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Evaluation of penetration depth of 2% chlorhexidine digluconate into root dentinal tubules using confocal laser scanning microscope

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Objectives: This study evaluated the penetration depth of 2% chlorhexidine digluconate (CHX) into root dentinal tubules and the influence of passive ultrasonic irrigation (PUI) using a confocal laser scanning microscope (CLSM). ***Materials and Methods:*** Twenty freshly extracted anterior teeth were decoronated and instrumented using Mtwo rotary files up to size 40, 4% taper. The samples were randomly divided into two groups (n = 10), that is, conventional syringe irrigation (CSI) and PUI. CHX was mixed with Rhodamine B dye and was used as the final irrigant. The teeth were sectioned at coronal, middle and apical levels and viewed under CLSM to record the penetration depth of CHX. The data were statistically analyzed using Kruskal-Wallis and Mann-Whitney U tests. ***Results:*** The mean penetration depths of 2% CHX in coronal, middle and apical thirds were 138 µm, 80 µm and 44 µm in CSI group, respectively, whereas the mean penetration depths were 209 µm, 138 µm and 72 µm respectively in PUI group. Statistically significant difference was present between CSI group and PUI group at all three levels ($p < 0.01$ for coronal third and $p < 0.001$ for middle and apical thirds). On intragroup analysis, both groups showed statistically significant difference among three levels ($p < 0.001$). ***Conclusions:*** Penetration depth of 2% CHX into root dentinal tubules is deeper in coronal third when compared to middle and apical third. PUI aided in deeper penetration of 2% CHX into dentinal tubules when compared to conventional syringe irrigation at all three levels. (*Restor Dent Endod* 2015;40(2):149-154)

Key words: Chlorhexidine digluconate; Passive ultrasonic irrigation; Penetration depth; Root dentinal tubules

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Introduction

Microorganisms are the main causative factor for pulpal and periapical diseases. Elimination of microorganisms from the root canal system is a challenging task because of its anatomic complexities. The main goal of endodontic treatment is to obtain complete root canal disinfection and to prevent re-infection.[1] Mechanical preparation of root canal with hand or rotary instruments result in a preparation, leaving 35% of untouched canal surface.[2] These untouched areas harbor microorganisms and may lead to a persistent periradicular infection. This highlights the importance of chemomechanical preparation for the removal of bacteria, their by-products, and the substrates necessary for its growth from the un-instrumented areas.

Sodium hypochlorite (NaOCl) and chlorhexidine digluconate (CHX) are the widely used root canal irrigants in endodontic therapy. CHX has a unique property of adsorbing

to the dentin for longer period of time and has a long lasting effect on microorganisms, termed substantivity. It has a wide range of antimicrobial activity against both Gram positive and Gram negative bacteria.[3,4] Bacteria can penetrate deeper into dentinal tubules to a variable range and that of *Enterococcus feacalis* was found to range even up to 1,000 μm.[5,6] Hence, an irrigant penetrating deep into the dentinal tubules will be beneficial to reach the deeply penetrated bacteria. On literature search, limited data are available regarding penetration depth of irrigants into root dentinal tubules. Penetration depth of NaOCl into root dentinal tubules has been studied earlier in dentin blocks.[7] Krithikadatta *et al*. has evaluated the extent of disinfection of CHX, when it was used as an intracanal medicament.[8] There are no studies evaluating the penetration depth of CHX when using as an irrigant.

Passive Ultrasonic Irrigation (PUI) oscillates at an ultrasonic frequency of 25 - 30 kHz. Due to this oscillation, it has the potential to create acoustic streaming and aids in the deeper penetration of irrigants laterally.[9,10] A review evaluated the literature on PUI and has described its efficient antibacterial effect, better removal of smear layer, better cleaning efficacy in curved root canals and canal isthmuses.[11] Previous studies have reported an increase in penetration of NaOCl irrigant into lateral canals using PUI by clearing the teeth and injection of an opaque material.[9,10] There is lack of data on the penetration depth of CHX irrigant into root dentinal tubules using PUI. Hence, the aim of this study was to evaluate the penetration depth of 2% CHX into root dentinal tubules and the influence of PUI on penetration depth using Confocal Laser Scanning Microscope (CLSM).

Materials and Methods

Ethics institutional review board approval was obtained for this research. Twenty freshly extracted human permanent maxillary anterior teeth with single canals and straight mature roots were used in this study. Teeth with previous coronal restorations, caries, resorption or root canal treatment were excluded. All the teeth were decoronated to 15 mm using safe diamond disc (Isomet, Buehler Ltd, Düsseldorf, Germany) to eliminate the coronal interferences and to obtain a flat reference for instrumentation.[12] An ISO size 10 K-file was inserted until visible at the apical foramen. Working length was determined by reducing 1 mm from this length.

To standardize the size and taper of the canal, root canal preparation was done using Mtwo larger sequence files up to ISO size 40, 4% taper (VDW, Munich, Germany) in a crown down manner. 1 mL of 5.25% NaOCl was used after each instrument. Apical patency was maintained using size 10 K-file. One mL of 17% ethylenediaminetetraacetic acid (EDTA) solution was used finally for 1 minute for smear layer removal, and the canals were rinsed with saline. Then the canals were dried using absorbent points.

About 0.1 g of Rhodamine B dye (Sigma Aldrich, Bangaluru, India) was mixed with 500 mL of 2% CHX solution, which is further referred as 2% CHX+R. Viscosity and surface tension of 2% CHX and 2% CHX+R were checked using viscometer (Brookfield HADV-11 +PRO, Middleboro, MA, USA) and goniometer (Digidrop GBX, Bourg de Peage, France), respectively. The 2% CHX+R was used as the final irrigant.

All the prepared samples were randomly divided into two groups (n = 10 each) by toss of a coin. In group 1, conventional syringe irrigation group (control group), irrigation was performed using 30 gauge side vented needle (Max-i-Probe, Dentsply Rinn, Elgin, IL, USA). The tip of the needle was placed 1 mm short of working length and was moved up and down with 2 mm amplitude during irrigation. The irrigant was delivered at a rate of 1 mL/min. A total of 3 mL of 2% CHX+R was used for final irrigation. In group 2, PUI group (experimental group), the root canal was filled with 2% CHX+R, using a 30 gauge side vented needle and then an ultrasonic file (Irri-Safe, Satelec, Acteon Group, Merignac cedex, France) of size 20, 2% taper was inserted in the root canal 1 mm short of the working length. The irrigant was ultrasonically activated for 1 minute at a power setting of 3 with a piezoelectric ultrasonic unit (Suprasson P5 Booster - Satelec).[13] This sequence was repeated thrice resulting in a total irrigation time of 3 minutes and a total irrigation volume of 3 mL. Finally the teeth were cut at 3 mm (apical), 6 mm (middle) and 12 mm (coronal) levels using diamond disc, to obtain dentin sections of 1 mm thickness.

Evaluation of penetration depth of 2% CHX+R using CLSM

The sections were viewed under CLSM (TCS-SP5, Leica, Mannheim, Germany) to evaluate the depth of penetration of 2% CHX+R. The depth of penetration of 2% CHX+R into root dentinal tubules was depicted by the fluorescence at x10 magnification. An excitation at 543 nm was performed to collect the emission produced in the 560 nm mode. The obtained image was divided into four equal parts and the penetration depth was measured at each part. The average value was recorded as the penetration depth of the sample. The measurement was done using the digital measuring ruler present in Leica microsystems software (LAS-AF, Leica). All the specimens were evaluated by a single operator, who was blinded to the groups.

Statistical analysis

Statistical analysis was performed using Mann-Whitney *U* test for intergroup analysis. Kruskal-Wallis and Mann-Whitney *U* test were used for intragroup analysis to

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compare the coronal, middle and apical values. The significance level was set at $p < 0.05$.

Results

The viscosity of 2% CHX and 2% CHX+R were 2.89 ± 0.25 cP and 3.04 ± 0.25 cP, respectively. The surface tension of 2% CHX and 2% CHX+R were 34.60 ± 0.5 mN/m and 34.65 ± 0.5 mN/m, respectively. On viewing under CLSM, all sections showed the presence of a consistent fluorescent irrigant penetrated layer (Figure 1).

The penetration depths of the groups are reported in Table 1. On intergroup analysis, the depth of penetration in PUI group was statistically superior in all three levels when compared to conventional syringe irrigation group ($p < 0.01$ for coronal third and $p < 0.001$ for middle and apical thirds). On intragroup analysis, a statistically significant difference was found among the three levels in both groups ($p < 0.001$).

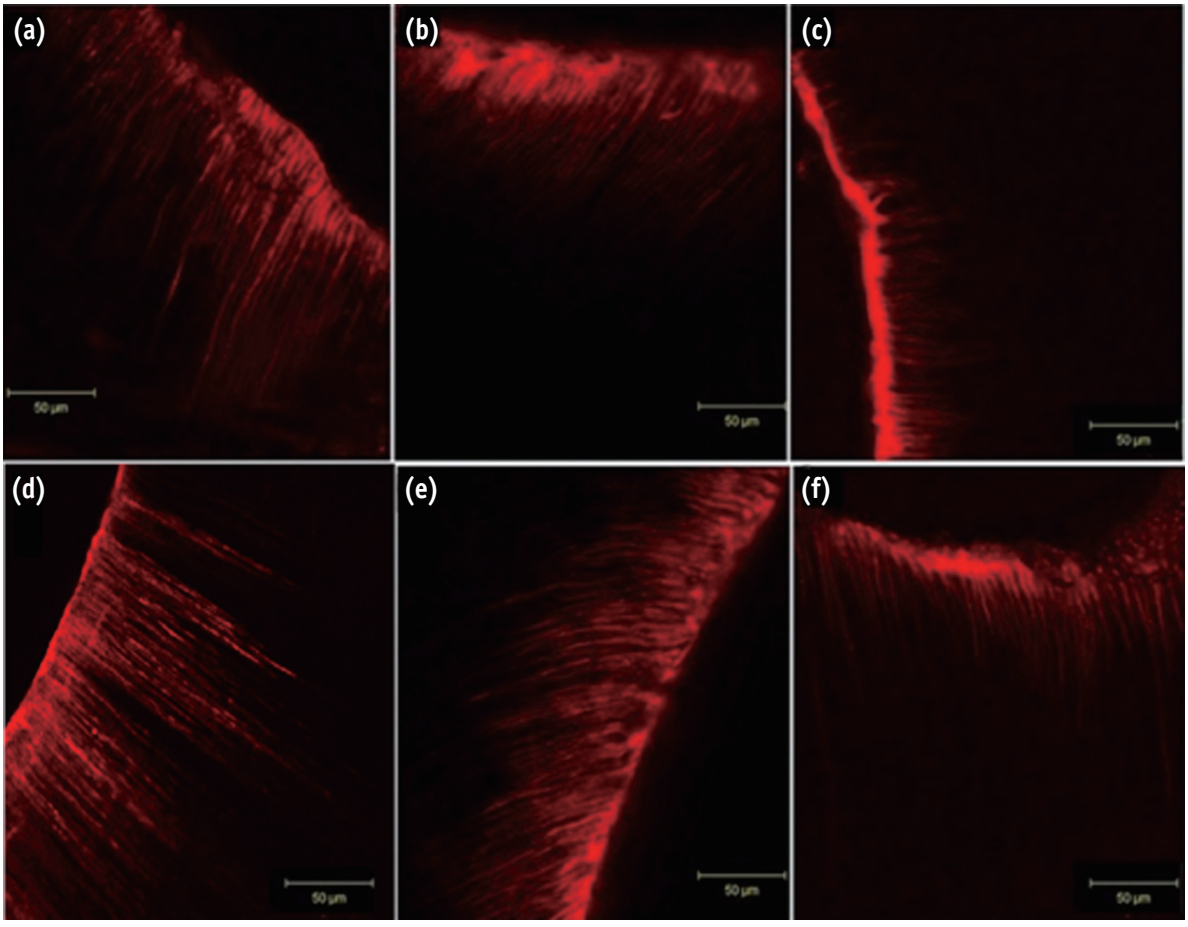

Figure 1. Representative confocal laser scanning microscopic images of samples. (a) Conventional syringe - coronal; (b) Conventional syringe - middle; (c) Conventional syringe - apical; (d) Passive ultrasonic irrigation (PUI) - coronal; (e) PUI - middle; (f) PUI - apical.

Table 1. Comparison of penetration depth (μm) of 2% chlorhexidine into root dentinal tubules

Group	Coronal level	Middle level	Apical level	*p* value
Group 1	132.16 (113.16 - 152.05)	77.07 (75.30 - 79.53)	44.69 (39.0 - 48.4)	< 0.001
Group 2	217.84 (185.24 - 246.56)	142.01 (118.65 - 151.29)	67.06 (63.88 - 74.69)	< 0.001
p value	< 0.01	< 0.001	< 0.001	

The numbers in the parentheses were the first and third quartile values.

Discussion

The success of endodontic treatment depends upon a number of factors, including appropriate instrumentation, irrigation and disinfection of the root-canal system.[1] CHX is one of the potential disinfectant solutions used for final irrigation.[3] It is a well-established fact that microorganisms can penetrate deeper into dentinal tubules and lead to persistent infection.[5] The benefit of CHX adhering to dentin and its substantivity can be beneficial if the irrigant penetrates deeper. Hence, the depth of penetration of irrigant into the dentinal tubules is a significant factor that needs to be taken into consideration. Even though 2% CHX is a commonly used root canal irrigant, data regarding the depth of penetration into dentinal tubules is lacking.

Activation of irrigants is one of many ways to increase the efficacy of irrigants. Various irrigant activation systems include sonic and ultrasonic agitation, manual activation with gutta-percha cones and agitation with brushes. A review of literature stated that PUI appears to be an adjunctive treatment for cleaning the root canal system and aids in better efficacy of irrigants when compared to other modes of irrigation. Hence, PUI was considered in this study. In PUI, after the enlargement of the canal up to the master apical file, a small file or smooth wire (*e.g.*, sizes 15 and 20) is used to ultrasonically activate the irrigants. The file or wire can vibrate freely in the enlarged root canal resulting in more powerful acoustic streaming and thus enhancing the lateral penetration of irrigants.[14] Previous studies have proved that PUI aids in deeper penetration of irrigants into simulated lateral canals.[9,10] Hence in this study, the depth of penetration of 2% CHX with conventional syringe irrigation and PUI was evaluated.

Lateral penetration of irrigants, medicaments and sealers into root dentinal tubules has been evaluated using various methods like bleaching, SEM, CLSM and recently with radiolabelling.[7,15-17] The penetration depth of NaOCl into dentinal tubules was assessed using bleaching of the dye technique.[7] However, CHX does not have the property of bleaching. In previous studies, Rhodamine B dye was added with root canal sealers to evaluate their penetration depth into dentinal tubules using CLSM.[16,18] Hence in this study, Rhodamine B dye was mixed with 2% CHX to visualize the penetration using CLSM. The CLSM has several advantages over other microscopy. The specimen processing for CLSM is relatively simple, enabling the observations to be made under near normal conditions. The sample preparation tends to produce fewer artifacts when compared to other microscopy.[18] It also aids in visualizing up to 10 μm below the surface of the specimen.[19] Hence, the methodology using CLSM was chosen for this study.

Viscosity and surface tension are the two main factors which influence the flow and penetration depth of the irrigants.[20,21] The wettability of the irrigating solution on dentin is related to its viscosity and surface tension. The surface tension is defined as 'the force between molecules that produces a tendency for the surface area of a liquid to decrease'.[22] This force tends to limit the ability of the liquid to penetrate in a capillary tube. The wettability of the solution governs the capability of its penetration both into the main and lateral canals, and into the dentinal tubules.[19,21] Previous studies showed an increase in penetration of the NaOCl irrigant with a decrease in viscosity and surface tension.[20] In our study, viscosity and surface tension of plain 2% CHX and 2% CHX mixed with Rhodamine B dye were evaluated and confirmed to be similar.

All the root canals were prepared to a size of 40, 4% taper to achieve a balance between adequate volume of irrigant and preservation of radicular dentin.[12] Removal of smear layer is one of the important criteria for achieving better penetration of irrigants.[23] In this study, smear layer was removed using 1 mL of 17% EDTA for 1 minute in all the samples to aid in deeper penetration of irrigant.[24] A 30 gauge needle was used in this study, so that the needle could penetrate till the apical third when the apical enlargement was to ISO size 40.[3] It has been proved that irrigant can be delivered only 1 mm deeper than the tip of the needle.[25] Hence in this study, the tip of the needle was placed 1 mm short of the working length. Ultrasonic file of ISO size 20 was used for PUI so that the file can freely oscillate inside the prepared canal.[14]

In a previous study, the penetration depth of NaOCl was studied in dentin block model immersed in NaOCl.[7] In the present study, CHX was irrigated into the root canal, to simulate the clinical scenario better than the dentin block model. The mean penetration depth of NaOCl into root dentinal tubules has been reported as 300 μm.[7] In this study, the mean penetration depth of 2% CHX in coronal, middle and apical thirds are 138 μm, 80 μm and 44 μm, respectively, for conventional syringe group, whereas the mean penetration depth in PUI group are 209 μm, 138 μm and 72 μm, respectively. However, the penetration depth of both studies cannot be compared directly because of the difference in methodology adopted. In this study, coronal third of root canals showed better penetration in both groups and apical third showed least amount of penetration. The density of dentinal tubules is less in apical third when compared to cervical areas, and the amount of peritubular dentin is also more in apical areas.[26] The nature of apical root dentin is different from that of coronal two thirds and exhibits sclerosis.[27,28] These reasons could be attributed for lesser penetration depth in apical thirds.

In this study, PUI group showed better penetration of 2% CHX irrigant into dentinal tubules than conventional syringe irrigation group, at all three levels. Acoustic microstreaming plays an important role in the efficacy of

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PUI.[14] A previous study have also highlighted the efficacy of passive ultrasonic irrigation as a final irrigation protocol.[29] Hence, 2% CHX along with PUI can be an effective final irrigation technique for better disinfection of the root canals.

Conclusions

Under the limitations of this study, it can be concluded that the penetration depth of 2% CHX into root dentinal tubules is deeper in coronal third when compared to middle and apical third. PUI has a positive effect on deeper penetration of 2% CHX irrigant into root dentinal tubules at all three levels.

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

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