Original Article

Comparison of penetrating depth of chlorhexidine and chitosan into dentinal tubules with and without the effect of ultrasonic irrigation

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Abstract

Background: Long term success of root canal treatment depends on complete removal of micro-organisms and their by-products. This can be effectively achieved by the ability of the irrigant to penetrate into the dentinal tubules, which is limited in the conventional mechanical debridement of the root canal system. Irrigant activation technique aids in movement of irrigants into the dentinal tubules.

Aim: To compare the depth of penetration of root canal irrigants into the dentinal tubules with and without ultrasonics using light microscope.

Materials and Methods: Forty noncarious mandibular premolars were used, all the tooth specimens were inoculated with an ATCC 29212 strain of E.faecalis and incubated under nutrient rich aerobic conditions at 37˚C. Teeth were sectioned below the cementoenamel junction to obtain a standard length of 8 mm and instrumented with K-files, irrigated with 5.25% sodium hypochlorite and a final rinse of 17% EDTA. Teeth were divided into four groups of ten each. Group IA was irrigated with 2% Chlorhexidine (CHX) and agitated ultrasonically, Group IB was irrigated with 2% Chlorhexidine, Group IC was irrigated with 2% Chitosan and ultrasonically agitated, Group ID was irrigated with 2 % Chitosan. The tooth specimens were sectioned and subjected to gram staining and viewed under 100X oil immersion microscope. A micrometer grid was attached to the eyepiece to enable measurement of the depth of penetration of the irrigants. Group IA (2% Chlorhexidine with ultrasonic agitation) showed better penetration into the dentinal tubules as compared to Groups IB, IC, ID.

Results: Irrigation with 2% Chlorhexidine with ultrasonic agitation had depth of penetration into the dentinal tubules upto 2350 μm. 2% Chlorhexidine without ultrasonic agitation penetrated upto 1800 μm. Chitosan with ultrasonic agitation penetrated upto1250 μm and Chitosan without ultrasonic agitation penetrated upto 44.80 μm.

Conclusion: 2% Chlorexidine as irrigant with ultrasonic agitation was found to have maximum depth of penetration into the dentinal tubules when compared with Chitosan.

Keywords: Chitosan, chlorhexidine, dentinal tubules, irrigants, ultrasonics

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INTRODUCTION

The elimination of microorganisms and their by-products is essential for the long-term success of the root canal treatment. This can be achieved by mechanical cleaning and shaping in combination with irrigants having antibacterial properties. The mechanical debridement of the root canal system fails to completely remove the debris from the root canal walls.[1] The ideal requirements for an efficient root canal irrigant[2-4] include excellent washing action, ability to dissolve organic and inorganic content and broad antimicrobial activity[5] against facultative and anaerobic microorganisms.

The penetrating ability of the irrigants and flushing action created by irrigation are dependent not only on the anatomy of the root canal system but also on the system of delivery, the depth of placement volume and fluid properties of the irrigants.[4,6,7] For a root canal irrigants to completely debride the root canal system, it must penetrate the dentinal tubules to a sufficient depth to eliminate the microbes colonizing the tubules.

Two percent chlorhexidine (CHX) digluconate is widely used in disinfection due to its high antibacterial activity.[1] It is a synthetic biguanide that consists of two symmetric 4-chlorophenyl rings. It is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and enters through some types of active or passive transport mechanism.[2-4,8-10] Two percent CHX has antibacterial activity against *Enterococcus faecalis*.[11] Chitin is the second-most abundant natural polysaccharide composed of β-(1,4)-linked N-acetyl glucosamine units. Partial deacetylation of chitin results in the production of chitosan. It is a naturally occurring polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine.[12] Chitosan has shown a large number of pharmaceutical applications. It has been used in drug delivery, peptide delivery, as an absorption enhancer and in gene delivery.[13,14] It has numerous biological properties such as hypocholestrolemic,[15] antibacterial,[12,16] antifungal[12] and wound-healing[17] properties. The antibacterial activity of 2% chitosan gel, 2% CHX gel and their combination against *Candida albicans* and *E. faecalis* was tested and found that the combination of 2% chitosan gel and 2% CHX gel had the highest antibacterial activity against *E. faecalis*.[12]

The use of ultrasonic energy for cleaning the root canal and to facilitate disinfection has a long history in endodontics.[18,19] Ultrasonic together with an irrigant contributes to a better cleaning of the root canal system than syringe irrigation.[20] Ultrasonic irrigation has shown a high cleaning efficacy of the root canal system.[21] This study used histological demonstration of bacteria under light microscopy to assess the depth of penetration of the root canal irrigants into the dentinal tubules. So far, no study has been conducted to evaluate the penetrating ability of CHX and chitosan with and without ultrasonics into the dentinal tubules. Hence, the current study was designed to compare the depth of penetration 2% CHX and chitosan into the dentinal tubules with and without the effect of ultrasonics using light microscopy.

MATERIALS AND METHODS

The Institutional Review Board of Sri Ramachandra University approved the collection and use of extracted teeth for this study. Forty intact noncarious human mandibular premolars free of cracks and cervical lesions and apical cracks were selected and stored in phosphate-buffered saline solution. The tooth specimens were sectioned below the cementoenamel junction to obtain the standard length of 8 mm. Tooth specimens were inoculated with *E. faecalis* (ATCC 29212) strain and incubated in a nutrient-rich medium (blood agar) at 37°C under aerobic conditions in laboratory facility to create a biofilm. The presence of *E. faecalis* and its penetration into the dentinal tubules was confirmed using light microscopy. Specimens were then subjected to a standard instrumentation protocol using K-files ranging from sizes #10 to #40 (Mani and Co., Japan). After instrumentation, the canal was irrigated with 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) to remove the smear layer. The tooth specimens were randomly divided into four equal experimental groups of ten each. Group IA specimens were irrigated with 2 ml of 2% CHX (Asep-RC, Anabond Stedman Pharma Research, India) for 2 min and ultrasonically agitated for 1 min. Ultrasonic agitation was carried out using a size #15 K-file driven by an ultrasonic device (Satelec, CA) at a frequency of 30 kHz with tip of the file placed 1 mm from the apical stop without binding the canal walls; Group IB tooth specimens were irrigated with 2 ml of 2% CHX for 2 min without any ultrasonic agitation; Group IC specimens were irrigated with 5 ml of 2% chitosan solution (Sigma Aldrich, Bengaluru, Karnataka, India) obtained by dissolving chitosan powder in 1% glacial acetic acid (pH: 2.4) for 2 min and then ultrasonically agitated for a period of 1 min. Group ID specimens were irrigated with 5 ml of 2% chitosan solution for 2 min.

Tooth specimens were cross-sectioned serially using a diamond disc mounted on a micromotor handpiece. Ten

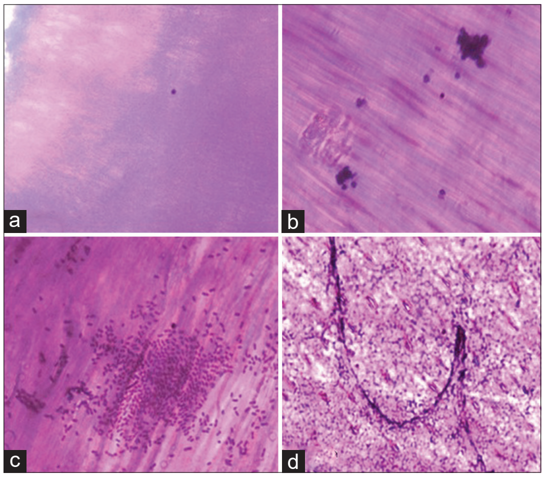

Figure 1: The light microscope images of samples. (a) Group I samples were irrigated with 2% chlorhexidine and ultrasonically agitated. (b) Group II samples irrigated with 2% chlorhexidine without ultrasonic agitation. (c) Group III samples irrigated with 2% chitosan and ultrasonically agitated. (d) Group IV samples irrigated with 2% chitosan without ultrasonic agitation

sections obtained from the root dentin (3 mm apical to the cementoenamel junction) were used to provide ideal thickness for the transmission in light microscopy (Nikon Eclipse 80i). Sections were Gram-stained and examined under an oil immersion microscope at ×100 magnification. The distance from the root canal to the highest penetrated cell in the dentinal tubule was measured using an objective micrometer grid (ERMA). Cementum was confirmed as a valid barrier against the penetration of bacteria. Statistical analysis was performed using the Kruskal–Wallis test, which showed a significant difference between the groups at $P < 0.05$.

RESULTS

Depth of penetration of irrigants (IA–ID) is shown in Figure 1. Figure 1a (2% CHX solution with ultrasonic agitation, 2350 μm) shows minimal bacterial colonies up to the cemental end of the dentinal tubules indicative of maximum penetration of the irrigants. Figure 1b (2% CHX solution without ultrasonic agitation, 1800 μm) shows the presence of bacterial colonies in the middle third of the dentinal tubules. Figure 1c and d (2% chitosan solution with ultrasonic agitation, 1250 μm; 2% chitosan solution without ultrasonic agitation, 44.80 μm, respectively) shows minimal penetration into the dentinal tubules.

DISCUSSION

The success of root canal treatment depends on the eradication of microbes from the root canal system and prevention of reinfection.[1] Cleaning and shaping of the root canal constitutes the most important phase of endodontic treatment and cannot be ignored. It aids in the removal of inflamed and necrotic tissue, microbes and other debris from the root canal system. NaOCl is an efficient antibacterial agent, which has shown various effects on the biofilm structure. It has shown a complete disruption and disintegration of the *E. faecalis* biofilm.[22,23] An increase in the concentration of sodium hypochlorite improves its efficacy, thus reducing the time period of usage.[16,24] Root canal instrumentation causes the formation of smear layer along the root canal walls. This smear layer has a granular appearance and harbors microorganisms, thus permitting their colonization.[25] Hence, to allow complete penetration of the antibacterial agent into the dentinal tubules, the smear layer must be eliminated using a suitable chelating agent such as EDTA.[26,27]

This study compared the penetrating ability of 2% CHX and chitosan, and the results showed that 2% CHX had maximal penetration of the dentinal tubules. Factors affecting the depth of penetration of root canal irrigants could be surface tension, viscosity and molecular size.[28]

Surface tension can be defined as "the force between molecules that produces a tendency for the surface area of a liquid to decrease."[28] This force tends to limit the ability of a liquid to penetrate a capillary tube. The increased penetration of 2% CHX can be attributed to the reduced surface tension of CHX (39.8 mN/m) as compared to chitosan with acetic acid as a solvent (2027 mN/m). The reduction in the surface tension could improve the intimate contact of irrigants with the dentinal walls of the root canal.[28] Hence, in our study, CHX penetrated deeper into dentinal tubules as compared to chitosan.

Viscosity is the ability of a liquid to flow. A liquid with reduced viscosity tends to have a higher penetration into the dentinal tubules than a highly viscous liquid. Reduced molecular size of an irrigant allows better penetration into the dentinal tubules, thus improving its antibacterial efficacy. The viscosity of CHX (2Cps) was found to be less than that of chitosan (39Cps). Molecular size of CHX in base form is <45 μm and that of powdered chitosan was 20 μm. It was found that ultrasonic agitation of an irrigant showed better penetration into the dentinal tubules. Ultrasonic agitation of the irrigants produces two main effects, namely acoustic streaming and cavitation.[20] The acoustic streaming produces a rapid vortex-like motion of the liquid and cavitation causes the formation of spontaneous cavities throughout the liquid contributing to the better penetration of the irrigants into the dentinal tubules.[21]

CONCLUSION

Irrigation with 2% CHX with ultrasonic agitation was found to have maximum depth of penetration into dentinal tubules.

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Conflicts of interest
There are no conflicts of interest.

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