

Tissue Dissolution by Sodium Hypochlorite: Effect of Concentration, Temperature, Agitation, and Surfactant

Sonja Stojicic, DDS, MSc,^{*†} Slavoljub Zivkovic, DDS, PhD,[†] Wei Qian, DDS, PhD,^{*}
Hui Zhang, DDS, PhD,^{*} and Markus Haapasalo, DDS, PhD^{*}

Abstract

Aim: Sodium hypochlorite is the most commonly used endodontic irrigant because of its antimicrobial and tissue-dissolving activity. The aim of this study was to evaluate and compare the effects of concentration, temperature, and agitation on the tissue-dissolving ability of sodium hypochlorite. In addition, a hypochlorite product with added surface active agent was compared with conventional hypochlorite solutions. **Methods:** Three sodium hypochlorite solutions from two different manufacturers in concentrations of 1%, 2%, 4%, and 5.8% were tested at room temperature, 37°C, and 45°C with and without agitation by ultrasonic and sonic energy and pipetting. Distilled and sterilized tap water was used as controls. Pieces of bovine muscle tissue (68 ± 3 mg) were placed in 10 mL of each solution for five minutes. In selected samples, agitation was performed for one, two, or four 15-second periods per each minute. The tissue specimens were weighed before and after treatment, and the percentage of weight loss was calculated. The contact angle on dentin of the three solutions at concentrations of 1% and 5.8% was measured. **Results:** Weight loss (dissolution) of the tissue increased almost linearly with the concentration of sodium hypochlorite. Higher temperatures and agitation considerably enhanced the efficacy of sodium hypochlorite. The effect of agitation on tissue dissolution was greater than that of temperature; continuous agitation resulted in the fastest tissue dissolution. Hypochlorite with added surface active agent had the lowest contact angle on dentin and was most effective in tissue dissolution in all experimental situations. **Conclusions:** Optimizing the concentration, temperature, flow, and surface tension can improve the tissue-dissolving effectiveness of hypochlorite even 50-fold. (*J Endod* 2010; ■:1–5)

From the *Division of Endodontics, Department of Oral Biological & Medical Sciences, Faculty of Dentistry, University of British Columbia, Vancouver, Canada; and †Department for Restorative Dentistry and Endodontics, Faculty of Dentistry, University of Belgrade, Belgrade, Serbia.

Address requests for reprints to Dr Markus Haapasalo, Division of Endodontics, Oral Biological & Medical Sciences, UBC Faculty of Dentistry, 2199 Wesbrook Mall, Vancouver, BC, Canada V6T 1Z3. E-mail address: markush@interchange.ubc.ca. 0099-2399/\$0 - see front matter

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Key Words

Agitation, Chlor-Xtra, sodium hypochlorite, surfactant, temperature, tissue dissolution

Success in endodontic treatment depends to a great extent on chemomechanical debridement of the canals. Although instruments remove most of the canal contents in the main root canal area, irrigation plays an indispensable role in all areas of the root canal system, in particular those parts that are inaccessible for instrumentation (1). The most favorable features of irrigants are their flushing action, tissue-dissolving ability, antimicrobial effect, and low toxicity (2, 3). Sodium hypochlorite is the most commonly used endodontic irrigant because of its well-known antimicrobial and tissue-dissolving activity (4–6).

The dissolving capability of sodium hypochlorite relies on its concentration, volume, and contact time of the solution but also on the surface area of the exposed tissue (7). However, high concentrations are potentially toxic for periapical tissue (8–10). Also, changes in mechanical properties such as decreased microhardness and increased roughness of radicular dentin have been reported after exposure to sodium hypochlorite in concentrations of 2.5% and 5.25% (11).

Possible ways to improve the efficacy of hypochlorite preparations in tissue dissolution are increasing the pH (12) and the temperature of the solutions, ultrasonic activation, and prolonged working time (13). Although there is a general consensus that increased temperature enhances the effectiveness of hypochlorite solutions, there are only a few published articles about this (14–16). It has been suggested that preheating low-concentration solutions improves their tissue-dissolving capacity with no effect on their short-term stability. Also, systemic toxicity is lower compared with the higher-concentration solutions (at a lower temperature) with the same efficacy (15). The impact of mechanical agitation of the hypochlorite solutions on tissue dissolution was found to be very important by Moorer and Wesselink (7) who emphasized the great impact of violent fluid flow and shearing forces caused by ultrasound on the ability of hypochlorite to dissolve tissue. However, the mechanisms involved are not completely understood (13). Despite several separate reports of the various ways to improve the effectiveness of tissue dissolution by sodium hypochlorite, the relative importance of temperature, concentration, and agitation remains unclear. In the present study, all these factors were examined under controlled conditions to allow comparison of their role. Finally, a hypochlorite product with an added surface active agent was compared with conventional products in the different experimental settings.

Materials and Methods

Solutions

Sodium hypochlorite solutions in concentrations of 1%, 2%, 4%, and 5.8% were tested. Stock solution of 6% sodium hypochlorite (Regular 1; EMD Chemicals Inc, Gibbstown, NJ) and 5.8% sodium hypochlorite (Regular 2; Inter-Med, Inc/Vista Dental Products, Racine, WI) were obtained from the manufacturers. Two different hypochlorite products with 5.8% sodium hypochlorite were included in the experiments: one conventional solution (Regular 2, Vista-Dental) and one with a surface active agent added (Chlor-Xtra, Vista-Dental). The amount of available chlorine was obtained by the manufacturers. The solutions were kept at 4°C following the recommendations of the manufacturer and brought to room temperature (RT) before use. One percent,

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2%, 4%, and 5.8% solutions of sodium hypochlorite were prepared by diluting the stock solution in distilled water. Distilled and sterilized tap water was used as controls.

Dissolution of Tissue

Bovine meat was used as a tissue sample in the experiment. It was kept frozen at -15°C in 100% humidity. Frozen tissue was cut into pieces of $4 \times 4 \times 2$ mm using stainless steel blade. Because the surface area has a great impact on the tissue dissolution, each sample had a similar size and shape. The samples had an original weight of 68 ± 3 mg with no significant difference between groups.

The experiments were done at RT and at 37°C and 45°C . A water bath (Water Bath Digital 10L; Fisher Scientific, Ottawa, Ontario, Canada) was used for the experiments at 37°C and 45°C . The temperature of the solutions was confirmed using a thermometer (Fisher Scientific).

Three different means of agitation were tested: ultrasonic, sonic, and pipetting. An ultrasonic system (Varios Lux 350; NSK, Japan) with a slender steel tip (E 7) at power setting 4 was used to deliver ultrasonic energy. Sonic vibration at 10,000 cpm was applied by EndoActivator (Dentsply, Tulsa, OK) using a flexible polymer tip size 25/04 (medium). The tips (ultrasonic and sonic) were immersed in the hypochlorite solutions into a depth of 10 mm, 5 mm away from the tissue specimens, without touching them. A transfer pipette (grad, 5.8 mL, Fisher Scientific) was used for mechanical agitation of the hypochlorite solutions; the tip of the pipette was at 5 mm distance from the tissue specimen. Agitation was performed 15 seconds per each minute during the 5-minute incubation period. The three hypochlorite solutions in selected concentrations were tested at RT and at 45°C with and without agitation. In addition, the effect of duration of agitation by pipetting was studied using the 2% and 5.8% solutions of the Regular 2 hypochlorite solution (Vista-Dental) at RT as follows: the tissue specimens were treated for 5 minutes in the hypochlorite solutions, pipetting was done as above either for one or two 15-second periods per each minute, or continuously throughout the 5-minute experiment.

TABLE 1. Tissue Weight Loss ($\% \pm$ Standard Deviation) After 5 Minutes of Exposure to Three Sodium Hypochlorite Products at Different Concentrations and Temperatures

% of weight loss	RT	37°C	45°C
Distilled water (control)	-0.87 ± 1.59	1.40 ± 2.33	10.91 ± 1.71
Sterilized water (control)	-2.26 ± 1.35	2.36 ± 1.14	8.26 ± 1.86
1%			
Regular 1	-3.21 ± 1.85	-0.27 ± 2.23	1.16 ± 1.92
Regular 2	-5.52 ± 1.38	-0.29 ± 1.24	1.72 ± 1.87
Chlor-Xtra	-6.51 ± 3.05	1.19 ± 1.26	4.74 ± 2.12
2%			
Regular 1	4.41 ± 1.79	$10.72 \pm 2.30^{\dagger}$	14.61 ± 5.27
Regular 2	3.95 ± 2.04	$8.25 \pm 2.30^{*}$	14.18 ± 3.45
Chlor-Xtra	$7.75 \pm 2.08^{\dagger}$	$13.07 \pm 0.80^{\dagger}$	$21.10 \pm 2.16^{*,\S}$
4%			
Regular 1	$20.52 \pm 3.00^{\dagger}$	$26.63 \pm 3.13^{\dagger}$	$36.26 \pm 2.95^{\dagger}$
Regular 2	$18.13 \pm 2.22^{\dagger}$	$24.93 \pm 1.32^{\dagger}$	$33.61 \pm 3.58^{\dagger}$
Chlor-Xtra	$28.19 \pm 3.27^{*,\dagger}$	$37.90 \pm 5.77^{*,\parallel}$	$49.17 \pm 5.20^{*,\parallel}$
5.8%			
Regular 1	$29.93 \pm 2.24^{\dagger}$	$40.68 \pm 2.64^{\dagger}$	$49.10 \pm 6.52^{\dagger}$
Regular 2	$27.28 \pm 4.47^{\dagger}$	$38.31 \pm 3.29^{\dagger}$	$48.18 \pm 4.45^{\dagger}$
Chlor-Xtra	$41.55 \pm 5.22^{*,\dagger}$	$59.05 \pm 6.20^{*,\parallel}$	$67.28 \pm 8.80^{*,\parallel}$

RT, room temperature; regular 1, sodium hypochlorite (EMD Chemicals Inc); regular 2, sodium hypochlorite (Inter-Med, Inc./Vista Dental Products); Chlor-Xtra, sodium hypochlorite with added surface active agent (Inter-Med, Inc./Vista Dental Products).

* $p < 0.05$.

$^{\dagger}p < 0.001$ versus distilled water.

$^{\ddagger}p < 0.05$.

$^{\S}p < 0.01$.

$^{\parallel}p < 0.001$ versus regular 1.

A&D Company, Ltd, Tokyo, Japan) before the hypochlorite treatments and placed each in 10 mL of preheated hypochlorite solution in separate beakers in the temperature-controlled water bath. Five parallel samples per group were included in all experiments. After 5 minutes in the solution, the samples were blotted dry and weighed again. The percentage of weight loss was calculated.

Contact Angle Measurement

Extracted human maxillary canine and mandibular premolars were used to prepare the dentin surfaces for contact angle measurements. After cutting off the crown and apical third of the root, each tooth was split in half labiopalatally using a low-speed diamond saw. Each cut surface was polished using a series of abrasive papers (CarbiMet; Buehler, Lake Bluff, IL) in the following sequence (120/P120, 180/P180, 240/P280, 320/P400, 400/P800, and 600/P1200). A 1.5- μL droplet of 1% and 5.8% hypochlorite solutions or distilled water (control) was placed on coronal root dentin using a 2- μL pipette. The contact angle was measured within 30 seconds using a NRL Contact Angle Goniometer (Ramé-hart, Netcong, NJ). Six parallel measurements were performed with each solution on dentin surfaces of both teeth.

Data Analysis

Weight loss was expressed as mean value \pm standard deviation of the percentage of the tissue weight loss. Data were analyzed using one-way analysis of variance followed by the Tukey post hoc test for multiple comparisons (SPSS Inc, Chicago, IL). Statistical significance was considered at $p < 0.05$.

Results

Tissue weight loss after 5 minutes in different concentrations of sodium hypochlorite at three different temperatures is shown in Table 1. Weight loss increased with increasing concentrations of sodium hypochlorite. A significant difference in weight loss was observed after exposure to 2% Chlor-Xtra and 4% and 5.8% ($p <$

TABLE 2. Effect of Three Different Methods of Agitation on Tissue Dissolution (% Tissue Weight Loss \pm Standard Deviation) by the Three Hypochlorite Products

	45°C						
	RT			45°C			
	No agitation	US	EA	P	US	EA	P
Distilled water	-0.87 \pm 1.59	2.35 \pm 1.26	1.86 \pm 1.33	2.18 \pm 1.44	10.50 \pm 4.38	10.90 \pm 3.71	11.91 \pm 2.39
Sterilized tap water	-2.26 \pm 1.35	1.78 \pm 1.19	2.67 \pm 2.08	1.48 \pm 1.06	10.03 \pm 3.55	8.58 \pm 2.06	11.04 \pm 1.74
2%							
Reg 1	4.41 \pm 1.79	11.76 \pm 1.95 [†]	12.76 \pm 1.63 [†]	20.56 \pm 1.67 [†]	29.85 \pm 2.89 [†]	29.01 \pm 1.05 [†]	37.31 \pm 2.25 [†]
Reg 2	3.95 \pm 2.04	11.39 \pm 3.98 [†]	12.83 \pm 2.47 [†]	17.80 \pm 0.85 [†]	29.15 \pm 3.66 [†]	26.07 \pm 1.06 [†]	33.95 \pm 2.37 [†]
Chlor-Xtra	7.75 \pm 2.08	16.96 \pm 3.28 [†]	16.25 \pm 0.88 [†]	20.65 \pm 1.68 [†]	38.37 \pm 3.72 [†]	35.17 \pm 5.47 [†]	40.90 \pm 1.80 [†]
5.8%							
Reg 1	29.93 \pm 2.24	36.28 \pm 3.89 [*]	34.19 \pm 3.52	35.94 \pm 3.02 [*]	74.99 \pm 7.17 [†]	69.96 \pm 4.78 [†]	73.00 \pm 4.12 [†]
Reg 2	27.28 \pm 4.47	34.00 \pm 3.41	32.76 \pm 3.38	39.16 \pm 3.60 [†]	75.51 \pm 7.96 [†]	68.82 \pm 7.09 [†]	71.68 \pm 5.90 [†]
Chlor-Xtra	41.55 \pm 5.22	48.03 \pm 6.48 [§]	47.76 \pm 6.14 [§]	48.91 \pm 7.62 [§]	81.38 \pm 2.88 [*]	78.26 \pm 2.98 [*]	87.93 \pm 7.13 ^{†,§}

US, ultrasound; EA, endoactivator; P, pipetting.

* $p < 0.05$.† $p < 0.01$.‡ $p < 0.001$ versus no agitation.§ $p < 0.001$ versus regular 1.

with controls at RT. Tissue specimens immersed in 1% sodium hypochlorite at RT increased in weight after 5 minutes.

Heating the hypochlorite solutions greatly increased tissue dissolution; the magnitude of the increase compared with RT varied from 30% to 300% depending on the concentration, temperature, and type of hypochlorite (Table 1). Of the three solutions, the one with added surface active agent dissolved significantly more tissue than the two other solutions in all temperature/concentration groups (Table 1).

The effect of the three different methods of agitation on the tissue-dissolving ability of 2% and 5.8% sodium hypochlorite is shown in Table 2. The tissue weight loss was significantly higher at both tested temperatures when sodium hypochlorite solutions were agitated than without agitation ($p < 0.001$ for 2% and $p < 0.01$ for 5.8% sodium hypochlorite, Table 2). Under agitation at RT, tissue weight loss was significantly higher with 5.8% Chlor-Xtra than with both 5.8% regular hypochlorite products (Table 2). Agitation experiments with an increased time of active agitation from 25% to 100% showed continuous increase in tissue dissolution as the relative time of agitation increased (Table 3). The increase of tissue dissolution from no agitation to 100% agitation (pipette) in Regular 2 hypochlorite at RT was 12.7-fold with 2% and 2.1-fold with 5.8% solution. Tissue weight loss was significantly higher after simultaneous action of temperature and agitation than by either one alone (Tables 1-3). Chlor-Xtra (5.8%) had the lowest contact angle of the three hypochlorite solutions. There was no significant difference in contact angle between the 1% solutions ($p > 0.05$) (Table 4).

Discussion

A great number of studies have focused on the tissue-dissolving ability of sodium hypochlorite. It has been found that the solvent capability of sodium hypochlorite depends on its concentration; time; volume; pH; temperature; agitation; and the type, amount, and surface area of the tissue (2, 5, 7, 12). However, great variations among these factors contribute to the difficulty of making comparisons between different studies and the relative importance of each factor (17). The present study evaluated the effect of concentration, temperature, and agitation on sodium hypochlorite ability to dissolve organic material in a standardized setting. Tissues from a number of different sources have been used in studies about sodium hypochlorite tissue-dissolving ability (6). Porcine muscle tissue (1, 12), rabbit liver (7), rat connective tissue (5), pig palatal mucosa (18), bovine muscle tissue (2), and bovine pulp (3) have been used to determine dissolution ability of different irrigants. The reasons for using different tissue instead of dental pulp have been availability and easier standardization of the surface area of each specimen (2). Tissue specimens used in the present study were prepared from bovine muscle tissue with a standardized weight of 68 ± 3 mg. The meat specimens were cubical in shape ($4 \times 4 \times 2$ mm) giving an equal surface area. Pilot experiments had shown that it was difficult to determine the endpoint of complete dissolution of the tissue because of a great number of bubbles (result of saponification reaction); therefore, fixed time was used instead, and the samples were weighed before and after exposure. Other methods have used different approaches (eg, measuring the changes in the solutions, such as the amount of available chlorine in the solution after completed dissolution [7] or the amount of hydroxyproline in the residual tissue after incubation with the solution [19]).

Previous studies have shown that the tissue-dissolving ability of sodium hypochlorite solution decreases if it is diluted (2, 5, 16). Results from the present study showed that 5.8% sodium

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TABLE 3. Effect of Agitation by Pipetting for 0%, 25%, 50%, and 100% of 5-minute Exposure Time on Tissue Dissolution (% \pm Standard Deviation) by 2% and 5.8% Regular (Reg 2) Hypochlorite at Room Temperature

Solutions	Agitation/no. of agitation per each minute			
	No agitation	15/45	15/15/15/15	Continuous
2% sodium hypochlorite	2.71 \pm 1.68	14.51 \pm 2.07 [†]	24.56 \pm 2.37 [†]	34.68 \pm 3.84 [†]
5.8% sodium hypochlorite	29.94 \pm 2.31	39.11 \pm 4.44*	50.36 \pm 2.86 [†]	63.14 \pm 6.90 [†]

*p < 0.05.

[†]p < 0.001 versus no agitation.

hypochlorite was the most effective in agreement with the previous studies. Interestingly, tissue weight loss after 5 minutes in 2% of sodium hypochlorite solution was equal to controls with distilled and sterilized tap water. It should be emphasized, however, that the weight loss in water did not change after 5 minutes, whereas it continued to increase in 2% sodium hypochlorite after the first 5-minute period (results not shown). Tissue specimens immersed in 1% solutions increased in weight during the 5-minute exposure. This can probably be explained by hydration of the tissue after the initial-dissolving action of sodium hypochlorite on the meat. Other studies have also reported a similar effect on tissue by low-concentration hypochlorite solutions (5). However, longer exposure times will cause weight loss also with mild hypochlorite solutions.

The present study showed that heating the sodium hypochlorite solution enhanced its ability to dissolve organic material. These results are in accordance with other studies (14–16). Kamburis et al (20) found that heated sodium hypochlorite solutions were more effective in removing organic debris from dentin shavings than unheated solutions at the same concentration. It has also been shown that sodium hypochlorite solutions were stable for a period of 4 hours when heated to 37°C (21). Sirtes et al (15) found that 1%, 2.62%, and 5.25% solutions had an unchanged quantity of available chlorine for 1 hour at 45°C and 60°C, respectively. Therefore, in the present study, all experiments were finished in less than 1 hour to exclude the possibility of the loss of activity of sodium hypochlorite during experimentation at higher temperatures.

The effect of the surface active agent to hypochlorite was first shown by Cameron (22) who showed that the addition of the surface modifiers enhanced the ability of sodium hypochlorite to dissolve organic material. Clarkson et al (6) tested the dissolution ability of three different brands of sodium hypochlorite available in Australia and reported that the products with surfactants dissolved porcine pulp in a shorter time than regular sodium hypochlorite at the same concentration. The present study not only confirmed these findings but also showed that the advantage over regular products remained also when the products were diluted, heated, or agitated. Although not known in detail, it is possible that the better performance of Chlor-Xtra over regular hypochlorite is based partly on better contact on the surface of the tissue as well as faster exchange with fresh solution, both facili-

tated by the surface active agent. The lower contact angle of 5.8% Chlor-Xtra as compared with the two regular hypochlorites supports the assumption of better wetting of the surface of the substrate. When diluted to 1%, there was no significant difference in contact angle between the products, obviously because of the higher proportion of the water.

The importance of agitation on the tissue-dissolving ability of sodium hypochlorite has been reported, but there are very few studies on the effect of agitation on this (7). Aqueous solution of sodium hypochlorite is a dynamic balance of sodium hydroxide and hypochlorous acid. When sodium hypochlorite is in contact with organic material, sodium hydroxide reacts with fatty acids creating soap and glycerol, which is known as saponification reaction. It also reacts with amino acids creating salt and water (neutralization). In addition, hypochlorous acid acts with amino acids creating chloramine and water. These reactions, which happen mostly at the surface, lead to liquefaction of the organic tissue (23). At the same time, molecules of sodium hypochlorite involved in the reactions are consumed, resulting in the decline of local activity. It is therefore important to supply active hypochlorite to the area and also remove the remnants of dissolved tissue. In this study, the effect of three different agitation methods on the tissue-dissolving ability of sodium hypochlorite was investigated. It was found that the agitation of the solution improved their dissolving ability; however, there were no significant differences between the three methods of agitation.

The use of ultrasound energy in root canal preparation and irrigation has for a long time been a matter for debate. Although some researchers have reported great success of ultrasonics in root canal cleaning (24–28), others did not find ultrasound superior compared with root canal irrigation using the syringe (29–31). The mechanism of passive ultrasonic action has been attributed to acoustic streaming (microstreaming) and cavitation (13, 32). A sonic action has a similar mechanism of action to ultrasonic although the pattern of the oscillating file is different. However, according to Lumley et al (33), cavitation is limited to the distance of less than 100 μ m. All experiments in the present study were performed in the beaker with the ultrasonic/sonic tip immersed in the solutions to a depth of 10 mm, 5 mm away from the tissue specimen without touching it. Therefore, the possibility to evaluate the direct impact of ultrasonic or sonic energy by cavitation was excluded. However, acoustic streaming developed by this

TABLE 4. Contact Angle (Mean \pm Standard Deviation) on Dentin of Three Sodium Hypochlorite Solutions (1% and 5.8%)

Distilled water	1%			5.8%		
	Reg 1	Reg 2	Chlor-Xtra	Reg 1	Reg 2	Chlor-Xtra
39.33 \pm 12.35	52.17 \pm 13.58	44.83 \pm 12.33	41.42 \pm 9.59	71.92 \pm 12.76 [†]	54.58 \pm 13.91* [‡]	36.25 \pm 8.25 [§]

*p < 0.05.

[†]p < 0.001 versus distilled water.[‡]p < 0.01.[§]p < 0.001 versus 5.8% Reg 1.

mechanism creates stirring action and rapid movements of the irrigant further away from the energy source. This is likely to be the mechanism by which ultrasonic affects cleaning of the peripheral parts in root canal in vivo. Within the limitations of the present study, all three methods of agitation improved tissue dissolution. In clinical endodontics penetration of irrigants to the most apical canal and to other peripheral area such as fins and webs remains a great challenge, and both ultrasonic and sonic agitation may in that environment have advantages which could not be demonstrated in the present study design.

In conclusion, an increase in concentration and temperature of sodium hypochlorite greatly increased its efficacy in tissue dissolution. Refreshing the hypochlorite solution at the site of dissolution by agitation, preferably continuous, also resulted in a marked increase of hypochlorite effect. High temperature and agitation had an additive effect on the tissue dissolution. The sodium hypochlorite product with added surface active agent was the most effective in tissue dissolution at all concentrations and temperatures.

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