Brazilian Dental Journal (2016) 27(2): 208-216
http://dx.doi.org/10.1590/0103-6440201600650
ISSN 0103-6440

Penetration of Sodium Hypochlorite Modified with Surfactants into Root Canal Dentin

Flavio Palazzi[1], Andrea Blasi[1], Zahed Mohammadi[2], Massimo Del Fabbro[3,4], Carlos Estrela[5]

[1]Department of Neuroscience, Reproductive and Odontostomatological Sciences, Federico II University of Naples, Naples, Italy
[2]Department of Endodontics, Hamedan University of Medical Sciences, Hamedan, Iran & Iranian Center for Endodontic Research (ICER), Tehran, Iran
[3]Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Milan, Italy
[4]IRCCS Istituto Ortopedico Galeazzi, Milan, Italy
[5]Department of Stomatological Sciences, Dental School, UFG - Universidade Federal de Goiás, Goiânia, GO, Brazil

Correspondence: Dr. Flavio Palazzi, Via Pansini 5, 80131 Napoli, Italia. Tel. +39-34-7544-2798. e mail: flaviopalazzi@gmail.com

The aim of this study was to evaluate the effect of concentration, exposure time and temperature of sodium hypochlorite (NaOCl) added with surfactants on its penetration into dentinal tubules. Sixty-five extracted human permanent maxillary anterior teeth with single canals were prepared by ProTaper SX hand-operated instruments. 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 and thirty stained blocks were further split into halves and treated by nine different types of NaOCl-based solutions. Three solutions were added with surfactants (Hypoclean, H6, Chlor-Xtra) and the others were regular hypochlorites at increasing concentrations (1%, 2%, 4%, 5.25%, <6%, 6% NaOCl) from different brands. The dentin blocks were exposed to the solutions for 2, 5, and 20 min at 20 °C, 37 °C and 45 °C, respectively. The depth of NaOCl penetration was determined by bleaching of the stain and measured by light microscopy at 20 × and 40×. Statistical comparisons were made by using a generalized linear model with Bonferroni's post-hoc correction. The shortest penetration (81±6.6 µm) was obtained after incubation in 1% NaOCl for 2 min at 20 °C; the highest penetration (376.3±3.8 µm) was obtained with Chlor-Xtra for 20 min at 45 °C. Varying NaOCl concentration produced a minimal effect while temperature and exposure time had a significant direct relationship with NaOCl penetration into dentinal tubules, especially those with lowered surface tension. The exposure time and temperature of sodium hypochlorite as well as the addition of surfactants may influence the penetration depth of irrigants into dentinal tubules.

Key Words: sodium hypochlorite, surfactant, penetration, temperature, time.

Introduction

Post-treatment apical periodontitis is caused mainly by bacterial persistence despite root canal treatment (1). Bacteria causing persistent infections are usually located in areas unaffected by instruments and antimicrobial substances, including lateral canals, apical ramifications and isthmuses (2).

Even if there is no convincing report on dentinal tubule infection as a cause of post-treatment disease, bacterial invasion of dentinal tubules has also been regarded as a potential source of persistent infection (3,4). Ando and Hoshino (5) found bacteria in the dentinal tubules of infected teeth at approximately half the distance between the main root canal and the cementodentinal junction. Furthermore, Haapasalo and Ørstavik (3) found that *Enterococcus faecalis* rapidly invaded dentinal tubules and when cementum was absent, the front of the infection reached 1000 µm after 3 weeks. Therefore, 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 root canal treatment.

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant because of its well-known antimicrobial and tissue-dissolving activity (6). An *ex vivo* study on the effectiveness of 5.25% NaOCl solution against *E. faecalis* (7) found that in most of the teeth irrigated with NaOCl, the drop in the bacterial load was rapid, but lasted only up to 48 h after irrigation. In fact, 70% of the samples were colonized again in the following 96 h with a bacterial load that raised the infection to its previous level. A stained dentin block model was developed by Zou et al. (8) for the measurement of NaOCl penetration into dentinal tubules varying concentration and temperature of the solution, and exposure time. Within the experimental setup, the depth of NaOCl penetration varied between 77 and 300 µm and deepest penetration was obtained when these factors were present simultaneously, suggesting an accumulated effect.

The major drawback of NaOCl is its high surface tension (9), which may affect its wettability. Wettability was shown to play an important role in the penetration of antimicrobial solutions into irregularities of the root canal system (RCS) and into the depth of dentinal tubules (10). The wettability of a solution depends on its surface tension, which is defined as the force between molecules tending to reduce the surface area of a liquid (11). The decrease of surface tension may be accomplished by the addition of chemicals known as detergents (12). Palazzi et al. (9) showed that two recently introduced NaOCl solutions modified with surfactants had surface tension significantly lower than 5.25% NaOCl and distilled water. In the main root canal, low surface tension is likely to help the spreading of a solution; in the narrow and long dentinal tubules, penetration can occur either by capillary forces or by diffusion/flow into the dentin. Different detergents added to antimicrobial solutions as surface active agents could improve their bactericidal efficacy, increasing their wettability over the dentin surface in the root canal (12), as a better adaption to dentin and penetration into the dentinal tubules (9,10) or merely added its own antibacterial effect to the disinfecting solution in the combined solutions (13).

Two sodium hypochlorite solutions modified to reduce their surface tension, Chlor-Xtra (Vista Dental Products, Racine, WI, USA) and Hypoclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) showed superior wettability against dentin in comparison to 5.25% NaOCl and distilled water (14); solutions with added surface active agents immediately spread on the dentin surface, yielding a zero-degree contact angle. In a recent study by Mohammadi et al. (15), Hypoclean solution demonstrated a more effective antibacterial action against *E. faecalis* than 5.25% NaOCl at all experimental periods (7, 14, 21 and 28 days) in bovine root dentin and displayed no bacterial growth at the first two cultures after treatment. Furthermore, Wang et al. (13) clearly indicated that the addition of a detergent in the disinfecting solutions can increase their antibacterial effect against bacteria in infected dentin. Nonetheless, no difference was shown between 6% NaOCl and two 6% NaOCl solutions with added surfactants regarding their ability to kill *E. faecalis* in dentin. This result supports the findings by Williamson et al. (16) who reported no statistically significant difference between 6% NaOCl and Chlor-Xtra (Vista Dental Products, Racine, WI, USA) in killing *E. faecalis* biofilm. Whether the concentration and temperature of the NaOCl solutions with added surface active agents and exposure time play a role in dentin penetration is not known.

The aim of the present study was to evaluate the effect of concentration, exposure time and temperature on the penetration of NaOCl solutions modified with surfactants, into dentinal tubules. The null hypothesis tested was that all these factors do not contribute to the penetration of NaOCl solutions modified to reduce their surface tension into root canal dentin.

Material and Methods

Sodium hypochlorite at concentrations of 1, 2, 4, 5.25 and 6% were tested. Stock solution of 15% NaOCl (Ogna Laboratori Farmaceutici, Muggiò, Italy) was obtained from the manufacturer. One percent, 2%, 4%, 5.25%, 6% solutions of NaOCl were prepared by diluting the stock solution in distilled water. Hypoclean (5.25% NaOCl modified with two surface tension modifiers, cetrimide and polypropylene glycol) and H6 (experimental 6% NaOCl solution with added cetrimide and polypropylen glycol) solutions were freshly prepared starting from stock solution and tested. The percentage of surfactants in the Hypoclean solution was like in the H6 solution (this product is not normally available in this form). Two different hypochlorite products with <6% NaOCl, purchased shortly before commencement of the project, were included in the experiments: one conventional solution (Sodium Hypochlorite, Vista-Dental) and one with non ionic (Triton X) surface active agent (with undisclosed concentration) added (Chlor-Xtra, Vista-Dental). All solutions were stored in amber colored bottles, kept at 4 °C following the recommendations of the manufacturer and brought to room temperature before use. Mean active chlorine content of all solutions was established from 3 iodometric titration readings at 20 °C and sodium thiosulfate was standardized each time. A digital pH meter (Metrohm model 827 pH LAB, Metrohm, Varese, Italy) was used to assess the pH of the solutions: a buffer at a pH of 10 was used as a control for each analysis and for each solution 3 measurements were taken at 20 °C and mean value was calculated (Table 1).

The methodology used for assessing the penetration of NaOCl solutions into dentin was adapted from the stained dentin block model developed by Zou et al. (8). Sixty-five extracted human permanent maxillary anterior teeth with single canals, straight mature roots, and no caries or resorption were used in this study. The age, sex and race of the donors were unknown. All teeth were kept in 0.5% NaOCl solution for up to 7 days. Teeth with previous coronal restorations or root canal treatment were excluded. This

Table 1. Chemical characteristics of the solutions tested

Solutions	Mean active chlorine %	pH
1% NaOCl	1.47 ± 0.01	12 ± 0.0
2% NaOCl	2.25 ± 0.0	12 ± 0.0
4% NaOCl	4.16 ± 0.01	12.4 ± 0.0
5.25% NaOCl	5.21 ± 0.01	12.7 ± 0.0
Hypoclean	5.21 ± 0.02	12.4 ± 0.0
6% NaOCl	6.03 ± 0.01	12.6 ± 0.0
H6	6.01 ± 0.02	12.5 ± 0.0
<6 NaOCl	5.60 ± 0.01	12.7 ± 0.0
Chlor-Xtra	5.42 ± 0.01	12.6 ± 0.0

study was approved by the institutional Research Ethics Committee.

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).

Altogether, 130 blocks were randomly divided into 2 groups of 100 and 30 blocks respectively according to the manufacturer of NaOCl solutions tested. Group 1 (n=100): during instrumentation, blocks were irrigated with 5.25% NaOCl (Ogna); after preparation, each block was immersed in 10 mL 6% NaOCl (Ogna) for 5 min followed by immersion in 10 mL 17% EDTA (Ogna) for another 5 min. Group 2 (n=30): during instrumentation blocks were irrigated with <6% NaOCl (Vista-Dental); after preparation, each block was immersed in 10 mL <6% NaOCl (Vista-Dental) for 5 min followed by immersion in 10 mL 17% EDTA (Vista-Dental) for another 5 min. All blocks were washed in distilled water and dried with paper towels. Thereafter, all samples were immersed into crystal violet (Gram Crystal Violet, Oxoid Ltd. Wade Road, Basingstoke, Hants, UK) at room temperature for 72 h to stain the dentin. The specimens were then rinsed under tap water for 30 min. The blocks were grooved on the mesiodistal surfaces along their entire lengths and split into halves with a blade and hammer.

Specimens that showed poor or no penetration of the stain were excluded. Altogether, 189 half-sections (Group 1) were randomly divided into 63 subgroups (n=3) to be exposed to 1, 2, 4, 5.25 and 6% NaOCl, Hypoclean and H6 for 2, 5 and 20 min at 20 °C, 37 °C and 45 °C, respectively. Sodium hypochlorite solutions (1%-6%, wt/wt) were prepared immediately before use by diluting 15% stock solution with distilled water. The 54 half-sections (Group 2) were randomly divided into 18 subgroups (n=3) to be exposed to <6% NaOCl and Chlor-Xtra for 2, 5 and 20 min at 20 °C, 37 °C and 45 °C, respectively. 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 min, and the surface of each dentin specimen was ground to remove a layer of approximately 100 µm by using 1000-grit abrasive papers (3M ESPE; St. Paul, MN, USA) to expose the dentin areas that were affected only by hypochlorite solutions that had penetrated the tubules from area of the root canal. This amount of removed dentin was controlled by measuring the specimens with a digital caliper (Mitutoyo, Tokyo, Japan).

The quantitative analysis of the penetration of the NaOCl solution into dentin was performed by measuring the depth of crystal violet stain that was bleached. The specimens were then observed with a light microscope (Olympus CX41, Olympus, Hamburg, Germany) and images of the specimens were subsequently produced with an Olympus C-5060 digital camera at a magnification of 20× and 40× (Fig. 1). Measurements were conducted from coded images for NaOCl penetration by the scale of analysis image software (Soft Imaging System GmbH, Münster, Germany). For each section, the extension of NaOCl penetration, in micrometers, was outlined and measured in at least 10 different areas, on both sides of the root canal. 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. Penetration into dentin and into dentinal tubules was expressed as mean (µm)±standard deviation of the depth of penetration. The mean of the measurements was considered as the final value for each specimen. A skilled observer, blinded to the treatment subgroups, performed the evaluations.

Statistical analysis were carried out by using IBM SPSS Statistics 20.0 software (IBM Corp, Armonk, NY, USA). Statistical comparisons were made by using a Generalized linear model (GLM) with Bonferroni's post-hoc correction. The significance level for all statistical tests was set at $p<0.05$.

Results

The mean values of penetration depth are shown in

Figure 1. Representative image of bleaching-penetration area. A microscope view (original magnification ×40) of stained root dentin treated by Hypoclean for 5 min at 45 °C (scale bar = 150 µm).

Table 2. The shortest penetration (81±6.6 μm) was measured after incubation with 1% NaOCl for 2 min at 20 °C; the highest penetration (376.3±3.8μm) was obtained with Chlor-Xtra for 20 min at 45 °C (Fig. 2). All 3 parameters evaluated, hypochlorite concentration, exposure time, and temperature, contributed to deeper penetration of NaOCl without surfactants addition. Only 2 of the evaluated parameters, exposure time and temperature, contributed to deeper penetration of NaOCl with lowered surface tension.

Surfactants

A deeper penetration was recorded in surfactant containing solutions. The differences were statistically significant (p<0.001) for Hypoclean *versus* 5.25% NaOCl and Chlor-Xtra *versus* <6% NaOCl except for results at all temperatures for 2 min. Moreover, significant differences were recorded for Hypoclean *versus* H6 and Chlor-Xtra *versus* H6 except for results at all temperatures for 2 min. No statistically significant differences were recorded for Hypoclean *versus* Chlor-Xtra and for H6 *versus* 6% NaOCl. Moreover, differences were not statistically significant for H6 *versus* 5.25% NaOCl except for results at 20 °C for 5 min (p<0.01).

Concentration

Depth of penetration of regular solutions generally increased within each group with increasing hypochlorite concentration. Nevertheless, in most cases, differences were not statistically significant; the differences were always statistically significant only for 1% *versus* 6% NaOCl. Depth of penetration decreased significantly (p<0.001) with increasing concentration from 5.25% (Hypoclean) to 6% (H6) with modified hypochlorite solutions when exposure time was 5 and 20 min.

Exposure time

Depth of penetration increased with increasing exposure time and the differences among times of exposure were significant (p<0.01 at least) for all solutions tested at all temperatures except for <6% NaOCl between 2 and 5 min (p>0.05) at 20 °C.

Temperature

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 5.25% NaOCl and 6% NaOCl for 20 min, results from treatments at the 3 temperatures were statistically different from each other (p<0.05). In all the groups treated for 2 min, results from treatments at the 3 temperatures were not statistically different from each other (p>0.05) except for values from treatment by 2% NaOCl at 20 °C and 45 °C (p<0.05).

In all the groups treated for 5 min, results from treatments at the 3 temperatures were not statistically different from each other (p>0.05) except for values from treatment by 4% NaOCl (p<0.001), 5.25% NaOCl (p<0.001), Hypoclean (p<0.01) and Chlor-Xtra (p<0.05) at 20 °C and 45 °C. In the groups treated for 20 min, for Hypoclean only results from treatments at 20 °C and 45 °C were statistically different (p<0.001). For H6, only results from treatments at 37 °C and 45 °C were not statistically different (p>0.05). For Chlor-Xtra, only results from treatments at 20 °C and 37 °C were not statistically different (p>0.05).

Discussion

The present study aimed to evaluate the effect of concentration, exposure time, and temperature on NaOCl penetration with and without surfactants into dentinal tubules. Measurement of NaOCl penetration into dentin

Table 2. Mean and standard deviations (μm) of penetration of different NaOCl solutions for all the groups at different experimental conditions

Temp. (°C)	Time	1% NaOCl	2% NaOCl	4% NaOCl	5.25% NaOCl	6% NaOCl	Hypoclean	H6	<6% NaOCl	Chlor-XTRA
20 °C	2 min	81 ± 6.6	81 ± 6.6	116.3 ± 4.6	130.7 ± 2.5	142.67± 4.2	158.3 ± 4.0	145 ± 4.6	157.7 ± 9.0	139.7 ± 6.5
20 °C	5 min	138 ± 8.0	145 ± 5.2	154 ± 4.6	162.7 ± 4.5	190 ± 9.0	241 ± 17.1	196.7 ± 10.0	172.8 ± 3.5	243.3 ± 2.5
20 °C	20 min	189.7 ± 7.5	196.7 ± 3.5	212 .7 ± 8.5	218 ± 3.0	222 ± 8.5	327.3 ± 6.4	234.3 ± 6.7	198 ± 4.8	307.7 ± 7.1
37 °C	2 min	82.7 ± 2.5	89.7 ± 9.0	116.3 ± 8.5	134.0 ± 5.3	140.3 ± 7.5	163 ± 4.0	145.7 ± 9.0	143 ± 1.0	154.3 ± 8.5
37 °C	5 min	141.3 ± 13.5	157.3 ± 2.5	172 ± 12.5	182.3 ± 4.2	190 ± 9.5	252.7 ± 14.0	196 ± 10.8	188.5 ± 6.9	261.7 ± 13.1
37 °C	20 min	194.7 ± 6.7	216 ± 5.0	224 ± 9.0	269 ± 4.0	278.7 ± 1.2	347.7 ± 3.2	287.7 ± 1.5	281 ± 9.3	336 ± 5.3
45 °C	2 min	85.7 ± 2.9	112 ± 3.5	141 ± 14.0	146.3 ± 6.7	158 ± 9.0	164.3 ± 0.6	162.7 ± 10.0	151 ± 3.6	162.3 ± 3.1
45 °C	5 min	146.7 ± 14.6	165 ± 4.6	190.3 ± 8.7	204.7 ± 5.7	217 ± 12.0	274.3 ± 15.0	222 ± 12.1	198.1 ± 3.6	272.7 ± 2.1
45 °C	20 min	208 ± 8.5	224.7 ± 12.0	234.7 ± 15.0	299.7 ± 1.5	309.3 ± 11.1	370.3 ± 7.5	315.7 ± 12.6	293.2 ± 3.34	376.3 ± 3.8

structure cannot be done in an *in vivo* study because of obvious ethical and practical limitations. The *ex vivo* model chosen was the same used by Zou et al. (8) in order to make comparisons easier: its simplified experimental setup allowed the absence of *in vivo* factors that could directly or indirectly have impact on the findings. However, the goal of the present study was not to simulate the clinical use of NaOCl solutions as irrigants, but to assess their potential penetration ability into dentinal tubules. Even if natural teeth may present an irregular distribution of tubules, depending on diverse factors, such as age, shape and thickness of cementum-like tissue, studies using natural teeth have a high value and are likely closer to clinical practice. The impact of variations in dentinal structure among different teeth was minimized by using numerous dentin blocks and their random distribution

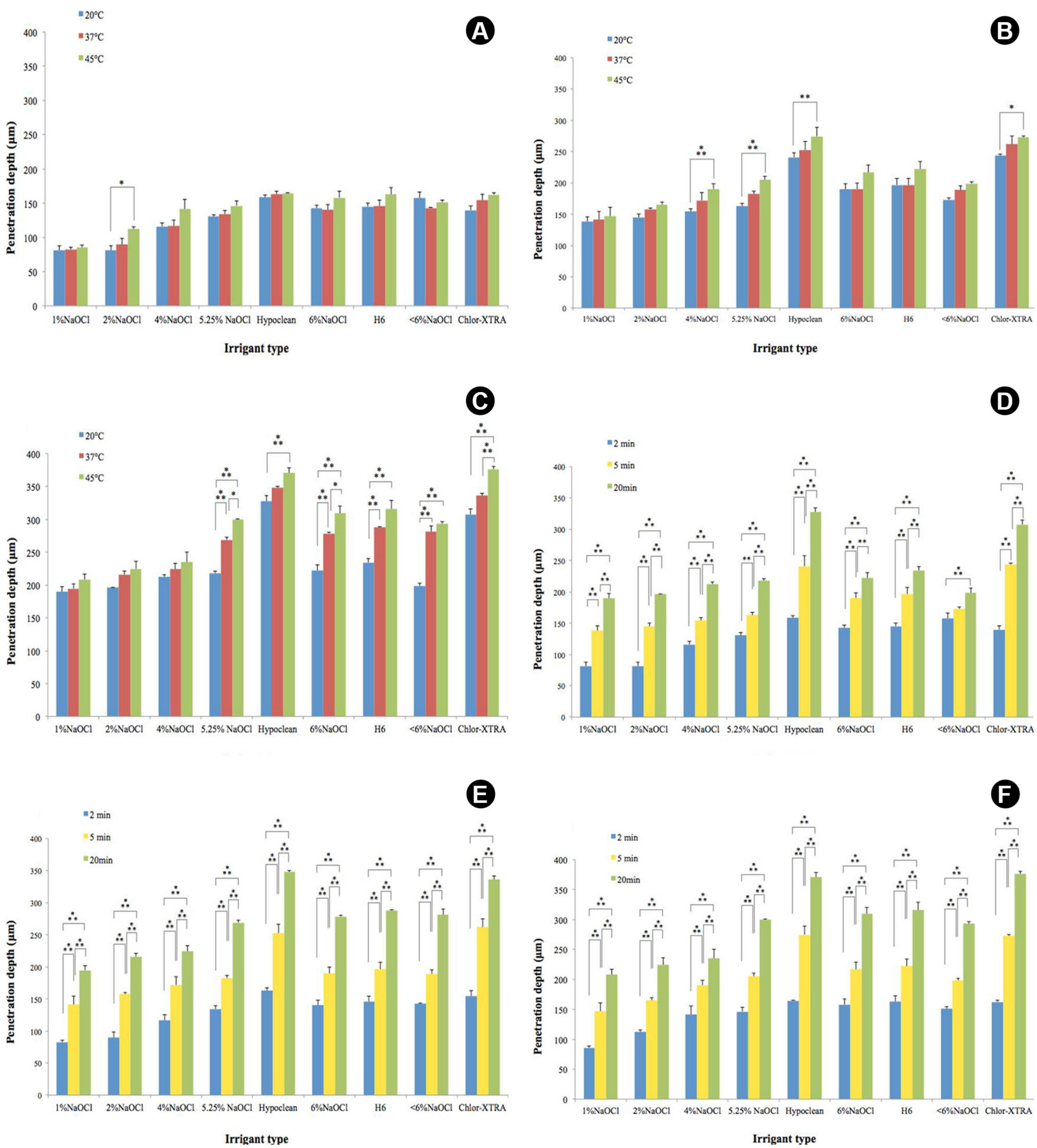

Figure 2. Depth of penetration of sodium hypochlorite into dentin, effect of time, concentration, temperature and surfactants. (A) 2 min, (B) 5 min, (C) 20 min, (D) 20 °C, (E) 37 °C, (F) 45 °C. *p<0.05; **p<0.01; ***p<0.001.

into different treatment groups. The influence of canal shape, as teeth with more ovoid shaped canals may have had less irrigant penetration due to higher debris levels in the canal, can be reduced by increasing the number of samples, as done in this study. The irregular penetration of crystal violet dye and the deeper penetration found in the buccolingual direction compared with the mesiodistal direction (24,25) suggested to split blocks following grooves on the mesiodistal surfaces. Because of dye penetration was significantly greater in the coronal and middle than in apical root third (25), the apical thirds of the root were excluded in the present study, as blocks that showed poor or no penetration of the dye.

The low standard deviations in all groups indicated that the material was homogenous enough for the study. The reason for dividing the teeth into two groups, also with different NaOCl solutions being used during canal preparation was to test NaOCl solutions added with surface active agents from different manufacturers in comparison with their regular counterparts. The rationale of using various NaOCl concentrations without surfactants was to verify the outcomes reported by Zou et al. (8). Even if the volume of NaOCl that is exposed to the dentin was much greater than during root canal treatment, an excess amount of hypochlorites was placed in the beakers with the dentinal pieces, without additional agitation, to minimize variations between different dentin blocks.

The two commercial products, Hypoclean and Chlor-Xtra, and the experimental solution H6 were used as surfactant-containing products to compare their penetration ability into dentinal tubules with their regular counterparts 5.25% NaOCl, <6% NaOCl and 6% NaOCl respectively. Despite their toxic concentration to the periapical tissue, 6% and <6% NaOCl solutions from different manufacturers were used in the present study to evaluate in comparison the penetration of highly concentrated NaOCl solutions modified with surfactants into the dentinal tubules. Data from the present study showed that the addition of surfactants increases the penetration of NaOCl solutions into dentinal tubules, except for Chlor-Xtra *versus* <6%NaOCl (% chlorine: 5.60 ± 0.01) at 20 °C for 2 min. The available chlorine levels of initial solutions could explain this result especially considering the chlorine loss due to interaction between chlorine and surfactants. However, the differences in penetration ability were statistically significant ($p<0.001$) for Hypoclean *versus* 5.25% NaOCl, and Chlor-Xtra *versus* <6% NaOCl, except for results at all temperatures for 2 min of exposure time.

The ability of surface-active agents to reduce the surface tension of NaOCl solutions has been recently demonstrated (9). Hypoclean and Chlor-Xtra solutions clearly showed superior wettability against dentin in comparison to 5.25% NaOCl at 22 °C (14): solutions immediately spread on the dentin surface, yielding a zero-degree contact angle. The wettability of NaOCl solutions modified with surfactants was completely controlled by capillary penetration: the combined effect of interface energetics and surface topography allowed solutions to readily penetrate into the dentin tubules and surface roughness. However, because the evidence available suggests that surface-active agents have no effect on their pulp tissue dissolution capability, the solubilizing abilities of hypochlorites with lowered surface tension may be reduced by contact with organic material like regular preparations. Thus it can be speculated that most of their activity are lost after 2 min.

The evaluation of the influence of physicochemical properties of irrigant solutions on their penetration depth must take into account surface tension, the spreading properties on dentin surfaces (17,18) and fluid viscosity, as it describes the internal resistance to irrigant flow (19) deformed by either shear or tensile stress. When adding a surfactant to an irrigant, the liquid surface tension decreases with increasing surfactant concentration, until reaching the critical micellar concentration (CMC) (9,18). Above the CMC, the addition of surfactant contributes to the formation of micelles in the liquid, and the surface tension remains relatively constant; the best wetting properties are achieved at this concentration (18). Nevertheless, the exact formulation of Chlor-Xtra is not known. According to the manufacturer's declaration, it contains proprietary surface modifiers to reduce viscosity, a non-ionic wetting agent (Triton X 100) with undisclosed concentration, and alkylating agents. Few data are available concerning the modification of irrigant viscosity through surfactant addition. Viscosity of a fluid is a measure of its resistance to flow under an applied force and has a direct influence on the nature of irrigant flow (20). Bukiet et al. (20) showed that mean viscosity (μ) (mPa s) of 2.4% NaOCl mixed with different concentrations of Triton X 100 at 37 °C, generally increased with the concentration of surfactant. However, for low surfactant concentrations, viscosity first decreased until a minimum value of μ was observed. The minimum value of μ was reached for 0.0001428% Triton X 100 concentration value, twice its CMC before μ increased with concentration. This decrease is likely related to the neutralization of surfactant charges by the sodium cations present in NaOCl solutions. As a result, the conformation of surfactants is more compact and viscosity decreases (21). The minimum μ value occurred for each surfactant at different concentrations. So, whilst viscosity decreases due to neutralization of surfactant charges, it then increases after formation of micelles. Adding a surfactant at high concentration larger than CMC increases irrigant viscosity. Moreover, dynamic viscosity of NaOCl solutions statistically increased with NaOCl concentration (from 0.6 to 9.6%) but decreased with increased temperature (from 22 to 37 °C) (20). This well-known phenomenon may be explained by thermal agitation of the molecules which move more easily at 37 °C than at 22 °C (22) and could explain the increase in penetration depth recorded for Chlor-Xtra *versus* <6% NaOCl at 37 °C and 45 °C with 2 min exposure time.

Nevertheless, the antibacterial effectiveness of NaOCl is highly dependent on the effective concentration, temperature, volume, refreshment rate and contact time into root canals and dentin tubules. After endodontic therapy, microorganisms may persist in areas such as isthmus, ramifications, lateral canals and dentinal tubules because these areas are difficult to clean mechanically and they provide protection from antimicrobial actions of irrigants and medicaments. An incomplete action of irrigant solutions may result into failure to heal or prevent inflammation of the periapical tissues, reflecting a persistent infection of the root canal system (1). There are insufficient data and conflicting evidence regarding the effectiveness of added surfactant on antimicrobial properties and cleaning. However, presence of surfactant showed to have no effect on pH and prevalent chlorine form in solution.

According to Wang et al. (13), the addition of surfactants did not significantly improve depth of penetration of regular 6% NaOCl solution. It was reported no statistically significant difference between 6% NaOCl, 6% NaOCl added with cetrimide and Chlor-Xtra regarding their ability to kill *E. faecalis* in root dentin. This result also supports the findings by Williamson et al. (16) who recorded no difference between 6% NaOCl and Chlor-Xtra in killing *E. faecalis* biofilm. Wang et al. (13) suggested that high concentration NaOCl even without surfactants reaches maximal killing within the timeframes (1-3 min) selected for disinfectant exposure; therefore, the added surfactants could not contribute to any increase in antibacterial activity. Nevertheless, some studies (13,16) did not control the content of free available chlorine in the NaOCl solutions tested. However, the findings by Wang et al. (13) could be explained starting from the absence of significant difference in penetration ability recorded in the present study for H6 (% chlorine: 6.01±0.02) *versus* 6% NaOCl (% chlorine: 6.03±0.01). Because penetration can occur either by capillary forces or by diffusion/flow into the root dentin, low surface tension could also limit the trend of penetration of modified NaOCl solutions into narrow and long dentinal tubules (16,17). Nonetheless, the differences in penetration ability recorded in the present study were statistically significant (p<0.001) for Hypoclean *versus* 6% NaOCl and Chlor-Xtra *versus* 6% NaOCl (different brand), except for results at all temperatures for 2 min of exposure. Moreover, Wang et al. (13) reported a better antibacterial performance by surfactant-containing solutions in all layers and not just in the deeper areas of dentin. Thus, the effect of the addition of surface active agents on available chlorine levels into NaOCl solutions and their stability over time should be investigated.

Concentration provided a lesser contribution to penetration in solutions with surfactants: depth of penetration decreased with increasing concentration from 5.25% (Hypoclean) to 6% (H6). The differences for Hypoclean *versus* H6 were statistically significant (p<0.001) except for results at 2 min. Depth of penetration of regular solutions increased with increasing hypochlorite concentration, except for increase from 1% to 2% at 20 °C for 2 min that resulted in the same values. Nevertheless, differences were small and in most cases, they were not statistically significant. Differences were not statistically significant at all times and temperatures for 1% *versus* 2% NaOCl and 5.25% *versus* 6% NaOCl. These results do not match those reported by Wong et al. (23) and Zou et al. (8). Wong et al. (23) indicated that, although the bactericidal action extended to 300 µm depth into the dentinal tubules, the action was more prominent for 3% *versus* 0.5% NaOCl there. Zou et al. (8) investigated the penetration depths of a 6% NaOCl stock solution and of 1%, 2%, 4% NaOCl immediately prepared before use by dilution with distilled water at different times of exposure and temperatures by observing bleaching action on stained dentin; penetration depth extended between 77 µm (1% NaOCl) and 300 µm (6% NaOCl) into the colored dentin. Actually both studies did not control the content of free available chlorine in the solutions. Because of alternative methods to reduce interferences from substances causing false high readings such as flame infrared emission and thermometric ammonium ion titration, are not widely available, in the present study the iodometric titration procedure was used for estimating free available chlorine for every formulation.

The appropriate contact time for root canal irrigants to achieve their goals of debridement and disinfection in clinical situations is unclear (1).

Under the conditions of the present study, longer exposure time resulted in deeper penetration of both hypochlorites with lowered surface tension and regular preparations. According to the findings by Zou et al. (8) the speed of penetration for both regular preparations and modified solutions declined sharply along time. Nonetheless, the speed of penetration resulted significantly higher for sodium hypochlorites with surfactants *versus* their regular counterparts except for H6 *versus* 6% NaOCl. Moreover, the additive effect of surfactants to the increase in penetration depth after another 18 min resulted more pronounced at 20 °C.

Heating sodium hypochlorites improves their antimicrobial action as well as their tissue and smear layer dissolution capacity. The efficiency of heated NaOCl results from an acceleration of the reaction rate and an enhancement in irrigant flow.

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 5.25%NaOCl and 6% NaOCl for 20 min, results from treatments at the 3 temperatures were statistically different from each other ($p<0.05$). However, in all the groups treated for 20 min, results from treatments at the 3 temperatures were not statistically different from each other ($p>0.05$) with the following exceptions: for H6, penetration at 37 °C was statistically higher ($p<0.05$) than at 20 °C; for Chlor-Xtra, penetration at 45 °C was statistically higher ($p<0.05$) than at 37 °C; finally for Hypoclean, H6 and Chlor-Xtra penetration at 45 °C was statistically higher than at 20 °C ($p<0.001$; $p<0.05$; $p<0.05$, respectively). As the temperature increases, surface tension values of regular sodium hypochlorites and their counterparts added with surfactants decrease (data not published). Higher temperatures may also result in lower viscosity of irrigant solutions. Apparently the diminution of viscosity due to increased temperature was not sufficient to significantly increase the penetration of irrigant solutions. In a clinical setting, the thermodynamic behavior of irrigant solutions inside root canals *in vivo* must be considered. The enhanced efficacy of preheated NaOCl could be limited to the time during which it is actively being injected into the canal. A heat loss of at least 5 °C inside the syringe and the heat loss while the liquid passed through the needle must also be considered. Moreover the temperatures of preheated NaOCl solutions are buffered inside the root canal and tend to rapidly reach equilibrium at 35.1 °C (±1.0 °C) in the apical part of the root canal. The periradicular vascular system, intratubular fluids and water content of hard and soft tissues have a key role in dissipating heat and buffering temperatures. If dentin is considered a good insulator, differences in dentin thickness along the root canal wall can result in nonhomogeneous temperature absorption depending on the location within the root canal. The effective volume into dentinal tubules will be very small and it will quickly reach body temperature or the temperature of the surrounding tooth structure. Even if the effect of temperature on the success rate of endodontic irrigation and clinical relevance of the preheating of root canal irrigants remain unclear, predictable increases in the temperatures of irrigants could be obtained by heating sodium hypochlorites inside the root canals with a heat source plugger/heat carrier; or through the use of ultrasonic devices. Therefore, it could be inferred that NaOCl penetration increases with the exposure time and use of surfactants. This would confirm that surfactants, which decrease the contact angle of irrigant solutions, contribute to the penetration of hypochlorites into dentinal tubules.

Under the present experimental framework, exposure time significantly improved penetration depth of sodium hypochlorites with surfactants added. Nonetheless, temperature and concentration provided a lesser contribution to the penetration of sodium hypochlorite modified with surfactants into root canal dentin.

Resumo

O objetivo deste estudo foi avaliar o efeito da concentração, tempo de exposição e temperatura de hipoclorito de sódio (NaOCl) acrescidos de tensioativos na penetração nos túbulos dentinários. Sessenta e cinco dentes superiores humanos uniradiculares extraídos foram preparadas usando instrumentos ProTaper SX operados manualmente. Os dentes foram seccionados perpendicularmente ao longo eixo. As coroas e o terço apical foram removidos. Os restantes das raízes foram transformadas em blocos de 4-mm de comprimento e coradas durante em violeta de cristal. Cento e trinta blocos foram posteriormente divididos em metades e tratados por nove tipos diferentes de soluções de NaOCl. Três soluções com tensioativos foram adicionadas (Hypoclean, H6, Chlor-Xtra); e os outros foram os hipocloritos regulares em concentrações crescentes (1, 2, 4, 5,25, <6 and 6% de NaOCl) de diferentes origens. Os blocos de dentina foram expostos às soluções de 2, 5 e 20 min a 20 °C, 37 °C e 45 °C, respectivamente. A profundidade de penetração do NaOCl foi determinada pelo branqueamento da mancha e medido em microscopia de luz com ampliações de 20× e 40×. Comparações estatísticas foram feitas usando um modelo linear generalizado com a correção de Bonferroni (post-hoc). A menor penetração (81±6,6 μm) foi medida após incubação com 1% de hipoclorito de sódio durante 2 min a 20 °C; a maior penetração (376,3±3,8 μm) foi obtida com Chlor-Xtra durante 20 min a 45 °C. Variando a concentração do NaOCl verificou-se um efeito mínimo, enquanto que a temperatura e o tempo de exposição teve uma relação significativa direta com a penetração de hipocloritos de sódio, especialmente aqueles com tensão superficial reduzido, nos túbulos dentinários. O tempo de exposição e temperatura do hipoclorito de sódio bem como a adição de agentes tensioativos pode influenciar significativamente a profundidade de penetração de soluções irrigantes nos túbulos dentinários.

References

1. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-1301
2. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol 2005;99:231-252.
3. Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-1379.
4. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002;13:171-183.
5. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. Int Endod J 1990;23:20-27.
6. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60-64.
7. Neglia R, Ardizzoni A, Giardino L, Ambu E, Grazi S, Calignano S, et al.. Comparative in vitro and ex vivo studies on the bactericidal activity of Tetraclean, a new generation endodontic irrigant, and sodium hypochlorite. New Microbiologica 2008;31:57-65.
8. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010;36:793-796.

9. Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L. Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. Int Endod J 2012;45:129-135.
10. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod 2006;32:1091-1093.
11. Tasman F, Cehreli ZC, Ogan C, Etikan I. Surface tension of root canal irrigants. J Endod 2000;26:586-587.
12. Abou-Rass M, Patonai FJ. The effects of decreasing surface tension on the flow of irrigating solutions in narrow root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol 1982;53:524-526.
13. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. J Endod 2012;38:948-953.
14. Giardino L, Morra M, Becce C, Pappen FG, Mohammadi Z, Palazzi F. Comparative wettability of different sodium hypochlorite solutions. G It Endod 2012;26:57-62.
15. Mohammadi Z, Mombeinipour A, Giardino L, Shahriari S. Residual antibacterial activity of a new modified sodium hypochlorite-based endodontic irrigation solution. Med Oral Patol Oral y Cir Bucal 2011;16:e588-e592.
16. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. J Endod 2009;35:95-97.
17. De Gennes PG, Brochard-Wyart F, Quere D. Capillarity and wetting phenomena. Drops, Bubbles, Pearls, Waves. New York, USA: Ed Springer.2004, pp. 1-31.
18. Bukiet F, Couderc G, Camps J, Tassery H, Cuisinier F, About I. Wetting properties and critical micellar concentration of benzalkonium chloride mixed in sodium hypochlorite. J Endod 2012;38:1525-1529.
19. Viswanath DS. Viscosity of liquids: theory, estimation, experiment, and data. New York: Ed Springer, 2007. pp. 1-8.
20. Bukiet F, Soler T, Guivarch M, Camps J, Tassery H, Cuisinier F, et al.. Factors affecting the viscosity of sodium hypochlorite and their effect on irrigant flow. Int Endod J 2013;46:954-961.
21. Onesippe C, Lagerge S. Study of the complex formation between sodium dodecyl sulphate and gelatin. Colloids Surf A 2009;337:61-66.
22. Guyon E, Hulin JP, Petit L, Mitescu CD. Physical hydrodynamics. Oxford, USA: Oxford University Press, 2001.
23. Wong DTS, Cheung GSP. Extension of bactericidal effect of sodium hypochlorite into dentinal tubules. J Endod 2014;40:825-829.
24. Ørstavik DD, Kerekes KK, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J 1991;24:1-7.
25. Paqué F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layers impedes dye penetration into the dentin of endodontically instruments root canals. Int Endod J 2006;39:18-25.

Received January 27, 2016
Accepted February 16, 2016