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REVIEW

Effect of root canal irrigants on cervical dentine permeability to hydrogen peroxide

P. Surapipongpuntr, W. Duangcharee, S. Kwangsamai & A. Ekka
Restorative Department, Faculty of Dentistry, Naresuan University, Phitsanulok, Thailand

Abstract

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Aim To examine the effects of various root canal irrigants on cervical dentine permeability by monitoring the diffusion of hydrogen peroxide (H_2O_2).

Methodology Sixty cervical dentine discs were prepared from human third molar teeth. After removal of enamel and cementum, the outer dentine surface was etched with 17% ethylenediamine tetraacetic acid (EDTA) for 1 min. The dentine discs were randomly assigned to five groups according to the irrigant used: A, saline solution; B, 2.5% sodium hypochlorite solution (NaOCl); C, 5% NaOCl; D, 17% EDTA and 2.5% NaOCl; E, 17% EDTA and 5% NaOCl. After irrigation on the inner dentine surface with the various solutions, the diffusion of 30% H_2O_2 was evaluated through each disc using a plastic-split chamber. H_2O_2 was applied to the inner-side chamber, while the outer-side chamber was filled with deionised water. After 30 min of application of H_2O_2, the solution in the outer-side chamber was collected to determine the concentration of H_2O_2 using a spectrophotometer.

Results The penetration of H_2O_2 through dentine in group E was significantly highest, followed by groups D, C, B and A respectively (one-way ANOVA, $P < 0.05$).

Conclusions Among the irrigants used, 17% EDTA and 5% NaOCl had the greatest effect in increasing dentinal permeability to H_2O_2.

Keywords: bleaching agent, dentine permeability, root canal irrigants.

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Introduction

Hydrogen peroxide (H_2O_2) is used for intracoronal bleaching of endodontically treated teeth (Baratieri *et al.* 1995). However, recent evidence suggests that this procedure can cause significant complications. Free radicals and active oxygen biologically released from H_2O_2 have been reported to possess bleaching effects and to damage human cells and tissues (Kashima-Tanaka *et al.* 2003). Cervical root resorption was attributed to the toxic nature of 30% H_2O_2 which diffused through the dentine (Fuss *et al.* 1989). It has been postulated that H_2O_2 denatured dentine at the cervical region (Lado *et al.* 1983), which further induced a foreign body reaction. Other authors claimed that the diffusion of H_2O_2 through dentinal tubules irritated the periodontium, causing an inflammatory resorptive process and external root resorption (Harrington & Natkin 1979, Friedman *et al.* 1988). An increase in permeability of cervical dentine may influence the diffusion of H_2O_2 into the periodontal tissue, leading to cervical root resorption.

During root canal treatment, various irrigants are used to enhance the efficacy of mechanical cleansing. Sodium hypochlorite (NaOCl), a well-known nonspecific proteolytic agent, is the most favoured endodontic

Correspondence: Peraya Surapipongpuntr, Restorative Department, Faculty of Dentistry, Naresuan University, Amphur Muang, Phitsanulok 65000, Thailand (Tel.: 61 55 261 934; fax: 61 55 261 934; e-mail: perayap@nu.ac.th or puapichat@hotmail.com).

irrigant owing to its tissue-dissolving, antibacterial and lubricant properties (Koskinen *et al.* 1980, Harrison & Hand 1981). Furthermore, a combination of irrigants has been reported to improve the potential for cleansing (Svec & Harrison 1981). Ethylenediamine tetraacetic acid (EDTA), a chelating agent, has been used to remove debris accumulating on the instrumented root canal walls (Hottel *et al.* 1999). Removal of smear layer has been suggested to improve the permeation of medicaments into dentinal tubules and to improve the sealing of root filling materials to dentinal walls (Kennedy *et al.* 1986, Foster *et al.* 1993). Application of 15% or 17% EDTA solution was found to be most effective in removing the smear layer both *ex vivo* and *in vivo* (McComb & Smith 1975, Yoshida *et al.* 1995). Moreover, investigators have shown that rinsing with 1 mL of EDTA for 1 min adequately eliminated the smear layer, opened the dentinal tubules, and provided a clean surface (Crumpton *et al.* 2005).

Dentine permeability may be altered after being exposed to NaOCl or the combination of EDTA and NaOCl because of the proteolytic effect of NaOCl and the chelating effect of EDTA. Previous study has demonstrated that treatment with 5% NaOCl for 1 h depleted the dentine of organic compounds and significantly increased the permeability of dentine (Barbosa *et al.* 1994). In addition, an application of 17% EDTA caused a significantly greater increase in dentine permeability compared with 1% NaOCl (Carrasco *et al.* 2004). As the irrigation of a root canal is an important step in root canal treatment, this study focused on the alteration in the permeability of cervical dentine to hydrogen peroxide after pre-treatments using various endodontic irrigants. Thus, the aim was to examine the effects of the various root canal irrigants on cervical dentine permeability by monitoring the diffusion of H_2O_2. The hypothesis was that there would be a difference in dentine permeability among groups.

Materials and methods

Dentine disc preparation

Sixty intact third molar teeth extracted for various clinical reasons were used. Donor patients, aged <25 years, gave informed consent for their teeth to be used. Each tooth was sectioned mesio-distally into two halves using a diamond blade (Isomet®, Buehler, IL, USA) under copious water. A dentine disc specimen (1.00 ± 0.05 mm thick) was prepared from the buccal half of each tooth by removing enamel and cementum and polishing the inner dentine into a flat surface using abrasive papers (numbers 500, 800 and 1000). The outer dentine was etched with 1 mL of 17% EDTA for 1 min to remove the smear layer (Crumpton *et al.* 2005) and then rinsed with 2 mL of de-ionised water. The specimens were randomly assigned to five groups ($n = 12$) according to the irrigant used: group A (control), physiological saline solution (4 mL for 2 min); group B, 2.5% NaOCl (4 mL for 2 min); group C, 5% NaOCl (4 mL for 2 min); group D, 17% EDTA (1 mL for 1 min) followed by 2.5% NaOCl (3 mL for 1 min); and group E, 17% EDTA (1 mL for 1 min) followed by 5% NaOCl (3 mL for 1 min). Irrigation was performed on the inner side of the dentine discs, whilst all dentine discs were rinsed with 2 mL de-ionised water. The sample size per group was determined using data from the pilot study. The value of the standardised range was (maximum mean − minimum mean)/sigma = (5932.8−12.3)/3208.2 = 1.84 (Woolson & Clarke 2002). Using the table provided by Kastenbaum *et al.* (1970), with $\alpha = 0.05$ and power equal 0.9, the group sample size was $n = 12$.

Hydrogen peroxide penetration test

Each dentine disc was inserted between two plastic chambers modified from a previous report and sealed with double O-rings (3 mm in diameter). The inner-side chamber was filled with 1 mL of 30% (W/W) H_2O_2, whereas the outer-side chamber was filled with 1 mL de-ionised water (Fig. 1). After 30 min of application of H_2O_2, the solution in the outer-side chamber was collected to evaluate the amount of diffused H_2O_2.

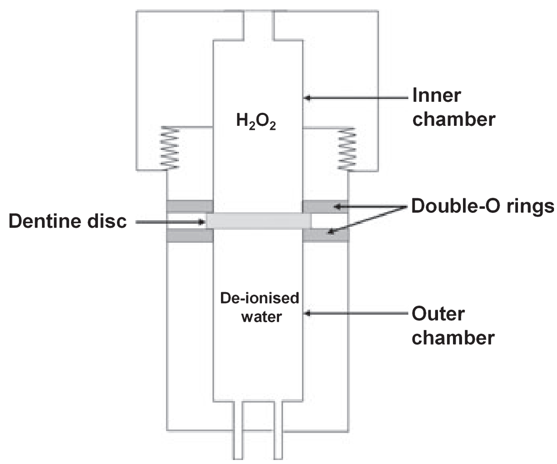

Figure 1 Diagram of the experimental setup for the H_2O_2 diffusion test.

Assay for H_2O_2

The concentration of H_2O_2 was assayed using the xylenol orange technique (Jiang *et al.* 1990). In this reaction, H_2O_2 oxidises ferrous (Fe^{2+}) to ferric ion (Fe^{3+}) in the presence of sorbitol, which acts as a catalyst. Ferric ion then forms a blue–purple complex (λ_{max} 560 nm) with xylenol orange. A 200 μL aliquot of sample was added to 100 μL ferrous ammonium sulphate (2.5 mmol L^{-1}), prepared fresh daily, 100 μL sorbitol (0.1 mol L^{-1}), 100 μL sulphuric acid (0.25 mol L^{-1}) and 100 μL xylenol orange. Reverse osmosis water was added to make up 1 mL. After shaking the sample for 45 min, the absorbance at 560 nm was determined and compared with a hydrogen peroxide standard curve, showing a relation between absorbance and H_2O_2 concentration for a dilution series. If the measured absorbance of the sample was higher than the standard curve, the sample was diluted with de-ionised water and re-examined.

Scanning electron microscopic observations

The inner surface of the dentine disc was observed by a scanning electron microscope (SEM; LEO1455vp, LEO Electron Microscopy Ltd, Cambridge, UK). Dentine discs were fixed in 2.5% glutaraldehyde in phosphate-buffered solution (pH 7.3) at 4 °C for 2 h. After dehydration through ascending concentrations of alcohol (30, 50, 70 and 90% for 5 min and 100% for 10 min), the specimens were dried by the carbon dioxide critical-point technique (Polaron CPD7501, Watford, UK) and coated with gold using the SPI-Module sputter coater (Structure Probe, Inc., West Chester, PA, USA).

Data analyses

The amounts of H_2O_2 diffused through dentine in all groups were compared by one-way analysis of variance (ANOVA). A probability value of $P < 0.05$ was considered as significant.

Results

The amounts of H_2O_2, which diffused through dentine discs, are shown in Fig. 2. All tested irrigants produced an increase in dentine permeability to H_2O_2 compared with the control (group A). The permeability of dentine measured as H_2O_2 diffusion was highest in group E, followed by group D, C, B and A, respectively. Statistical analysis of the data showed significant difference among groups (One-way ANOVA, $P < 0.05$). *Post hoc* test (Dunnett's T3) showed a significant difference for dentine permeability to hydrogen peroxide in all pairs except for one pair (C and D).

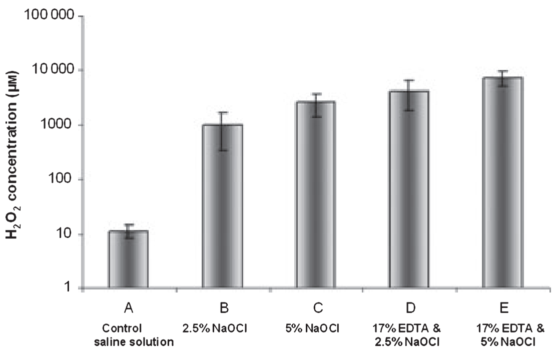

Figure 2 Graph showing mean concentration of H_2O_2 diffusing through cervical dentine discs in each group (n = 12 per group).

SEM observations

The results of SEM analysis of each group at ×5000 were as the followings.

Group A: irrigation with physiologic saline solution

The specimen showed an amorphous layer of polishing debris, a smear layer (Fig. 3).

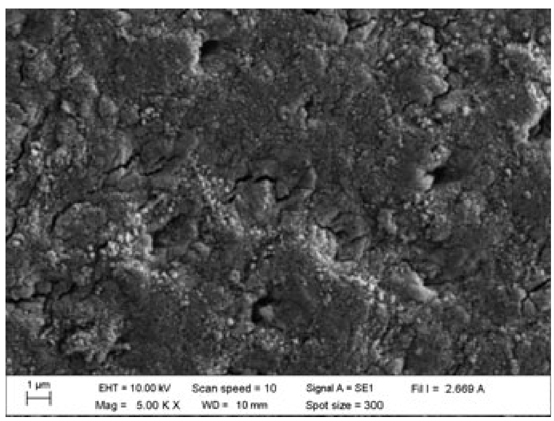

Figure 3 Group A: Irrigation with saline solution: the surface was covered with an amorphous smear layer. Bar: 1 μm, ×5000.

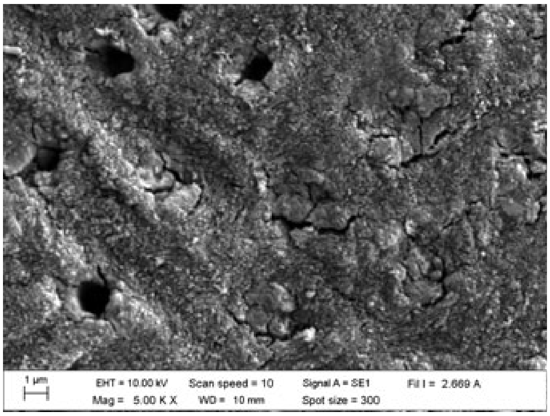

Figure 4 Group B: Irrigation with 2.5% NaOCl: some amorphous debris was removed and a number of dentinal tubules were observed. Bar: 1 μm, ×5000.

Group B: irrigation with 2.5% NaOCl

The dentine surface was covered with the smear layer. Some amorphous debris was removed and dentinal tubules were observed (Fig. 4).

Group C: irrigation with 5% NaOCl

Some superficial organic debris was removed, resulting in a smoother surface compared to group B. The dentinal tubules were partially obstructed by smear layer (Fig. 5).

Group D: irrigation with 17% EDTA followed by 2.5% NaOCl

The combination of EDTA and NaOCl completely removed the smear layer covering the dentine surface. The dentinal tubules were opened and the collagen fibrils were exposed (Fig. 6).

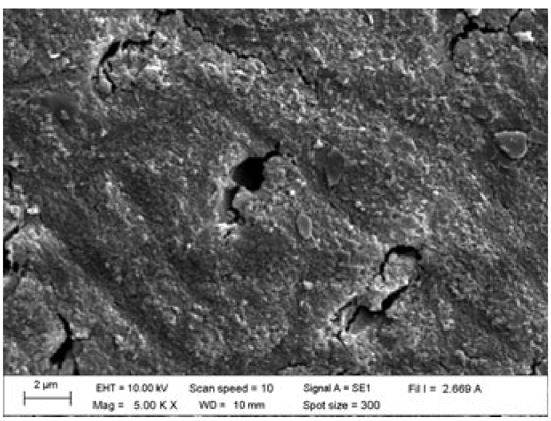

Figure 5 Group C: Irrigation with 5% NaOCl produced a smoother surface compared with group B. Dentinal tubules were incompletely occluded with the smear layer. Bar: 2 μm, ×5000.

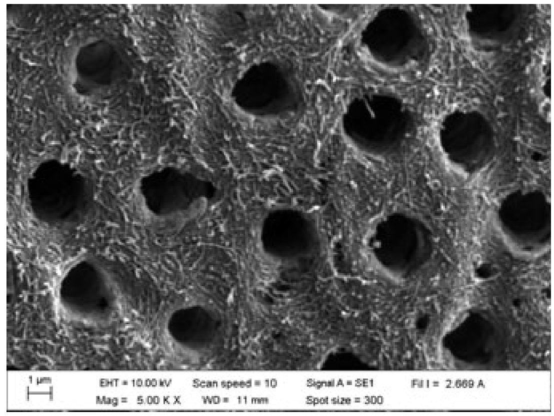

Figure 6 Group D: Irrigation with 17% EDTA followed by 2.5% NaOCl removed the smear layer and opened dentinal tubules. The collagen fibrils and secondary tubules on the intertubular dentine were observed. Bar: 1 μm, ×5000.

Group E: irrigation with 17% EDTA followed by 5% NaOCl

This combination removed almost all collagen fibrils similar to group D. The secondary tubules on the intertubular dentine were presented and the peritubular dentine was destroyed (Fig. 7).

Discussion

Irrigants used to improve the efficacy of mechanical cleansing during root canal treatment may alter the integrity of dentine and increase its permeability. The

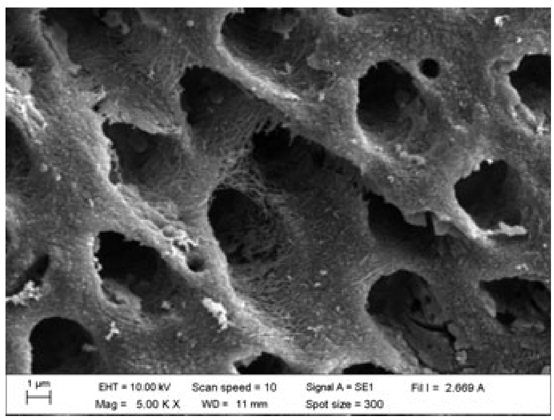

Figure 7 Group E: Irrigation with 17% EDTA followed by 5% NaOCl removed the collagen fibrils shown in group D. The secondary tubules on the intertubular dentine were revealed and the peritubular dentine was destroyed. Bar: 1 μm, ×5000.

present results revealed the effects of different root canal irrigants on cervical dentine permeability to 30% H_2O_2. The dentine permeability was significantly highest in group E (17% EDTA and 5% NaOCl), followed by group D, C, B and A, respectively.

In group A, physiological saline solution, which has no demonstrated demineralising or tissue solvent activity, cleaned the surface poorly. SEM images of dentine surfaces irrigated with this solution revealed an amorphous layer of polishing debris and occluded dentinal tubules. As a result, group A was significantly lower in permeability to 30% H_2O_2 than the other groups.

In group B and C, the action of the NaOCl solution on dentine permeability was examined. NaOCl significantly increased the permeability of the dentine to 30% H_2O_2 compared with physiologic saline solution. In addition, an increase in dentine permeability seemed to depend on the concentration of NaOCl. The amount of diffused H_2O_2 was higher in group C (5% NaOCl) compared with group B (2.5% NaOCl). This is likely because of the proteolytic action of concentrated sodium hypochlorite on the organic phase of dentine (Barbosa *et al.* 1994). SEM study of the dentine surface applied with the higher concentration of NaOCl revealed enhanced cleanliness. However, NaOCl alone was not able to remove the smear layer as reported previously (Yamada *et al.* 1983).

The effects of the combined use of 17% EDTA followed by 2.5% or 5% NaOCl were evaluated in groups D and E. The dentine permeabilities in these groups were significantly higher than those in groups treated with NaOCl alone. The use of EDTA resulted in a considerable increase in dentine permeability and corroborates previous findings (Carrasco *et al.* 2004). In that study, 17% EDTA significantly increased the permeability of cervical dentine to copper ion penetration compared with 1% NaOCl. It is possible that the mineral element in smear layer can protect the organic phase in dentine and as a result using NaOCl alone is not able to remove the smear layer. After first rinsing, EDTA removes the mineral phase, thus exposing the organic components lining the dentinal tubules. The subsequent deproteinisation then exposes the tubules (Marshall *et al.* 2001) therefore increasing dentine permeability. In this study, the combination of 17% EDTA with 5% NaOCl caused the greatest increase in dentine permeability to 30% H_2O_2.

SEM images of the dentine surfaces in group E revealed the removal of smear layer and complete removal of collagen fibrils, which was also evident in dentine surfaces of group D (17% EDTA and 2.5% NaOCl). Additionally, the secondary tubules on the inter-tubular dentine and irregular inter-tubular structure were observed (Fig. 7). It may consider that deproteinisation after demineralisation is dependent on the concentration of NaOCl (Correr *et al.* 2006).

There is no clear clinical consensus as to whether the smear layer should be removed before canal filling (Moss *et al.* 2001). The removal of smear layer may improve the permeation of medicaments into dentinal tubules, the penetration of sealer, and the surface contact between filling material and the dentine wall (Kennedy *et al.* 1986, Foster *et al.* 1993). On the other hand, it has been shown that the smear layer can act as a barrier, inhibiting bacterial colonisation on dentinal tubules (Drake *et al.* 1994). In the present findings, the combination of 17% EDTA and 5% NaOCl revealed the greatest alteration of dentinal surfaces and the greatest increase in dentine permeability. It has been suggested that the combined use of high concentration of sodium hypochlorite and EDTA during root canal treatment may affect negatively the integrity of the dentine, thereby allowing increased access of caustic fluids to cervical vital tissues and consequently causes cervical root resorption. Therefore, the concentration of NaOCl should be carefully considered, particularly in cases where intra-coronal bleaching may be indicated. The antibacterial and tissue dissolution actions of NaOCl increases with its concentration (Yesilsoy *et al.* 1995, Spano *et al.* 2001), but the high concentration is accompanied by increased toxicity. Thus, the desirable concentration should demonstrate low toxicity, yet with adequate antibacterial and tissue dissolving effects. Additionally, previous report has concluded that 30% H_2O_2 caused alterations in the chemical structure of pulverised human dentine and cementum (Rotstein *et al.* 1992). The dentine irrigated with EDTA and NaOCl might be more susceptible to hydrogen peroxide damage. However, in this study no negative control group was used to compare the permeability of dentine before and after application of hydrogen peroxide; further study is required to investigate this issue.

Although bleaching discoloured teeth is usually performed on anterior teeth, molar teeth were selected for this study because dentine discs can be readily prepared from the cervical part into similar thickness and area. Intact human third molar teeth from the same age range (<25 years old) were used in order to reduce the morphologic difference among groups. However, differences in dentine permeability of each tooth might influence the results as shown in group C and D. In group D, dentine permeability was higher than that in group C but there was no statistical difference between them. The group of 12 teeth might be too small to reach definite statistical conclusions.

In the clinical situation, cervical dentine is covered with enamel or cementum, and consequently the permeability of both mineralised tissues influences the diffusion of H_2O_2. Additionally, it should be kept in mind that the bleaching agent used in this study was in a liquid form, which is suitable for the diffusion test. However, the standard agent for internal bleaching is sodium perborate mixed with water or H_2O_2 that may reduce the acidity of H_2O_2 and any adverse effects on the biomechanical properties of the dentine (Chng *et al.* 2002, Attin *et al.* 2003). Using the standard treatment method, the amounts of H_2O_2 diffusing through dentinal tubules may be less than those presented in this study.

Conclusions

Among the irrigants used, 17% EDTA and 5% NaOCl had the greatest effect on increasing dentinal permeability to H_2O_2.

Acknowledgements

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