ORIGINAL RESEARCH

Effects of Different NaOCl Concentrations Followed by 17% EDTA on Dentin Permeability

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Abstract

Aim: The aim of this study is to evaluate the permeability of root dentin after immersion in sodium hypochlorite (NaOCl) at different concentrations for 30 minutes and a final wash with 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes.

Materials and methods: Twenty 1 × 5 mm dentin fragments from the middle third of the root were prepared from 10 bovine teeth and divided into four groups; three of these groups were immersed in 1%, 2.5%, or 5.25% NaOCl for 30 minutes, while group IV was immersed in the saline solution. All dentin fragments were subjected to a final wash with 17% EDTA for 3 minutes. The fluid transport study model was used to measure the fluid conductance induced by hydrostatic pressure. The measurements were repeated twice to confirm the values, and in the case of a discrepancy, the procedure was performed again.

Results: The control group (saline solution) presented lower hydraulic conductance mean values with (0.25 ± 0.12). The 5.25% NaOCl followed by 17% EDTA produced the highest mean conductance value (1.18 ± 0.18) followed by 2.5% NaOCl and 17% EDTA with (0.81 ± 0.09) and (0.48 ± 0.02), respectively. The results of the hydraulic conductance evaluation of dentin were different for each NaOCl concentration. After data analyses, there were significant differences from analysis of variance (ANOVA) between all the groups ($p < 0.0001$).

Conclusion: The NaOCl solution concentration affects dentin permeability.

Keywords: Dentin permeability, Root canal irrigants, Sodium hypochlorite.

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Introduction

The mechanical action of endodontic instruments alone does not adequately clean and disinfect the root canal system.[1,2] Therefore, the use of irrigating solutions during biomechanical preparation is essential for the clinical success of endodontic treatment. The chemical behavior of these substances in the debridement of the root canal promotes cleaning and disinfection while eliminating bacteria, dissolving organic components, and lubricating the dentin walls.[3–5]

NaOCl is the solution most commonly used in endodontics due to its organic matter dissolution properties and antimicrobial activity. However, this solution also promotes adverse changes in the chemical and structural properties of dentin by modifying the mineral components of this tissue, such as calcium and phosphorus.[2,6] This modification of the mechanical properties of dentin tissue caused by irrigation generates undesirable effects such as reduced dentin microhardness, flexural strength, modulus of elasticity,[3] permeability, and solubility,[6] in addition to erosion in dentin tissue.[2]

During the irrigation process, NaOCl promotes changes in the local pH when exposed to dentin tissue.[3] Proteolytic activity is responsible for the destruction of collagenous components of the mineralized dentin matrix, establishing a phantom mineral matrix surrounded by collagen with lower flexural strength.[2,3] However, this action depends on the volume of the irrigant used, the application time, and the solution concentration.[7]

Despite the effectiveness of NaOCl, this irrigant is not able to dissolve the inorganic components of dentin or to decrease the formation of the smear layer during preparation.[4,8] Chelating agents such as 17% EDTA are recommended to complement root canal treatment; however, the destructive effect of NaOCl on mineralized dentin is known to be irreversible and occurs regardless of the use of EDTA as a final irrigant.[9]

The preparation efficiency provided by automated systems provokes discussion about the effects required from irrigating agents due to the decreased contact time between these solutions and the dentin substrate. Automated systems with a single instrument indicate the use of NaOCl solutions at higher concentrations to ensure that the maximum effect is achieved, but the use of higher concentrations of this solution is related to a greater destructive effect on the collagen fibers of dentin tissue, in addition to the increased toxicity of NaOCl.[3,5,9] The increase in NaOCl concentration has been related to increased dentin permeability. This condition may modify the biomechanical properties of dentin, compromising the success of rehabilitating clinical procedures

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that depend on the formation of interfaces between resinous or adhesive materials and the dentinal walls of the root canal.[9–11] Furthermore, to the best of our knowledge, there are no reports in the literature on how much dentin permeability would need to be altered to achieve cleaning and disinfection objectives.

The objective of this study is to evaluate the permeability of root dentin after immersion in NaOCl for 30 minutes at different concentrations and a final wash with 17% EDTA for 3 minutes, considering that is a similar clinical period.

Materials and Methods

Specimen Preparation

This study was approved by the Research Ethics Committee of the Institute of Health Sciences, the Federal University of Pará (no. 4893020218). Ten bovine incisors were used, which were immersed in 1% NaOCl solution to eliminate any remaining tissue and disinfect the root surface and later stored in physiological solution under refrigeration for a period not exceeding 3 months.

The specimens were prepared according to the method proposed by Akisue et al.[12] and Mena-Serrano et al.,[13] with modification. The crowns were removed at 1 mm below the cementoenamel junction and cross-sectioned using a 0.10 × 22 mm double-sided cutting blade (KG Sorensen, Cotia, SP, Brazil). The remaining roots were sectioned longitudinally, removing all the cementum, and, subsequently, cross-sectioned to select the middle third of the root, forming 5 dentin bars for each group. This process resulted in samples with initial dimensions of a 1.2 mm thickness and 5 mm height.

After sectioning, each sample was polished using a Politriz Metaserv 250 Simples sander (Instrumental Brasil, SP, Brazil) with 600- and 1000-grit water sandpaper (3M Brazil, Sumaré, SP, Brazil). The polishing process, in addition to removing waste and leveling the surface, resulted in samples with dimensions of a 1-mm thickness and 5 mm height. The specimens were stored in plastic containers at room temperature during immersion in NaOCl and EDTA and randomly allocated into four groups: G1—1% NaOCl; G2—2.5% NaOCl; G3—5.25% NaOCl; G4—saline solution. Specimens from G1, G2, and G3 were immersed in NaOCl for 30 minutes, and then all dentin fragments ($n = 20$) were subjected to a final wash with 17% EDTA for 3 minutes (Table 1).

Permeability Test

Each specimen was placed in a THD-02c permeability apparatus (Odeme Equipamentos Médicos e Odontológicos Ltda, Joaçaba, SC, Brazil). The hydrostatic pressure and the injection time of the irrigating liquid were controlled, with the hydrostatic pressure calibrated to 200 cm H_2O and measured for 5 minutes. The permeability was measured by the displacement of an air bubble measured using a digital caliper mounted next to a 100 glass capillary tube with a length of 116 mm. The measurements were repeated twice to confirm the values, and in the case of a discrepancy, the procedure was performed again.

Table 1: Division of groups according to solution regimes ($n = 5$)

Group	*Initial solution (30 minutes)*	*Final solution (3 minutes)*
G1	1% NaOCl	17% EDTA
G2	2.5% NaOCl	17% EDTA
G3	5.25% NaOCl	17% EDTA
G4	Saline	17% EDTA

The dentin filtration index (*Q*) was evaluated using the following formula:

$$Q = \frac{V_s \times D}{L \times T}$$

where *Q* is the filtration index (μL/minutes); hydraulic conductance of dentin; V_s is the standardized volume/total internal volume of the glass capillary (100 μL); *D* is the displacement of the bubble in the capillary tube (mm) (given by the caliper); *T* is the time in minutes (5 minutes); *L* is the capillary length (116 mm).

Data Analysis

The final results were expressed as the hydraulic conductance of dentin (*Q* = filtration index). After confirming distribution normality, the data were subjected to a one-way analysis of variance (ANOVA) test with Tukey's *post hoc* test, adopting an α of 0.05.

Results

All the data analyses were performed using GraphPad Prism 8 (GraphPad Software, Inc, La Jolla, CA). The solutions investigated in the present study were the following: NaOCl 1%, NaOCl 2.5%, NaOCl 5.25%, and saline solution as the control. Different concentrations of NaOCl were selected considering that the deleterious effects of NaOCl are related to the concentration used in endodontic treatment. The hydraulic conductance values of dentin (mean ± SD) for the four groups investigated after the use of irrigation solutions are presented in Table 2. The control group, G4 (saline), presented lower hydraulic conductance mean values with 0.25 ± 0.12. The G3 group (5.25% NaOCl and 17% EDTA) presented higher hydraulic conductance values with 1.18 ± 0.18, followed by G2 (2.5% NaOCl and 17% EDTA) with 0.81 ± 0.09 and G1 (1% NaOCl and 17% EDTA) with 0.48 ± 0.02. After data analyses, there were significant differences from ANOVA between all the groups ($p < 0.0001$).

Discussion

The study of dentin permeability has always been significant for endodontists, since the cleaning and the disinfection of root canals require the use of chemical substances that act in areas not touched by instruments, representing sites of contamination such as isthmus areas, recesses, and dentin tubules.[11,13,14] Another factor is the fact that obtaining dentin tissue free of debris leads to better diffusion of the intracanal medication and an improvement in the mechanical bonding of the sealing materials.[10,11] The hydraulic conductance is a measure of the ease with which fluid, under hydrostatic or osmotic pressure, can pass through a permeable barrier (in this case, dentin) under defined conditions.[15] From a clinical point of view,

Table 2: Mean, standard deviation, minimum, maximum values for filtration status of dentin specimens after immersion in solutions (μL/min) and *p* values (Tukey's test)

Solutions	*M ± SD*	*Minimum*	*Maximum*	*p values*
G1: 1% NaOCl	$0.48^{a} \pm 0.02$	0.46	0.51	<0.01
G2: 2.5% NaOCl	$0.81^{b} \pm 0.09$	0.70	0.95	<0.01
G3: 5.25% NaOCl	$1.18^{c} \pm 0.18$	0.97	1.36	<0.01
G4: Saline	$0.25^{d} \pm 0.12$	0.22	0.30	<0.05

M, mean; SD, standard deviation. Different alphabetic superscript letters (a–d) denote mean values with statistical differences among groups the irrigating solutions modify the integrity of the dentin tissue, bringing about changes in the permeability and, consequently, changes in the hydraulic conductance of these tissues.[15] In the present study, the effect of the NaOCl solution concentration on dentin permeability was evaluated.

NaOCl is the irrigating agent most commonly used in endodontic practice, due to its broad antimicrobial activity [2–6,16] and dissolution of organic matter remaining in the root canal.[6,7] Although all concentrations of NaOCl have effective antimicrobial properties, the effect on the organic matter is directly related to the concentration.[2–7,9,17] NaOCl is characterized by its proteolytic action on tissue, which negatively affects dentin and causes depletion of the organic components formed mainly by type I collagen and proteoglycans.[2,3,5] The collagen matrix is the main component and is organized into a fibrillar frame around the peritubular dentin, whereas the proteoglycans connect one or more glycosaminoglycan chains and are responsible for the regulation of water content and intratubular permeability.[5,14] This side effect is highly undesirable and irreversible due to the alteration of dentin structure, which causes a reduction in moisture, consequently, leading to tooth fragility.[18–21]

The high surface tension of NaOCl affects its ability to penetrate dentin, thereby reducing its antibacterial efficacy in canal irregularities and in the deeper regions of the dentinal tubules.[22] However, NaOCl at high concentrations has low surface tension, facilitating action at a greater depth in the dentin.[3,22] This effect was confirmed in the present study by increasing the NaOCl solution concentration, which significantly altered dentin permeability. In G3, greater hydraulic conductance was observed compared with G1, G2, and G4, revealing that the action of NaOCl is directly proportional to its concentration. In the present study, saline solution (G4) was used as the control. Although this solution presented lower values than the group treated with the lowest concentration of NaOCl (G1), saline solution is not able to replace the clinical use of NaOCl. Saline solution is harmless to dentin tissues, showing no organic dissolution and antimicrobial action and these properties are fundamental for the clinical success of endodontic treatment.

The smear layer is formed by residual organic and inorganic components from dentin cutting procedures, such as in biomechanical preparations using rotary or manual instruments on the root dentin wall.[23,24] This layer binds to the intertubular dentin and penetrates the dentinal tubules, reducing permeability, increasing the presence of microorganisms in canal space, and decreasing the ideal seal.[23–29]

Removal of the smear layer with acid solutions results in increased fluid flow within the exposed dentin.[23] The use of chelators such as EDTA is recommended as an irrigating solution to complement root canal therapy, promoting the removal of the smear layer.[2,3,5,28]

The group treated with saline solution and 17% EDTA presented lower values than the group treated with the lowest concentration of NaOCl. This result agrees with the findings of Zhang et al.,[9] indicating that dentin degradation in endodontics is not due to the chelating effect of 17% EDTA but rather to erosion caused by the use of NaOCl as the initial irrigant and that the deleterious effects are related to its concentrations and contact time with intact root dentin.[9] In the present study, a time of exposure to 17% EDTA used was of 3 minutes to reach similarity to the clinical time of use of this solution.

The results of Gu et al.[3] demonstrated that the effects of the interaction of mineralized dentin with the NaOCl solution facilitate the penetration of EDTA due to the increased permeability.[3,9] EDTA then dissolves the apatite layer, exposing the collagen fibers, which increases the infiltration and proteolytic activity of NaOCl.[2,3,9,23] The degradation of mineralized dentin occurs via diffusion in a concentration- and time-dependent manner. In this process, the release of the OCl^- anion acidifies the medium during the contact of the solution with the dentin walls, where a "phantom mineral layer" scarce in collagen is formed, generating a friable mineral matrix, resulting in reduced flexural strength as a deleterious effect, which may reflect the susceptibility of the root to fracture.[2,3,5,9]

In the present study, the use of the low concentration of NaOCl also resulted in increased dentin permeability. From a clinical point of view, this concentration seems to have advantages relative to the other concentrations because it is more biocompatible and produces smaller dentin structural and morphological alterations.[4,19,29] The antimicrobial action would also not be compromised by using lower concentrations of NaOCl. The antibacterial effects produced by irrigation with NaOCl at 1%, 2%, and 5.25%, compared by Siqueira et al.,[16] showed that the frequency of irrigation and the amount of irrigant used during endodontic treatment rendered the antibacterial effectiveness equal for all three solutions.[16] This means that regular exchange and the use of large amounts of irrigators should maintain the antibacterial efficacy of the NaOCl solution, compensating for the effects of the concentration.[16] The antimicrobial activity is balanced by the different concentrations and by the time required for irrigation, which is greater with lower than with higher concentrations.[4,29]

Finally, the actual mechanism of action of NaOCl in the depletion of collagen in mineralized dentin remains an object of study. NaOCl readily infiltrates the collagen water compartments, oxidizes the organic matrix, and denatures the collagen components of the mineralized dentin. The deleterious effects of collagen degradation in mineralized dentin are dependent on the concentration and application time of this substance.[3,6,9,22] It should be noted that there are no reports in the literature on how much dentin permeability would need to be altered to achieve cleaning and disinfection objectives; thus, the possibility of using a less-concentrated NaOCl solution should be considered due to the adverse effects of higher concentrations. In view of these results, 1% NaOCl showed lower conductance values between the tested concentrations; however, there is no consensus in the literature about the ideal concentration of NaOCl to be used.[6] In endodontics, the search for the ideal irrigation regime aims to maintain the integrity of the mechanical properties of dentin tissue while promoting tissue dissolution and antimicrobial activity;[5] however, further studies are needed to conclude which NaOCl concentration may be satisfactory to exert these properties with minimal interference to root dentin integrity.

Due to the *in vitro* nature of this experiment, some clinical conditions become impossible to simulate as the submission of the specimens under pressure generated during the endodontic treatment. Permeability of dentin surfaces was evaluated in a study about adhesive systems and reported that there is an outward fluid flow across exposed dentin in response to the low but positive pulpal tissue pressure, which is completely absent in extracted teeth[13] or dentin bars and was not simulated in the present study. If this factor is able to interfere or not these results is not determined.

Conclusion

NaOCl at low concentrations is capable of promoting changes in dentin permeability. NaOCl modifies the permeability of root dentin directly proportional to its concentration.

Clinical Significance

The possibility of using a less-concentrated NaOCl solution should be considered due to the adverse effects of higher concentrations.

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