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Research Article

EVALUATION OF ANTIBACTERIAL PROPERTIES OF CHITOSAN-CITRATE SOLUTION-AN *IN VITRO* STUDY

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ABSTRACT

Introduction: Chitosan has high antibacterial properties, chelating ability, biocompatibility and biodegradability. Sodium hypochlorite (NaOCl) is considered the gold standard irrigant solution. The aim of this in vitro study was to assess the antibacterial effectiveness of chitosan-citrate 0,6% as an irrigant and to compare with the antibacterial effectiveness of NaOCl 2,5%.

Materials and Methods: A total of thirty extracted single-rooted human teeth were used for the purpose of this in vitro assessment. The teeth were allocated to each group: Group I (n=15) – after inoculation with E.faecalis disinfection with NaOCl 2,0% was done and Group II (n=15) - after inoculation with E.faecalis the disinfection was with chitosan-citrate 0,6%. Teeth were prepared to evaluate the colony-forming units (CFU/mL) after disinfection and for SEM analysis to observed Enterococcus faecalis biofilm removal.

Results: The assessment of CFU/mL showed significant reduction of the survival rate of E. faecalis from biofilms after disinfection with chitosan-citrate solution 0,6% compared to the disinfection with NaOCl 2,0% The analysis of scanning electron microscopy (SEM) after disinfection with chitosan-citrate 0,6% illustrated dentin walls with no bacterial biofilm or with minimum bacterial biofilm.

Conclusion: Chitosan-citrate 0,6% showed high antibacterial activity and enabled removal of bacterial biofilm.

INTRODUCTION

The success of endodontic treatment depends on thorough disinfection of the root canal system. Removal of the bacterial biofilm from root canal is essential for prevention of endodontic diseases[10]. Copious irrigation is required to remove debris and reduce microorganisms in the complex root canal system. The ultimate irrigating solution should eliminate both organic and inorganic phases of the smear layer and not have an erosive effect on dentin surfaces[26,27].

Bacterial biofilms in the root canal are highly resistant to disinfecting agents used in endodontic treatment. The complex and unpredictable nature of root canal anatomy and the multi-species biofils amplify the difficulty in eradication of the microbial biomasses from there[1,24]. It is believed that microorganisms found in the root canal system after treatment are responsible for treatment failure[2]. Microbiota found in secondary infections are able to survive in nutrient - limited conditions with a wide pH range. Microbial phenotypes in secondary infections have been reported to be predominated by gram-positive bacteria[3,16]. Microorganisms such as Enterococci, Streptococci, Lactobacilli, Actinomyces and fungi (e.g. Candida) are presented in teeth with post-treatment infection[17,28]. *Enterococcus faecalis* is species commonly isolated from persistent root canal infection - found in 22-77% of cases[18]. *E. faecalis* is considered to be a transient bacterium of the oral microbiota that can settle in the root canal system by easily adapting to the changed conditions[13]. *E. faecalis* is able to survive in non-nutrient conditions over a long period of time, with the bacterium being in a state of minimal metabolic activity, and under new, more favorable conditions, it can be activated[5,21]. *E. faecalis* is resistant to most intra-canal medications, including Ca $(OH)_2$. It can withstand a high

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alkaline environment, most likely due to the *E. faecalis* proton pump, which regulates the intracellular pH [6].

Although sodium hypochlorite (NaOCl) is considered the gold standard irrigant solution, there is no solution at present capable of acting simultaneously on the organic and inorganic elements of the smear layer [4]. NaOCl at a concentration of 1-5.25% is most commonly used because of its bactericidal effectiveness and ability to dissolve organic tissue [29]. However, NaOCl was reported to exhibit cytotoxicity when injected into periradicular tissue [8]. It was also reported to have an unpleasant taste, in addition to potential for caustic corrosion and allergic reactions [8]. Some Researches also demonstrated that it had no effect on the inorganic part of the smear layer [7]. The superficial destructive effect of NaOCl on mineralized dentin is irreversible which results in increased microlekage.

Chitosan is a natural polysaccharide obtained by the deacetylation of chitin, which is found in crab and shrimp shells [14]. It has attracted a great deal of attention in dental research because of its broad spectrum of antibacterial properties [25], biocompatibility, biodegradability, bioadhesion and lack of toxicity [22]. Due to its high chelating ability for various metal ions in acidic conditions, it has been applied widely in the removal or recovery of metal ions in different industrial areas [15]. The structure of chitosan is similar to that of extracellular matrix proteins, such as proteoglycans and glycosaminoglycan. Previous research demonstrated its ability to enhance the mechanical properties of dentin collagen and to reinforce collagen constructs[11]. Studies also showed that chitosan nanoparticles and their derivatives interacted with and neutralised matrix metalloproteinases (MMPs) or bacterial collagenase and improved the resistance of dentin to degradation [12,20].

The highly complex nature of *E. faecalis* poses a great challenge to an endodontist. Therefore, the effective irrigant plays a key role in the root canal treatment. Based on the current literature, we hypothesised that chitosan-citrate 0,6% solution would show significant reduction of the survival rate of *E. faecalis* from biofilms - colony-forming units (CFU/mL) and would effectively and safely remove the bacterial biofilm.

The aim of this in vitro study was to assess the antibacterial effectiveness of chitosan-citrate 0,6% as an irrigant used during the endodontic treatment and to compare it with the antibacterial effectiveness of NaOCl 2,0%. Scanning electron microscopy (SEM) was used to evaluate the bacterial biofilm removal from the dentine walls of canals treated with the chitosan-citrate solution and NaOCl.

MATERIALS AND METHODS

A total of thirty extracted single-rooted human teeth were used for the purpose of this in vitro assessment. The teeth were extracted due to orthodontic or periodontal reasons and were instrumented with rotary crown-down technique-№ F4 *(ProTaper Universal,* Dentsply, Maillefer, Switzerland*)*.

The teeth were allocated to each group: Group I (n=15) - after inoculation with *E.faecalis* disinfection with NaOCl 2,0% was done and Group II (n=15) - after inoculation with *E.faecalis* the disinfection was with chitosan-citrate 0,6%. All teeth from Group I and Group II were placed in a transparent Eppendorf tube, autoclaved at 121°C for 20 min, and stored in sterile phosphate-buffered saline (PBS) until use. The bacterial strains were grown in a Brain Heart Infusion (BHI) agar (Oxoid Microbiology Products, Cambridge, UK) overnight at 35°C. All roots (both groups) were separated longitudinally in two halves, so in a group 30 samples are obtained. When the bacterial density reached at least 1.5-2 x 10^8 CFU/mL, all samples were inoculated with 200 lL of a pure microbial culture, and the suspension was incubated for 48 h at 35°C. Half of the samples in each group were prepared to evaluate the colony-forming units (CFU/mL) after disinfection with NaOCl 2,0% and chitosan-citrate 0,6%. The second half of the samples of both groups are prepared for SEM analysis to observed *Enterococcus faecalis* biofilm removal. All samples were placed in both disinfecting solutions for 5 min. For biofilm density was used five-degree scale and for biofilm removal - four degree scale (Eick *et al*).

RESULTS

Table 1 Calculation of CFU / mL of *E. faecalis* after disinfection with NaOCl 2.0% and with chitosan citrate 0.6%

CFU/mL Bacterial strain	Before disinfection with NaOCl 2%	After disinfection with NaOCl 2%	Before disinfection with chitosan-citrate 0,6%	After disinfection with chitosan-citrate 0,6%
E.faecalis ATCC 29212	28,7 x 10^4	8 x 10^1	28,7 x 10^4	0,7 x 10^1
E. faecalis 1917	26 x 10^4	0	26 x 10^4	0

The assessment of CFU/mL showed significant reduction of the survival rate of *E. faecalis* from biofilms after disinfection with chitosan-citrate solution 0,6% compared to the disinfection with NaOCl 2,0%

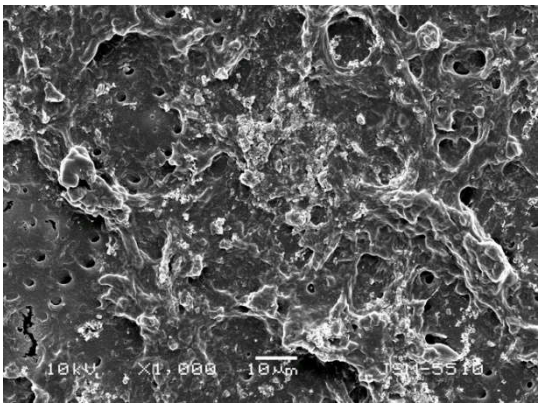

Figure 1 SEM of prepared radicular dentin after disinfection with NaOCl 2,0% (magnification x 1000)

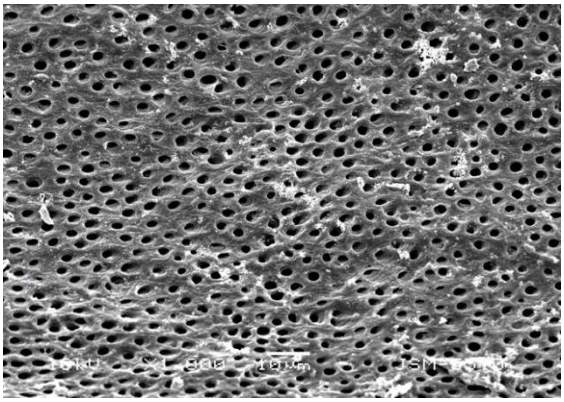

Figure 2 SEM of prepared radicular dentin after disinfection with chitosan-citrate solution 0.6% (magnification x 1000)

The analysis of scanning electron microscopy (SEM) after disinfection with chitosan-citrate 0,6% illustrated dentin walls with no bacterial biofilm or with minimum bacterial biofilm.

DISCUSSION

The purpose of this study was to evaluate the antibacterial activity against bacterial biofilm of *E. faecalis* when using chitosan-citrate solution. The concentration of citric acid is an important factor. The antibacterial effectiveness of citric acid is directly proportional to its concentration[23]. Consequently we used citric acid 1% to dissolve the chitosan. 1% concentration of citric acid is too small to occur its the antibacterial effectiveness and removal the bacterial biofilm, so in the present study we focused on the activity of chitosan.

Sodium hypochlorite (NaOCl) in 1% to 5.25% concentration is a commonly used irrigant solution because of its bactericidal effectiveness and ability to dissolve organic tissue[30]. NaOCl is reported to exhibit cytotoxity when injected into periradicular tissue, unpleasant taste, potential for caustic corrosion and allergic reaction. Furthermore, it has no effect on the inorganic part and is not capable to remove the bacterial biofilm[7].

There are several reports that chitosan has antibacterial effect against *E. faecalis* [25]. Chitosan-citrate solution 0,6% resulted in significantly greater reduction of CFU/mL than NaOCl 2,0% ($0,7 \times 10^1$ CFU/mL after disinfection with chitosan-citrate 0,6% and 8×10^1 CFU/mL after disinfection NaOCl 2,0%) at 5 min. An almost complete bactericidal effect was obtained with chitosan-citrate. *E. faecalis* was found to be resistant to acid and alkalis [19]. Because chitosan is bacteriostatic rather than bactericidal, the most acceptable model is the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes [9].

Another advantage of chitosan-citrate is removal the smear layer and the bacterial biofilm due to its high chelating ability [15]. Our scanning electron microscopy study revealed wide-opened and well-cleaned dentine tubules without erosions using chitosan-citrate solution 0,6%.

CONCLUSION

In conclusion chitosan-citrate 0,6% showed high antibacterial activity and enabled removal of bacterial biofilm. Chitosan-citrate solution has been indicated as a root canal irrigant with a mild and good antibacterial effect on the root dentine walls.

Aknowlegdement

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