**Effects of oxidative irrigants on root dentin structure: Attenuated total reflection Fourier transform infrared spectroscopy study.**

**ATABEKDidem1, BODUR Haluk 1, YALÇIN Gözde 1, KALAYCI Şükrü 2**

*1 DDS, PhD, Gazi University Faculty of Dentistry Department of Pediatric Dentistry, Ankara, TURKEY*

*2 PhD, Gazi University Faculty of Chemistry, Ankara, TURKEY*

**Corresponding Author:**

Didem ATABEK, PhD

Gazi University, Faculty of Dentistry,

Department of Pedodontics, Ankara/TURKEY,

E-mail: dtdidem@hotmail.com

Tel: +903122034088

Fax: +903122239226

**Effects of oxidative irrigants on root dentin structure: Attenuated total reflection Fourier transform infrared spectroscopy study.**

**Abstract**

**Aims:** The aim of this study was to compare the effect of oxidative irrigants on the collagen degradation of root canal dentin. **Methods:** Fifty human 2nd premolar roots were used in the study. The dentin specimens prepared from those teethwere 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 surfaces. 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). Insignificant differences were observed between ozone and 5.25% NaOCl-treated groups (p>0.05). **Conclusions:** The highest collagen degradation occurred in the ozone-treated samples because of the high oxidizing ability. The structural composition of human dentin is very much affected by the use of oxidative irrigants at higher concentrations.

**Key-words:** Ozone, NaOCl,FT-IR

**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 (4), 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 affectsdentin 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 (O3) 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 (1,13-17). 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 collagen degradation of root canal dentin.

**Material and Methods**

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 devoid of enamel, 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 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. 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 triplicatefor each solution concentration and time period (3, 7, 18-20).

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’s were statistically analyzed by using Kruskall-Wallis analysis of variance.

**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 v3 peak). Smaller ratios of amide:phosphate values correspondedto greater extent of dentin deproteination. The carbonate:phosphate ratio (the ratio of absorbance of carbonate v2 peak to phosphate v3 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 andaninsignificant 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 chemomechanical 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 (7,9,21).In the study, the effects of different irrigation regimes on the amide/phosphate, carbonate/phosphate, and apatite/collagen ratios of root dentin structure were analyzed by ATR FT-IR.

Based on its unique capacity todissolve necrotic tissue remnants, NaOCl remains the most widely recommended irrigant with concentrations ranging from 0.5% to 5.25% (22). 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 (7,23). 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 (1,7,16). Although the antibacterial efficiency of ozone has beenevaluated by previous studies (1,12-17), 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 (3,7,18-20).

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:phosphateand carbonate:phosphate ratios) of root dentinin the following order: Ozone, 5.25% NaOCl,2.25% NaOCl.

The result of this paper is in agreement with the other previous studies of NaOClthat higher concentration associated with the oxidizing capacity had the highest effect on dentin degradation (3,7,18,23). This result can also be explained by the knowledge that the thermal stability of collagen is reduced by the oxidants (23). 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, 19, 24). 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 (3,7,18,19,24). Toour limited knowledge, this is the first study in whichozone application as an endodontic irrigant was evaluated on this subject. Based on the results of the study, ozone resulted inthe most dentin deproteination thanksto its high oxidizing capacity.

**Conclusions**

\*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.

**References**

1. Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. An in vitro evaluation of the ability of ozone to kill a strain of Enterococcus faecalis. Int Endod J. 2005 Jan;38(1):22-9.
2. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol. 1983 Mar;55(3):307-12.
3. Hu X, Peng Y, Sum CP, Ling J. Effects of concentrations and exposure times of sodium hypochlorite on dentin deproteination: attenuated total reflection Fourier transform infrared spectroscopy study. J Endod. 2010 Dec;36(12):2008-11.
4. Spangberg L, Pascon EA. The importance of material preparation for the expression of cytotoxicity during in vitro evaluation of biomaterials. J Endod. 1988 May;14(5):247-50.
5. Busslinger A, Sener B, Barbakow F. Effects of sodium hypochlorite on nickel-titanium Lightspeed instruments. Int Endod J. 1998 Jul;31(4):290-4.
6. Kaufman AY, Keila S. Hypersensitivity to sodium hypochlorite. J Endod. 1989 May;15(5):224-6.
7. Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, Ling JQ, Pashley DH, Tay FR. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod. 2010 Jan;36(1):105-9.
8. Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int Endod J. 2001 Mar;34(2):113-9.
9. Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J. 2001 Mar;34(2):120-32.
10. Estrela C, Silva JA, de Alencar AH, Leles CR, Decurcio DA. Efficacy of sodium hypochlorite and chlorhexidine against Enterococcus faecalis--a systematic review. J Appl Oral Sci. 2008 Nov-Dec;16(6):364-8.
11. Tirali RE, Bodur H, Ece G. In vitro antimicrobial activity of sodium hypochlorite, chlorhexidine gluconate and octenidine dihydrochloride in elimination of microorganisms within dentinal tubules of primary and permanent teeth. Med Oral Patol Oral Cir Bucal. 2012 May 1;17(3):e517-22.
12. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dent. 2008 Feb;36(2):104-16.
13. Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, Welsch U, Miethke T, Brand K, Hickel R. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J. 2009 Jan;42(1):3-13.
14. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dent. 2008 Feb;36(2):104-16.
15. Baca P, Junco P, Arias-Moliz MT, González-Rodríguez MP, Ferrer-Luque CM. Residual and antimicrobial activity of final irrigation protocols on Enterococcus faecalis biofilm in dentin. J Endod. 2011 Mar;37(3):363-6.
16. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004 Nov;30(11):778-81.
17. Cardoso MG, de Oliveira LD, Koga-Ito CY, Jorge AO. Effectiveness of ozonated water on Candida albicans, Enterococcus faecalis, and endotoxins in root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Mar;105(3):e85-91.
18. Zhang K, Tay FR, Kim YK, Mitchell JK, Kim JR, Carrilho M, Pashley DH, Ling JQ. The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dent Mater. 2010 Jun;26(6):514-23.
19. Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: I. Demineralization. Biomaterials. 2001 Apr;22(8):787-92.
20. Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials. 2001 Apr;22(8):793-7.
21. Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine--mechanical, chemical and structural evaluation. Int Endod J. 2007 Oct;40(10):786-93.
22. Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389-98.
23. Komsa-Penkova R, Koynova R, Kostov G, Tenchov B. Discrete reduction of type I collagen thermal stability upon oxidation. Biophys Chem. 2000 Jan 24;83(3):185-95.
24. Mountouris G, Silikas N, Eliades G. Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin. J Adhes Dent. 2004 Autumn;6(3):175-82.

**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** | 14.659a,b,c  (±4.22) | 15.968a,b  (±4.62) | 12.856a,b  (±1.79) | 14.22a,b  (±2.46) | 12.548a,b  (±3.45) | 14.084a,b  (±1.50) | 6.146  (±1.22) | 6.766  (±1.02) | 6.41  (±0.22) | 6.43  (±0.12) |
| **Amide/**  **Phosphate** | 0.15A,B,C  (±0.32) | 0.124A,B,C  (±0.22) | 0.55A,B  (±0.22) | 0.238A,B  (±0.27) | 0.282A,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).