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Evaluation of permeability of root dentin after different irrigation protocols

Испитивање пермеабилности коренског дентина после испирања различитим протоколима иригације

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SUMMARY

Introduction/Objective This study was aimed to evaluate dentin permeability after irrigation with sodium hypochlorite (NaOCl) and final rinse with chlorhexidine (CHX), ethylenediamine tetraacetic acid (EDTA) + CHX, and new combination products: QMiX or MTAD.

Methods Roots of 60 maxillary incisors were randomly divided into five groups (n=12) before instrumentation and irrigation with NaOCl according to the final irrigation regimen: CHX (2% CHX), EDTA + CHX (17% EDTA + 2% CHX), QMiX, MTAD, and control group (distilled water). After final irrigation, ten roots of each group were horizontally sectioned and dye penetration was evaluated in the coronal, middle, and apical thirds. Remaining samples were subjected to scanning electron microscopy. Data were analyzed with ANOVA/Tukey's test.

Results Less dye penetration was found in CHX group compared with control as well as with QMiX and MTAD group in all thirds ($p < 0.05$). Significant difference between the control and EDTA + CHX, QMiX or MTAD group was observed only in the apical root third ($p < 0.05$).

Conclusion Dentin permeability was significantly reduced after final irrigation with CHX, but not after use of other final irrigation solutions, except in the apical third of the root canal.

Keywords: dentinal tubule cleansing; intra-canal disinfectants, irrigants; chlorhexidine; EDTA; MTAD; sodium hypochlorite; QMiX

САЖЕТАК

Увод/Циљ Циљ овог истраживања је био да се испита пермеабилност коренског дентина после иригације натријум хипохлоритом (NaOCl) и финалне иригације хлорхексидином (CHX), етилендиамино-сирћетном киселином (EDTA) + CHX и нових комбинација: QMiX или MTAD.

Метод Корени 60 горњих централних секу-тића су, пре инструментације и иригације NaOCl, методом случајног узорка подељени у 5 група (n=12) на основу финалног протокола иригације: CHX (2% CHX), EDTA+CHX (17% EDTA+2% CHX), QMiX, MTAD и контролна група (дестилвана вода). Након финалне иригације 10 коренова из сваке групе су хоризонтално пресечени и пенетрација боје одређена у круничној, средњој и апексној трећини. Преостали узорци су испитивани методом електронске микроскопије. Подаци су анализирани примјеном ANOVA/Tukey's теста.

Резултати Пенетрација боје у CHX групи је била мања у свим трећинама у односу на контролу као и у односу на QMiX и MTAD групу ($p < 0,05$). Значајна разлика између контролне и група EDTA+CHX, QMiX и MTAD је забележена само у апексној трећини корена ($p < 0,05$).

Закључак Пермеабилност дентина је значајно смањена после финалне иригације CHX, али не и након иригације другим растворима, осим у апексној трећини.

Кључне речи: чишћење зубних тубула; интраканални дезинфицијенси; хлорхексидин; EDTA; MTAD; натријум хипохлорит; QMiX

INTRODUCTION

Irrigation is essential for successful debridement of the root canals with mechanical procedures [1]. Sodium hypochlorite (NaOCl) is the most commonly used irrigation solution due to its antimicrobial action and tissue-dissolving potential [2,3]. However, NaOCl is not sufficient for total cleaning of the root canal system from microorganisms, debris, tissue remnants and the smear layer. For optimal irrigation, a combination of irrigation solutions has to be used. Therefore, NaOCl has been used in combination with demineralizing agents such as ethylenediamine tetraacetic acid (EDTA), for effective removal of smear layer. Chlorhexidine (CHX) a chemical with substantive antimicrobial properties has been studied as a final irrigation solution after NaOCl and EDTA [2]. Recently, new combination products QMiX and MTAD have been aimed for removing inorganic smear layer and disinfecting the root canal system following NaOCl irrigation [4-6]. QMiX and MTAD contain

antibacterial agent with known prolonged antimicrobial action (substantivity) (CHX and doxycycline, respectively) [7, 8], demineralizing agent (EDTA and citric acid, respectively) and a detergent.

Although a combination of irrigants may enhance its antimicrobial and cleaning effectiveness, the possible chemical reaction between them has to be considered. This is especially evident when CHX is combined with NaOCl. The chemical interaction between these two solutions results in the color change of mixture to brown and formation of precipitate [9]. When associated with EDTA, CHX produces white precipitate [10,11]. In QMiX, this interaction is avoided by its chemical design [5]. The combination of QMiX with NaOCl produced inconsistent results. While some authors found orange-brown precipitation [12], others found visually detectable color change but without precipitate formation in the interaction of these two solutions [13]. When MTAD was added to NaOCl yellow precipitate formed [14].

The clinical significance of the precipitate formed in interaction with NaOCl and CHX is that it may contain substance harmful to the general health [15]. Concern has been raised that color change could compromise esthetics [16]. Furthermore, it can act as a chemical layer occluding dentinal tubules and altering dentin permeability [17,18]. Subsequently, diffusion of intracanal medicaments and sealing of root canal could be compromised [19]. The penetration of the precipitate into dentinal tubules formed in interaction between either NaOCl or CHX with other irrigation solutions has not yet been clarified.

Therefore, this study was aimed to evaluate dentin permeability after irrigation with NaOCl and final rinse with CHX, EDTA + CHX, QMiX or MTAD. The null hypothesis was that there would be no differences in permeability of root dentin between different final irrigation solutions.

METHODS

Sample selection and treatment

This study was conducted after approval from the Institutional Ethics Committee (No. 01-3-88/2015). Sixty intact human maxillary incisors with single straight and mature roots, and single canals extracted from 18-30-year-old subjects were included in the study. Teeth with caries, restorations, calcifications, intraradicular resorption or complicated root canal anatomy were excluded. Root canal anatomy was verified with radiographs. The root surface was cleaned with scalpel, ultrasonic instruments and brushes. Then teeth were stored in 0.9% saline with a 0.2% thymol solution at 4°C until use.

The crown of each tooth was cut to standardize the root lengths to 14 mm. Before chemomechanical preparation root canals were divided into five groups (n=12) according to the final irrigant solution used: CHX (2% CHX solution, Consepsis, Dentsply Tulsa Dental, Tulsa, OK, USA), EDTA + CHX (17% EDTA, ENDO-SOLution, CerKamed, PPH CerKamed, Stalowa Wola, Poland and 2% CHX solution, Consepsis, Dentsply Tulsa Dental, Tulsa, OK, USA), MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA), QMiX (Dentsply Tulsa Dental) and distilled water (control group). The

working length was established 1 mm short of the apical foramen by #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland). After that, apical foramen of each root was sealed with wax. Root canal preparation was carried out with Pro-Taper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to F4 file (40/0.06) as the master apical file. The canals were irrigated with 1 mL 5.25% NaOCl, after each instrument, except in the MTAD group where canals were irrigated with 1 mL 1.3 % NaOCl (recommended manufacturer's protocol). Irrigation was performed with 27 gauge stainless steel needles (Endo-Eze, Ultradent, South Jordan, UT, USA), which tip was placed 1 mm from the working length and then was moved up and down during irrigation. At the end of preparation the 5 mL of 17% EDTA was used in the canals for five minutes for smear layer removal followed by distilled water, to remove traces of EDTA. Then, 5 mL of 5.25% (CHX, EDTA + CHX, and QMiX groups) or 1.3% NaOCl (MTAD group) was delivered into the canals for two minutes followed by 10 mL distilled water for two minutes to minimize the potential reaction between NaOCl and final irrigant solutions. Final rinse was performed with 5 mL of 2% CHX, 17% EDTA followed by 2% CHX, QMiX, or MTAD for two minutes. In EDTA + CHX group canals received intermediate flush between two solutions with 10 mL of distilled water for two minutes to prevent interaction. Finally, all canals were dried with paper points.

Dentin permeability analysis

Ten roots of each group were externally coated with fast polymerizing epoxy resin (Brascola Ltda, SP) leaving the root canals free and immersed in 0.2% Rhodamine B solution. After 24 hours, specimens were rinsed continuously under tap water during next 24 hours. A sharp blade was used to remove resin coatings, and the teeth were embedded in a polyester resin. Each root was horizontally sectioned using a slow-speed water-cooled cut machine (Extec Labcut 1010, Enfield, CT) to obtain 1-mm-thick slice. All slices were polished with silicon carbide papers to obtain a flat surface. A slice from each third was randomly chosen, mostly from each third's middle portion, and scanned (Epson Perfection 1240U scanner; Epson Corp, Tokyo, Japan) with resolution at 400 dpi and analyzed with the software ImageLab 4.1 (Bio Red, Tokyo, Japan) to assess dye penetration. Dye penetration in dentin was expressed as percentage of the dye penetrated area in relation to the total root-third area.

Scanning electron microscopy (SEM) analysis

Two roots of each group were prepared for scanning with an electron microscopy (SEM) analysis. Roots were transversely sectioned at 3, 6, and 9 mm from the apex using diamond disc at slow-speed. The specimens were dehydrated using ascending grades of ethanol (25%, 50%, 75% and 100%), mounted on aluminum holder, sputter coated with gold and then examined with SEM (JEOL-JSM-6610LV, Tokyo, Japan). Specimens were examined at a magnification between 3,700 x and 6,500 x and 20 kV to detect precipitate formation on the root dentin surfaces and inside of the dentin tubules.

Statistical analysis

The statistical analyses were performed using SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). The results obtained for dye penetration (Kolmogorov-Smirnov test $p > 0.05$) were submitted to the one-way analysis of variance (ANOVA) and Tukey's post hoc. The significance levels were set at 5 %.

RESULTS

Results of percentage of dye penetration are shown in table 1 and figures 1, 2 and 3. MTAD, QMiX and control group ($p < 0.05$) showed significantly higher dye penetration than CHX group in the coronal third. In the middle third, all groups showed more dye penetration compared to CHX group ($p < 0.05$). And finally in the apical third, control group showed significantly more dye penetration than other groups ($p < 0.05$), while both MTAD and QMiX groups showed significantly greater dye penetration than CHX group ($p < 0.05$). The highest dye penetration was recorded in the coronal thirds of all groups with significant differences between the thirds ($p < 0.05$) (Table 1).

Table 1. The mean \pm SD of dye penetration (%) in dentinal tubule at the coronal, middle and apical third of root dentin.

Root level	CHX	EDTA + CHX	QMiX	MTAD	Distilled water
Coronal	61.61 \pm 14.56 ^{Aa}	76.72 \pm 10.71 ^{Aba}	81.33 \pm 6.74 ^{Ba}	79.28 \pm 7.81 ^{Ba}	83.77 \pm 17.65 ^{Ba}
Middle	25.80 \pm 9.03 ^{Ab}	52.95 \pm 15.28 ^{Bb}	62.24 \pm 15.37 ^{Bb}	58.52 \pm 13.30 ^{Bb}	65.50 \pm 18.28 ^{Bb}
Apical	14.43 \pm 3.85 ^{Ac}	16.80 \pm 4.91 ^{ABc}	24.57 \pm 5.23 ^{Bc}	25.87 \pm 6.56 ^{Bc}	36.01 \pm 12.19 ^{Cc}

CHX – chlorhexidine; EDTA – ethylenediamine tetraacetic acid

Mean values represented with same superscript uppercase letters (row) or lowercase letters (column) are not significantly different ($p > 0.05$).

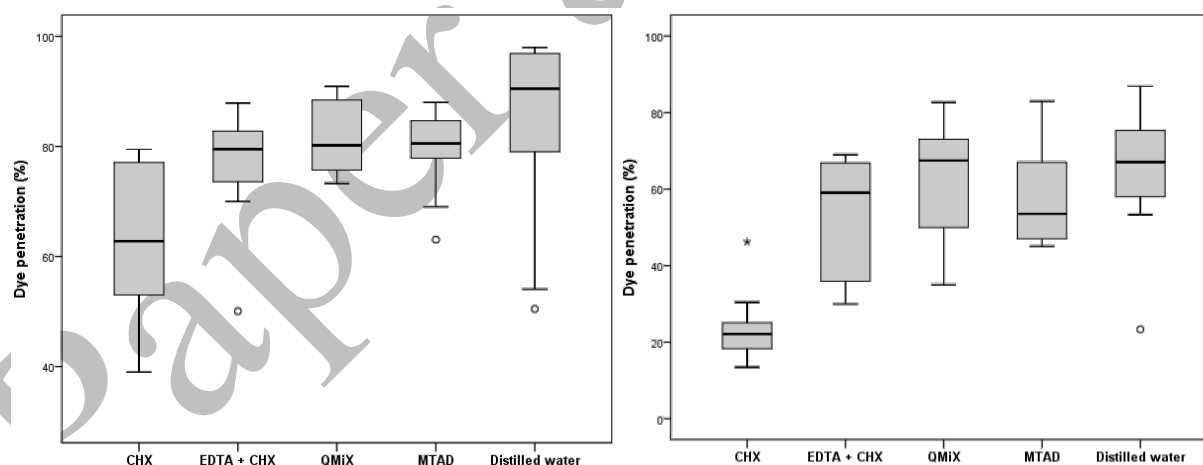


Figure 1. Box plots of dye penetration (%) in coronal third. Horizontal bars-medians; box-boundaries- the 25th and 75th percentiles; whiskers- the 25th and 75th percentiles; whiskers- maximum and minimum observed values; 0- and minimum observed values; 0-outliers; asterisk (*)-extreme values.

Representative SEM images of samples irrigated with different final irrigation protocols are shown in Figure 4. Precipitate was found in samples irrigated with CHX, EDTA + CHX, QMiX and MTAD while the control group revealed root canals without precipitate formation.

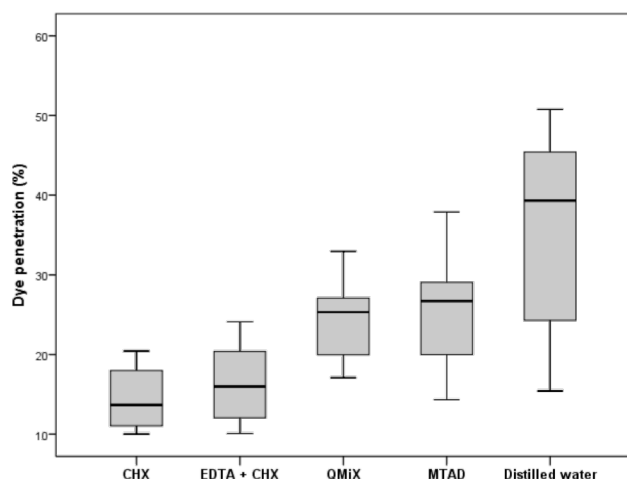


Figure 3. Box plots of dye penetration (%) in apical third. Horizontal bars-medians; box-boundaries- the 25th and 75th percentiles; whiskers- maximum and minimum observed values.

DISCUSSION

The present study evaluated the interaction between NaOCl and CHX, EDTA + CHX, QMiX or MTAD on dentin permeability. The products formed in these interactions hindered with dentin permeability after final rinse with CHX, but not after use other solutions, except in the apical third.

A closed-end canal model was used in the current study to mimic clinical situation. Distilled water was used between NaOCl

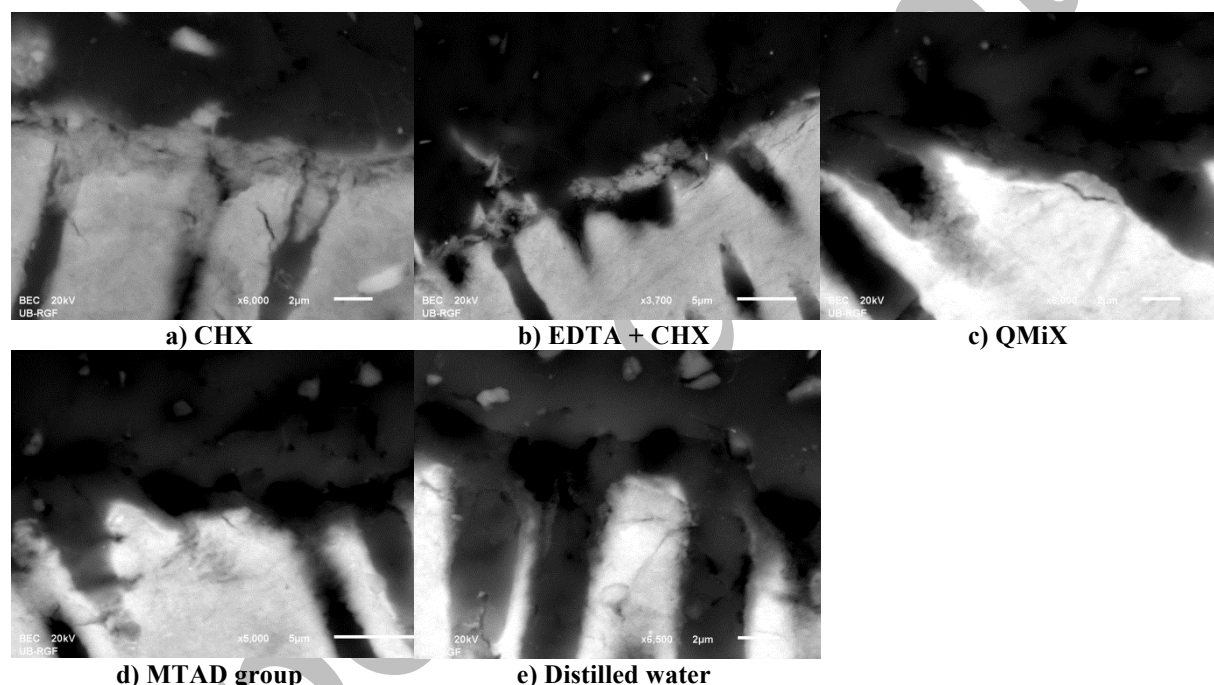


Figure 4. SEM images (magnification between 3,700 x and 6,500 x) of the sectioned specimens showing precipitate formation on the root dentin surfaces and inside of the dentin tubules after final irrigation with CHX (a), EDTA + CHX (b), QMiX (c) and MTAD (d). In control group (distilled water) precipitate was not found (e).

and final irrigation solution (CHX, EDTA + CHX, QMiX and MTAD) as well as between EDTA and CHX in order to prevent precipitation, as it has been recommended in clinical conditions [4]. Moreover, the operational sequence used was aimed to exclude effect of smear layer on dentin permeability.

In the present study irrigation with CHX after NaOCl significantly reduced dentin permeability in all third of root canals compared to control group. This result indicate that product formed in interaction between NaOCl and CHX, characterized as brown precipitate [9], is present in dentinal tubules, as has been shown previously [13]. This can be explained by the ability of both solutions to diffuse into tubules up to 500 μ m, according to the results of studies that have used dyes or measured

their antibacterial penetration [18, 20-22]. Akisue et al. [18] employing the same methodology for specimens analysis as in the present study, found that precipitate formed between 1% NaOCl and 2% CHX caused reduction of dentin permeability only in the apical third, when compared to no irrigation control group and group irrigated with 15% citric acid followed by 2% CHX. Discrepancy in the results between our and mentioned study could be attributed to the concentration of NaOCl used (5.25 vs. 1%). Namely, it has been shown that precipitation is concentration dependent [9]. The precipitate was formed into dentinal tubules although attempt was made to prevent its formation by introducing intermediate flush with distilled water in the main canal. This raise some concerns because this precipitate may act as reservoir of toxic [23] and carcinogenic [24] substance, known as para-chloranilin (PCA), even after removal of precipitate form main canal. In addition, this precipitate acting as a chemical smear layer may limit the effective disinfection of dentinal tubules by preventing intracanal medicaments from penetrating the dentinal tubules [17, 18]. Namely, in infected root canals viable bacteria have been found deep within dentinal tubules, up to 375 μm [25] and it persistence after chemomechanical procedures may be responsible for root canal reinfection and treatment failure. The precipitate may compromise adaptability of the root filling materials to the root canal walls [19] and may reduce the sealer penetration into dentinal tubules, as well. The sealer penetration into dentinal tubules increases the surface contact between dentin walls and filling materials, which may improve retention of the filling material by mechanical locking and may exerts antibacterial effect on bacteria remaining in dentinal tubules after canal preparation by isolating them from essential nutrients sources [26]. Moreover, the precipitate may provide a path through which leakage could take place between the root canal filling and the dentinal walls. Namely, Vivacqua-Gomes et al. [19] found that combination of 1% NaOCl with 2% CHX favor coronal microleakage of root-filled teeth. Staining potential of this insoluble dark brown precipitate is also of relevance [16].

In root canals irrigated with EDTA + CHX, QMiX or MTAD after NaOCl dentin permeability was reduced but did not significantly differ from control group in the coronal and middle third. But in the apical third these groups showed significant less dye penetration than control group. Also, QMiX and MTAD exhibited more dye penetration than CHX in all root thirds. These results indicate that precipitation probably occurs in dentinal tubules, but not in amount that could affect dentin permeability in coronal and middle thirds, in contrast to interaction between NaOCl and CHX. Stereomicroscope study showed that QMiX had significant lower scores of precipitate associated with 2.5% NaOCl than 2% CHX in root canals, probably due to lower concentration of CHX in QMiX [12]. On the other hand, Kolosowski et al. [13] found no precipitation neither on dentin surfaces nor dentinal tubules after immersion of dentin discs in 2.5% NaOCl followed by saline and QMiX, measured by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Although direct comparisons could not be made due to differences in methodology, it can be argued that intermediate flush with distilled water in our study prevent interaction between QMiX and 5.25% NaOCl in dentine tubules, as did saline in mentioned study [13]. Moreover, lack of significant differences in

dentin permeability of EDTA + CHX and MTAD group with control specimens also suggest that distilled water have significant impact on precipitate prevention in dentinal tubules except in apical sections. Limitation of irrigation modality used and impaired delivery of irrigants into apical third, including distilled water, constitute possible reasons that could explain lower apical dentin permeability in EDTA + CHX, QMiX and MTAD group. Also, influence of anatomical factors on dentin permeability should also be considered. Namely, tubular sclerosis that starts in the third decade of life in the apical region interferes the penetration of root canal irrigants [27]. Moreover, dye penetration into dentinal tubules at the apical region is strongly dependent on the group of teeth [28]. In order to standardize dentin pattern among the specimens in the present study only maxillary incisors of subjects under the age of 30 were included.

In agreement with the previous studies [18, 27, 29] we found the highest dye penetration in the coronal and lowest in the apical third of root canal in all groups, including controls with significant differences between the thirds. This may be due to the irregularity and lower size and density of dentinal tubules in apical area [27]. Namely, the number of dentinal tubules decreases from coronal near the pulp from 40 000 mm⁻² to 14 400 mm⁻² in the apex [30]. Moreover, lower efficacy of the irrigants in these portions of the root canal, cleared out the dentinal tubules less thoroughly.

CONCLUSION

Final irrigation with CHX after initial NaOCl rinse significantly reduced dentin permeability at all root levels. Interactions between NaOCl and EDTA + CHX, QMiX or MTAD exert no significant effect on dentin permeability, except in the apical section of the root canal. Based on the current results, final irrigation with CHX after NaOCl should be avoided in order to prevent precipitate formation which reduces dentin permeability, subsequently compromising sealing of the root canal system. On the other hand, EDTA + CHX, QMiX or MTAD might be recommended as reasonable solutions for final irrigation. Further studies are necessary to better clarify influence of different final irrigants on the dentin permeability.

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