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Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants

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Abstract

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. *International Endodontic Journal*.

Aim To investigate the surface tension characteristics of 5.25% sodium hypochlorite and three recently introduced sodium hypochlorite solutions, which had been modified to reduce their surface tension: Chlor-Xtra, Hypoclean A and Hypoclean B.

Methodology Freshly produced MilliQ water was used as a reference liquid. All measurements of surface tension were taken by the Wilhelmy plate technique, using a Cahn DCA-322 Dynamic Contact Angle Analyzer at the temperature of 22 °C. A glow-discharge cleaned glass slide was immersed in 5 mL of the test liquid in a beaker cleaned with hot chromic acid, rinsed with MilliQ water and finally air plasma-cleaned in a glow-discharge reactor. The force on the glass slide was recorded continuously by the instrument software as the beaker was raised and withdrawn at the constant speed of 40 micron/s, until at least 1 cm of the glass slide was immersed. The typical accuracy was 0.5 mJ m^{-2}. For each sample, fifteen measurements were taken, and mean values were calculated. A Kruskal–Wallis ANOVA analysis, followed by Mann–Whitney's *U* rank sum test for pair-wise comparisons, was used to compare surface tension values. Statistical significance was set at $\alpha = 0.05$.

Results MilliQ water (72.13 mJ m^{-2}) and 5.25% sodium hypochlorite (48.90 mJ m^{-2}) had the highest surface tension values ($P < 0.01$) compared to Chlor-Xtra (33.14 mJ m^{-2}), Hypoclean B (30.00 mJ m^{-2}) and Hypoclean A (29.13 mJ m^{-2}). Hypoclean A had the lowest surface tension ($P < 0.01$).

Conclusions The new 5.25% sodium hypochlorite solutions modified with surfactants, Hypoclean A and Hypoclean B, had surface tension values that were significantly lower ($P < 0.01$) than Chlor-Xtra and 5.25% NaOCl. Because of their low surface tension and increased contact with dentinal walls, these new irrigants have the potential to penetrate more readily into uninstrumented areas of root canal system as well as allow a more rapid exchange with fresh solution, enabling greater antimicrobial effectiveness and enhanced pulp tissue dissolution ability.

Keywords: chlor-Xtra, hypoclean, irrigants, sodium hypochlorite, surface tension, tetraclean.

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Introduction

The ideal outcome of root canal treatment is the eradication of microorganisms from the root canal system (RCS), or at least their significant reduction to levels compatible with periradicular tissue healing (Siqueira & Rocas 2008). After chemomechanical debridement and disinfection, negative bacterial culture only occurs in 40–60% of cases (Byström & Sundqvist 1985), and bacterial persistence has been shown to be a risk factor for post-treatment apical periodontitis. *Enterococcus faecalis* is a bacterial species

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often detected in cases of post-treatment disease with periapical lesions, occasionally as a monoinfection (Foschi *et al.* 2005).

Amongst currently used solutions, sodium hypochlorite (NaOCl) appears to satisfy most of the requirements of a root canal irrigant (Zehnder 2006). It dissolves both the necrotic tissue (Naenni *et al.* 2004) and the organic components of the smear layer (Haikel *et al.* 1994). It has also been shown to disintegrate endodontic biofilms in laboratory conditions (Clegg *et al.* 2006, Dunavant *et al.* 2006) and inactivate endotoxins (Sarbinoff *et al.* 1983). An *ex vivo* study on the effectiveness of 5.25% NaOCl solution and Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) against *E. faecalis* (Neglia *et al.* 2008) found that in most of the teeth irrigated with NaOCl, the drop in the bacterial load was rapid, but only lasted up to 48 h after irrigation. In fact, 70% of samples were colonized again in the following 96 h with a bacterial load that raised the infection to its previous level.

High surface tension (Giardino *et al.* 2006) could affect the ability of NaOCl to penetrate into dentine and thus reduce its antibacterial effectiveness within dentinal tubules. When cementum is absent, *E. faecalis* can penetrate dentinal tubules of root canal walls to a depth of 800–1000 μm after 3 weeks of incubation (Haapasalo & Ørstavik 1987), whereas the greatest penetration of NaOCl into dentinal tubules has been reported as being 300 μm with a 6% solution for 20 min at 45 °C (Zou *et al.* 2010).

To obtain a suitable contact time of NaOCl with root canal dentinal walls, a major role is played by the wettability of the irrigant on dentine. Wettability is correlated with surface tension (Pecora *et al.* 1991) on ideal surfaces (chemically homogeneous, flat, nonreactive, undeformable and not swollen by the wetting liquid) and then with the surface properties of dentine (Eick *et al.* 1972, Erickson 1992).

To achieve optimal wettability, the surface energy of the substrate must be as high as possible, and the surface tension of the liquid contacted with the substrate must be as low as possible. Surface tension as a condition of intramolecular attraction at the liquid surface prevents the spreading of the solution over a surface (Cameron 1986). When this intramolecular attraction is destroyed, the surface tension decreases. A low surface tension could increase the penetration of irrigants into the uninstrumented areas of the RCS, lateral canals and dentinal tubules and thus increase their contact with the dentine walls (Abou-Rass & Patonai 1982). Surface tension might be reduced by using heat or adding chemicals known as surfactants (Abou-Rass & Patonai 1982, Cameron 1986, Pecora *et al.* 1998, Williamson *et al.* 2009, Stojicic *et al.* 2010). Surface-active agents can consist of monomers, polymers or complex mixtures (containing an active portion within an inactive adduct phase or base). Surfactant molecules are characterized by a hydrophobic portion, organic/oil soluble or water insoluble, and a hydrophilic region (often polar), water soluble. Moreover, each molecule may contain a positively charged group (cationic class), a negatively charged one (anionic class), both (amphoteric class) or no electric charge (nonionic class). Nonionic agents do not ionize but contain hydrophillic polar groups and/or hydrogen bonding capabilities, which can provide strong interactions with water molecules, improving solubility (McNaught & Wilkinson 1997).

The aim of this study was to compare the surface tension of 5.25% NaOCl solution (Niclor 5; Ogna Laboratori Farmaceutici) with three recently introduced NaOCl solutions modified to reduce their surface tension, Chlor-Xtra (Vista Dental Products, Racine, WI, USA), Hypoclean A and Hypoclean B (Ogna Laboratori Farmaceutici). The null hypothesis tested was that there is no significant difference in the surface tension values of the solutions examined.

Materials and methods

The endodontic irrigants tested were: (i) 5.25% NaOCl solution (Niclor 5; Ogna Laboratori Farmaceutici); (ii) Chlor-Xtra (Vista Dental Products), 6% NaOCl with surface tension modifiers; (iii–iv) Hypoclean A and Hypoclean B (Ogna Laboratori Farmaceutici), two 5.25% NaOCl solutions modified with two tension modifiers, cetrimide and polypropylen glycol (the percentage of surfactants in the solution ''A ''was 1% more than in solution ''B''). Freshly produced MilliQ water (distilled water) was used as reference liquid. The surface tension of the test liquids was measured using the Wilhelmy plate technique (Fig. 1b), with a Cahn DCA-322 Dynamic Contact Angle Analyzer [Gibertini Elettronica, Novate (MI), Italy] (Fig. 1a). A glow-discharge cleaned glass slide was immersed in 5 mL of the test liquid in a beaker carefully cleaned with hot chromic acid mixture, rinsed with MilliQ water and finally air plasma-cleaned in a glow-discharge reactor. The force on the glass slide was recorded continuously by the instrument software as the beaker was raised and withdrawn at the constant speed of 40 micron/s, until at least 1 cm of the glass slide appeared immersed.

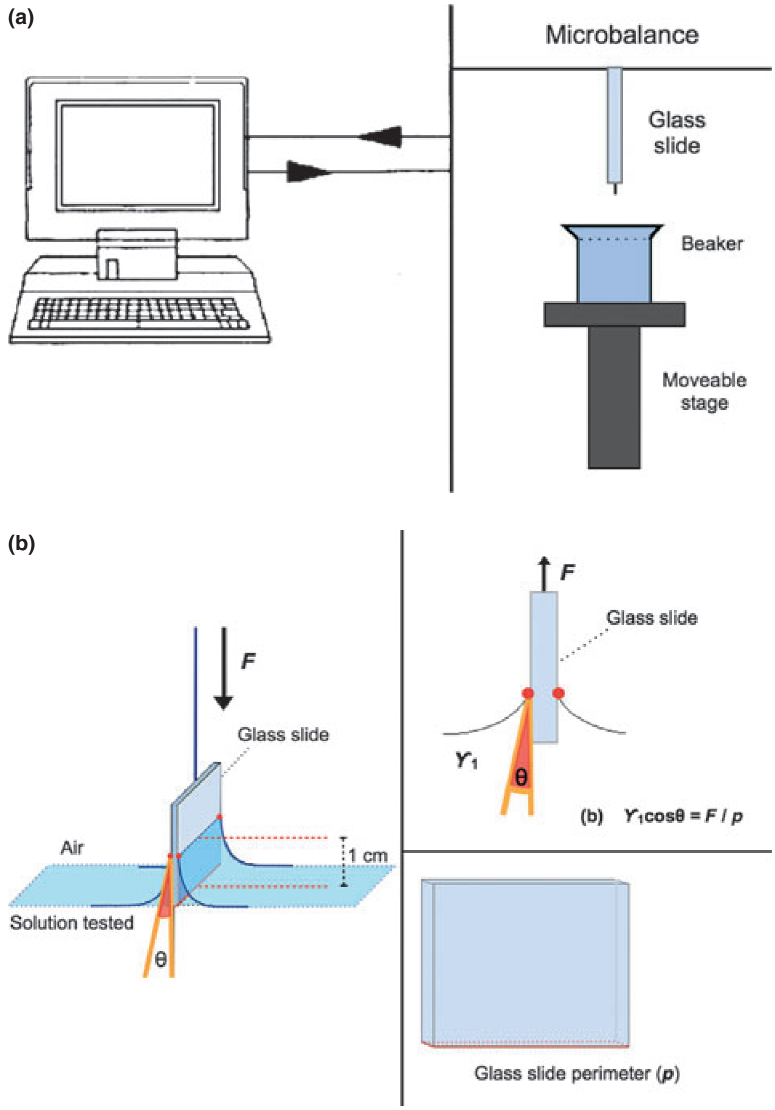

Figure 1 (a) Dynamic contact angle analyzer (Cahn DCA-322). (b) Wilhelmy plate technique.

The liquid surface tension was calculated by the general equation:

$$F = mg + \gamma_1 p\cos\,\theta + \mathrm{Fb} \qquad (1)$$

where F is the force measured by the instrument, m the weight of the glass slide, g the gravity constant, p the glass slide perimeter, γ_1 the liquid surface tension, θ the contact angle and Fb the buoyancy force. The last contribution (buoyance force) on the right hand side of equation (1) can be eliminated by linear extrapolation to zero depth of immersion, whilst the first contribution (mg) is zeroed by the instrument software, yielding on rearrangement:

$$\gamma_1 = F/p\cos\theta \qquad (2)$$

In case of complete wetting, $\theta = 0$ and e $\cos\,\theta = 1$, so that:

$$\gamma_1 = F/p \qquad (3)$$

and the liquid surface tension (γ_1) can be obtained by the ratio between the force (F) at zero depth of immersion and the sample perimeter (p). Measurements were taken at 22 °C, using 5 mL of the test liquids. Samples were contained in plastic vials, which were opened immediately before the experiments. The typical accuracy is 0.5 mJ m^{-2}. For each sample, fifteen measurements were taken and mean value was calculated.

Statistical analysis

Kruskal–Wallis ANOVA analysis, followed by Mann–Whitney's *U* rank sum test for pair-wise comparisons, was used to compare surface tension values, because of the lack of homogeneity of variance, proved with Levene test. All the statistical analyses were set with a significance level of $\alpha = 0.05$.

Results

Figure 2 shows the mean values of surface tension registered for the different irrigant solutions and MilliQ water. The Kruskal–Wallis ANOVA (Table 1) revealed significant differences amongst the different liquids surface tensions ($P < 0.01$), which were all lower ($P < 0.01$) than that of distilled water (72.13 mJ m^{-2}). MilliQ water and 5.25% NaOCl (48.90 mJ m^{-2}) surface tension values were significantly different ($P < 0.001$) (Table 2). Hypoclean A and Hypoclean B solutions yielded the lowest values ($P < 0.01$), 29.13 and 30.00 mJ m^{-2}, respectively, and were significantly different (Mann–Whitney's *U* test $P < 0.05$) from 5.25% NaOCl ($P < 0.001$). Finally, the surface tension of Chlor-Xtra Vista was higher than Hypoclean A ($P < 0.001$) and Hypoclean B ($P < 0.001$).

Discussion

In this study, MilliQ water was used as reference liquid as, owing to the interfacial adsorption of ubiquitous hydrocarbons from the atmosphere, even the purest water undergoes contamination on storage. Thus, the use of freshly prepared ultrapure water avoids the possibility that when water is stored, the surface tension decreases below the accepted value of 71 mJ m^{-2} (Adamson & Gast 1997). MilliQ water surface tension value recorded in the present study was 72.13 mJ m^{-2}, which was significantly higher than those of all the liquids tested. The surface tension of 5.25% NaOCl (48.90 mJ m^{-2}) was consistent with that commonly reported in the literature (Adamson & Gast 1997, Taşman *et al.* 2000). According to manufacturers, Hypoclean A and B are NaOCl solutions containing 5.25% NaOCl and two different surfactants: cetrimide (cationic surfactant) and polypropylene glycol (PPG), the nonionic polymer of propylene glycol. Surface tension of Hypoclean A (29.13 mJ m^{-2}) solution was significantly lower than 5.25% NaOCl ($P < 0.001$) and Chlor-Xtra ($P < 0.001$). Thus, the null hypothesis tested in the study had to be rejected as differences could be detected in surface tension values between all of the examined solutions and distilled water. The antimicrobial efficacy of NaOCl could be improved by better wetting of the surface of the substrate and deeper intratubular penetration. New generation endodontic irrigants combine a powerful detergent effect with a strong antimicrobial efficacy (Shabahang & Torabinejad 2003, Torabinejad *et al.* 2003, Neglia *et al.* 2008). When the antibacterial action of Tetraclean (Ogna Laboratori Farmaceutici), MTAD (BioPure MTAD; Dentsply Tulsa Dental, Tulsa, OK, USA) and formula modifications was tested in a laboratory setting (Pappen *et al.* 2010), the association of cetrimide in different concentrations improved MTAD antibacterial performance on the biofilm and in direct exposure tests at different exposure times. In a recent study (Mohammadi *et al.* 2011), a modified NaOCl (Hypoclean A)

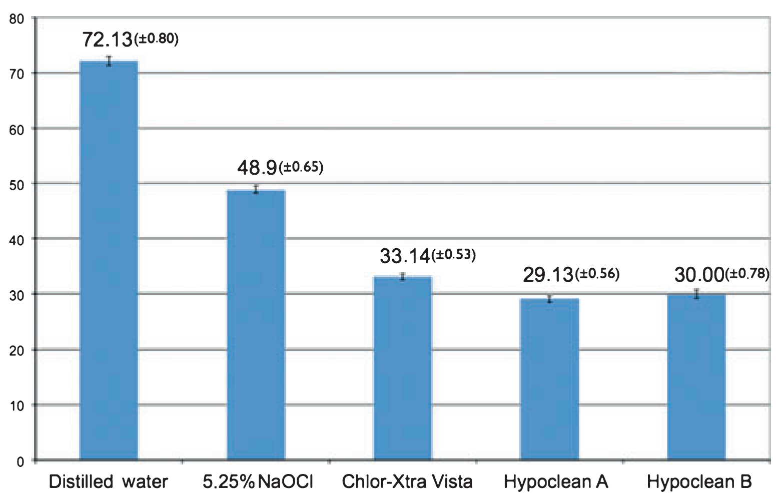

Figure 2 Average surface tension values expressed in mJ m^{-2} (±SD) of the four irrigants examined and MilliQ water (distilled water).

Table 1 Kruskal–Wallis analysis of surface tension values (mJ m^{-2}) for solutions and distilled water

Liquids tested*	N	Mean (SD)	Median	Mean rank	*P* value
(MilliQ water)[a]	15	72.13 (0.80)	72.30	68.00	$P < 0.01$
(5.25% NaOCl)[b]	15	48.90 (0.65)	48.90	53.00	
(Chlor-Xtra)[c]	15	33.14 (0.53)	32.90	38.00	
(Hypoclean A)[d]	15	29.13 (0.56)	29.10	10.70	
(Hypoclean B)[e]	15	30.00 (0.78)	30.10	20.30	

*Liquids with same superscript are not statistically significant ($P < 0.05$).

Table 2 Mann–Whitney rank sum test for pair-wise comparisons of surface tension values for solutions and distilled water

	P value
MilliQ water versus	
NaOCl 5.25%	$P < 0.001$
Chlor-Xtra	
Hypoclean A	
Hypoclean B	
NaOCl 5.25% versus	
Chlor-Xtra	$P < 0.001$
Hypoclean A	
Hypoclean B	
Chlor-Xtra versus	
Hypoclean A	$P < 0.001$
Hypoclean B	
Hypoclean A versus	
Hypoclean B	$P < 0.01$

Significance level of $a = 0.05$.

demonstrated a more effective antibacterial action against *E. faecalis* than 5.25% NaOCl at all experimental periods (7, 14, 21, 28 days) in bovine root dentine and displayed no bacterial growth at the two-first cultures after treatment. It can be assumed that low surface tension increases NaOCl penetration into inaccessible areas of the RCS and dentinal tubules, improving its antimicrobial efficacy. By improving wettability (Torabinejad *et al.* 2002), endodontic irrigants should have a low surface tension and also increased protein solvent power in lateral canals and dentinal tubules. Several studies have examined the tissue-dissolving abilities of NaOCl solutions modified with surfactants sometimes with conflicting results. Nonetheless, few studies have examined the effectiveness of surface-active agents in reducing surface tension whilst taking into account stability of surface tension reduction over time, stability of available chlorine, protein solvent ability and antimicrobial effectiveness of modified solutions. The environment in which surface-active agents are mixed with NaOCl is highly alkaline and oxidizing. Therefore, sodium hypochlorite interacts with surfactants resulting in their consumption and degradation. This interaction must occur gradually preserving long-term efficacy of NaOCl throughout a slow decline in the percentage of active chlorine in solution. Cameron (1986) reported that the efficacy of an endodontic irrigant could be improved by reducing its surface tension: the addition of a fluorocarbon surfactant, with proven ability to withstand aggressive oxidizing media and efficiency as a surfactant in low concentrations, enhanced the ability of NaOCl to dissolve organic material. Clarkson *et al.* (2006) tested the dissolution ability of three different brands of NaOCl available in Australia and reported that the products with surfactants dissolved porcine pulp in a shorter time than regular NaOCl at the same concentration. In the present study, the surface tension of Chlor-Xtra was 33.14 mJ m^{-2}, significantly lower than that of 5.25% NaOCl, consistent with better performance in tissue-dissolving effectiveness of 5.8% Chlor-Xtra as compared with the two regular hypochlorites (Stojicic *et al.* 2010). Moreover, Stojicic *et al.* (2010) showed that the advantage over regular products remained even when the products were diluted, heated or agitated. Aqueous solutions of NaOCl are a dynamic balance of sodium hydroxide (NaOH) and hypochlorous acid (HOCl). When NaOCl comes in contact with organic material, it acts as an organic and fat solvent, and several chemical reactions take place: NaOH reacts with fatty acids transforming them into fatty acid salts (soap) and glycerol (alcohol), which reduces the surface tension of the remaining solution (saponification reaction). Sodium hydroxide also neutralizes amino acids creating salt and water (neutralization reaction). In addition, active chlorine, HOCl and hypochlorite ions (OCl^-) react with amino acids creating chloramine and water (chloramination reaction) (Estrela *et al.* 2002). These reactions happen mostly at the surface and occur simultaneously and synergistically (Spanó *et al.* 2001). Barbin (1999) studied under laboratory conditions the dissolution of bovine pulp tissue by NaOCl (0.5%, 1.0%, 2.5%, 5.0%) and found that the velocity of dissolution of bovine pulp fragments was directly proportional to the concentration of the NaOCl solution and was greater in the solutions without surfactant; solutions without surfactant had a decrease in surface tension and those with surfactant an increase. Barbin (1999) also registered the highest percentage of residual chlorine for NaOCl solutions without surfactant even with high concentrated solution. More concentrated NaOCl solutions present a greater availability of reagents (free chlorine) and the dynamic chemical equilibrium diverted to the formation of NaOH, providing greater appearance of saponification and neutralization reactions without significant consumption of OCl^{-}. Spanó *et al.* (2001) also showed a directly proportional relation between NaOCl concentration and residual chlorine. In colloidal and surface chemistry, the critical micelle concentration (CMC) is defined as the concentration of surfactants above which micelles form and almost all additional surfactants added to the system go to micelles. Before reaching the CMC, the surface tension changes with the concentration of the surfactant. After reaching the CMC, the surface tension remains relatively constant or changes with a lower slope and the surfactant molecules form aggregates (micelles). This transition from premicellar to micellar solutions at the CMC occurs over a narrow range of concentration (McNaught & Wilkinson 1997). The ability of surfactants to reduce surface or interfacial tension is expected to be directly related to the CMC (Huibers *et al.* 1996). The micelles, together with the production of air bubbles in the surfactant, would reduce the contact surface between NaOCl solution and organic materials. The involvement of only the superficial lipid fragments could explain the findings of Barbin (1999). Moreover, an excess of surfactant would divert the dynamic chemical balance of the saponification reaction to the reagents (NaOH and fatty acids), with decreased intensity of reaction. The formation of chloramines occurs mainly with increased consumption of OCl^{-}, as in the cases of less concentrated solutions. All NaOCl solutions were strongly alkaline, and after pulp dissolution, the pH decreases, changing the dynamic chemical equilibrium with increased formation of HOCl and decreased velocity of tissue dissolution (Spanó *et al.* 2001, Rossi-Fedele *et al.* 2011). Nonetheless, Spanó *et al.* (2001) suggested an inverse relationship between the concentration of NaOCl solution and the percentage reduction in pH from the beginning to end of pulp dissolution. In the study of Barbin (1999), pH values before pulp tissue dissolution were higher than 12 for all NaOCl solutions tested, with and without surfactant, and the presence of surfactant did not affect the degree of percentage reduction in pH after dissolution. When the addition of surfactants does not lead to a significant decrease in pH value, ideal environmental conditions are preserved for saponification and neutralization reactions. Thus, a tissue dissolution proportionately less than the consumption of hydroxyl ions in neutralization of the surfactant could explain the increase in surface tension after dissolution reported by Barbin (1999) for solutions with surfactant. The consumption and degradation of surface-active agents must be slow enough to ensure a significant and long-lasting reduction in surface tension whilst preserving the stability of available chlorine. The CMC of surfactants needs to be determined, whereas the value of the CMC for a given dispersant in a given medium depends on temperature, pressure and on the presence and concentration of other surface-active substances and electrolytes. In a system with nonionic polymers, the CMC of surfactant is lower than that of a nonionic polymer-free system: different types of interactions between polymer and individual surfactant molecules or polymer and micelles were assumed as the possibility that the surfactant acts as a bridge through the hydrophobic ends of polymer chains (Huibers *et al.* 1996).

Conclusions

Both the new 5.25% sodium hypochlorite solutions modified with surfactants, Hypoclean A and Hypoclean B, had surface tension values that were significantly lower ($P < 0.01$) than Chlor-Xtra and 5.25% NaOCl. Further investigations into biological and physical experimental models should be performed to demonstrate higher penetration, protein solvent power and bacterial decontamination in uninstrumented areas of RCS, lateral canals and dentinal tubules than 5.25% NaOCl.

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