

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

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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;36:1558–1562)

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, Kanuma, 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.

The specimens were weighed on an electronic balance (FX-300; 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 labiolatally 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 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.

$^{\S}p < 0.05$.

$^{\dagger}p < 0.01$.

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

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