Shelf life, dissolving action, and antibacterial activity of a neutralized 2.5% sodium hypochlorite solution

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Objectives. The aim was to evaluate the shelf life and the dissolving and antibacterial properties of a neutralized 2.5% NaOCl solution.
Study design. The loss of available chlorine and the pH of the neutralized 2.5% NaOCl solution were recorded to determine its shelf life. The dissolving action on bovine dental pulp was assessed measuring weight loss, pH variation, and decrease in available chlorine content. The antibacterial activity was evaluated on artificially infected human teeth. The roots were endodontically prepared, sterilized, and inoculated with *Enterococcus faecalis* before irrigation with the neutralized solution. The presence of intracanal bacteria after irrigation was recorded.
Results. The neutralized solution presented a shelf life of 2 hours, dissolving capacities equivalent to control for the first 5 minutes, and a better antibacterial efficiency.
Conclusion. The neutralized 2.5% NaOCl solution must be used within 2 hours after mixing, should be frequently renewed to maintain its dissolving capacities, and presented enhanced antibacterial properties. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e66-e73)**

It is widely accepted that periapical inflammation is due to bacterial infection. Apart from the classic Kakehashi germ-free study,[1] it has been shown on dogs that periapical lesions heal spontaneously after root canal preparation without any root canal filling as long as the access cavity is correctly sealed.[2] It has also been demonstrated that no periapical inflammation occurrs in the absence of bacteria regardless of the quality of the root canal filling.[3] Therefore, the complete elimination of the intracanal bacteria seems to be a prerequisite to root canal filling to achieve a successful endodontic treatment.

The mechanical cleaning of the root canal does not seem to be sufficient to ensure a proper disinfection. It has been shown that even a large final apical preparation size only reduces the number of intracanal bacteria.[4,5] It has also been demonstrated in vivo[6] and more recently in vitro[7] that the chemical cleaning with saline does not allow the elimination of endodontic pathogens. This leads to the conclusion that the root canal irrigant must present antibacterial properties that ensures a satisfactory chemomechanical preparation of the root canal when associated with the mechanical preparation.

Recent surveys in different countries showed that sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant.[8] The antibacterial properties of this solution are well known and many studies have evaluated its effectiveness against bacteria from endodontic origin. However, to the best of our knowledge, no root canal irrigant has been shown to be able to eliminate all the intracanal bacteria in a reproducible manner. This is confirmed by the lower success rate reported when treating infected teeth than when performing initial root canal therapy.[9]

The concentration of a commercial NaOCl solution represents its concentration in available chlorine. Because hypochlorous acid (HOCl) is a weak acid, available chlorine in the NaOCl solution takes several chemical forms according to the pH: $Cl_2$is the acidic form, HOCl the neutral form, and ClO^- the alkaline form.[10] Because HOCl is a much better antibacterial agent than ClO^-, adjusting the pH of an NaOCl solution to the pKa of hypochlorous acid (around pH 7.5) would the-

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oretically increase its antibacterial properties.[11] Buffering NaOCl solutions to increase its efficiency was developed by Dr. Henry Drysdale Dakin during World War I to treat gas gangrene. Some properties of buffered NaOCl solutions have already been explored in endodontics. The decrease to pH 9 by sodium carbonate addition did not enhance the antibacterial activity of a 0.5% NaOCl.[12] However, the HOCl concentration of an NaOCl solution at pH 7.5 is twice as high as at pH 9, and this should hypothetically increase the antibacterial properties of the solution. On the other hand, the decrease to pH 6.5 and 7.5 of a 4.2% NaOCl solution statistically increased its antibacterial effectiveness against *Enterococcus faecalis* in artificially infected teeth.[13]

However, besides the abovementioned positive aspect, adjusting the pH of an NaOCl solution to its pKa is likely to have 2 negative consequences. The manufacturers add sodium hydroxide to the commercial NaOCl solutions to ensure a long shelf life.[14] Reducing the pH of the NaOCl solution by addition of an acid leads to the neutralization the OH^- ions. Therefore, the shelf life of the solution may be reduced because of the depletion in OH^-. In addition, the neutralized solution may have lost its dissolving properties on organic material, because the cleaning effectiveness of a NaOCl solution is related to the concentration of ClO^-.[15] Because the optimal pH region of the antibacterial activity of NaOCl differs from that of its cleaning activity, the neutralized NaOCl solution may no longer be able to dissolve dental pulp.[16] The dissolution of pulpal organic content is of primary importance in endodontics.[17] The effects of pH reduction on these properties of a NaOCl solution have not so far been investigated in endodontics.

The purpose of the present study was to assess: 1) the shelf life; 2) the dissolving properties on bovine dental pulp; and 3) the antibacterial properties of a neutralized 2.5% sodium hypochlorite solution.

MATERIALS AND METHODS

Determination of the shelf life of a neutralized 2.5% NaOCl solution

A 10% NaOCl (w/w molecular weight NaOCl 74.44) unbuffered stock solution (Sigma Chemical Co., St. Louis, MO) was used. Because the purpose was to achieve a final concentration of 2.5% NaOCl of pH 7.5 at the same time, 1 volume of the 10% NaOCl solution was mixed with 3 volumes of HCl (Sigma Chemical Co.) at various concentrations. The pH of the solutions were recorded with a pH meter at 25°C (Hanna instruments, Tanneries, France). This showed that when 3 volumes of 0.2 mol/L HCl was mixed with 1 volume of 10% NaOCl, a 2.5% NaOCl solution at pH 7.5 was obtained. This concentration was used for the following studies.

The pH of the modified 2.5% NaOCl solution was recorded each hour for 5 hours at 25°C using the same device. The solution was allowed to stand statically at room temperature and was protected from light to mimic clinical conditions. At the end of each period, the available chlorine content was measured by titration with sodium thiosulfate. The neutralized solution was considered to be unsuitable for root canal irrigation when it was too acidic (pH <6), because of the risk of Cl_2 evaporation, or when the available chlorine content was too low (loss of 50%), because of the loss of effectiveness.

Dissolving action on bovine dental pulp

Bovine incisors were obtained from freshly slaughtered animals. They were stored at −6°C for 2 hours during transportation. A groove was made on each side of the roots using a diamond burr in a high-speed handpiece, without entering the pulp chamber. The roots were then split into 2 halves using a knife and a hammer, and the dental pulps were carefully removed with cotton pliers. The pulp specimens were stored in saline solution at room temperature before use. Samples weighing 0.1 g were placed into microfuge tubes containing 1 mL freshly prepared Dakin (0.5%) (n = 20), 2.5 % unmodified NaClO (n = 20), neutralized 2.5% NaOCl solution (n = 20), or saline solution as control (n = 20). The tubes were closed and kept rotating in an agitator at 150 rpm at 25°C for 5 minutes (n = 5 per liquid), 10 minutes (n = 5 per liquid), 15 minutes (n = 5 per liquid), and 20 minutes (n = 5 per liquid).

During each period, 3 parameters were recorded:

Pulp weight: After removal from the tubes, the samples were washed in saline, blotted dry, and weighed using a precision balance (Mettler, Greifensee, Switzerland).

pH: The pH of the liquid was recorded using a pH meter calibrated at 25°C (Hanna Instruments, Tanneries, France). A small magnetic stirrer was used to homogenize the solutions.

Available chlorine content: The chlorine content of the liquid was measured using the same titration method described with sodium thiosulfate.

The results were submitted to a statistical analysis:

Pulp weight variations over time between the 3 liquids and the saline solution were compared using a 2-way analysis of variance (ANOVA) (way 1 liquid and way 2 time). Post hoc comparisons were

performed using a Duncan test at the end of each period.

pH variations over time were analyzed using an ANOVA for each of the 3 liquids.

Available chlorine content values were expressed as percentages of the initial values and also compared using a 2-way ANOVA. Post hoc comparisons were performed using a Duncan test at each period of time. The significance level was set at 5% for all statistical analyses.

Antibacterial activity on *Enterococcus faecalis* in artificially infected human teeth

Specimen preparation. With informed patient consents, 120 extracted permanent human maxillary monoradicular front teeth were stored in phosphate-buffered saline solution + penicillin 100 IU/mL + streptomycin 100 μg/mL at 4°C until use. All of the teeth were intact with a mature apex. A slow-speed diamond saw under saline irrigation was used to decoronate them so that all of the roots were 13 mm long. A size-10 K file was introduced into the canal to radiographically measure the working length and to check the patency of the apical foramen. The root canals were prepared by the same operator to the cementodentinal junction with a ProFile device (Dentsply Maillefer, Ballaigues, Switzerland) using a reduction handpiece coupled to an electric motor until a ProFile size 30 taper 06 reached the working length. A size-10 K file was used between each ProFile to ensure apical patency. Two milliliters of 2.5% NaOCl was delivered with a 27-gauge needle between each file, totaling 16 mL per root. After using 5 mL 17% EDTA for 1 min, a final rinse was performed with 5 mL 1% sodium thiosulfate and 5 mL saline. The teeth were then sterilized at 135°C for 35 minutes.[18] After sterilization, the 120 roots were divided randomly into 6 subgroups of 20 teeth each according to the 2 contact times (5 and 20 minutes), and the 3 root canal irrigants (Dakin [Cooper, Melun, France], unmodified 2.5% NaOCL solution, and neutralized 2.5% NaOCl solution).

Specimen infection. Pure cultures of *Enterococcus faecalis* (CIP 103015; Institut Pasteur, Lyon, France) were prepared according to the recommendations of the Institut Pasteur and then suspended in brain-heart infusion (BHI) for 24 hours. The sterile roots were placed 5 by 5 in glass tubes filled with 15 mL of the BHI with the turbidity adjusted to 1×10^8 CFU/mL (equivalent to 0.5 McFarland). The contaminated broth was vortexed for 30 seconds to enhance the penetration of the bacteria into the roots and incubated for 5 days at 37°C. Because the incubation time varies according to the conditions of the study, a preliminary work showed, by optical density evaluation of the medium, that this period was sufficient to ensure a proper contamination of the prepared roots but not too long to reach broth saturation. This was confirmed by the negative and positive control samples.

Controls. Sixty additional teeth were endodontically prepared and used for control. The efficiency of tooth sterilization was verified by storing 20 sterilized roots in BHI broth for 1 week and inoculating agar plates with the storage medium. Specimen infection was checked using 20 additional teeth which were irrigated with sterile saline (negative control) and 20 teeth which were irrigated with 10% NaOCl (positive control).

Specimen irrigation. After the incubation period, all the roots were irrigated according to the same protocol. A sterile cotton pellet was placed into the canal and the orifice was sealed with Cavit (3M Espe, St Paul, MN). The outer surface was cleaned with a sterile gauze, and the apex was sealed with hot wax to simulate clinical conditions. The Cavit and cotton pellets were removed and the canal irrigated with 2 mL saline, using a 28-gauge endodontic needle, to eliminate the medium and the planktonic bacteria. According to its group, each root was then irrigated with 5 mL of one of the 3 liquids, which was left in place for 5 or 20 minutes. The root canal was rinsed with 5 mL 1% sodium thiosulfate to stop the effects of chlorine and finally with 5 mL sterile saline. Lack of effects of 1% sodium thiosulfate on bacteria was tested separately. A sterile size-50 K file was then gently placed in the canal at 1 mm from the working length and turned clockwise to cut dentin chips. Each file was placed in a sterile vial containing 1 mL BHI broth and incubated for 3 days. The vials were labeled to analyze the results in a blind manner. After incubation this broth was used to inoculate agar plates (brain-heart agar under aerobic conditions) that were cultivated for 48 hours. After these 48 hours, the presence or absence of bacteria on the agar plates was recorded and used for statistical analysis. Microscopic observation of gram-positive cocci arranged in a cross-chain pattern was used to regularly verify the purity of the strains.

Statistical analysis. A chi-squared test was performed, for the 5- and 20-minute contact times, to look for statistical differences among the 3 liquids. The significance level was set at 95%.

RESULTS

Determination of the shelf life of the neutralized 2.5% NaOCl solution

The chlorine content of the neutralized 2.5% NaOCl solution decreased to 93% of the initial value after 1 hour, and decreased respectively to 72% after 2 hours, 52% after 3 hours, 29% after 4 hours, and no longer measurable after 5 hours. Similarly, pH decreased from

Table I. Variation of pH and available chlorine content over time of the neutralized 2.5% NaOCL solution

Time (h)	pH	Available chlorine (%)
0	7.3	2.48
1	7.2	2.30
2	6.4	1.77
3	5.5	1.42
4	5.1	0.71
5	4.9	0.36

After 2 h, the pH fell below 6.4 and the solution should no longer be used.

7.3 to 4.9 after 5 hours (Table I). It can be concluded that the neutralized 2.5% NaOCl solution should be used within 2 hours after mixing.

Dissolving action on bovine dental pulp

The negative control showed that saline solution had no effect on pulp weight, which remained stable for 20 minutes. Similarly, no pH variation was observed.

Pulp weight variations. The 2-way ANOVA showed a statistically significant difference for both parameters, showing the influence of time ($P < .001$) and liquid ($P < .001$). The Duncan test failed to show a statistically significant difference between the 3 liquids at 5 minutes. But the difference was statistically significant at 10, 15, and 20 minutes: The unmodified 2.5% NaOCl solution was more effective than the neutralized 2.5% NaOCl solution, which was in turn more effective that Dakin (Table II).

pH variations. The ANOVA failed to show a statistically significant difference among the pH levels recorded at 0, 5, 10, 15, and 20 minutes for each liquid. Therefore, it can be concluded that the pH did not vary during the first 20 minutes despite the presence of dental pulp (Table III).

Variation in available chlorine content percentage. The 2-way ANOVA showed a statistically significant difference for both parameters, showing the influence of time ($P < .001$) and liquid ($P < .001$). The Duncan test failed to show a statistically significant difference between the 3 liquids at 5 minutes. However, there was a statistically significant difference at 10, 15, and 20 minutes. It can be concluded that some liquids lost more available chlorine than others over time: the unmodified 2.5% NaOCl solution lost less chlorine, in percentage, than the 2 other solutions. The Dakin lost the highest percentage of available chlorine (Table IV).

Antibacterial study

The controls showed that the sterilization procedure was adequate (no contaminated tooth), 1% sodium thiosulfate had no effect on bacterial growth, and the inoculation protocol led to 100% of infected teeth.

The chi-squared tests showed a statistically significant difference among the 3 groups of teeth ($P = .03$). This showed the influence of the liquid used for root canal irrigation. It can be concluded from the present work that Dakin (0.5% NaOCl) was less effective than the unmodified 2.5% NaOCl, which was in turn less effective than the neutralized 2.5% NaOCl solution (Table V).

DISCUSSION

This work showed that neutralizing a 10% NaOCl solution with 0.2 mol/L HCl produced a neutralized 2.5% NaOCl solution which needed to be used within 2 hours. This neutralized solution was as efficient at dissolving dental pulp as the unmodified 2.5% NaOCl solution during the first 5 minutes of contact, and it presented a better antibacterial activity against *Enterococcus faecalis*.

Shelf life

Mixing 1 volume of 10% NaOCl solution and 3 volumes of 0.2 mol/L HCl solution diluted the chlorine content so that the final concentration of available chlorine was 2.5%. It also modified the pH of the solution, decreasing it from 13 to 7.5. The pH of the solution decreased slowly during the first 2 hours (7.3 to 6.4) and then decreased more rapidly during the next 3 hours (6.4 to 4.9). This pH variation may be due to 2 combined phenomena: first, the content of free sodium hydroxide being very low in the 10% NaOCl solution, a small quantity of acid could easily neutralize the OH^- ions; second, HOCl, which is predominant in the neutralized 2.5% NaOCl solution, is easily transformed in H^+ and Cl^- because the decomposition of HOCl into chlorine (Cl_2) then chloride (Cl^-) is very rapid. Because the solubility of Cl_2 in water is very low, chloride escapes from the solution. This is not the case for the H^+ ions, which leads to an accelerated decrease in pH. In addition, the air at the surface of the solution contains CO_2, which can be dissolved in the solution to form carbonic acid $(HCO_3)^- + H^+$. These combined phenomena could explain the decrease in pH and the very poor available chlorine content after 5 hours. These results indicate that unlike the alkaline classic NaOCl solutions that can remain quasi stable for weeks[19] or months,[20] even if exposed to light,[21] the experimental liquid should be used within the first 2 hours after mixing.

It was decided to use HCl to neutralize the NaOCl solution to obtain sodium chloride, a biocompatible corresponding salt, but other possibilities might be considered. The addition of buffer such as sodium carbon-

Table II. Variation of weight (mg) of 0.1 g bovine dental pulp immersed in 1 mL root canal irrigant

	0 min	*5 min*	*10 min*	*15 min*	*20 min*
Dakin	102 ± 5	75 ± 12[a]	65 ± 12[a]	62 ± 15[a]	55 ± 12[a]
Unmodified 2.5% NaOCl	105 ± 5	62 ± 9[a]	32 ± 9[b]	17 ± 6[c]	5 ± 0[c]
Neutralized 2.5% NaOCl	103 ± 6	72 ± 5[a]	55 ± 11[a]	50 ± 5[b]	35 ± 10[b]
Saline	105 ± 5	105 ± 5	105 ± 5	105 ± 5	105 ± 5

The analysis of variance showed a statistically significant difference among the 3 liquids ($P < .001$).
[a,b,c]Within the same column (time), the groups with the same superscript letter were not statistically different according to the Duncan test.

Table III. Variation of pH of 1 mL root canal irrigants containing 0.1 g bovine dental pulp

	0 min	*5 min*	*10 min*	*15 min*	*20 min*
Dakin	10.3 ± 0.05	10.07 ± 0.09	10.05 ± 0.05	10.02 ± 0.15	9.92 ± 0.05
Unmodified 2.5% NaOCl	12.5 ± 0.15	12.05 ± 0.23	12.12 ± 0.09	12.17 ± 0.12	12.25 ± 0.23
Neutralized 2.5% NaOCl	7.4 ± 0.07	7.17 ± 0.05	7.17 ± 0.05	6.92 ± 0.05	7.40 ± 0.08
Saline	7.5 ± 0	7.5 ± 0	7.5 ± 0	7.5 ± 0	7.5 ± 0

The analysis of variance failed to show a statistically significant pH variation over time.

Table IV. Available chlorine content (%) of 3 root canal irrigants containing a bovine dental pulp sample after 5, 10, 15, and 20 minutes

	0 min	*5 min*	*10 min*	*15 min*	*20 min*
Dakin	0.5	0.39 ± 0.01	0.32 ± 0.01	0.25 ± 0.01	0.14 ± 0.01
Unmodified 2.5% NaOCl	2.5	2.06 ± 0.21	1.72 ± 0.26	1.50 ± 0.48	1.38 ± 0.54
Neutralized 2.5% NaOCl	2.5	1.78 ± 0.21	1.13 ± 0.19	0.90 ± 0.19	0.90 ± 0.17
Saline	0	0	0	0	0

Table V. Number of sterile roots per group

	Contact 5 min	*Contact 20 min*
Dakin	7/20	11/20
Unmodified 2.5% NaOCl	10/20	15/20
Neutralized 2.5% NaOCl	14/20	19/20
Saline (negative control)	0/10	0/10
10% NaOCl (positive control)	10/10	10/10

The chi-squared tests performed at 5 and 20 minutes showed a statistically significant influence of the liquid and the contact time ($P = .03$): The neutralized 2.5% NaOCL solution was more efficient at eliminating intracanal bacteria than the 2 other solutions.

ate[12] and neutralizer such as acetic acid[13] has been investigated, but phosphoric acid, a triacid having 3 different pKa, and all of the polycarboxylic acids are good candidates.

Dissolving action on bovine dental pulp

The neutralized 2.5% NaOCl solution showed the same dissolving efficiency as the others within the first 5 minutes. The pulp samples lost 31% of their weight in the neutralized 2.5% NaOCl solution and 40% in the unmodified 2.5% NaOCl solution. It was only after 10 minutes that a statistically significantly difference was found between both liquids, showing a greater efficiency of the unmodified 2.5% NaOCl solution. It should be borne in mind that this difference is clinically irrelevant, because the root canal irrigant must be frequently renewed. Therefore, it can be concluded that the experimental liquid dissolves dental pulp as well as the 2.5% NaOCl solution under clinical conditions, i.e., when frequently renewed.

All studies evaluating the dissolving ability of root canal irrigants use the same protocol of immersing a biologic sample in the liquid. However, some variations in protocol are worth noticing. Some authors used bovine dental pulp,[22] whereas others used porcine dental pulps,[21] human dental pulps,[23] palatal mucosa,[24,25] porcine muscle,[26] and bovine tendon collagen.[27] It seems reasonable to prefer dental pulp, because it differs from other tissues from an histologic point of view. Bovine dental pulp is also preferable, because large samples are necessary to undertake all of the the experimentations on the same day to ensure a reliable comparison among the liquids. Some authors record the time necessary to completely dissolve the dental pulp sample,[23] but this does not correspond to the real chair

time. It was therefore decided to weigh the samples every 5 minutes and to stop after 20 minutes, because a preliminary study had shown that the unmodified 2.5 % NaOCl solution dissolved the bovine dental pulp samples within 20 minutes. However, comparing studies is often difficult because of variations in protocols. Along with the exposure time, the ratio between the weight of the dental pulp sample and the volume of dissolving liquid is probably of prime importance but varies greatly among studies. Therefore, for the sake of standardization, the results should be expressed not as percentage weight loss but as mg/mL/min. For example, the present study showed that Dakin, the neutralized 2.5% NaOCl solution, and the unmodified 2.5% NaOCl solution dissolved 2.25, 3.25, and 5.0 mg/mL/min, respectively. The dissolving capacity of NaOCl solutions increased with chlorine content. Dakin solution, which contains 0.5% NaOCl, was the less effective dissolving agent. This confirmed the results of earlier studies.[22] Another study demonstrated the loss of available chlorine and the stability of pH of the 2.5% NaOCl in a similar experiment.[28] However the efficiency of the irrigant is not strictly proportional to available chlorine content, because the unbuffered 2.5% NaOCl solution is fivefold more concentrated but only 2.2 times more effective than Dakin. Therefore, the ratio $HOCl/ClO^-$ between the 2 chemical forms of available chlorine must be considered. According to Fukuzaki,[16] the ClO^- concentration seems to be the major factor determining the dissolving action of NaOCl. This is why the unmodified 2.5% NaOCl solution showed a better dissolving action than the neutralized 2.5% NaOCl solution despite the same available chlorine content.

Antibacterial efficiency

This work, performed on teeth artificially infected with *Enterococcus faecalis*, demonstrated that, under the conditions of the study, the 2.5% neutralized NaOCl had a better antibacterial activity than the 2.5% unmodified solution, which was in turn more efficient than Dakin.

A recent review clearly explained the problems of the investigations performed on studies with bacteria in a planktonic form.[29] Artificially infected teeth were used in this work to be more clinically relevant. All of the the roots had the same length, were cleaned, endodontically prepared, and sterilized before inoculation with a known bacteria to standardize infection. Some authors used bovine teeth, but human teeth are generally preferred.[30] *Enterococcus faecalis* is frequently used in bacteriologic studies because of its pathogenic potential,[31] its resistance to endodontic treatments,[32] and its presence in cases of endodontic failure.[33] However, even using artificially infected human teeth and common bacteria, it is very difficult to compare the results of the present work with those of previous studies owing to variations in the protocols and the criteria. Canal sampling may be done using paper points[34] or aspirating the irrigant remaining in the root.[35] In the present study, using large K files to cut dentin chips just at the apex of the roots would permit a more reliable evaluation of the remaining bacteria because it should allow the collection of all the bacteria present at the apex of the root, including the intratubular bacteria. This virtually eliminates the false negative results reported when sampling does not take into account the bacteria adherent to the canal walls or entrapped within dentin tubuli. It seems to be preferable to use a dichotomous criterion rather than counting the bacteria cultivated from sampling the canal, because it is impossible to collect all of the bacteria attached to dentin walls or entrapped within the tubules. In other words, counting the bacteria underestimates the results because it is impossible to know which percentage of bacteria is collected or left in the canal. To compare several root canal irrigants, as in the present study, some authors use various concentrations of irrigant to determine the concentration necessary to completely eliminate the bacteria.[10] This is not satisfying when working with NaOCl solutions, because diluting the solution 10- or 100-fold decreases the pH of the solution. This modifies the ratio between ClO^- and HOCl and therefore changes the outcome of the study. Some authors use endodontic irrigants for various periods and record the time necessary to achieve bacteria-free samples.[35] This technique seems to be more satisfying and was already used with long contact times, such as 24 hours,[36] but this is clinically irrelevant. Consequently, it was decided to use clinically relevant contact times such as 5 and 20 minutes, to use the number of bacteria-free teeth as criterion, and to harvest apical dentin chips using sterilized large K files.

According to this study, the unmodified 2.5% NaOCl solution was more efficient than Dakin. This result was expected, because it has already been demonstrated that the cytoxicity of the NaOCl solution,[37] as well as its antibacterial effects, increased with concentration.[38] This work also showed that the 2.5% neutralized NaOCl solution was more efficient than the unmodified 2.5% NaOCl solution. This confirms the hypothesis at the basis of the present work.[39] The mechanisms of action of NaOCl vary according to the state of available chlorine.[40] Unlike ClO^- ion, HOCl easily penetrates cell membrane because it is electrically neutral and because the spatial organization of the molecule is close to that of water. Once the cell membrane is penetrated, HOCl has a bacteriostatic effect, reacting with DNA, RNA, and all nucleotides[41] and oxidizing sulfhydral

groups to form disulfide bonds.[42] Hypochlorous acid also has a bactericidal effect, reacting with amino acids to make organic chloramines that are themselves cytotoxic,[43] with lipids resulting in chlorohydrin.[44] In addition, the molecule may also inhibit glucose oxidation, leading to respiration loss.[45] These published data may explain the good antibacterial properties of the neutralized 2.5% NaOCl solution reported in the present work. In contrast, another study evaluated the antibacterial properties of a 0.5% NaOCl solution buffered by sodium carbonate at pH 9 and concluded that buffering did not increase the antibacterial properties of the solution.[12] The difference between the outcomes is likely due to the fact that the pH of the solution used in the present work was closely adjusted to the pKa, thus containing the highest percentage possible of hypochlorous acid. In addition, the variation in pH between the modified and unmodified solutions is higher in the present study (pH 12.5-7.5) than in the earlier study (pH 10.5-9). This leads to a larger modification of the $ClO^-/HOCl$ ratio.

The results of this study are promising but are limited to laboratory conditions. They should be confirmed, because it is difficult to extrapolate the in vitro results to clinics. In human infected teeth, the bacteria may be protected by a smear layer,[46] entrapped deeply within the dentinal tubules,[47] and associated in resistant biofilms.[48]

CONCLUSIONS

1. The neutralized 2.5% NaOCl solution must be used within 2 hours after mixing. Because endodontic procedures rarely exceed 2 hours, this drawback can be easily overcome by preparing the mixture shortly before use.
2. The neutralized 2.5% NaOCl solution showed the same dissolving efficiency on bovine dental pulp as the unmodified 2.5% NaOCl solution within the first 5 minutes. Therefore, the neutralized 2.5% NaOCl solution should be frequently renewed to prevent a loss of clinical efficiency.
3. Although none of the solutions used in the present study were sufficient to eliminate 100% of the bacteria in 100% of the roots, the 2.5% neutralized NaOCl solution showed a higher antibacterial effectiveness than the unmodified 2.5% NaOCl solution.

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