Restor Dent Endod. 2020 Nov;45(4):e47
https://doi.org/10.5395/rde.2020.45.e47
pISSN 2234-7658·eISSN 2234-7666

Restorative Dentistry & Endodontics RDE

Research Article

Check for updates

Physicochemical properties, cytotoxicity and penetration into dentinal tubules of sodium hypochlorite with and without surfactants

Hernán Coaguila-Llerena,[1] **Isadora Barbieri**,[1] **Mário Tanomaru-Filho**,[1] **Renato de Toledo Leonardo**,[1] **Ana Paula Ramos**,[2] **Gisele Faria** [1*]

[1]Department of Restorative Dentistry, São Paulo State University (UNESP), Araraquara School of Dentistry, Araraquara, SP, Brazil
[2]Department of Chemistry, São Paulo University (USP), Ribeirão Preto College of Philosophy Sciences and Letters, Ribeirão Preto, SP, Brazil

OPEN ACCESS

Received: Apr 3, 2019
Revised: Jun 6, 2020
Accepted: Jun 24, 2020

Coaguila-Llerena H, Barbieri I, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G

***Correspondence to**
Gisele Faria, PhD
Professor, Department of Restorative Dentistry, São Paulo State University (UNESP), Araraquara School of Dentistry, 1680 Humaitá Street, Araraquara, SP 14801-903, Brazil.
E-mail: gisele.faria@unesp.br

Funding
This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP under Grant 2018/24662-6; and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq under Grant 51759.

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

ABSTRACT

Objectives: The aim of this study was to assess the physicochemical properties, cytotoxicity and penetration into dentinal tubules of ChlorCid™ Surf (3% sodium hypochlorite [NaOCl] with surfactant) in comparison to ChlorCid™ (3% NaOCl without surfactant).
Materials and Methods: The physicochemical properties evaluated were pH, surface tension, free available chlorine (FAC) and contact angle. Cytotoxicity was evaluated in L929 fibroblasts exposed to the solutions by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide and neutral red assays. Assessment of penetration into dentinal tubules was performed by staining single-rooted permanent human teeth with crystal violet ($n = 9$), which were irrigated with the solutions and analyzed in cervical, middle and apical segments. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's *post*-test, 2-way ANOVA and Bonferroni's *post*-test or *t*-test ($\alpha = 0.05$).
Results: ChlorCid™ Surf and ChlorCid™ FAC values were close to those indicated by the manufacturer. ChlorCid™ Surf showed lower surface tension and contact angle on dentin, and higher pH than ChlorCid™ ($p < 0.05$). The penetration of ChlorCid™ Surf was higher in cervical and middle segments, compared with ChlorCid™ ($p < 0.05$). There was no difference in irrigant cytotoxicity ($p > 0.05$).
Conclusions: ChlorCid™ Surf showed lower surface tension, lower contact angle on root canal dentin, higher penetration into dentinal tubules and more alkaline pH, compared with ChlorCid™. However, both solutions showed similar cytotoxicity and FAC content.

Keywords: Dentin permeability; Materials testing; Physicochemical analysis; Sodium hypochlorite; Surface-active agents

INTRODUCTION

The complex anatomy of the root canal system hinders the elimination of microorganisms in areas untouched by endodontic files, including the dentinal tubules [1,2]. Therefore, the penetration depth of irrigants into dentinal tubules may affect the effectiveness and

Author Contributions

Conceptualization: Coaguila-Llerena H, Faria G, Ramos AP; Data curation: Coaguila-Llerena H, Barbieri I, Faria G; Formal analysis: Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G; Funding acquisition: Coaguila-Llerena H, Barbieri I, Faria G; Investigation: Coaguila-Llerena H, Ramos AP, Faria G; Methodology: Coaguila-Llerena H, Barbieri I, Ramos AP, Faria G; Project administration: Faria G, Ramos AP; Resources: Coaguila-Llerena H, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G; Software: Coaguila-Llerena H, Faria G, Ramos AP; Supervision: Faria G, Ramos AP; Validation: Coaguila-Llerena H, Barbieri I, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G; Visualization: Coaguila-Llerena H, Barbieri I, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G; Writing - original draft: Coaguila-Llerena H, Barbieri I, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G; Writing - review & editing: Coaguila-Llerena H, Barbieri I, Tanomaru-Filho M, Leonardo RDT, Ramos AP, Faria G.

ORCID iDs

Hernán Coaguila-Llerena
https://orcid.org/0000-0002-9991-718X
Isadora Barbieri
https://orcid.org/0000-0003-3413-531X
Mário Tanomaru-Filho
https://orcid.org/0000-0002-2574-4706
Renato de Toledo Leonardo
https://orcid.org/0000-0002-0672-9981
Ana Paula Ramos
https://orcid.org/0000-0001-6200-8989
Gisele Faria
https://orcid.org/0000-0001-7030-3718

consequent prognosis of root canal treatment [3]. The penetration of sodium hypochlorite (NaOCl) into dentinal tubules is reportedly affected by its concentration, contact time, temperature, ultrasonic activation, gel form and surface tension [3-5].

Surface tension is defined as the force between surface molecules, which causes a drop of liquid to spread or concentrate when placed on a surface [6]. This property is related to the contact angle, whose measurement may provide a better understanding of the interaction between solids and liquids, and, consequently, irrigant wettability [7]. It has been reported that the addition of surfactants can decrease NaOCl surface tension, contact angle and improve its penetration into the root canal system [3,8-14]. However, there is still no definitive consensus regarding the improvement of the NaOCl penetration into dentinal tubules, since some studies did not report this effect [5,15].

NaOCl contains hypochlorite ions and hypochlorous acid in different proportions. Together, they constitute free available chlorine (FAC), which promotes organic dissolution and antimicrobial activity [16,17]. The pH of NaOCl influences its organic dissolution and antimicrobial activity; organic dissolution is greater at an alkaline pH [18]. More research is warranted on the effect of a surfactant on the physicochemical properties of NaOCl, such as surface tension, contact angle, FAC and pH, as well as on NaOCl penetration into dentinal tubules. The cytotoxicity of new endodontic irrigants should also be evaluated, since NaOCl may come into contact with periapical tissues, and the tissue response to the irrigant may influence the root canal treatment prognosis [19]. It was previously reported that the addition of benzalkonium chloride, a cationic surfactant, did not alter the cytotoxicity of 2.4% NaOCl [10].

Recently, 3% NaOCl with a surfactant, called ChlorCid™ Surf (Ultradent Products, South Jordan, UT, USA), was introduced. The manufacturer does not declare what surfactant is contained in its composition but claims that it modifies the surface tension, thus allowing better penetration into anatomical irregularities [20]. To the best of our knowledge, no studies have evaluated the physicochemical properties, cytotoxicity and penetration into dentinal tubules of ChlorCid™ Surf. Therefore, this study aimed to assess the physicochemical properties, cytotoxicity and penetration into dentinal tubules of ChlorCid™ Surf (Ultradent Products) compared with surfactant-free 3%NaOCl (ChlorCid™, Ultradent Products). The null hypothesis was that there would be no difference between the solutions regarding the parameters evaluated.

MATERIALS AND METHODS

Physicochemical properties

1. pH

Two 3% NaOCl solutions were stirred, one with surfactant (ChlorCid™ Surf, Ultradent Products) and the other surfactant-free (ChlorCid™, Ultradent Products). The 10 mL of each solution was placed in plastic flasks and the pH of each solution ($n = 5$ per group) was measured using a digital pH-meter (DM-22, Digimed, São Paulo, SP, Brazil), which was previously calibrated according to manufacturer's instructions. The readouts were obtained at 22°C room temperature according to the requirements of the European Pharmacopoeia [21]. Each sample was measured 3 times and the mean value was considered as the value of each sample.

2. FAC content

The FAC content of the solutions was determined following the Standard Methods for the Examination of Water and Wastewater (SMEWW) using the N,N-diethyl p-phenylene diamine (DPD) colorimetric method [22]. The 10 mL of each solution was mixed with 500 µL of phosphate buffer and 500 µL of DPD was added. The FAC reacts immediately with DPD and forms a pink color. The results were obtained using a calibrated spectrophotometer (DR6000, HACH, Loveland, CO, USA) at 515 nm, and expressed in percentage (%) of mass/mass (m/m). Five samples of each group were used for the assay ($n = 5$).

3. Surface tension

An optical technique, the pendant drop method, was performed at 22°C–24°C room temperature. Each solution was placed in a syringe coupled to OCA-20 system (DataPhysics Instruments GmbH, Filderstadt, Germany), wherein it forms a drop, which is digitally captured by a camera, and the surface tension is calculated automatically using SCA-20 software (DataPhysics Instruments GmbH), as described by Andrade *et al.* [23]. This test was performed in triplicate and repeated at 3 times.

4. Contact angle

The sample size was calculated using the G*Power 3.1 software for Windows (Franz Faul, Christian-Albrechts-Universität zu Kiel, Kiel, Germany). The calculation, based on an effect size = 1.01 (based on pilot studies), test power (β) = 0.95 and α = 0.05, using "F-test family", showed that 21 specimens (7 per group) were needed. However, since losses could occur during the experimental phase, 10 teeth per group were used. After approval by the research ethics committee (CAAE: 09798919.3.0000.5416), 15 single-rooted permanent human teeth stored in 0.1% thymol solution at 4°C were used to assess the contact angle. The teeth were decoronated and sectioned longitudinally to obtain 30 hemisections. Each hemisection was mounted on a 20-mm diameter and 15-mm wide poly vinyl chloride ring, observing that the dentin surface of the root canal was left facing the bottom of the device, so that it could later be filled with acrylic resin. After the filling set, the root canal dentin surface and the resin were polished with sequential abrasive paper granulations (120 to 1,200-grit) (3M ESPE, St. Paul, MN, USA) to obtain a smooth surface. The hemisections were distributed into the following 3 groups ($n = 10$): ChlorCid™, ChlorCid™ Surf and water. The specimens were kept in 4°C distilled water until the test. Before performing each measurement, the dentin surface was dried with absorbent paper to remove excess water [10]. The sessile drop method was performed using the OCA-20 system (DataPhysics Instruments GmbH) and SCA-20 software (DataPhysics Instruments GmbH) to determine the contact angle (Θ). Three 5 µL aliquots of each solution were pipetted at 3 different points on the dentin surface, and the images were captured after 30 seconds until each drop was stable [24]. The average of the 3 values was considered the final value for each specimen (**Figure 1**).

Cytotoxicity

ChlorCid™ and ChlorCid™ Surf solutions were considered grade 1 dilutions, serially diluted in saline using a dilution factor of 1.5 [25]. The cells were incubated with the solutions at dilutions from 1/250 to 1/1,898, which corresponded from 0.4% to 0.05% doses/concentrations. L929 mouse fibroblasts were cultured (5×10^4 cells/mL) in 96-well culture plates (Corning, Corning, NY, USA) containing Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin (100 U/mL penicillin, 100 mg/mL streptomycin) (Sigma-Aldrich, St. Louis, MO, USA). The cells were kept in an oven for 24 hours and incubated with the solutions at different doses for 10

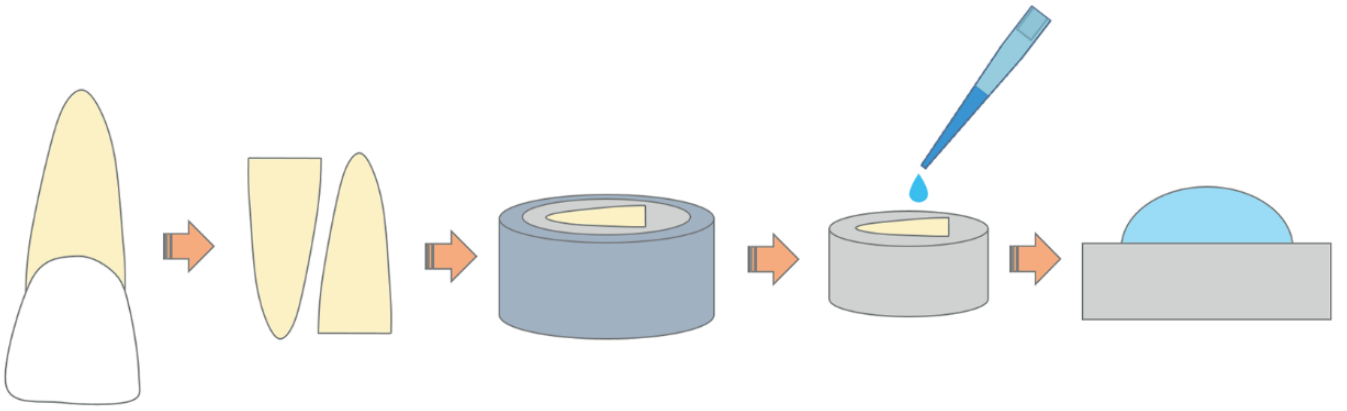

Figure 1. Schematic representation of the contact angle evaluation of ChlorCid™ and ChlorCid™ Surf on root canal dentin. First, each tooth hemisection was mounted on PVC rings with acrylic resin. After resin curing, the rings were removed and the specimen was polished to avoid surface irregularities. Thereafter, 5 μL-drops of each solution were placed on the specimens to measure the contact angle using the OCA-20 system.
PVC, poly vinyl chloride.

minutes to simulate clinical contact time during chemo-mechanical procedures [25]. Saline and DMEM were used as controls. After this first incubation, the solutions were removed from the oven, and the cells were incubated with DMEM for 4 hours [25].

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed by adding 100 μL of 0.5 mg/mL MTT solution (Sigma-Aldrich) to each well, and incubating the cells for 3 hours. Afterward, 100 μL of acidified isopropyl alcohol was added. The neutral red (NR) assay was performed by adding 100 μL of the 0.05 mg/mL NR solution (Sigma-Aldrich) to each well, and incubating the cells for 3 hours. Then, 100 μL of 1% acetic acid solution in 50% ethanol was added to each well. The optical densities were measured using a spectrophotometer (ThermoPlate, Shenzhen, China) with a 570 nm wavelength filter. The percentage of cell viability was calculated from the absorbance of the control (saline), considered as 100%. The experiments were performed in triplicate and repeated at 3 different times.

Penetration into dentinal tubules

The sample size was calculated using the G*Power 3.1 software for Windows. The calculation, based on an effect size = 1.5 (based on pilot studies), test power (β) = 0.80 and α = 0.05, using "*t*-test family" (differences between 2 independent groups), showed that 14 specimens (7 per group) were needed. However, since losses could occur during the experimental phase, 9 teeth per group were used. The methodology was performed according to Faria *et al.* [5], with modifications. The working length of 20 single-rooted permanent human teeth was established 1 mm short of the apical foramen using a size 10 type K-file. Then, the root apex was sealed with composite resin, and the root canals were instrumented using the ProDesign Logic system (Easy Equipamentos Odontólogicos, Belo Horizonte, MG, Brazil) up to a 40/.05 file at 350–600 rpm speed and 1–4 Ncm torque, using an electric motor (VDW Silver, VDW GmbH, Munich, Germany). The root canals were irrigated with 2 mL of 2.5% NaOCl for 1 minute at each instrument change, followed by irrigation with 5 mL of 17% EDTA for 3 minutes and 5 mL of distilled water. The root canals were then filled with 1% crystal violet solution (Labsynth, Diadema, SP, Brazil) and kept at 37°C and 95% humidity for 3 days,

https://doi.org/10.5395/rde.2020.45.e47

changing the crystal violet every 12 hours. Afterward, the root canals were irrigated with 20 mL of distilled water. The roots were randomly distributed into 2 groups (n = 9): ChlorCid™ Surf and ChlorCid™. Two teeth were irrigated with distilled water, and served as the control of the reaction. The specimens were irrigated with 5 mL of the irrigants for 2 minutes using a 5 mL syringe (Ultradent Products) attached to a 27 G side-vented needle (Endo-Eze®, Ultradent Products) positioned 2 mm short of the working length. Next, the root canals of all the groups were irrigated with 5 mL of distilled water, and were sectioned transversely along their longitudinal axis at 3, 7 and 12 mm from the apex. The cervical surface of each segment was polished using 1,000-grit abrasive paper (3M ESPE). A stereomicroscope (LeicaM80, Leica Microsystems, Wetzlar, Germany) and Leica Application Suite EZ 3.0 software (Leica Microsystems) were used to obtain the images. Penetration depth analysis was performed by measuring crystal violet bleaching effects in the cervical surface of each segment, using Image J software (National Institutes of Health, Bethesda, MD, USA). Linear measurements, from the root canal border to the maximum extent of crystal violet bleaching, were made in 10 equidistant regions. The mean value, in micrometers (μm), of 10 linear measurements was considered the final value of the penetration depth for each specimen. A previously calibrated and blinded examiner conducted the analysis twice at a 2-week interval (intraclass correlation coefficient > 0.9).

Statistical analysis

The data were analyzed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) and SPSS 20.0 (IBM, Armonk, NY, USA) statistical software. The statistical tests consisted of one-way analysis of variance (ANOVA) and Tukey's *post*-test (surface tension and contact angle), 2-way ANOVA and Bonferroni's *post*-test (cytotoxicity and penetration into dentinal tubules) or *t*-test (pH and FAC content), at a significance level of 5%.

RESULTS

ChlorCid™ and ChlorCid™ Surf solutions had an alkaline pH of 12.27 ± 0.05 and 13.08 ± 0.02, respectively, and were significantly different ($p < 0.05$). ChlorCid™ had 2.78% ± 0.047% FAC, whereas the ChlorCid™ Surf FAC was 2.85% ± 0.044% (**Table 1**). Deionized water had the highest surface tension (71.95 ± 0.46 mN/m), followed by ChlorCid™ (70.20 ± 0.38 mN/m) and ChlorCid™ Surf (46.10 ± 0.22 mN/m), with significant difference among the 3 solutions ($p < 0.05$). Regarding the contact angle, deionized water had the highest values (50.78° ± 8.85°), followed by ChlorCid™ (36.99° ± 3.34°) and ChlorCid™ Surf (29.18° ± 3.75°) ($p < 0.05$) (**Figure 2**).

There was no difference in the cytotoxicity between ChlorCid™ and ChlorCid™ Surf according to both MTT and NR assays ($p > 0.05$). There was no difference between saline and DMEM groups (**Figure 3**). It was found that the higher the dose, the higher the cytotoxicity.

Table 1. Mean and standard deviation of pH and free available chlorine in percentage (%) of weight/mass (w/w) of ChlorCid™ and ChlorCid™ Surf

Solution	pH	Free available chlorine (% [m/m])
ChlorCid™	12.27 ± 0.05*	2.788 ± 0.047
ChlorCid™ Surf	13.08 ± 0.02	2.854 ± 0.044

*$p < 0.0001$.

https://doi.org/10.5395/rde.2020.45.e47

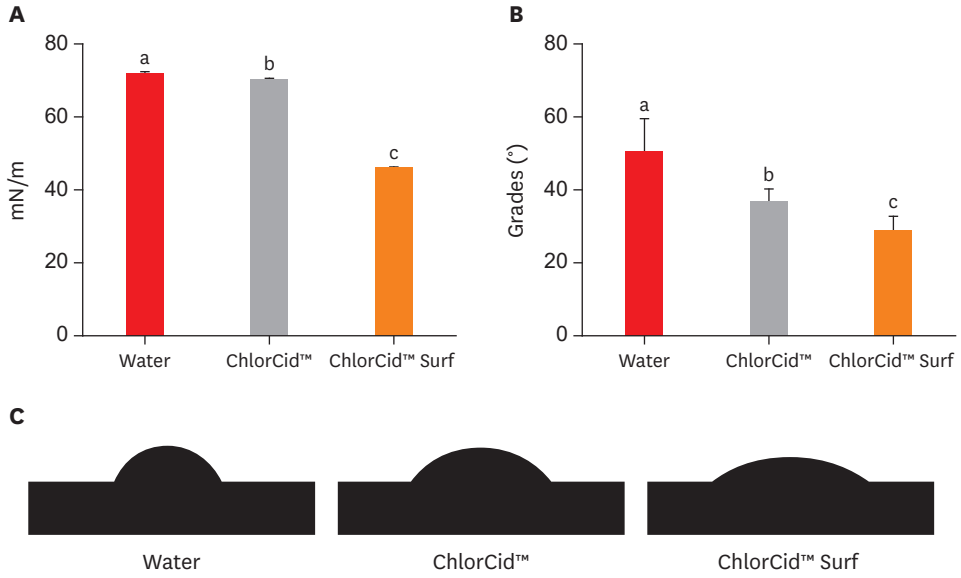

Figure 2. Mean and standard deviation of (A) surface tension in millinewton/meter (mN/m) and (B) contact angle (Θ) of ChlorCid™, ChlorCid™ Surf and deionized water (control). Different letters in columns indicate a significant difference between solutions. (C) Representative images of the contact angle on the dentin surface.

In cervical and middle segments, ChlorCid™ Surf had higher penetration depth (156.22 ± 24.40 μm cervical, 127.52 ± 31.89 μm middle) than ChlorCid™ (92.86 ± 34.44 μm cervical, 88.61 ± 35.34 μm middle) ($p < 0.05$). In the apical segment, penetration depth was 50.47 ± 17.60 μm for ChlorCid™ and 51.44 ± 14.42 μm for ChlorCid™ Surf, with no significant difference ($p > 0.05$). Comparison between the segments showed that both ChlorCid™ and ChlorCid™ Surf had higher penetration depth in the cervical and middle segments than the apical segment ($p < 0.05$), and that there was no difference between cervical and middle segments ($p > 0.05$) (**Figure 4**).

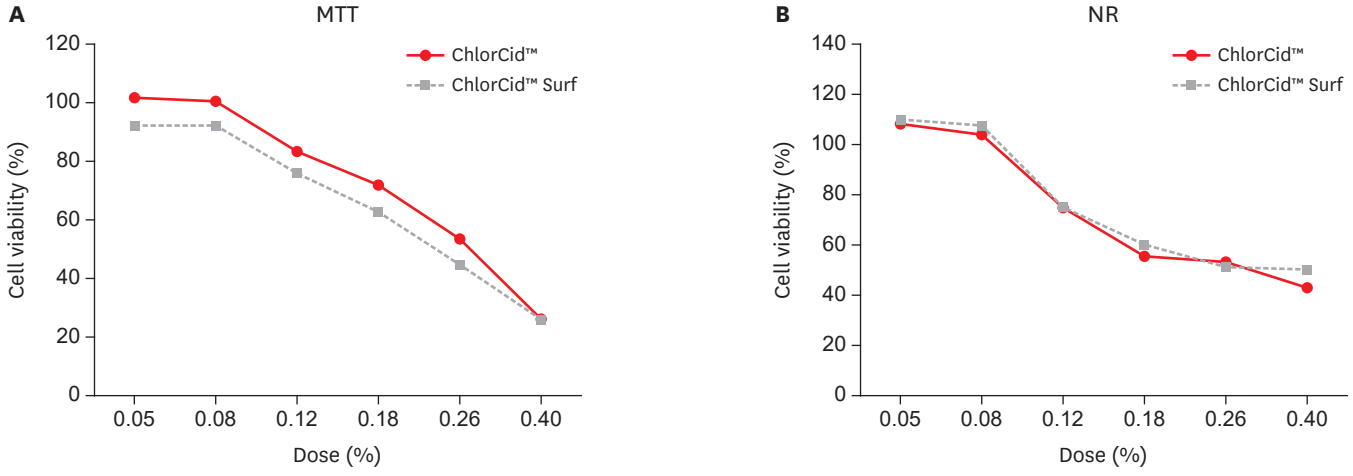

Figure 3. Viability of L929 fibroblasts after exposure to ChlorCid™ and ChlorCid™ Surf solutions at different doses by (A) MTT and (B) NR assays. No significant differences were founded between the solutions ($p > 0.05$).
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NR, neutral red.

https://doi.org/10.5395/rde.2020.45.e47

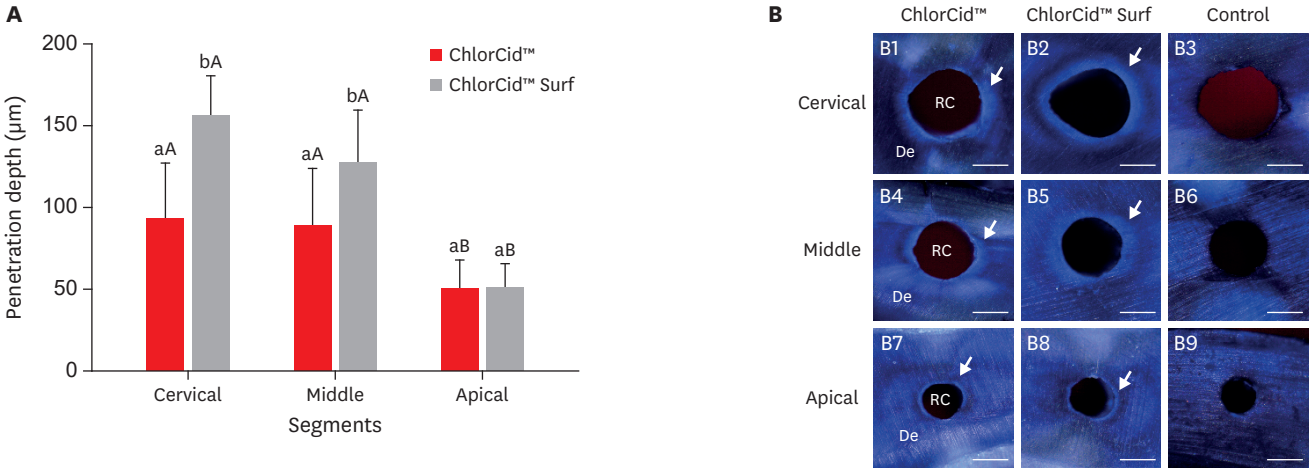

Figure 4. Penetration depth of ChlorCid™ and ChlorCid™ Surf solutions into dentinal tubules. (A) Mean and standard deviation in micrometers (μm) of penetration depth. Different lowercase letters in columns of each segment indicate a significant difference between the solutions. Different capital letters in columns indicate a significant difference between segments of each solution. (B) Representative images of penetration depth of ChlorCid™, ChlorCid™ Surf and water in cervical (B1-B3), middle (B4-B6) and apical (B7-B9) segments, respectively. The bleached crystal violet represents the penetration depth of irrigants into the dentin (arrow) (bar = 500 μm).
De, dentin; RC, root canal.

DISCUSSION

This study aimed to assess the physicochemical properties, cytotoxicity and penetrability into dentinal tubules of a 3% NaOCl solution with surfactant (ChlorCid™ Surf), compared with surfactant-free 3% NaOCl (ChlorCid™). The null hypothesis was rejected because ChlorCid™ Surf had lower surface tension, lower contact angle on dentin, and higher pH and penetration into dentinal tubules than ChlorCid™.

The determination of surface tension of NaOCl solutions has been performed using the ring, glass slide, or pendant drop methods [9-12,26]. Studies that performed the pendant drop method reported that 2.4% NaOCl and 2.5% NaOCl showed a surface tension similar to water, which is approximately 72 mM/m, and equivalent to dyn/cm or mJ/m^2 [10,26]. ChlorCid™ Surf showed lower surface tension than ChlorCid™ (46.10 ± 0.22 and 70.20 ± 0.38 mN/m, respectively). Previous studies showed that the addition of surfactants reduced surface tension of NaOCl solutions, whether the NaOCl concentration was higher or lower than 3% [10-12,14,27,28].

The contact angle evaluation was performed by storing the specimens in distilled water and immediately removing the excess water before the assay since dentin is always wet during root canal treatment [10,14]. ChlorCid™ Surf showed a lower contact angle on root canal dentin than ChlorCid™. This observation agrees with studies showing that the addition of surfactants to NaOCl solutions can reduce this value [10,13]. The contact angle of ChlorCid™ on dentin was lower than that of water. Studies have shown that contact angles of 1% and 2.5% NaOCl on dentin show no difference from the contact angle of water on dentin [13,14]. Differences can be attributed to the methodology, considering that Iglesias *et al.* [14] polished the specimens up to 180-grit abrasive paper, whereas the present study specimens were polished up to 1,200-grit abrasive paper, as previously described [29]. Different granulations may affect dentin surface irregularities, and interfere in contact angle evaluation [30]. Differences from the study by Stojicic *et al.* [13] may be related to

the low water contact angle on dentin observed in their study (39.33°), adopted to make comparisons with NaOCl solutions.

The crystal violet staining technique was used because NaOCl bleaching ability to act on this dye allows penetration depth evaluation [5]. Dentin exposure time to NaOCl was 2 minutes as previously reported [5]. ChlorCid™ Surf penetration was higher in cervical and middle segments, compared with ChlorCid™, indicating that the surfactant in the ChlorCid™ Surf composition led to higher penetration into dentinal tubules. A higher penetration depth of NaOCl with surfactants was also observed in root canal dentin blocks stained with crystal violet [3]. Conversely, Faria *et al.* [5] showed that the addition of surfactant did not influence the penetration of 2.5% NaOCl into dentin. The difference between their study and ours is that they [5] left the irrigants in contact with dentin for a longer time (10 and 15 minutes). Our hypothesis is that the greater exposure time in their study produced an effect in which the results for the solutions with and without surfactant were the same. ChlorCid™ penetration depth is reported to be similar at both 3 and 7 mm from the apex, using manual irrigation for 2 minutes [5]. In sum, the addition of surfactants to 3% NaOCl (ChlorCid™ Surf) resulted in decreased surface tension and contact angle on dentin. Consequently, in increased penetration into dentinal tubules.

The assessment of pH is important since it may have a critical effect on the reactivity of chlorine in NaOCl solutions [31]. Additionally, commercially available NaOCl products, at different concentrations, have an alkaline pH [3,12,16], which is directly related to a higher organic dissolution capacity [18]. ChlorCid™ Surf and ChlorCid™ pH is alkaline, above 12; however, ChlorCid™ Surf showed significantly higher pH than ChlorCid™, indicating that the surfactant influenced this parameter. Previous studies reported that the surfactant had no significant influence on NaOCl pH at different concentrations [3,12,14]. These divergent results may be attributed to the surfactant type and/or concentration in the ChlorCid™ Surf composition.

The determination of FAC content was performed according to the most recent edition of the SMEWW [22], which includes all the recognized methods, among them the iodine/sodium thiosulfate titration method (SMEWW 4500-Cl B) and DPD colorimetric method (SMEWW 4500-Cl G). The DPD colorimetric method is considered simple and fast [22], which is an advantage. Although the iodine/sodium thiosulfate titration method has been widely performed [3,10,12,16,32], the DPD colorimetric method has also been performed in endodontic research [25,33]. ChlorCid™ Surf and ChlorCid™ had a lower FAC content than that reported by the manufacturer (2.85% and 2.78%, respectively). Comparatively, that declared by the manufacturer of ChlorXtra was also lower (6% NaOCl with Triton X-100 surfactant), but that established by the manufacturer of Hypocelle was higher (4% NaOCl with an undeclared surfactant) [12]. This demonstrates that FAC may be irregular in commercial presentations of NaOCl. Regarding the influence of surfactant on the FAC of NaOCl, similar values were observed with and without formulations containing cetrimide [14]. On the other hand, the gradual loss of FAC in NaOCl solutions is reported to be significantly higher in the presence of surfactants; however, this was not observed in the present study [12].

The cytotoxicity of irrigating solutions is important since toxic effects may harm cells in the periapical region and prejudice the repair process [34]. In this regard, the MTT assay has been widely used [10,25,33]; however, it is necessary for more than one assay to evaluate the cytotoxicity [35]. The evaluation of different cellular parameters allows a more reliable interpretation of the cytotoxic effect of the material [36]. For this reason, 2 assays were

performed, the MTT, which allows evaluation of cellular metabolism, and the NR assay, which allows evaluation of cell viability based on the ability of live cells to incorporate NR in the membrane of their lysosomes [37,38]. ChlorCid™ Surf and ChlorCid™ induced similar cytotoxicity of L929 cells in both MTT and NR assays, corroborating a study showing that 2.4% NaOCl in association with benzalkonium chloride showed cytotoxicity similar to 2.4% NaOCl [10]. Although ChlorCid™ Surf showed higher penetration into dentinal tubules than ChlorCid™, further research is needed to assess whether the 2 products are different regarding their antimicrobial effect along with the extension of dentinal tubules.

CONCLUSIONS

It can be concluded that ChlorCid™ Surf showed lower surface tension, lower contact angle on root canal dentin, higher penetration depth into dentinal tubules and more alkaline pH, compared with ChlorCid™. However, both showed similar cytotoxicity and FAC content.

REFERENCES

1. 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 Endod 2005;99:231-252.
PUBMED | CROSSREF
2. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36:1277-1288.
PUBMED | CROSSREF
3. Palazzi F, Blasi A, Mohammadi Z, Del Fabbro M, Estrela C. Penetration of sodium hypochlorite modified with surfactants into root canal dentin. Braz Dent J 2016;27:208-216.
PUBMED | CROSSREF
4. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010;36:793-796.
PUBMED | CROSSREF
5. Faria G, Viola KS, Coaguila-Llerena H, Oliveira LRA, Leonardo RT, Aranda-García AJ, Guerreiro-Tanomaru JM. Penetration of sodium hypochlorite into root canal dentine: effect of surfactants, gel form and passive ultrasonic irrigation. Int Endod J 2019;52:385-392.
PUBMED | CROSSREF
6. Pécora JD, Guimarães LF, Savioli RN. Surface tension of several drugs used in endodontics. Braz Dent J 1992;2:123-127.
PUBMED
7. Kontakiotis EG, Tzanetakis GN, Loizides AL. A comparative study of contact angles of four different root canal sealers. J Endod 2007;33:299-302.
PUBMED | CROSSREF
8. Abou-Rass M, Patonai FJ Jr. The effects of decreasing surface tension on the flow of irrigating solutions in narrow root canals. Oral Surg Oral Med Oral Pathol 1982;53:524-526.
PUBMED | CROSSREF
9. 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.
PUBMED | CROSSREF
10. Bukiet F, Couderc G, Camps J, Tassery H, Cuisinier F, About I, Charrier A, Candoni N. Wetting properties and critical micellar concentration of benzalkonium chloride mixed in sodium hypochlorite. J Endod 2012;38:1525-1529.
PUBMED | CROSSREF
11. 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.
PUBMED | CROSSREF

12. Guastalli AR, Clarkson RM, Rossi-Fedele G. The effect of surfactants on the stability of sodium hypochlorite preparations. J Endod 2015;41:1344-1348.
PUBMED | CROSSREF
13. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod 2010;36:1558-1562.
PUBMED | CROSSREF
14. Iglesias JE, Pinheiro LS, Weibel DE, Montagner F, Grecca FS. Influence of surfactants addition on the properties of calcium hypochlorite solutions. J Appl Oral Sci 2019;27:e20180157.
PUBMED | CROSSREF
15. Giardino L, Cavani F, Generali L. Sodium hypochlorite solution penetration into human dentine: a histochemical evaluation. Int Endod J 2017;50:492-498.
PUBMED | CROSSREF
16. Clarkson RM, Kidd B, Evans GE, Moule AJ. The effect of surfactant on the dissolution of porcine pulpal tissue by sodium hypochlorite solutions. J Endod 2012;38:1257-1260.
PUBMED | CROSSREF
17. Wong DT, Cheung GS. Extension of bactericidal effect of sodium hypochlorite into dentinal tubules. J Endod 2014;40:825-829.
PUBMED | CROSSREF
18. del Carpio-Perochena A, Bramante CM, de Andrade FB, Maliza AG, Cavenago BC, Marciano MA, Amoroso-Silva P, Duarte MH. Antibacterial and dissolution ability of sodium hypochlorite in different pHs on multi-species biofilms. Clin Oral Investig 2015;19:2067-2073.
PUBMED | CROSSREF
19. Yasuda Y, Tatematsu Y, Fujii S, Maeda H, Akamine A, Torabinejad M, Saito T. Effect of MTAD on the differentiation of osteoblast-like cells. J Endod 2010;36:260-263.
PUBMED | CROSSREF
20. Ultradent Products. ChlorCid® Surf [Internet]. South Jordan, UT: Ultradent Products; 2018 [cited 2020 Sep 3]. Available from: https://assets.ctfassets.net/wfptrcrbtkd0/4TD20QySslMLYQ7krTGsHX/fcc08568844faa1a5ac6b149a927537f/ChlorCid-Surf-Sales-Sheet-1005815AR03.pdf.
21. European Directorate for the Quality of Medicines & HealthCare. European pharmacopoeia. Strasbourg Cedex: European Directorate for the Quality of Medicines & HealthCare; 2014. p24-25.
22. Baird R, Eaton A, Rice E. Standard methods for the examination of water and wastewater. Washington, D.C.: American Public Health Association; American Water Works Association; Water Environment Federation; 2017. p11-12.
23. Andrade MA, Favarin B, Derradi R, Bolean M, Simão AM, Millán JL, Ciancaglini P, Ramos AP. Pendant-drop method coupled to ultraviolet-visible spectroscopy: a useful tool to investigate interfacial phenomena. Colloids Surf A Physicochem Eng Asp 2016;504:305-311.
PUBMED | CROSSREF
24. Tartari T, Wichnieski C, Bachmann L, Jafelicci M Jr, Silva RM, Letra A, van der Hoeven R, Duarte MA, Bramante CM. Effect of the combination of several irrigants on dentine surface properties, adsorption of chlorhexidine and adhesion of microorganisms to dentine. Int Endod J 2018;51:1420-1433.
PUBMED | CROSSREF
25. Viola KS, Rodrigues EM, Tanomaru-Filho M, Carlos IZ, Ramos SG, Guerreiro-Tanomaru JM, Faria G. Cytotoxicity of peracetic acid: evaluation of effects on metabolism, structure and cell death. Int Endod J 2018;51 Suppl 4:e264-e277.
PUBMED | CROSSREF
26. Yilmaz Z, Basbag B, Buzoglu HD, Gümüsderelioglu M. Effect of low-surface-tension EDTA solutions on the wettability of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:109-114.
PUBMED | CROSSREF
27. Guneser MB, Arslan D, Dincer AN, Er G. Effect of sodium hypochlorite irrigation with or without surfactants on the bond strength of an epoxy-based sealer to dentin. Clin Oral Investig 2017;21:1259-1265.
PUBMED | CROSSREF
28. Dragan O, Tomuta I, Casoni D, Sarbu C, Campian R, Frentiu T. Influence of mixed additives on the physicochemical properties of a 5.25% sodium hypochlorite solution: an unsupervised multivariate statistical approach. J Endod 2018;44:280-285.e3.
PUBMED | CROSSREF
29. Ballal NV, Ferrer-Luque CM, Sona M, Prabhu KN, Arias-Moliz T, Baca P. Evaluation of final irrigation regimens with maleic acid for smear layer removal and wettability of root canal sealer. Acta Odontol Scand 2018;76:199-203.
PUBMED | CROSSREF

30. Milosevic A. The influence of surface finish and *in-vitro* pellicle on contact-angle measurement and surface morphology of three commercially available composite restoratives. J Oral Rehabil 1992;19:85-97.
PUBMED | CROSSREF

31. Rossi-Fedele G, Guastalli AR, Doğramacı EJ, Steier L, De Figueiredo JA. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J 2011;44:792-799.
PUBMED | CROSSREF

32. Vogel A. A textbook of quantitative inorganic analysis. London: Longmans; 1962.

33. Coaguila-Llerena H, Rodrigues EM, Tanomaru-Filho M, Guerreiro-Tanomaru JM, Faria G. Effects of calcium hypochlorite and octenidine hydrochloride on L929 and human periodontal ligament cells. Braz Dent J 2019;30:213-219.
PUBMED | CROSSREF

34. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod 2003;29:654-657.
PUBMED | CROSSREF

35. Peters OA. Research that matters - biocompatibility and cytotoxicity screening. Int Endod J 2013;46:195-197.
PUBMED | CROSSREF

36. Fotakis G, Timbrell JA. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 2006;160:171-177.
PUBMED | CROSSREF

37. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
PUBMED | CROSSREF

38. Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc 2008;3:1125-1131.
PUBMED | CROSSREF