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ORIGINAL ARTICLE

Surface tension and wetting ability comparison of sodium hypochlorite and ethylenediaminetetraacetic acid with and without surfactants

ABSTRACT

Aim: *To evaluate the surface tension and wetting ability (contact angle) of endodontic irrigants as 5.25% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA) with and without addition of surfactants.*
Methodology: *Eighty halves of single-root teeth were randomly distributed into five groups (n=16) to investigate surface tension and wetting ability of: 5.25% NaOCl; 5.25% NaOCl containing surface-active agents (Hypoclean); 17% EDTA; 17% EDTA with surfactants (EDTA Plus) and freshly obtained distilled water MilliQ as the control group. The surface tension was calculated by the "pendant drop method" and the wettability of the surface by the "sessile drop method". All measurements were taken at room temperature (20 °C). Data were analyzed by one-way analysis of variance (ANOVA).*
Results: *The highest surface tension and the least wetting ability were observed for distilled water and 5.25% sodium hypochlorite (p>0.05). EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and a higher wetting ability than distilled water at room temperature (p<0.001). The addition of surfactants reduced the surface tension and the contact angle of sodium hypochlorite (as in Hypoclean, p<0.001), but not those of EDTA (as in EDTA Plus, p>0.05).*
Conclusions: *Addition of surfactant agents reduces the surface tension and increases the wetting ability of the sodium hypochlorite; surfactants did not affect surface tension and wetting ability of EDTA.*

Giusy Rita Maria La Rosa[1*]
Cristina Scolaro[2]
Giuseppe Leanza[1]
Silvia Rapisarda[1]
Gaetano Isola[1]
Maria Cutroneo[3]
Lorenzo Torrisi[4]
Eugenio Pedullà[1]

[1]Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy

[2]Department of Engineering, University of Messina, Messina, Italy

[3]Department of Neutron Physics, Nuclear Physics Institute, Rez, Czech Republic

[4]Department of Mathematics, Computer Sciences, Physics and Earth Sciences, University of Messina, Messina, Italy

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Corresponding author
Giusy Rita Maria La Rosa | Via Plebiscito, 628, 95124 Catania | Italy
Phone: +39 0957435360 | email: g_larosa92@live.it

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Introduction

Success and prognosis of root canal therapy have both been shown to be dependent on many factors, including the quality of instrumentation and irrigation (1). Mechanical debridement failure may occur due to residual organic tissue, not completely removed from the root canal walls. Therefore, instruments and chemical irrigants are used in conjunction to achieve endodontic debridement especially in cases of teeth presenting complex anatomy in which the morphological irregularities are commonly untouched by instrumentation (1, 2).
The irrigation effectiveness depends on the working mechanisms of the irrigants and the ability to bring the irrigants in contact with microorganism and tissue debris in the root canal (1, 3). The intimacy of this contact is associated to the wettability of the dentin surface where the drop of irrigant is applied. The term "wetting ability" refers to the ability of a liquid to wet a solid surface while the term "wettability" is defined as the ability of the surface of a material to be wet in a uniform and stable way by a liquid substance (4, 5). The wetting ability of a solution influences its penetration ability into the main and lateral canals as well as into the dentinal tubules. It strictly correlated to its surface tension (6, 7) which could be considered as "the force between molecules which produces a tendency for the surface area of a liquid to decrease" (8), limiting the capacity of a liquid solution to penetrate into a capillary hole. Thus, a low surface tension and a high wetting ability represent ideal properties for an endodontic irrigant (8, 9).
The sequential protocol for irrigation includes the use of sodium hypochlorite (NaOCl) during mechanical preparation to dissolve the organic matter and kill microorganisms followed by a strong chelating agent like the ethylenediaminetetraacetic acid (EDTA) solution to remove the inorganic components of the smear layer and leave an adequate substrate for optimal efficacy of the final irrigant (9-12). However, both solutions have high surface tension, which could limit their penetration into dentinal tubules as well as into irregularities of the root canal system such as fins and isthmuses (9).
The effectiveness of an endodontic irrigant could be improved by reducing its surface tension and enhancing its wetting ability using surfactants (13) which act as detergents, wetting and foaming agents, emulsifiers and dispersants (6). Thus, surfactants improve wetting properties of irrigants by diffusing in water and adsorbing interfaces between air and water (dentinal fluid), reducing water's surface tension and increasing their wetting ability on surface. Moreover, surfactants should induce a greater protein solvent power in lateral canals and dentinal tubules due to their capacity to improve wetting ability (14).
Hypoclean (Ogna Lab Srl, Muggiò, Milan, Italy) is a commercial available 5.25% NaOCl solution added with two surface-active agents (1) which presents an optimized ability to kill bacteria compared to a pure 5.25% NaOCl solution and a greater penetration into inaccessible areas of the root canal system and dentinal tubules (15). EDTA Plus (Essential Dental Systems, S. Hackensack NJ, USA) is a 17% ethylenediaminetetraacetic acid sodium salt solution in water with surfactants, presenting, according to the manufacturer, an improved canal diffusion due to the reduced surface tension (16).
No study has examined the effectiveness of surface-active agents in reducing both surface tension and wetting ability of two common irrigants, as NaOCl and EDTA, at the most common concentration of 5.25% and 17%, respectively. Therefore, aim of this study was to evaluate the surface tension and wetting ability of sodium hypochlorite and ethylenediaminetetraacetic acid with and without surfactants. The null hypothesis tested was that surfactants did not affect the surface tension and wetting ability of 5.25% NaOCl and 17% EDTA solutions.

Material and Methods

Four endodontic irrigant solutions were tested:

- 5.25% NaOCl (Niclor 5, Ogna Lab Srl, Muggiò, Milan, Italy);
- 5.25% NaOCl containing surface-active agents (Hypoclean, Ogna Lab Srl, Muggiò, Milan, Italy);
- 17% ethylenediaminetetraacetic acid (17% EDTA, Ogna Lab Srl, Muggiò, Milan, Italy);
- 17% ethylenediaminetetraacetic acid with surfactants (EDTA Plus, Essential Dental Systems, S. Hackensack NJ, USA).

Freshly obtained distilled water MilliQ (MilliQ 18 MOhm, Millipore Corporation, Billerica, MA, USA) was used in the control group.

The study is in accordance with principles of Declaration of Helsinki and it was approved by the Institutional Review Board. A pilot study was performed to establish the sample size calculation. The minimum sample size was fixed at 16 samples for each group (n=16) to ensure a test power of 0.80 (G*Power 3.1.9.2 software, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) with α=0.05.

Therefore, forty fully formed human single-root teeth extracted for periodontal or orthodontic reasons were assessed under 20 magnification using a dental operating microscope (OPMI pico Dental Microscope; Carl Zeiss, Oberkochen, Germany) to exclude those with open apices, resorptive defects, and longitudinal fractures. All included teeth were stored in a 0.2% thymol water solution at 6 °C and used within 30 days of extraction (17). After cutting off the crown and the apical third of each tooth, the remaining root was longitudinally split using a low-speed saw (Isomet, Buhler, Ltd, Lake Bluff, NY, USA) with a diamond disc (Ø 125×0.35×12.7 mm at 330 °C) under cooled distilled water to prevent overheating. Eighty sectioned root halves were obtained. Each cut surface was polished using a series of abrasive papers (CarbiMet; Buehler, Lake Bluff, IL, USA) in the following sequence: 180/P180, 240/P280, 320/P400, 400/P800, and 600/P1200 to obtain a flat wide dentin surface (1, 18). Five root halves were discarded because were damaged during the longitudinally split face and were replaced with other samples. Thus, the eighty root halves were used as substrate for the wetting ability measurements and randomly divided (n=16) in 1 control group and 4 test groups on the basis of the tested liquid:

Group 1 (Control) = freshly produced MilliQ water (MilliQ, 18 MOhm)
Group 2 = 5.25% NaOCl (Niclor 5)
Group 3 = Hypoclean
Group 4 = 17% EDTA
Group 5 = EDTA Plus

All measurements of surface tension and wetting properties were carried out at 20 °C, in order to replicate the conditions of previous assays, as this value is often described as equivalent to "room temperature" (19).

The tested liquids were contained in glass vials which were opened immediately before the experiments.

Measurement of the surface tension

An optical method was performed to calculate the surface tension of endodontic irrigants, the so called "pendant drop method" (8), using the Phoenix 300 system (goniometer and software by SEO-Surface Electro Optics; Suwon City, Gyunggido, South Korea) following the manufacturer's instructions (19, 20-22).

The test liquid was automatically expelled (computer controlled) out of a syringe needle until it almost separated from the tip. Afterwards, the drop shapes were taken by a self-contained digital charge-coupled device camera just before the gravitational force exceeded the interfacial force that caused the drop release from the tip. The surface tension of every tested irrigants and fresh distilled water was automatically evaluated by the apparatus. Pure water was used for calibration before the assays (22).

The equations explaining the drop profile are obtained from the Young-Laplace equation. Using the selected plane method, the surface tension is rapidly derived. This method estimates the surface tension by measuring the equatorial diameter, d_e, and the diameter d_s of a pendant drop in a plane located at a distance d_e from the tip of the droplet. Surface tension (ST) can be mathematically determined by the following equation:

$$ST=(\Delta\varrho \cdot g \cdot d_e^{\,2})/H \qquad [1]$$

where $\Delta\varrho$ is the fluids density difference, g is the gravitational acceleration, and H is the correction factor. Distilled water was used for calibration prior to the assays. Sixteen measurements per test solution were made.

Measurement of wetting ability

The method used for the measurement of the angles of wettability is called "sessile drop" (4, 5); it consists in measuring the contact angle "θ" between a solid interface and the tangent to the profile of a drop.

Contact angles of the tested irrigants or distilled water (control) were measured by depositing 1l drops on the dentinal surface of root halves using a calibrate syringe. The drop images were captured by a webcam (Philips CamSuite 2.0, Milan, Italy) aligned to the eyepiece of an optical microscope which records video images and pictures of the system formed by the solid sample (root dentine) and drop (Figure 1). The eyepiece resolution was of 10× while the lens one could vary between 4× and 2×. The images were scanned in color with a resolution of 640×480 pixels and processed by a computer with an image editing software (Capture One Pro 7, PhaseOne, Cambrige, MA, USA) and appropriate software for the angles measurement (Image measurement, Klonk SmBA, Ringsted, Denmark) (23). Dental samples were dried with compress air before their use for wettability measurement. Sixteen measurements were carried out at room temperature (T=20 °C, UR=35%, P=1 atm) for every tested solution and distilled water, one for each root halve. The average was calculated; for each liquid one microliter was tested. The droplet of the liquid was distributed in thermal equilibrium on the horizontal surface of the samples. Contact angles values less than 90° indicate that the wettability of the surface is favorable (hydrophilic), while angles greater than 90° means that the wettability of the surface is unfavorable (hydrophobic) (Figure 2).

The contact angle θ (degree) was calculated from the height h (mm), the base diameter d (mm), the arctangent (arctg) and the arccosine ($\cos^{-1}$) of the deposited drop on the surface, according to the relations:

$$\theta=2\ \mathrm{arctg}\ (2h/d) \qquad [2]$$

$$\text{or } \theta=90° + \cos^{-1} (4\ hd/4h^2+d^2) \qquad [3]$$

Equation [2] was used for θ<90 °C (hydrophilic), while equation [3] was used when θ>90 °C (hydrophobic).

Statistical analysis

After verifying the normality of the data through the Shapiro-Wilk test, the parametric one-way analysis of variance (ANOVA) followed by Tukey's post hoc was applied to identify significant differences among the irrigant solutions, using a statistical software (Prism 7.0; GraphPad Software, Inc, La Jolla, CA). The significance level was set at $p<0.05$.

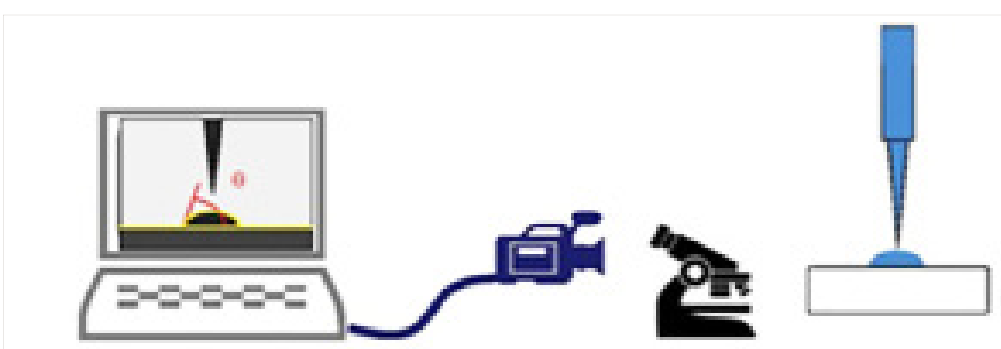

Figure 1
Setup used to record the wetting ability measurements: sketch of the experimental setup.

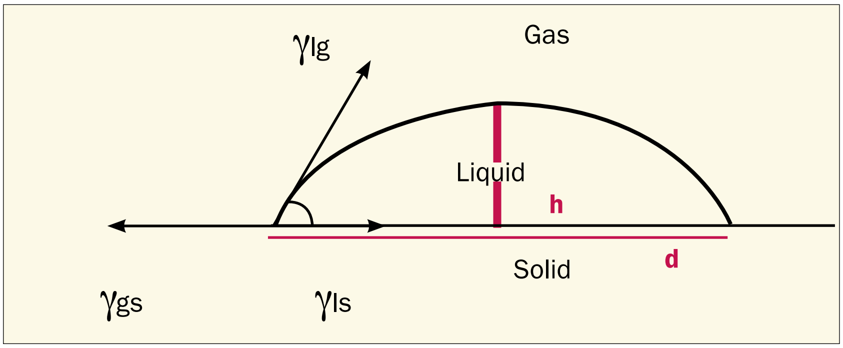

Figure 2
Sketch of the θ angle. This angle is an index of wetting ability of a liquid on a surface.

Results

Table 1 shows the mean, the standard deviation and the median values of surface tension and contact angle on dentin observed for the different irrigants and distilled water. Inferential analysis showed no significant difference between distilled water and sodium hypochlorite for the parameters analyzed (>0.05). EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and contact angle (which means higher wetting ability) than distilled water and sodium hypochlorite ($p<0.001$).
The addition of surfactants agents to sodium hypochlorite, as in Hypoclean, reduced the surface tension and the contact angle significantly ($p<0.001$). Instead, EDTA Plus (17% EDTA with surfactants) not showed a significant reduction of its wetting ability and surface tension than 17% EDTA without surfactants ($p>0.05$).

Discussion

This study compared the superficial tension and the wetting ability (evaluated by means of the contact angle θ) of different endodontic irrigants with and without surfactant agents. On the basis of the present outcomes, the null hypothesis was partially rejected because the results showed that in sodium hypochlorite, the surface tension was reduced and the wetting ability was increased by the addition of surfactants, while these parameters did not significantly change in 17% modified EDTA (EDTA Plus). Even the purest water incurs in contamination on storage, consequently fresh MilliQ water was chosen as control liquid avoiding the interfacial adsorption of ubiquitous hydrocarbons from the atmosphere that could decrease its surface tension (24). Sodium hypochlorite and EDTA, as Niclor 5 and EDTA 17% respectively, were chosen because they are the most common irrigants used in endodontic clinical practice. More specifically, Hypoclean and EDTA Plus were selected because they have the same concentration of Niclor 5 and 17% EDTA respec-

Table 1
Mean, standard deviation and median of surface tension (DINE/cm^2) and contact angle (wetting ability) alues (grades) for tested irrigant solutions and distilled water

Group	Liquids tested	n	Surface tension			Contact angle (wetting ability)		
			Mean	*Standard Deviation*	*Median*	*Mean*	*Standard Deviation*	*Median*
1	MilliQ water	15	70.80^a	1.75	69.75	82.72^a	7.31	82.84
2	5.25% NaOCl	15	67.65^a	1.31	67.88	77.99^a	8.91	78.84
3	Hypoclean	15	27.60^b	1.81	27.89	57.31^b	7.53	61.64
4	EDTA 17%	15	30.57^b	1.01	30.13	67.32^b	5.43	66.12
5	EDTA Plus	15	32.94^b	1.69	33.47	65.95^b	5.78	65.94

Liquids with same superscript letter are not statistically significant ($p>0.05$).

tively but they are added with surfactant agents. There are different surfactants that could be added to endodontic irrigant solutions: Hypoclean uses cetrimide (cationic surfactant) and polypropylen glycol (nonionic polymer of propylene glycol) in an unknown concentration (2) while the surface-active agent of EDTA Plus is not declared.

The methodology used to assess the superficial tension as well as the wetting ability of endodontic irrigants tested is currently accepted in the literature and straightforward to reproduce (5, 8). Several methods have been developed to measure surface tension such as the "ring method" of Du Noüy adopted in a recent study (18), the Wilhelmy plate, the capillary rise, the shape analysis of pendant drop, the maximum bubble pressure, the drop weight. However, when different methods are used, values are not necessarily comparable (22). The pendant drop method using a goniometer and the same apparatus have previously been employed and consequently validated in endodontic research (19-22).

In this study, the detected values of irrigants wetting ability were obtained on dentin in order to simulate the surface that takes contact with the irrigants in clinical practice. A possible limit of this methodology, however, is that the dentin surface was gridded and dry according to Stojicic et al. (25). As a consequence, the tested surfaces are not completely comparable to the dentin surfaces found in clinical practice. Moreover, dentin has a complex structure: it possesses roughness and dentinal tubules and could present chemical interactions with some of the tested liquids in vivo (1, 26). In addition, the results obtained could be affected by the type and concentrations of irrigants tested. Therefore, considering these limitations, the results must be applied in clinical practice with extreme caution.

According to our results, the NaOCl solution with surfactants tested (Hypoclean) showed a significant lower surface tension and higher wetting ability than sodium hypochlorite alone (Niclor 5), as previously reported (6, 25, 27). Regarding EDTA, the solution with surfactant tested (EDTA Plus) showed values of the wetting ability (contact angle's values) and superficial tension not significantly different from the 17% EDTA alone. Nevertheless, it is previously reported the ability of surfactants to reduce the surface tension and increase dentine wetting ability of 17% EDTA (2). These contrasting findings could be due to the possible differences in type and concentration of surface-active agents used in the solutions with surfactants tested in the different studies (27-30). Consequently, the present results showed that the surfactants reduce the surface tension and contact angle (wetting ability increased) of sodium hypochlorite, but not that one's of 17% EDTA. It is possible to hypothesize that this result is due to the different chemical composition of two solutions as well as to the different kind and/or concentration of surfactants agents employed.

Moreover, EDTA with or without surfactants and Hypoclean showed a significant lower surface tension and contact angle values than distilled water. These outcomes are in agreement with some of the previous studies (25, 28-30).

The penetration of irrigating solutions into the complex anatomy of the root canals and dentinal tubules could be increased by the addition of surfactants that reduce the surface tension and increase the wetting ability of irrigating solutions as sodium hypochlorite and EDTA (1, 24, 29). Improving wetting ability of an irrigant solution is clinically important because it could increase its solvent capability and consequently enhance its bactericidal ability through a better penetration into the non-instrumented areas of the root canal system (1, 6). Anyway, in literature, there is no common consensus on the higher dissolution capability and antibacterial action of the sodium hypochlorite with surfactants than the sodium hypochlorite without them. Some studies showed that the addition of surfactants did not significantly improve these capabilities (6, 21, 30) while other authors have shown a significant improvement (17, 18, 31-33). Interestingly, Estevez et al. (17) suggested that these contrasting results can be explained by several methodological dif-

ferences regarding the irrigation protocol. More specifically, the greater irrigation times and irrigant volumes could compensate for the effects associated to the lowered surface tension (17).
Due to their low surface tension and increased contact with dentinal walls, the new irrigants have the potential to penetrate more readily into instrumented areas of root canal system (34) as well as allow a more rapid exchange with fresh solution, enabling greater antimicrobial effectiveness and enhanced pulp tissue dissolution ability (29).
There is also disagreement on the better clinical efficacy of the EDTA with surfactants than that one without surface active agents (12, 27). These differences could be related to the different study designs, type of commercially available irrigant solution tested and kind and/or concentration of surface-active agent added to the irrigants (28). Moreover, some previous ex-vivo studies reported that a reduced chelating power was detected when EDTA was associated with a wetting agent (21, 35). Despite the chelating agents could differ for type and concentration and thus a generalization is not possible, extreme caution is necessary to clinically evaluate these in vitro results.
Further research into the chelating ability of different irrigating solutions used in combinations and/or with the incorporation of surfactants would be needed to take the advantage of maximum irrigant penetration into the uninstrumented areas of the root canal system with maximum chelating efficiency.

Conclusions

Sodium hypochlorite solution modified with surfactants showed a lower surface tension and a higher wetting ability than sodium hypochlorite alone and distilled water.
However, the addition of surfactants to EDTA (as in EDTA Plus) did not result in better wetting ability and lower surface tension compared with EDTA alone.
Further investigations into biological and physical experimental models should be performed to demonstrate higher penetration, protein solvent power and bacterial decontamination in instrumented areas of root canal system of endodontic irrigants with surfactants than that one's without surfactants.

Clinical Relevance

Improving wetting ability of an irrigant solution could be clinically relevant because it may increase its solvent capability and ability penetration into instrumented areas of root canal system.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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