Sapotaceae family. Gutta percha is rigid at room temperature and becomes malleable between 25 and 30°C, softens at 60°C and melts at 100 °C with partial decomposition.[9] The sterilization of gutta-percha cones is hampered by the fact that they do not tolerate high temperatures.[10]

To overcome this difficulty, investigators recommend the use of chemical solutions for cold sterilization.[11] Literature revealed several methods for rapid decontamination of gutta percha cones chair side. Among others, these include the following chemical agents: Polyvinylpyrolidone iodine, Glutaraldehyde, Sodium hypochlorite, Hydrogen peroxide, Chlorhexidine, Iodine, MTAD and Ethyl alcohol. The chemical agents used in this study were, QMix ™ 2 in 1(Dentsply,Tulsa Dental), 3% Sodium hypochlorite(Prevest DenPro; India) and Chitosan(Everest Bio; India). Sodium hypochlorite (NaOCl) solution is a broad spectrum antimicrobial agent and it is recognized as efficient, inexpensive and reliable.[9]

QMix ™ 2 in 1 is an irrigating solution, contains a mixture of a bisbiguanide antimicrobial agent, polyamine carboxylic acid calcium chelating agent, saline, and a surfactant. It was evaluated as an effective irrigant similar to 17% ethylenediaminetetraacetic acid (EDTA) in removing canal wall smear layer after the use of 5.25% NaOCl as the initial rinse.[12] Chitosan, a versatile hydrophilic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible.[13]

The efficacy of QMix ™ 2 in 1 and chitosan in disinfecting the GP has not been investigated. The aim of the study was to evaluate the efficacy of QMix ™ 2 in 1, Sodium hypochlorite and Chitosan in disinfecting the GP cones contaminated with *Enterococcus faecalis* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Artificial contamination of gutta-percha cones

A total 90 Gutta percha cones (2%, Size:80, Dentsply) have been used for the study which were divided into five groups. Groups I, II, III, and IV further subdivided into sub group A and sub group B of 10 cones each, according to bacterial strain used. Group V contains 10 cones which act as negative control. Microbial suspensions of *E. faecalis (*ATCC29212) and *S. aureus (*ATCC6538) of approximately 10^8 CFU/ml in Trypticase soy broth (HiMedia Laboratories) were used for this study. Sub group A was contaminated with *E. faecalis (*ATCC29212) and sub group B was contaminated with *S. aureus (*ATCC6538) for 30 minutes (Fig-1). The cones were then transferred to sterile paper pads and allowed to air dry for 10 minutes.

Disinfection of gutta-percha cones

After artificial contamination, all gutta-percha cones of Group IA and Group IB were disinfected with Q Mix 2 in 1, while Group IIA and Group IIB was disinfected with 3% Sodium hypochlorite, and Group IIIA and Group IIIB with Chitosan. For disinfection of the cones, the individual cones were immersed in sterile test tubes containing 5ml of the respective disinfectant solutions for 1 minute (Fig- 2). Group IVA and Group IVB were positive control where cones were not disinfected after bacterial contamination and group V was negative control where no bacterial contamination and no disinfection was done. The cones of all groups were then individually transferred to sterile test tubes containing 10 ml of thioglycolate media (HiMedia Laboratories) and incubated at 37°C for 7 days. Later after 7 days, a micropipette (Precica) was used to transfer the thioglycolate media to a petridish containing brain heart infusion agar. A sterile cotton tip was used to

spread the thioglycolate media in a thin layer over BHI agar. The plates were then incubated for 48 hours aerobically at 37°C and the colony forming units were counted with a digital colony counter.

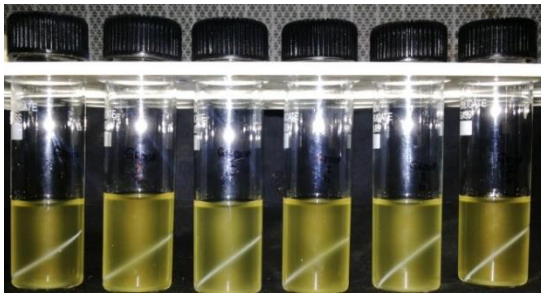


Fig 1: Contamination of GP.

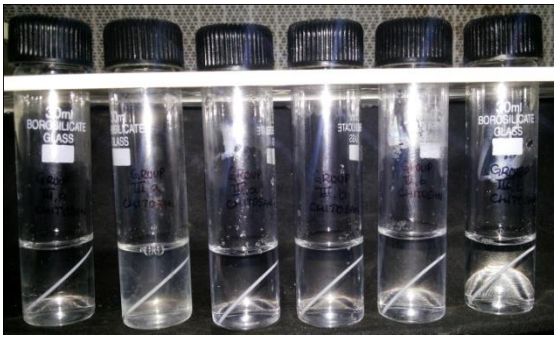


Fig 2: Disinfection of GP

Statistical analysis

The data was statistically amazed by one-way ANOVA (analysis of variance) using SPSS 17.0 software.

RESULTS

The mean value bacterial count of *E. faecalis* and *S. aureus* on the gutta percha cones tested directly from boxes (Group V) were the least (1.6). Among the disinfecting solutions, the mean bacterial count of *E. faecalis* and *S. aureus* were found to be lesser after treating the cones with QMix ™ 2 in 1 when compared with other disinfecting solutions. 3% NaOCl was found to be the second most effective disinfecting solution while chitosan was the least effective among the solutions tested (Table 1 & 2, Graph 1). Fig- 3,4,5 shows the bacterial colonies after disinfection with QMix ™ 2 in 1, Hypo and Chitosan respectively.

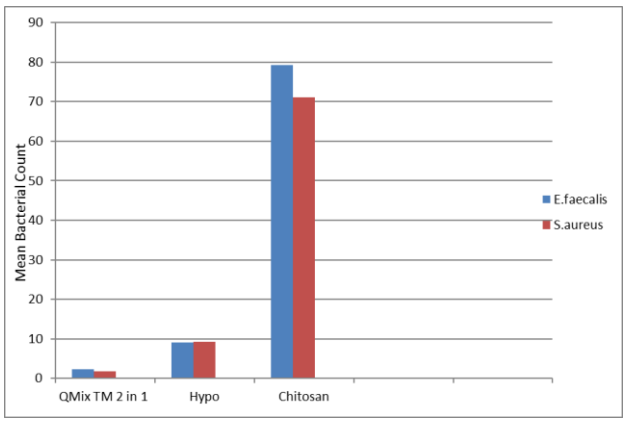
Table 1: Statistical values of all groups for *E. faecalis.*

ORGANISM	GROUP	MEAN	SD	P VALUE
E. faecalis	GROUP IA	2.3	2.1	<0.001
	GROUP IIA	9.0	1.77	<0.001
	GROUP IIIA	79.2	12.2	<0.001
	GROUP IVA	154.3	23.0	----
	GROUP V	1.6	1.99	----

Table 2: Statistical values of all groups for S.aureus

ORGANISM	GROUP	MEAN	SD	P VALUE
S. aureus	GROUP IB	1.8	1.99	<0.001
	GROUP IIB	9.3	1.87	<0.001
	GROUP IIIB	71.1	12.1	<0.001
	GROUP IVB	142.9	16.5	-----
	GROUP V	1.6	1.9	----

Graph 1: Comparison of mean values of all three tested groups



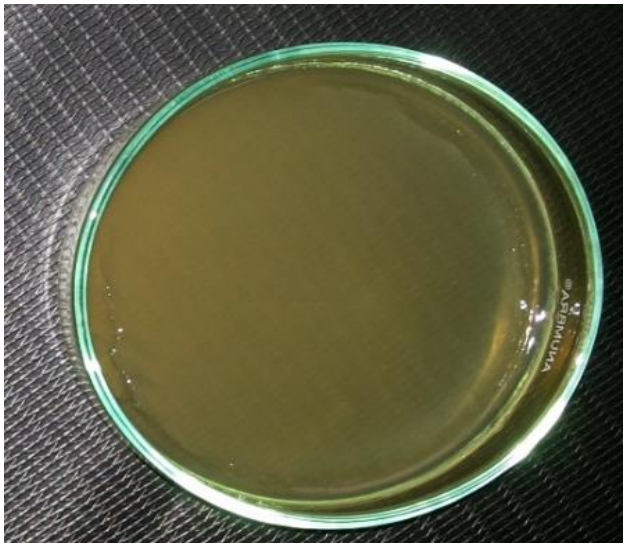


Fig 3: Bacterial colonies after disinfection with QMix ™ 2 in 1

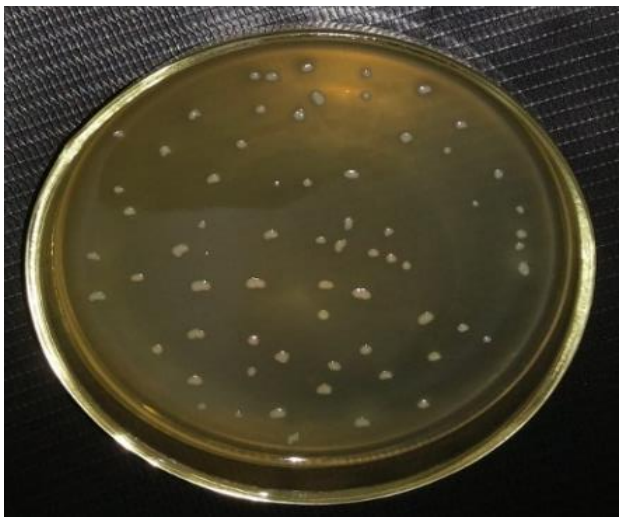


Fig- 4: Bacterial colonies after disinfection with Sodium hypo chlorite

Fig-5: Bacterial colonies after disinfection with Chitosan

DISCUSSION

The obturation of prepared root canals with hermetic seal is one the most critical steps in the root canal treatment procedure. For the treatment to succeed, it is imperative that a breakdown in the asepsis chain doesn't occur. Therefore, the sterility of the obturating material is most important. The results of this study showed very less mean value of bacterial colonies of the brand new gutta-percha cones that were cultured. This corroborates others, who also found contamination (5-8%) in sealed new gutta-percha cones (Mongtomery *et al*. 1971, Gomes *et al.* 2005). However, our study is in contradiction to several studies (Dolittle 1975, Pereira *et al*. 2010) which did not found contamination on gutta-percha cones from new packaging.[14, 15]

Decontamination with respective decontaminating solutions is been done for one minute, as it is the minimum chair side time required for the same, more ever disinfection methods used in this study mirrored the University of Connecticut Dental School accepted protocol for disinfection of gutta-percha. In the present study QMix ™ 2 in 1 has shown good antimicrobial efficacy and statistically superior to all other disinfecting solutions ($P < 0.001$). The reason could be effective functioning of its individual constituents. Surface active agent lowers the surface tension of solution and increases their wettability. The potential benefit of bisbiguanide in this mixture is that it prevents the microbial colonization. A polyaminocarboxylic acid-Calcium chelating agent

can cause cell wall damage in gram negative bacteria by chelating and removing divalent cations (Mg^{+2} and Ca^{+2}) from bacterial cell membrane and increasing its permeability. Wang *et al*., concluded that QMix and 6% NaOCl had stronger antibacterial effects against *E. faecalis* than 2% NaOCl and 2% CHX. Stojicic S et al. also concluded in their study that QMix and 6% NaOCl had stronger antibacterial effect against *E. faecalis* which resides deep in dentin than 1% and 2% NaOCl and 2% CHX. [16, 17]

NaOCl has effectively killed *E.faecalis* and *S.aureus*. NaOCl exerts its antibacterial effect by inducing the irreversible oxidation of Sulfhydryl groups of essential bacterial enzymes, resulting in disulfide linkages, with consequent disruption of the metabolic function of bacterial cells. [18] Many *in vitro* and *in vivo* studies have proved NaOCl has good antimicrobial efficacy against *E. faecalis*. [19, 20] Fidalgo *et al.*, evaluated the antimicrobial activity of NaOCl, Citric acid, and EDTA against *E. faecalis*, *C. albicans*, *S. aureus* and concluded that 2.5% and 5.25% NaOCl are microbiocides against *S. aureus*, while 0.5% and 1% NaOCl are only microbiostatic against the tested *E. faecalis* and *C. albicans*. In this study there was statistically significant difference present between QMix ™ 2 in 1 and NaOCl ($P < 0.001$).

In this study chitosan is been shown to least effective against both E.feacalis and S.aureus. Chitosan have been investigated as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeasts and fungi in experiments involving *in vivo* and *in vitro* interactions. The exact antibacterial mechanism of chitosan is not fully understood, several factors may contribute to the antibacterial action. [21, 22] The most acceptable antibacterial mechanism of chitosan is being the interaction between positively charged chitin/ chitosan molecules and negatively charged microbial cell membranes. In this model the interaction is mediated by the electrostatic forces between the protonated NH^{3+} groups and the negative residues, presumably by competing with Ca^{2+} for electronegative sites on the membrane surface.[23] This electrostatic interaction results in two fold interference: i) by promoting changes in the properties of membrane wall permeability, thus provoke internal osmotic imbalances and consequently inhibit the growth of microorganisms.[24] and ii) by the hydrolysis of the peptidoglycans in the microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions and other low molecular weight proteinaceous constituents (e.g. proteins, nucleic acids, glucose, and lactate dehydrogenase)[25].

CONCLUSIONS

Within the limitations of this study it can be concluded QMix ™ 2 in 1 is most effective in disinfecting the gutta percha cones followed by 3% Sodium hypochlorite. Chitosan is least effective in disinfection of gutta percha cones. Further studies have to be done to understand antibacterial mechanism of chitosan.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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