

# Penetration of Sodium Hypochlorite into Dentin

Ling Zou, DDS, PhD, <sup>\*†</sup> Ya Shen, DDS, PhD, <sup>†</sup> Wei Li, DDS, PhD, <sup>\*</sup>  
and Markus Haapasalo, DDS, PhD<sup>†</sup>

## Abstract

**Introduction:** Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant. The aim of this study was to evaluate the effect of concentration, time of exposure, and temperature on the penetration of NaOCl into dentinal tubules. **Methods:** Thirty extracted human permanent maxillary anterior teeth with single canals were instrumented by using ProTaper rotary files. The teeth were then sectioned perpendicular to the long axis. The crowns and apical thirds of all the teeth were removed. The remaining roots were processed into 4-mm-long blocks and stained overnight in crystal violet. One hundred eight stained blocks were treated by 1%, 2%, 4%, and 6% NaOCl for 2, 5, and 20 minutes at 20°C, 37°C, and 45°C, respectively. The depth of penetration of NaOCl was determined by bleaching of the stain and measured by light microscopy at magnifications of 20× and 40×. Statistical comparisons were made by using one-way analysis of variance, and Dunnett T3 tests were made for multiple comparisons. **Results:** The shortest penetration (77 μm) was measured after incubation with 1% NaOCl for 2 minutes at room temperature. The highest penetration (300 μm) was obtained with 6% NaOCl for 20 minutes at 45°C. After the initial penetration during the first 2 minutes, the depth of penetration doubled during the next 18 minutes of exposure. Temperature had a modest effect within each group on the depth of penetration and in most cases was not statistically significant ( $P > .05$ ). Depth of penetration increased with increasing hypochlorite concentration, but the differences were small. Within each time group, depth of penetration with 1% NaOCl was about 50%–80% of the values with the 6% solution. **Conclusions:** Temperature, time, and concentration all contribute to the penetration of sodium hypochlorite into dentinal tubules. (*J Endod* 2010;36:793–796)

## Key Words

Concentration, dentin, penetration, sodium hypochlorite, temperature, time

Studies on the microbiology of endodontic infections have clearly demonstrated that the bacteria present in the necrotic root canal system can be found in the main root canal space, lateral canals, and dentinal tubules (1). Eradication of the microbes during treatment relies on effective instrumentation, irrigation, and use of intracanal medicaments (2). Several studies have reported that with currently available instrumentation systems and protocols, large areas of the canal walls might remain untouched by the instruments (3–5), emphasizing the importance of irrigation for the removal of debris, bacteria, toxic products, and substrates necessary for bacterial growth from the inaccessible, uninstrumented surfaces (6).

Studies on the effectiveness of irrigation have focused on the effect in the main root canal (7). The dentin block model (8) has allowed evaluation of the antibacterial effect of some locally used medicaments in dentin surrounding the main root canal (9). However, very little is known about the penetration into dentinal tubules of endodontic irrigants, including sodium hypochlorite. According to literature, the number of infected tubules and the depth of penetration of bacteria are highly variable, ranging from 150 μm to half the distance between the main root canal and the cementodentinal junction (10). Regarding the practice of one-appointment endodontic treatment of teeth with root canal infection, information about the penetration of hypochlorite into dentin and the factors influencing the depth of penetration would be most valuable. Concentration, time of exposure, and temperature of the solution all have been shown to facilitate dissolution of organic tissue by hypochlorite (11–13). Whether the same factors play a role in dentin penetration is not known. Perhaps the main reason for the lack of such information is the lack of a reliable method to measure the penetration of hypochlorite into dentin. In the present study, a stained dentin block model was developed and used for the measurement of hypochlorite penetration.

The purpose of this study was to evaluate the effect of concentration, time of exposure, and temperature on the penetration of NaOCl into dentinal tubules. The hypothesis tested was that all these factors contribute to the penetration of NaOCl into dentin.

## Materials and Methods

Thirty extracted human permanent maxillary anterior teeth with single canals, straight mature roots, and no caries or resorption were used in this study. Teeth with previous coronal restorations or root canal treatment were excluded.

The crowns and apical thirds of all the teeth were removed, and the remaining roots were equally divided into coronal and middle segments. To avoid contamination of the canals by dentin cut during the splitting process, grooves were made by using a slow speed silicon carbide disk around the root surfaces horizontal to the long axis, not penetrating the canal space. To standardize the size and taper of the canals, they were all prepared by using the ProTaper SX hand-operated instruments (Dentsply Maillefer, Ballaigues, Switzerland). During instrumentation, each block was irrigated with 5.25% NaOCl. After preparation, each block was immersed in 10 mL 6% NaOCl

<sup>\*</sup>From the \*State Key Laboratory of Oral Diseases, West China College of Stomatology, West China Hospital of Stomatology, Sichuan University, Chengdu, China; and <sup>†</sup>Division of Endodontics, Department of Oral Biological & Medical Sciences, UBC Faculty of Dentistry, Vancouver, BC, Canada.

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

Copyright © 2010 American Association of Endodontists.  
doi:10.1016/j.joen.2010.02.005

(EMD Chemicals Inc, Darmstadt, Germany) for 5 minutes followed by immersion in 10 mL 17% ethylenediaminetetraacetic acid (Merck Co, Darmstadt, Germany) for another 5 minutes. Thereafter, they were washed in distilled water and dried with paper towel.

All samples were immersed into crystal violet (Benex Limited, Shannon, Ireland) overnight. After rinsing under tap water for 30 minutes, the blocks were grooved on the mesiodistal surfaces along its entire length and split into 2 halves with a blade and hammer. Specimens that showed poor or no penetration of the stain were excluded. Altogether, 108 half-sections were randomly divided into 36 groups ( $n = 3$ ) to be exposed to 1%, 2%, 4%, and 6% NaOCl for 2, 5, and 20 minutes at 20°C, 37°C, and 45°C, respectively. Each half-section was treated by 10 mL NaOCl in a beaker individually.

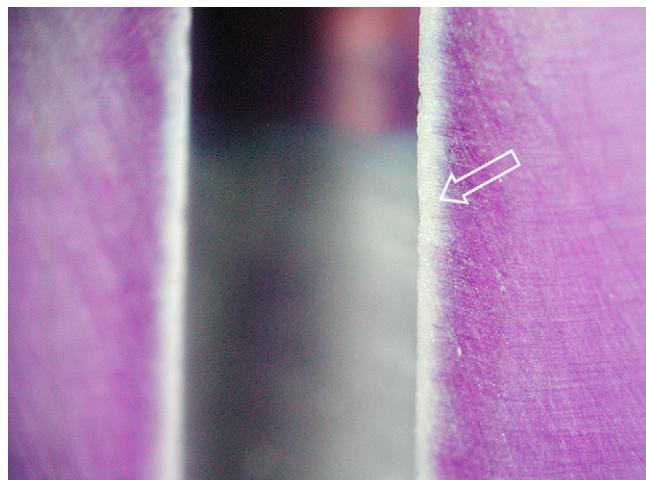
Sodium hypochlorite solutions (1%–4%, wt/wt) were prepared immediately before use by diluting a 6% stock solution (EMD Chemicals Inc) with distilled water. Stained and split dentin blocks were placed in beakers each with 10 mL of hypochlorite for the indicated times. No agitation of the samples/solutions was done. A Water-Jacket Incubator was used to heat the solutions until they reached 37°C and 45°C, respectively. It also provided constant temperature during the experiments at these temperatures.

After exposure to NaOCl, the specimens were washed in distilled water for 1 minute, and the surface of each dentin specimen was ground to remove a layer of about 100 µm by using abrasive papers (no. 1000; Struers, Copenhagen, Denmark) to expose a dentin area that was affected only by hypochlorite that had penetrated the tubules from area of the root canal. The specimens were then observed with a light microscope (Nikon Eclipse 50i; Nikon, Tokyo, Japan) at a magnification of 20 $\times$  and 40 $\times$ . For each section, at least 10 areas on both sides of the root canal were measured from coded images for NaOCl penetration by the scale of analysis software in Nikon digital sight DS-L2 (Nikon). After this, the samples were ground 2 more times to measure hypochlorite penetration (bleaching of the stain) at different areas of dentin surrounding the root canal.

Statistical evaluations were performed by using SPSS 11.0 software (SPSS Inc, Chicago, IL). The normal distribution of the data was tested with the Kolmogorov-Smirnov test. Statistical comparisons were made by using one-way analysis of variance, and Dunnett T3 tests were made for multiple comparisons. The level of significance for all statistical tests was set at  $P < .05$ .

## Results

Penetration of sodium hypochlorite into dentin was detected as a bleached zone from the root canal toward the periphery (Fig. 1). The values of penetration depth are shown in Fig 2. All 3 parameters evaluated, hypochlorite concentration, time of exposure, and temperature, contributed to deeper penetration as revealed by the bleaching of

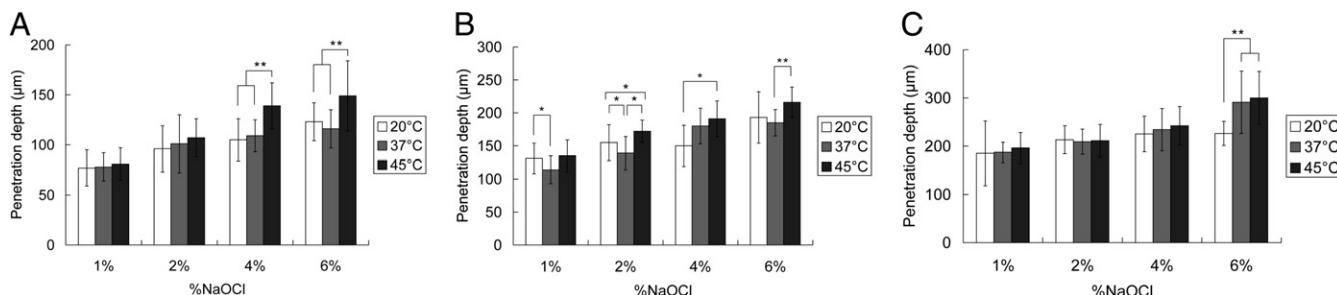


**Figure 1.** A microscope view (original magnification,  $\times 20$ ) of stained root section treated by 1% sodium hypochlorite for 2 minutes.

the stained dentin. The shortest penetration (77 µm) was measured after incubation with 1% NaOCl for 2 minutes at room temperature. The highest penetration (300 µm) was obtained with 6% NaOCl for 20 minutes at 45°C. After the initial penetration during the first 2 minutes, the depth of penetration doubled during the next 18 minutes of exposure. Temperature had little effect within each group on the depth of penetration, and in most cases the differences were not statistically significant. Only in the groups treated by 2% NaOCl for 5 minutes, results from treatments at the 3 temperatures were statistically different from each other ( $P < .05$ ). Depth of penetration increased with increasing hypochlorite concentration, but the differences were small. Within each time group, depth of penetration with 1% NaOCl was about 50%–80% of the values with the 6% solution.

## Discussion

The well-known challenges of the *in vivo* studies in endodontics, many of which are related to difficulties in standardization and recruitment of patients, are reasons for the effort to develop experimental models that would reflect the reality of the *in vivo* situation and avoid some of the common confounding factors. A great deal of *in vitro* and *ex vivo* research on endodontic microbiology, disinfection, and irrigation involves use of dentin (1, 2, 9). From the extracted teeth, dentin can be processed into specimens with standardized size and shape, even the root canals can often be standardized better than what is possible to achieve *in vivo*. Manipulation of dentin (eg, sterilization) might, however, affect its properties and behavior such as bacterial binding



**Figure 2.** Depth of penetration of sodium hypochlorite into dentin, effect of time, concentration, and temperature. (A) 2 minutes, (B) 5 minutes, and (C) 20 minutes. \* $P < .05$ ; \*\* $P < .01$ .

and other surface properties (14). Also, simplifying the experimental setup too much might result in the absence of some *in vivo* factors that, although not targeted in the study, could directly or indirectly have a major impact on the results. Measurement of hypochlorite penetration in dentin cannot be done in an *in vivo* study because of obvious ethical and practical limitations. The chosen *ex vivo* approach with numerous dentin blocks allowed random distribution of the block segments in different treatment groups. This was done in hope that the impact of variations in dentin structure between different teeth could be minimized. Furthermore, blocks that showed poor or no penetration of the dye were excluded from the study. The low standard deviations in all groups indicate that the material was homogenous enough for the study. It has been shown that the extent and depth of bacterial invasion are significantly less in apical dentin than in coronal and middle parts of the root canal (15). Paque et al (16) reported that dye penetration was significantly greater in the coronal and middle than in apical root third. Therefore, apical thirds of the root were excluded in the present study.

Previous studies and our pilot experiments showed that certain dyes easily penetrate through the whole depth of dentin, even when the root surface cement is left untouched (16). Crystal violet and safranin red were both tested in pilot experiments, giving equal results for penetration of the dye and hypochlorite. Crystal violet was chosen because of its better visibility under light microscopy. As a powerful oxidizing agent, hypochlorite bleached the violet or red color (safranin), revealing the normal light color of dentin. The width of the stained area could be easily recorded and was regarded to represent the penetration depth of sodium hypochlorite activity.

The present study is the first report in which hypochlorite penetration into dentin has been measured in such accuracy (micrometers). Studies with sampling of infected dentin cannot be done with such precision, because typically zones of 100  $\mu\text{m}$  are removed with each sample, and the microbiologic challenges of the studies are many, including detection limit and contaminations. It can be speculated that staining of the dentinal tubule walls by a dye might change their surface properties, which in turn might be reflected in a changed pattern of fluid penetration. Although this remains an open question, the observation that crystal violet and safranin red produced similar results is an indication of the validity of the results. Another factor that might have an impact on dentin penetration is agitation of the hypochlorite solution. In the present study, an excess amount of hypochlorite was placed in a beaker with the dentin pieces, but no additional agitation was done. This was done to minimize variations between different dentin blocks. It should be emphasized that agitation with different methods could have changed the results. The main goal of the present study, however, was to establish and evaluate a method that allows measuring the depth of penetration into dentin of sodium hypochlorite, the most important irrigant in endodontics. Future studies will address the various factors impacting hypochlorite penetration.

Within the experimental setup, the depth of hypochlorite penetration varied between 77 and 300  $\mu\text{m}$ . Ando and Hoshino (10) found bacteria in the dentinal tubules of infected teeth at approximately half the distance between the root canal and the cementodentinal junction. Haapasalo and Ørstavik (8) found that *Enterococcus faecalis* rapidly invaded the tubules, and the front of the infection reached 1000  $\mu\text{m}$  in some blocks after 3 weeks of incubation. Simple comparison of microbiologic penetration studies and the present study might indicate that it is difficult to reach all bacteria in every case by hypochlorite irrigation. However, the present study is the first one of its kind, and caution must be exercised regarding the conclusion that can be drawn at this stage. However, the depth the irrigants can penetrate into dentinal tubules is potentially an important factor that can affect their effectiveness and contribute to the outcome of the treatment.

The 3 parameters potentially affecting hypochlorite penetration that were evaluated in the present study were concentration, time, and temperature. All of these did have an impact on the penetration, but the effect was generally less than anticipated. Perhaps the most surprising observation was that increasing the concentration from 1% to 6% did not result in more than 30%–50% increase in penetration (Fig. 2). It is possible, therefore, that increased temperature has a more pronounced effect in tissue dissolution and bacterial killing in the main canal (17) than in dentin penetration. For instance, it has been reported that the capacity of 1% NaOCl at 45°C in dissolving human pulp tissue was equal to that of a 5.25% solution at 20°C (12).

Disinfecting solutions require an adequate working time to reach their potential (18). Longer exposure time in the present study resulted in deeper penetration of hypochlorite, although the speed of penetration declined sharply along time. For example, at 20°C, penetration depth of 1% NaOCl in 2 minutes was about 77  $\mu\text{m}$ ; after another 18 minutes at the same temperature, the depth reached about 185  $\mu\text{m}$ . Because the solubilizing abilities of NaOCl solutions are reduced by contact with organic material, it can be speculated that most of its activity is lost after 2 minutes, and continuous replenishment of fresh solution will be needed (19). The 10-mL hypochlorite reservoir in which the dentin blocks were placed probably provided for exchange of fresh, active hypochlorite for the deeper parts of the tubules.

The antibacterial effectiveness of NaOCl is dependent on its concentration, temperature, and volume and contact time in the root canal (12, 20). In the present study, we evaluated the effect of these factors on the penetration ability of NaOCl, except for volume. The results showed that the 3 variables all had an effect on NaOCl penetration, but the effect was not very pronounced for any of the factors alone. The penetration depths of 1%, 2%, 4%, and 6% solutions after 2 minutes at room temperature were 77, 96, 105, and 123  $\mu\text{m}$ , respectively. The highest values, 291 and 300  $\mu\text{m}$ , were found in the groups treated by 6% NaOCl at 37°C and 45°C for 20 minutes.

Within the limitations of this study, temperature, time, and concentration all play a role in determining the depth of hypochlorite penetration into dentinal tubules. Deepest penetration was obtained when these factors were present simultaneously, suggesting an additive effect.

## Acknowledgments

*The work of Ling Zou was supported by China Scholarship Council as a visiting Scholarship.*

## References

- Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol* 1990;6:142–9.
- Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. *Endodontic Topics* 2005;10:77–102.
- Rossi-Fedele G, De Figueiredo JA. Use of a bottle warmer to increase 4% sodium hypochlorite tissue dissolution ability on bovine pulp. *Aust Endod J* 2008;34:39–42.
- Mannan G, Smallwood ER, Gulabivala K. Effect of access cavity location and design on degree and distribution of instrumented root canal surface in maxillary anterior teeth. *Int Endod J* 2001;34:176–83.
- Peters OA, Laib A, Gohring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. *J Endod* 2001;27:1–6.
- Gomes-Filho JE, Aurélio KG, de Moraes Costa MMT, Bernabe PFE. Comparison of the biocompatibility of different root canal irrigants. *J Appl Oral Sci* 2008;16:137–44.
- van der Sluis LW, Wu MK, Wesselink PR. The efficacy of ultrasonic irrigation to remove artificially placed dentine debris from human root canals prepared using instruments of varying taper. *Int Endod J* 2005;38:764–8.
- Haapasalo M, Ørstavik D. *In vitro* infection and disinfection of dentinal tubules. *J Dent Res* 1987;66:1375–9.
- Haapasalo HK, Siren EK, Waltimo TMT, Ørstavik D, Haapasalo MPP. Inactivation of local root canal medicaments by dentine: an *in vitro* study. *Int Endod J* 2000;33:126–31.

10. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. *Int Endod J* 1990;23:20–7.
11. Zehnder M. Root canal irrigants. *J Endod* 2006;32:389–98.
12. Sirtes G, Waltimo T, Schatzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. *J Endod* 2005;31:669–71.
13. Kamburis JJ, Barker TH, Barfield RD, Eleazer PD. Removal of organic debris from bovine dentin shavings. *J Endod* 2003;29:559–61.
14. Chivatxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by *Enterococcus faecalis*. *J Endod* 2008;41:873–82.
15. Love RM. Regional variation in root dentinal tubule infection by *Streptococcus gordonii*. *J Endod* 1996;22:290–3.
16. Paque F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. *Int Endod J* 2006;39:18–25.
17. Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980;49:175–7.
18. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999;12:147–79.
19. Clarkson RM, Moule AJ, Podlich H, et al. Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. *Aust Dent J* 2006;51:245–51.
20. Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue *in vitro*. *J Endod* 2008;34:449–52.