Effects of Oxidative Irrigants on Root Dentin Structure: Attenuated Total Reflection Fourier Transform Infrared Spectroscopy Study

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Abstract

Aim: The aim of this study was to compare the effect of oxidative irrigants on the organic and inorganic structure of root canal dentin.

Methodology: Fifty human 2nd premolar roots were used in the study. The dentin specimens prepared from those teeth were immersed in liquid nitrogen for 15 min. The frozen composition was titrated in a mixer and the obtained dentin powder was kept frozen at -70°C until use. Ten groups of 50mg dentin powder were immersed in agents (A: Ozone for 100 or 200 sec, B: 5.25% NaOCl, C: 2.25% NaOCl, D: 2% Chlorhexidine, E: 0.9% NaCl (control)) for 5 or 10 min. An Attenuated Total Reflection Fourier Transform Infrared Spectrophotometer (ATR FT-IR) was used to analyze dentin powder. The data were statistically analyzed by using Kruskall-Wallis analysis of variance.

Results: In all groups, collagen degradation was significantly increased compared to the control and 2% CHX groups ($p<0.05$). The use of ozone increased collagen degradation significantly compared to the use of 2.25% NaOCl and 2% Chlorhexidine for 5 min ($p < 0.05$). No significant differences were observed between ozone and 5.25% NaOCl-treated groups ($p > 0.05$).

Conclusions: The structural composition of human dentin was significantly affected by the use of oxidative irrigants at higher concentrations.

Introduction

The successful treatment of an infected root canal involves a combination of mechanical and chemical means [1]. Using mechanical instrumentation alone might reduce the number of bacteria in the root canal system by 50%. Several irrigating solutions, such as Chlorhexidine (CHX) and sodium hypochloride (NaOCl), are used during endodontic treatment [2]. With different exposure times and concentrations, NaOCl is the most widely recommended irrigation solution on the basis of its antimicrobial potency [1,3,4] and capability to dissolve remnant necrotic tissue [5,6]. However, there are still some concerns with respect to the toxic effects [2], bad smell and taste [5], and allergic reactions [6]. In addition, as a nonspecific oxidizing and proteolytic agent, NaOCl oxidizes the organic matrix, denatures the collagen component of the smear layer, and affects dentin mechanical properties [3,7]. Therefore, root-treated teeth are becoming more susceptible to deformation and fractures [8,9]. General agreement regarding the optimal concentration and duration of NaOCl treatment does not exist. Today, researchers are still looking for an alternative endodontic antiseptic with high antimicrobial potential and fewer side effects.

CHX has been suggested as an endodontic irrigant because of its antibacterial effects, lower cytotoxicity, and greater substantivity than NaOCl [10,11]. However for CHX the effects on the structural integrity of dentin have not been evaluated.

Ozone (O_3) is a naturally occurring compound consisting of three oxygen atoms. As a gas, ozone has diffusion capacity in the deeper layers of dentin and dentinal tubules [12]. It has been proposed as an alternative oral antiseptic in dentistry. Further, results of studies have shown that ozone in the gaseous or aqueous phase has strong oxidizing power with reliable microbial effects [1,12-16]. It has been reported that oxidation mediated by ozone destroys the cell walls and cytoplasmic membranes of bacteria and fungi. After the membrane is damaged by oxidation, the permeability increases and ozone molecules can enter the cells and cause microorganisms to die [13]. Nowadays, the gaseous or aqueous phases of ozone have been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses [12]. In this context, ozone is a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power and low likelihood of drug resistance. On the other hand, it is also a very powerful oxidizing agent and the effect of gaseous ozone on the collagen degradation of root canal dentin has not been evaluated yet.

The aim of this study was to compare the exposure, time-dependent, and concentration-dependent effects of oxidative irrigants (NaOCl and gaseous ozone) on the organic and inorganic structure of root canal dentin.

Materials and Methods

The study was approved by the Ethics Committee of the Gazi University, Faculty of Dentistry and fifty human 2nd premolar roots were used in the study. The teeth were stored in 0.9% NaCl with 0.02% sodium azide at 4ºC for no more than 1 month. By using tungsten carbide burs, barbed broaches, and stainless steel files, radicular dentin samples, cementum, and pulpal tissues were prepared and immersed in liquid nitrogen for 15 min. The frozen composition was titrated in a mixer and the obtained dentin powder was kept frozen at -70°C until use. Ten groups of 50mg dentin powder were immersed in 50 ml of agents (A: Ozone for 100 or 200 sec., B: 5.25% NaOCl, C: 2.25% NaOCl, D: 2% Chlorhexidine, E: 0.9% NaCl (control)) for 5 and 10 minutes. Each aliquot of treated dentin powder

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was washed 3 times with deionized water, and air-dried at 37°C. Ozonated water was prepared using an ozone generator (Ozone, model MVO – UV, Anceros) The ozone generator was connected to a cylinder of pure oxygen that was calibrated to release oxygen at 0.4 mg/L per min. For the production of the ozonated water, 250 mL of autoclaved distilled water was placed in the system with a glass tube coupled to the ozone generator. Next, O_3 was bubbled through the water for 20 min, thereby producing O_3 at a concentration of 10 mg/L/min. A Fourier Transform Infrared Spectrophotometer (FT-IR) with a diamond Attenuated Total Reflection (ATR) setup was used to obtain infrared spectra for analysis and characterization of dentin specimens. FT-IR spectra of dentin powder were collected in triplicate for each solution concentration and time period [3,7,17-19]. Spectra were obtained between 400 and 4000 cm^{-1} at 4 cm^{-1} resolution by using 32 scans.

For each irrigation solution tested in the study, amide bands I, II, and III from the intact collagen component of mineralized dentin and phosphate and carbonate bands from the apatite component were revealed by ATR FT-IR. The peaks in these spectra (800-2.000/cm^{-1}) have been assigned according to the literature [3].

The data was statistically analyzed by using Kruskall-Wallis analysis of variance on ranks. Post hoc comparisons were performed with Dunn multiple comparison tests.

Results

Concentration-dependent and time-dependent effects of solutions on collagen depletion were evaluated using the collagen and apatite ratio (the ratio of absorbance of amide I peak to phosphate v_3 peak) (*Figure 1*). Smaller ratios of amide: Phosphate values corresponded to greater extent of dentin deproteination. The carbonate: Phosphate ratio (the ratio of absorbance of carbonate v_2 peak to phosphate v_3 peak) revealed the effects of solutions on inorganic structure of dentin (*Table 1*).

No difference was observed in the structure of samples exposed to 0.9% NaCl (control). When compared to the control group in terms of dentin degradation, statistically significant differences were found in NaOCl and ozone groups ($p<0.05$). CHX revealed statistically insignificant differences compared to control group ($p > 0.05$).

Each solution's time-dependent effects revealed an insignificant decrease in the apatite/collagen ratio and an insignificant increase in the amide/phosphate and carbonate/phosphate values ($p>0.05$). The use of ozone resulted in a significant decrease in the apatite/collagen ratio as a result of apatite dissolution and creation of a demineralized collagen matrix compared with 5 min application of 2.25% NaOCl ($p<0.05$). Insignificant differences were observed between ozone- and 5.25% NaOCl-treated groups ($p>0.05$).

The comparison between groups in terms of effecting the most dentin degradation is, respectively, ozone, 5.25% NaOCl, 2.25% NaOCl.

Discussion

The common principle of endodontic treatment is to keep the pulp chamber and root canals flooded with irrigants during the entire period of chemo mechanical preparation to maximize its instrument lubricant, tissue dissolution, and antimicrobial effects [7]. On the other hand, root canal treatment with different irrigants causes alterations in the chemical and structural composition of human dentin [7,9,20]. In the study, the effects of different irrigation regimes on the amide/

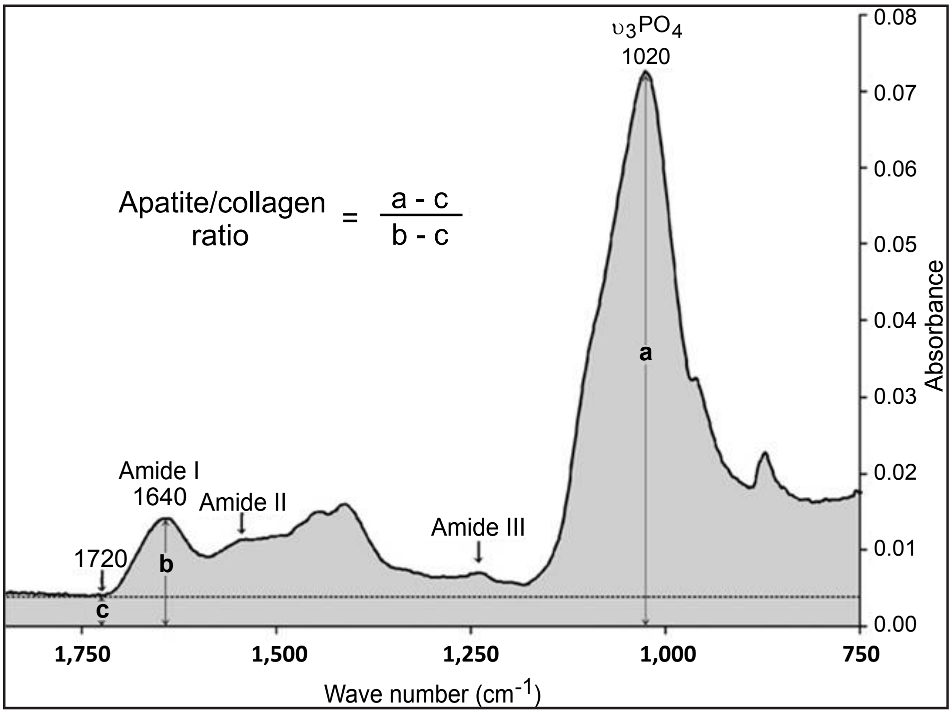

Figure 1*. A schematic depicting how the apatite/collagen ratio was obtained from each infrared spectrum. With the use of both NaOCl, the v_3 PO4 peak of apatite at 1020cm^{-1} could not be used as a reference for examining changes in height of the amide I peak of intact collagen. Hence, the apatite/collagen ratio was used as the substitute by first normalizing the apatite v_3PO4 peak [a] and the collagen amide I peak at 1640cm^{-1} [b] with the base of the spectrum at 1720cm^{-1} [c], and then obtaining the quotient between the normalized v_3PO4 peak [a−c] and the normalized amide I peak [b−c].*

***Table 1.** Comparison of the apatite/collagen, amide/phosphate and Carbonate/Phosphate ratios derived in the groups.*

	Ozone		5.25 % NaOCl		2.25 % NaOCl		2% CHX		Control (0.9% NaCl)	
	100 sec	**200 sec**	**5 min**	**10 min**	**5 min**	**10 min**	**5 min**	**10 min**	**5 min**	**10 min**
Apatite/ Collagen	14.659[a,b,c] (± 4.22)	15.968[a,b] (± 4.62)	12.856[a,b] (± 1.79)	14.22[a,b] (± 2.46)	12.548[a,b] (± 3.45)	14.084[a,b] (± 1.50)	6.146 (± 1.22)	6.766 (± 1.02)	6.41 (± 0.22)	6.43 (± 0.12)
Amide/ Phosphate	0.15[A,B,C] (± 0.32)	0.124[A,B,C] (± 0.22)	0.55[A,B] (± 0.22)	0.238[A,B] (± 0.27)	0.282[A,B] (± 0.31)	0.236[A,B] (± 0.42)	0.468 (± 0.52)	0.416 (± 0.72)	0.54 (± 0.64)	0.52 (± 0.58)
Carbonate/ Phosphate	0.108[*,‡,#] (± 0.11)	0.094[*,‡,#] (± 0.50)	0.172[*,‡] (± 0.23)	0.152[*,‡] (± 0.02)	0.186[*,‡] (± 0.23)	0.156[*,‡] (± 0.31)	0.384[#,‡] (± 0.60)	0.344 (± 0.56)	0.34 (± 0.42)	0.33 (± 0.54)

a = When compared with the control group, all apatite/collagen ratios from different time periods were significantly higher (p<0.05).
b =When compared with the 2% CHX group, all apatite/collagen ratios from different time periods were significantly higher (p<0.05).
c = When compared with the 5 min application of 2.25% NaOCl group, apatite/collagen ratio was significantly higher (p<0.05).
A= When compared with the control group, all amide/phosphate ratios were significantly increased (p<0.05).
B = When compared with the 2% CHX group, all amide/phosphate ratios were significantly increased (p<0.05).
C = When compared with the 5 min application of 2.25% NaOCl group, amide/phosphate ratios were significantly increased (p<0.05).
[*] = When compared with the control group, all carbonate/phosphate ratios were significantly increased (p<0.05).
[‡] = When compared with the 2% CHX group, all carbonate/phosphate ratios were significantly increased (p<0.05).
[#] = When compared with the 5 min application of 2.25% NaOCl group, carbonate/phosphate ratio was significantly increased (p<0.05).

phosphate, carbonate/phosphate, and apatite/collagen ratios of root dentin structure were analyzed by ATR FT-IR.

Based on its unique capacity to dissolve necrotic tissue remnants, NaOCl remains the most widely recommended irrigant with concentrations ranging from 0.5% to 5.25% [21]. On the other hand, as a nonspecific oxidizing and proteoliytic agent, NaOCl oxidizes the organic matrix, denatures the collagen and affects the mechanical properties of dentin like as reduced flexural strength in relation to decreased fracture resistance and reduced clinical success [7,22]. Given the demand for relative non-toxicity toward periapical and oral mucosal tissue for endodontic irrigants, gaseous ozone is currently used in endodontics as an alternative oral antiseptic [1,7,15]. Although the antibacterial efficiency of ozone has been evaluated by previous studies [1,12-16], the effect on root canal structure has not been evaluated yet.

Herein, an Attenuated Total Reflection Fourier Transform Infrared Spectrophotometer (ATR FT-IR) was used to analyze dentin surfaces. ATR FT-IR has previously been used to characterize the chemical composition of dentin. As a simple, effective direct, nondestructive and sensitive technique ATR FT-IR technique presents several advantages over other IR techniques [3,7,17-19].

The selected concentrations of irrigants (NaOCl: 5.25%/2.25%; CHX: 2%; ozone:100 sec/200 sec) represent extreme concentrations commonly reported as used clinically in the literature or recommended by the manufacturer. In addition 0.9% NaCl was used as standard control group, which is not considered to be different in its effect on dentin from that exerted by distilled or tap water [9].

The spectra analysis from the present study demonstrated that, in NaOCl and ozone groups, apatite/collagen ratios were increased compared to the 0.9%NaCl and CHX-treated dentin specimens. This indicates that there was a slow, continuous degradation of intact collagen from the mineralized dentin by ozone and NaOCl with insignificant differences, respectively. In addition, similarly, all oxidative irrigant groups influenced the inorganic phase (amide: Phosphate and carbonate: Phosphate ratios) of root dentin in the following order: Ozone, 5.25% NaOCl, 2.25% NaOCl.

The result of this paper is in agreement with the other previous studies of NaOCl that higher concentration associated with the oxidizing capacity had the highest effect on dentin degradation [3,7,19,22]. This result can also be explained by the knowledge that the thermal stability of collagen is reduced by the oxidants [22]. In addition, intrafibrillar and extrafibrillar apatite crystallites protect the collagen matrix from thermal denaturation but the apatite crystallites were unable to protect the collagen matrix from oxidative chemical degradation [7].

An important finding of the paper was that the different exposure time of each irrigant with the same concentration produced insignificantly different spectra analysis. This supports the findings of previous studies that the exposure times produced negligible differences of dentin deproteination [3,17,18,23]. These results report that the extent of deproteination of irrigants is not related to the exposure time.

The previous studies about dentin deproteination were focused on NaOCl in particular [3,7,17-19,23]. To our limited knowledge, this is the first study in which ozone application as an endodontic irrigant was evaluated on this subject. Based on the results of the study, ozone resulted in the most dentin deproteination thanks to its high oxidizing capacity.

Conclusion

Because the exposure time of irrigants does not produce a significant difference in extent of dentin deproteination, it is recommended to prolong the exposure time but not increase the concentration in order not to affect the dentin structure yet obtain better antimicrobial activity during root canal therapy. Further studies, especially those employing ozone, are required to develop safe usage limits for these materials.

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