Tissue Dissolution Ability of Sodium Hypochlorite Activated by Photon-initiated Photoacoustic Streaming Technique

Mehmet Burak Guneser, DDS, PhD, * Dilara Arslan, DDS, PhD, * and Aslihan Usumez, DDS, PhD^{\dagger}

Abstract

Introduction: The aim of this study was to evaluate the effect of the photon-initiated photoacoustic streaming (PIPS) technique on the pulp tissue-dissolving capacity of sodium hypochlorite (NaOCl) and compare it with the EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK) and the Er:YAG laser with an endodontic fiber tip. Methods: Bovine pulp tissue samples (45 \pm 15 mg) and dentin powder (10 mg) were placed in 1.5-mL Eppendorf tubes with 1 mL 5.25% NaOCI (Wizard; Rehber Kimya, Istanbul, Turkey) or distilled water (control) for 5 minutes with activation by the EndoActivator System, the Er:YAG laser with an endodontic fiber tip, and the PIPS technique. Nonactivated NaOCI served as the positive control. All testing procedures were performed at room temperature. The tissue samples were weighed before and after treatment, and the percentage of weight loss was calculated. The differences were statistically analyzed. Results: The highest rate of tissue dissolution was observed in the NaOCI + Er:YAG group (P < .05). The NaOCI + PIPS group dissolved more bovine pulp tissue than the nonactivated NaOCl group (P < .05). There was no statistically significant difference between the rates of tissue dissolution of the NaOCl + EA and the nonactivated NaOCl groups (P > .05). Conclusions: NaOCI activation with the Er:YAG laser with an endodontic fiber tip was the most effective in bovine pulp tissue dissolution. The PIPS technique also promoted superior tissuedissolving effects when compared with no activation. However, the EndoActivator System had no direct effect on tissue dissolution. (J Endod 2015; ■:1-4)

Key Words

Dentin, EndoActivator, Er:YAG laser, photon-induced photoacoustic streaming, pulp tissue dissolution, sodium hypochlorite **S** uccess in endodontic treatment depends mainly on complete removal of the pulpal debridement and bacterial population from the root canal system by means of chemomechanical preparation (1). Sodium hypochlorite (NaOCl) remains the most recommended and popular irrigant for root canal treatment because it has a superior tissue-dissolving activity (2–7) and antimicrobial effect compared with most other irrigants used in endodontics (8, 9). However, the root canal system often has a very complex anatomy, with lateral canals, isthmuses, complex branching, and deltas making complete debridement and disinfection impossible (10). Thus, irrigant activation is suggested to increase the efficacy of irrigant delivery and improve root canal cleanliness (6, 7, 11–15).

The EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK) has been shown to safely clean the complex root canal system by sonic activation of the root canal irrigants with a flexible, noncutting polymer (16) that does not cause detectable canal transportation. On the other hand, it has been shown to have no effect on necrotic pulp tissue dissolution in simulated accessory canals (17).

Laser-activated irrigation has also been proposed as an alternative to the conventional debridement and disinfection procedures (11-14, 18). Among several laser devices, the Er:YAG laser is promising because of its cleaning mechanism within the root canal, which depends on rapid fluid motion caused by expansion and implosion of laser-induced bubbles (13). The Er:YAG laser with an endodontic fiber tip effectively removes the smear layer and intracanal debris (19), especially on the apical thirds, without causing any structural damage or anatomic alteration inside the root canal or periodontal tissues (20).

Recently, photon-initiated photoacoustic streaming (PIPS), a light energy phenomenon, has been introduced to improve irrigation. The PIPS technique differs from other agitation techniques in that only the tip is placed into the pulp chamber, thereby preventing contact with the root canal wall (12, 21). This technique is attributed to photoacoustic and photomechanical activities, which make it different than other techniques. In this method, an Er:YAG laser is used with a newly designed tapered tip with a radial firing end and 3 mm of the polyamide sheath. When activated in a limited volume of irrigant, the high absorption of the Er:YAG wavelength in water, combined with the high peak power achieved from using subablative parameters (0.3 W, 20 mJ at 15 Hz), results in a photomechanical phenomenon. The strong photoacoustic shock wave promotes 3-dimensional movement of the irrigation solutions (21). Therefore, the PIPS technique shows better root canal debridement than conventional irrigation modalities (11, 12). Peeters and Mooduto (22) reported that using a plain fiber tip in the coronal portion can drive the irrigation solution to the end of the canal without any harmful effects on the apical tissues. In another study, PIPS was more effective in the removal of antibiotic pastes from the root canal compared with the EndoActivator System (23). At present, however, data on its organic tissuedissolving capacity are lacking. Therefore, the aim of the present study was to compare the effectiveness of the EndoActivator System, the Er:YAG laser with an endodontic fiber tip, and the PIPS technique on the pulp tissue-dissolving capacity of NaOCl.

Materials and Methods Bovine Pulp Tissue Preparation

Eighty intact, freshly extracted, young bovine maxillary central incisors were used. This investigation was not classified as an animal study because our work had no

From the Departments of *Endodontics and [†]Prosthodontics, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey.

Address requests for reprints to Dr Mehmet Burak Guneser, Bezmialem Vakif University, İstanbul, Turkey. E-mail address: bguneser@hotmail.com

^{0099-2399/\$ -} see front matter

Copyright © 2015 American Association of Endodontists. http://dx.doi.org/10.1016/j.joen.2015.01.014

Basic Research—Technology

influence on the premortal fate of the animals or the slaughtering process. The teeth were extracted within 24 hours after slaughter and immediately placed in glass vials with distilled water. Two longitudinal grooves were cut on the proximal surfaces of teeth with a diamond bur (MANI Inc, Tochigi, Japan), and the teeth were then split in half. Pulp tissue was removed carefully with a cotton plier, washed with distilled water to remove excess blood, and then blotted dry. All pulps were combined to create a random mix of tissue. The pulp tissue samples were adjusted to similar weights of 45 \pm 15 mg each with a no. 15 surgical blade.

Dentin Powder

Dentin powder was obtained using spherical dental burs #4 (MANI Inc) inside the root canals of previously split bovine teeth without water coolant and in a low-speed handpiece. All dentin powder was stored in plastic flasks.

NaOCI Solution

A stock solution of 5.25% NaOCl solution (Wizard; Rehber Kimya, Istanbul, Turkey) was tested. The pH of the solution was measured using a pH meter (HI 2211 pH-ORP Meter; HANNA Instruments, Woonsocket, RI) at room temperature (21°C) and adapted to a pH of 12 with 1 N HCl. The amount of final active chlorine content was also verified just before starting each test using an iodine/thiosulfate titration method as previously described (24).

Experiment

The initial weight of each pulp sample was measured with a precision balance (ME204; Mettler-Toledo, Columbus, OH). After the weights were recorded, the pulp samples were randomly divided into 4 experimental groups (n = 10) and 4 control groups (n = 10). The samples were then individually placed in 1.5-mL Eppendorf tubes (volume = 1.5 mL, diameter = 2.5 mm, taper = 4%, length = 25 mm).

The experimental groups (n = 10) were as follows:

- 1. 5.25% NaOCl + EndoActivator System activation (NaOCl + EA)
- 2. 5.25% NaOCl + Er:YAG laser with an endodontic fiber tip activation (NaOCl + Er:YAG)
- 3. 5.25% NaOCl + PIPS activation (NaOCl + PIPS)
- 5.25% NaOCl + no activation (nonactivated NaOCl, positive control group)

The negative control groups were as follows (n = 10):

- 1. Distilled water + EndoActivator System activation (distilled water + EA)
- 2. Distilled water + Er:YAG laser with an endodontic fiber tip activation (distilled water + Er:YAG)
- 3. Distilled water + PIPS activation (distilled water + PIPS)
- 4. Distilled water + no activation (nonactivated distilled water)

One milliliter 5.25% NaOCl solution and 10 mg dentin powder were added to tubes containing tissue samples for the experimental groups. For negative control groups, 40 Eppendorf tubes were prepared with an additional 1 mL distilled water and 10 mg dentin powder. All testing procedures were performed at room temperature.

The EndoActivator System Activation. The EndoActivator System was used for passive sonic activation of 5.25% NaOCl. It was performed using the EndoActivator handpiece set at 10,000 cycles per minute with a medium polymer tip (#25/.04).

Er:YAG Laser with an Endodontic Fiber Tip Activation. Er:-YAG laser activation was performed with a wavelength of 2,940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia) and an R14 handpiece with a $300-\mu$ m endodontic fiber tip (Preciso, Fotona). The fiber tip was used with an output power of 1 W, energy of 50 mJ, and a frequency of 20 Hz as specified by the manufacturer. The water and air on the laser system were turned off.

PIPS Activation. The PIPS protocol was performed with an Er:YAG laser with a wavelength of 2.940 nm (Fidelis AT). A 12-mm-long, 400- μ m quartz tip was tapered and had 3 mm of the polyamide sheath stripped back from its end. The tip was applied with 0.3 W, 15 Hz, and 20 mJ per pulse as specified by the manufacturer without water/air spray.

For all devices tested, the tips were immersed in Eppendorf tubes containing irrigating solutions throughout their working length. All samples were activated for 30 seconds, with resting times of 45 seconds after activation. The application was repeated 4 times. In between these activation procedures, the 5.25% NaOCl solution and distilled water in each Eppendorf tube were removed, and 1 mL fresh solution was added. Fresh dentin powder was also added to Eppendorf tubes for each irrigant application. Consequently, the total solution exposure time was 5 minutes with a total volume of 4 mL irrigant and 40 mg dentin powder for each sample in all groups.

After an exposure time of 5 minutes, the pulp samples were removed and washed with distilled water to remove dissolved/suspended tissue remnants or dentin powder. The samples were blotted dry and weighed again. The difference in weights of the tissue sample before and after exposure to 5.25% NaOCl solution or distilled water was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss. The data were then analyzed statistically using 1-way analysis of variance and Tukey post hoc tests with a 95% confidence level (P = .05).

Results

The comparison of the rates of tissue dissolution for all groups with 5.25% NaOCl and distilled water is shown in Table 1. Because no pulp tissue dissolution was observed in any negative control groups with distilled water, statistical analysis was applied only to the NaOCl groups. One-way analysis of variance showed statistically significant differences between the NaOCl groups (P < .05). The highest rate of tissue dissolution was obtained with the NaOCl + Er:YAG group (P < .05). The NaOCl + PIPS group dissolved more bovine pulp tissue than the nonactivated NaOCl group (P < .05), whereas there was no statistically

TABLE 1. Effect of 3 Different Methods of Activation on Tissue Dissolution (% Tissue Weight Loss \pm Standard Deviation) by the 5.25% NaOCI Solution and DistilledWater

	EndoActivator	Er:YAG	PIPS	No activation
5.25% NaOCl Distilled water	$\begin{array}{c} 47.77 \pm 8.17^{bc} \\ 0.17 \pm 1.08 \end{array}$	$\begin{array}{c} \textbf{71.59} \pm \textbf{6.95}^{\texttt{a}} \\ \textbf{0.42} \pm \textbf{0.83} \end{array}$	$\begin{array}{c} 57.29 \pm 14.41^{b} \\ 0.36 \pm 0.32 \end{array}$	$\begin{array}{c} \textbf{43.41} \pm \textbf{8.31^c} \\ -1.12 \pm 1.65 \end{array}$

NaOCl, sodium hypochlorite; PIPS, photon-initiated photoacoustic streaming.

Groups identified by different superscript letters are significantly different (P < .05). Groups identified by the same superscript letters are not significantly different (P > .05).

significant difference between the rates of tissue dissolution of the NaOCl + EA group and the nonactivated NaOCl group (P > .05).

Discussion

In the current study, the dissolution capacities of the irrigant activation methods were evaluated using a test tube model (2-4, 6) with dentin powder (25), as has been done in many studies. This type of experimental design cannot exactly reflect the clinical conditions; however, it did allow comparative quantitative evaluations regarding hypochlorite activation that are difficult to achieve in natural teeth. The standardization of pulp tissue samples and irrigants prevented the confounding factors related to solution concentration, pH, temperature, volume, mass, and tissue surface area, which are known to influence the results of tissue dissolution studies (3, 6). In addition, the irrigant was regularly exchanged for fresh solution throughout the experiment to simulate the repeated use of irrigant after each file.

This study was based on bovine tissues for ethical reasons and because of the unavailability of human teeth (25). It is also difficult to obtain standardized human pulp tissue samples (3). Bovine pulp tissue is more similar to human pulp than other animal tissues and has been used in previous studies to evaluate the dissolution capacities of endodontic irrigants (2, 7, 17, 24).

It is well-known that dentin has a considerable buffering effect against acid and alkali materials (26) and thereby reduces the tissue dissolution capacity of NaOCI (5). The small particles of dentin powder allow the use of standardized quantities and greater control of the mixture with the irrigants (25). Furthermore, it has been reported that the chemical composition and pH of bovine dentin are not different from human dentin (27). Thus, bovine dentin powder was obtained from root canal walls and added to all test groups to reflect the clinical conditions.

The distance between the tips of tested devices and the pulp samples that could alter the tissue dissolution capabilities (6) was 1 of the limitations of this study. For all devices tested, each tip could be immersed to the depth of its entire length without touching the pulp samples. Therefore, the distance was not the same for all groups because of the different lengths of the tips used. For example, the distance between the end of the PIPS tip and the pulp sample in the test tube was more than the distance of the other tips tested. Nevertheless, it is recommended to place PIPS only into the coronal access opening of the pulp chamber; therefore, the experimental design may somewhat reflect the clinical conditions even in test tubes.

The EndoActivator System is a safe sonic activation method because of its plastic tip against root canal transportation. However, NaOCl + EndoActivator System activation did not significantly improve the bovine pulp tissue dissolution compared with the nonactivated NaOCl irrigation. This is consistent with a recent study (7) that reported that NaOCl solution activated with the EndoActivator System had no effect on tissue dissolution. Al-Jadaa et al (7) suggest 2 possible explanations for the unsatisfactory results with sonic activation:

- The wavelength of a sonic setup is too long to induce sufficient streaming of the irrigant
- 2. The sonic energy is too low to activate the irrigant

Thus, we may recommend that the insufficient improvement with sonic activation of NaOCl irrigation for pulp tissue dissolution should be taken into consideration when attempting to dissolve the residual pulp tissue in anatomically complex teeth, such as dens invaginatus, C-shaped molars, and teeth with internal resorption.

Based on our results, the tissue dissolution rate of NaOCl + Er:-YAG laser with an endodontic fiber tip activation was superior to the

Basic Research—Technology

NaOCl + PIPS or NaOCl + EndoActivator System activation methods. The Er:YAG laser has bactericidal activity (28) and the ability to remove the smear layer and dentin debris (19) with minimal side effects in root canals (20). Although the laser is usually used to clean the root canal system after conventional root canal instrumentation, laser systems can also be applied to dissolve the residual pulp tissue remnants (29), especially in areas in which instruments cannot reach, even in well-shaped canals (30). To date, there has been only 1 study evaluating the effect of Er:YAG laser-activated NaOCl irrigant on tissue dissolution. Kuhn et al (29) found that Er:YAG laser activation of NaOCl revealed highly effective soft tissue dissolution, as is consistent with our results. This may be because of the ability of the laser-activated irrigation to create collapse shock waves and rapid streaming caused by laser-induced vapor and bubbles within the irrigant (12, 13). Moreover, our findings suggest that the Er:YAG laser itself has no effect on pulp tissue because the tissue was not dissolved in distilled water activated with the laser. Therefore, the improved tissue dissolution of the Er:YAG laser in conjunction with NaOCl irrigation may have been achieved directly by the activated NaOCI irrigant.

In the PIPS technique at low power, each impulse interacts with the water molecules, creating expansion and profound shock waves that induce the formation of a powerful streaming fluid with no thermal damage on the root surfaces (21). The vapor bubble begins to expand and produce a void in front of the laser light (13) and may increase the efficacy of the tissue dissolution ability of the irrigant. However, in the present study, PIPS did promote pulp tissue dissolution but significantly less so than the Er:YAG laser with an endodontic fiber tip. Similarly, Deleu et al (14) compared the laser-activated irrigation methods on the removal of the smear layer. They showed that the Er:YAG laser was more efficient than PIPS. It has also been shown that smaller fiber diameters and higher pulse energies produce higher fluid flow and might enhance the formation of vapor or a cavity that contains bubbles inside the irrigant (14). Hence, the higher tissue dissolution ability of the Er:YAG laser in this study may be explained by the use of higher pulse energy (50 mJ) and a smaller fiber diameter (300 μ m) compared with the parameters and tips of the PIPS technique. Consequently, the cleaning mechanism of PIPS is not vet clarified; therefore, because there are limited data in the literature, our results should be confirmed in future studies.

Conclusions

The results from this *in vitro* study indicate that NaOCl activation with the Er:YAG laser with an endodontic fiber tip was the most effective in bovine pulp tissue dissolution. The tissue dissolution effect of NaOCl could also be enhanced by PIPS when compared with no activation. However, the EndoActivator System had no direct effect on tissue dissolution.

Acknowledgments

We would like to thank Dr Pinar Cebe for supporting the laser tips.

The authors deny any conflicts of interest related to this study.

References

- Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291–301.
- Cobankara FK, Ozkan HB, Terlemez A. Comparison of organic tissue dissolution capacities of sodium hypochlorite and chlorine dioxide. J Endod 2010;36:272–4.
- Stojicic S, Zivkovic S, Qian W, et al. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod 2010;36: 1558–62.

Basic Research—Technology

- Rossi-Fedele G, Steier L, Dogramaci EJ, et al. Bovine pulp tissue dissolution ability of HealOzone®, Aquatine Alpha Electrolyte® and sodium hypochlorite. Aust Endod J 2013;39:57–61.
- Slutzky-Goldberg I, Hanut A, Matalon S, et al. The effect of dentin on the pulp tissue dissolution capacity of sodium hypochlorite and calcium hydroxide. J Endod 2013; 39:980–3.
- Haapasalo M, Wang Z, Shen Y, et al. Tissue dissolution by a novel multisonic ultracleaning system and sodium hypochlorite. J Endod 2014;40:1178–81.
- Al-Jadaa A, Paqué F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod 2009;35:1408–11.
- Morgental RD, Singh A, Sappal H, et al. Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants. J Endod 2013;39:406–10.
- 9. Zehnder M. Root canal irrigants. J Endod 2006;32:389-98.
- Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms. Int Endod J 2002;35:660–7.
- DiVito E, Peters OA, Olivi G. Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. Lasers Med Sci 2012;27:273–80.
- Peeters HH, Suardita K. Efficacy of smear layer removal at the root tip by using ethylenediaminetetraacetic acid and erbium, chromium: yttrium, scandium, gallium garnet laser. J Endod 2011;37:1585–9.
- Matsumoto H, Yoshimine Y, Akamine A. Visualization of irrigant flow and cavitation induced by Er:YAG laser within a root canal model. J Endod 2011;37:839–43.
- Deleu E, Meire MA, De Moor RJ. Efficacy of laser-based irrigant activation methods in removing debris from simulated root canal irregularities. Lasers Med Sci 2015; 30:831–5.
- Berkhoff JA, Chen PB, Teixeira FB, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod 2014;40:1172–7.
- Ruddle CJ. Hydrodynamic disinfection: tsunami endodontics. Dent Today 2007;26: 110. 112, 114–7.
- Al-Jadaa A, Paqué F, Attin T, Zehnder M. Necrotic pulp tissue dissolution by passive ultrasonic irrigation in simulated accessory canals: impact of canal location and angulation. Int Endod J 2009;42:59–65.

- Blanken J, De Moor RJ, Meire M, Verdaasdonk R. Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 1: a visualization study. Lasers Surg Med 2009;41:514–9.
- De Moor RJ, Meire M, Goharkhay K, et al. Efficacy of ultrasonic versus laseractivated irrigation to remove artificially placed dentin debris plugs. J Endod 2010;36:1580–3.
- Guidotti R, Merigo E, Fornaini C, et al. Er:YAG 2,940-nm laser fiber in endodontic treatment: a help in removing smear layer. Lasers Med Sci 2014;29:69–75.
- DiVito E, Lloyd A. ER: YAG laser for 3-dimensional debridement of canal systems: use of photon-induced photoacoustic streaming. Dent Today 2012;31:122. 124–7.
- Peeters HH, Mooduto L. Radiographic examination of apical extrusion of root canal irrigants during cavitation induced by Er,Cr:YSGG laser irradiation: an in vivo study. Clin Oral Investig 2013;17:2105–12.
- Arslan H, Akcay M, Capar ID, et al. Efficacy of needle irrigation, EndoActivator, and photon-initiated photoacoustic streaming technique on removal of double and triple antibiotic pastes. J Endod 2014;40:1439–42.
- Macedo RG, Wesselink PR, Zaccheo F, et al. Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. Int Endod J 2010;43:1108–15.
- Freire LG, Carvalho CN, Ferrari PH, et al. Influence of dentin on pH of 2% chlorhexidine gel and calcium hydroxide alone or in combination. Dent Traumatol 2010;26: 276–80.
- Wang JD, Hume WR. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. Int Endod J 1988;21:17–26.
- Camargo CH, Bernardineli N, Valera MC, et al. Vehicle influence on calcium hydroxide pastes diffusion in human and bovine teeth. Dent Traumatol 2006;22:302–6.
- Noiri Y, Katsumoto T, Azakami H, Ebisu S. Effects of Er:YAG laser irradiation on biofilm-forming bacteria associated with endodontic pathogens in vitro. J Endod 2008;34:826–9.
- Kuhn K, Rudolph H, Luthardt RG, et al. Er:YAG laser activation of sodium hypochlorite for root canal soft tissue dissolution. Lasers Surg Med 2013;45:339–44.
- **30.** Davis SR, Brayton SM, Goldman M. The morphology of the prepared root canal: a study utilizing injectable silicone. Oral Surg Oral Med Oral Pathol 1972;34: 642–8.