
Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions

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Objective. The goal of this study was to compare the dissolving potential of Dakin's solution with that of equivalent buffered and unbuffered sodium hypochlorite solutions on fresh and decayed tissues. In addition, the antimicrobial effect of Dakin's solution and equivalent unbuffered hypochlorite was tested.

Study design. Tissue specimens were obtained from freshly dissected pig palates. Unbuffered 2.5% and 0.5% sodium hypochlorite solutions and 0.5% solutions buffered at a pH of 12 and a pH of 9 (Dakin's solution) were tested on fresh and decayed tissue. Tissue decay was assessed histologically. Antimicrobial testing was performed with *Enterococcus faecalis* in dentin blocks and on filter papers.

Results. The 2.5% NaOCl solution was substantially more effective than the three 0.5% solutions in dissolving the test tissues. Buffering had little effect on tissue dissolution, and Dakin's solution was equally effective on decayed and fresh tissues. No differences were recorded for the antibacterial properties of Dakin's solution and an equivalent unbuffered hypochlorite solution.

Conclusions. In contrast to earlier statements, the results of this study do not demonstrate any benefit from buffering sodium hypochlorite with sodium bicarbonate according to Dakin's method. An irrigation solution with less dissolving potential may be obtained by simply diluting stock solutions of NaOCl with water.

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Sodium hypochlorite was introduced to medicine during World War I to clean open wounds.¹ It is the most widely used irrigation solution in endodontic practice today—mainly for its good antimicrobial and tissue-dissolving properties. Most endodontists use commercially available bleach (5.25%) to irrigate root canals, sometimes diluted with water or saline solution. The pH of unbuffered hypochlorite is 12, and at 5.25%, it is very hypertonic.² Although this solution is quite safe for household use,³ concentrated unbuffered sodium hypochlorite is not unproblematic in dentistry. Serious incidents have occurred when concentrated hypochlo-

rite solution was inadvertently introduced into the periapical tissues during endodontic irrigation.⁴

The solution originally used by Dakin was 0.5% sodium hypochlorite buffered with sodium bicarbonate to a pH of 9.¹⁻⁵ It was found to be more aggressive on necrotic than on vital tissues.⁶ Indeed, 0.5% buffered sodium hypochlorite has a considerably reduced cytotoxicity compared with commercially available unbuffered 5.25% bleach, but the antimicrobial effect is maintained.⁵ The latter findings spurred the conclusion that Dakin's solution was preferable to unbuffered bleach as an endodontic irrigation.⁷ Yet, neither the aggressiveness on tissues nor the antimicrobial potential in root canals has ever been directly assessed with Dakin's solution or unbuffered sodium hypochlorite at the same concentration of available chlorine.

Therefore, in this study, unbuffered sodium hypochlorite was tested versus solutions buffered at different pH levels to determine the dissolving potential on fresh and decayed tissues by using a histologically verified in vitro model for tissue decay. In addition, the killing efficacy of Dakin's solution and unbuffered sodium hypochlorite against *Enterococcus faecalis* was compared.

MATERIAL AND METHODS

Solutions

Four different sodium hypochlorite (NaOCl) solutions were prepared from a pure unbuffered 9% stock

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solution: 2.5% unbuffered (pH 12), 0.5% unbuffered (pH 12), and 0.5% buffered at pH 12 and pH 9 (Dakin's solution). The 0.5% solution buffered at pH 12 was prepared by adding 1 part 5% sodium hypochlorite to 9 parts of a 5% sodium carbonate solution (w/w). Dakin's solution was prepared accordingly: 1 part 5% sodium hypochlorite to 9 parts 1% sodium bicarbonate (w/w).⁸ The available chlorine content of the sodium hypochlorite solutions was verified by using a standard iodine/thiosulfate titration method. All percent values of hypochlorite solutions indicated content of free available chlorine (w/w). The buffer solutions (ie, 1% sodium bicarbonate [NaHCO₃] and 5% sodium carbonate [Na₂CO₃]) served as controls. The osmolarity of all test and control solutions was measured with an osmometer according to the manufacturer's guidelines (One-Ten; Fiske, Needham Heights, Mass).

Tissue specimens

Full-thickness palatal mucosa was dissected from 4 pigs within 30 minutes after slaughtering. These pigs were raised and slaughtered for food production according to the Swiss standards for animal welfare. The study protocol did not in any way influence the pre-mortem fate of the animals or the slaughtering process. Therefore, this investigation was not classified as an animal study, and the institutional ethics committee did not have any objections to the protocol. Immediately after dissection, small pieces of mucosa were cut for subsequent histologic analysis. The rest of the tissue samples were shock-frozen on dry ice and kept at -27°C until further analysis.

Assessment of in vitro tissue decay

Frozen-tissue specimens of defined surface area and similar weight (80 ± 5 mg) were obtained by using a round punch with a 5-mm inner diameter applied at the crest of the rugae. These blocks were unfrozen and kept in an oven at 37°C and 100% humidity. Two specimens each were taken for histologic analysis immediately after unfreezing and after 1, 4, 7, and 10 days of nonsterile incubation in ambient air. Selected tissue samples were transferred to half-strength Karnovsky's fixative for 1 day at 4°C. After an overnight wash in 0.185mol/L sodium-cacodylate buffer (pH 7.4), they were postfixed in 1.33% OsO₄ buffered with 0.067M s-collidine (pH 7.4) for 2 hours at room temperature and washed again. Then blocks were dehydrated in ascending grades of alcohol, transferred to propylene oxide, and embedded in an epoxy medium (Epon; Fluka Chemie GmbH, Buchs, Switzerland). Sections 2-μm-thick were cut with an ultramicrotome (Reichert Ultracut E; Leica, Glattbrugg, Switzerland) and diamond knives. They were stained by using the periodic

acid-Schiff/methylene blue-Azur II method and examined with a light microscope (Leitz Dialux 20; Leica) at magnifications of ×100 and ×400.

Tissue-dissolution assay

Fresh tissue specimens were thawed in 30 mL of isotonic saline solution at room temperature for 30 minutes. Based on the results of the histologic analysis (see RESULTS), decayed specimens were prepared by incubating punched tissue samples at 37°C for 7 days as described earlier and then transferring them to saline solution for 15 minutes. After the wash in saline, the 8 specimens were incubated in 30 mL of the test and control solutions in a water bath at 32°C, the average intracanal temperature.⁹ At the start of the incubation and after 15, 30, 60, 90, and 120 minutes of exposure to the solution, samples were blotted dry and weighed by using a precision balance in an air-tight container (AT 261; Mettler, Greifensee, Switzerland). Results were expressed as percent of original tissue weight. In addition, the pH was measured at each weighing point in all hypochlorite solutions and the available chlorine was assessed before the experiment and at 120 minutes of exposure.

Antimicrobial tests

Two well-established methods, a filter paper test¹⁰ and a dentin block test,¹¹ were performed to compare the antimicrobial activity of Dakin's solution with that of unbuffered sodium hypochlorite. *E faecalis* ATCC 29212 was used as a test organism in both methods.

Filter paper test. Briefly, after an overnight incubation in tryptic soy broth (TSB; Oxoid, Hampshire, England) at 37°C, the suspension of *E faecalis* was washed twice in phosphate-buffered saline (PBS) and spectrophotometrically standardized to a final cell density of approximately 1 × 10⁸ colony-forming units (CFUs)/mL. Sterilized filter paper disks, 4 mm in diameter, were immersed in this suspension at room temperature for 30 minutes and then placed into test tubes containing 1 mL of either Dakin's solution or unbuffered sodium hypochlorite in concentrations varying from 0.00005% to 0.5% available chlorine. The filter disks were then incubated in these solutions at 37°C for 3, 30, and 300 minutes. Bacteria-containing filter paper disks incubated in 1 mL of PBS served as positive controls. The antimicrobial activity of 1% sodium bicarbonate was tested accordingly. After incubation the filter papers were transferred to vials containing 1 mL of PBS and 20 glass beads (diameter, 3 mm; KEBO Lab, Oslo, Norway) which were then vigorously shaken on a Vortex mixer for 60 seconds. Serial 10-fold dilutions in PBS were performed. Droplets of 20 μL from each of the 4 parallel dilutions were inoculated

on tryptic soy agar (Oxoid) plates and incubated in air at 37°C for 24 hours. The total number of CFUs was calculated from dilutions yielding 5 to 50 colonies. The detection limit of the method was 50 CFUs per milliliter. Each experiment was performed in triplicate.

Dentin block test. Dentin test specimens were prepared according to the method used by Haapasalo and Ørstavik¹¹: Dentin blocks approximately 4-mm high and 6-mm wide with a central pulpal lumen diameter of 3.1 mm were created. The root cement was saved, but organic and inorganic debris—including smear layer—were removed by ultrasonic treatment in 17% EDTA for 10 minutes. The test pieces were then sterilized by autoclaving (121°C, 15 minutes) in TSB. The dentin blocks were subsequently infected by using *E faecalis* ATCC 29212 and then kept in 50 mL of TSB at 37°C for 2 weeks, during which time the broth was changed at 2- to 3-day intervals. Strict asepsis was used to prevent contamination, and the purity of the cultures was checked regularly in terms of colony morphology and cellular appearance of subcultures on tryptic soy agar. The infected dentin specimens were incubated in 5 mL of unbuffered sodium hypochlorite and Dakin's solution at concentrations of 0.5, 0.25, and 0.05% at 37°C for 15 minutes. In addition, sodium bicarbonate 1% was tested separately. Incubation in PBS served as positive control. All tests were repeated twice.

The bacteriologic samples were taken by drilling with round burs ranging in size from ISO 033 to 040 through the canal lumen. The dentin powder was collected in separate test tubes containing 2 mL of TSB and incubated for up to 3 days for the detection of bacterial growth. The purity of the growth was controlled by subculturing 100 µL from each test tube on tryptic soy agar and by subsequent examination of colony morphology and cellular appearance.

Statistics

Values obtained from the tissue-dissolution assay were compared by using 1-way analysis of variance (ANOVA). Post hoc analysis was performed by using the *t* test, followed by Bonferroni adjustment for multiple comparisons. All data are presented as means and SDs. Analyses were computed with a statistics program (StatView; SAS Institute, Cary, NC). The significance level for rejection of the null hypothesis (ie, that there was no difference in tissue-dissolving abilities among the tested solutions) was set at $P < .05$.

RESULTS

In vitro tissue decay

Histologic analysis of mucosa fixed immediately after dissection revealed excellent preservation of cells

and extracellular matrix (Fig 1, A and D). After 4 days of incubation, the epithelium had disappeared completely, leaving connective tissue papillae exposed at the specimen surface (Fig 1, B). In addition, cells were no longer visible in the interstices between the collagen fibers of the lamina propria (Fig 1, E). At day 7, the collagen of the connective tissue appeared condensed and the interstices were filled with unstructured debris (Fig 1, C). In an approximately 150- to 250-µm-thick zone at the specimen surface, numerous densely stained minute globules interpreted as colonies of microorganisms could be observed (Fig 1, F). At day 10, such colonies occurred throughout the entire preparations, but the appearance of the remaining connective tissue constituents was similar to that at day 7. Fresh and 7-day-old decayed tissue specimens were used to assay tissue dissolution.

Tissue dissolution

The unbuffered 0.5% sodium hypochlorite solution was only slightly hypertonic (330 mOsm/kg), whereas Dakin's solution and 0.5% NaOCl buffered with 5% Na₂CO₃ were found to be increasingly hyperosmotic (680 mOsm/kg and 1360 mOsm/kg, respectively). The highest hypertonicity was measured for the 2.5% unbuffered NaOCl solution, 2460 mOsm/kg.

After the incubation, the weight of the decayed tissue samples had decreased to 40 to 70 mg as compared with approximately 80 mg of fresh samples. As shown in Fig 2, the 2.5% NaOCl solution was substantially more effective than all 0.5% hypochlorite solutions with respect to dissolving both decayed and fresh tissues (ANOVA, $P < .05$). After 90 minutes, all tissue was dissolved. Both unbuffered 0.5% NaOCl and 0.5% NaOCl buffered with 5% Na₂CO₃ dissolved decayed tissue better than fresh tissue at all times (ANOVA, $P < .05$). In contrast, Dakin's solution was equally effective in dissolving decayed and fresh tissues at 15, 30, 60, 90, and 120 minutes (ANOVA, $P < .05$). The effect of the buffer solutions did not differ in fresh tissues ($P > .05$). In decayed tissue, use of the bicarbonate buffer resulted in a significant tissue weight increase compared with the carbonate (Na₂CO₃) buffer at all times (ANOVA, $P < .05$), which may explain the somewhat reduced tissue-dissolving properties of Dakin's solution on decayed tissue. At 60, 90, and 120 minutes, unbuffered 0.5% sodium hypochlorite dissolved decayed tissue significantly more effectively than Dakin's solution (ANOVA, $P < .05$). The other differences in mean values among the three 0.5% sodium hypochlorite solutions were not significant (ANOVA, $P > .05$). The pH of the unbuffered 0.5% NaOCl solution dropped from 12 to 10.5 after 120 minutes of tissue incubation, whereas the pH values for

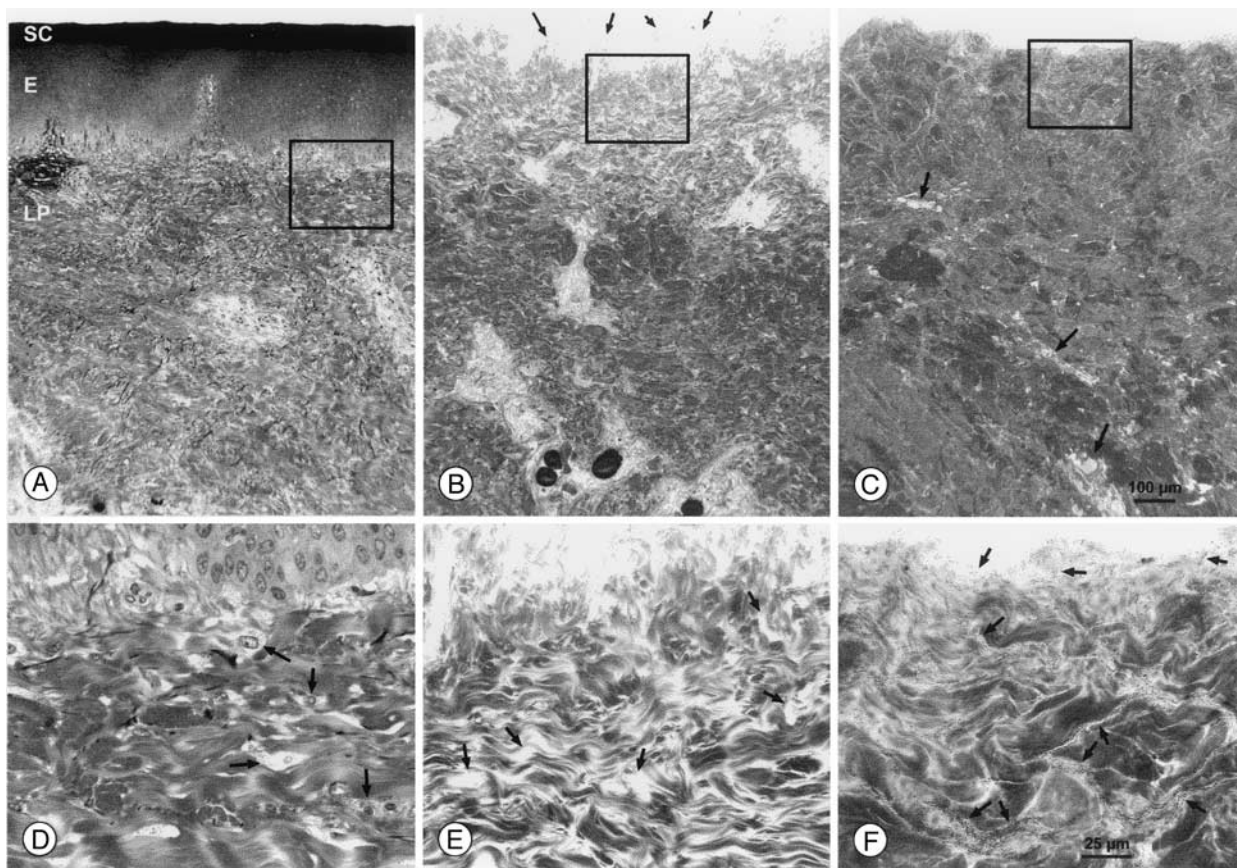


Fig 1. Histologic appearance of porcine palatal mucosa immediately after dissection (A, D) and after 4 days (B, E) and 7 days (C, F) of incubation at 37°C and 100% humidity. Note that in the 4-day-specimen, the exposed connective tissue papillae (B, arrows) and the absence of cells from the lamina propria (E, arrows), contrasting with the excellent cellular preservation in the fresh sample (D, arrows). In the 7-day-old specimen, interstices between the condensed collagen contain debris (C, arrows) and numerous minute colonies of microorganisms (F, arrows). E, epithelium; LP, lamina propria; SC, stratum corneum. Rectangles in A-C mark the position of the details enlarged in D-F. Periodic acid-Schiff/methylene blue-Azur II stain. A-C, Original magnifications are $\times 55$ (C, bar = 100 μm). D-F, Original magnifications are $\times 280$ (F, bar = 25 μm).

the buffered 0.5% and for the 2.5% hypochlorite solutions remained stable. At 120 minutes, the percentage of free chlorine dropped to 2.3% in the 2.5% solution and to 0.4% in the 0.5% solutions.

Antimicrobial tests

Filter paper test. Both unbuffered sodium hypochlorite and Dakin's solution showed eradication of *E faecalis* at a free-chlorine concentration of 0.0005% after 3 minutes of incubation. Lower concentrations had no or only a minimal effect. A 0.00005% sodium hypochlorite solution was slightly more effective on the bacteria than an equal concentration of Dakin's solution. Incubation in PBS, which served as the positive control, did not yield any change. Similarly, 1% sodium bicarbonate did not have any effect on the survival of *E faecalis* ATCC 29212 (Fig 3).

Dentin block test. In general, both unbuffered sodium hypochlorite and Dakin's solution disinfected the cultivated dentin layers at concentrations of 0.5% and 0.25%, whereas 0.05% had no or minimal effects on the bacteria. The incubation in PBS, which served as a positive control, and in 1% sodium bicarbonate yielded similar growth. All growth positive cases showed pure cultures of *E faecalis* (Table).

DISCUSSION

This study was undertaken to test the influence of buffering on some desired—and also some untoward—effects of sodium hypochlorite solutions, namely the effectiveness in dissolving decayed connective tissue and the aggressiveness on fresh mucosa. The results suggest that it is the amount of available chlorine, not the osmolarity, pH, or buffer capacity, which is respon-

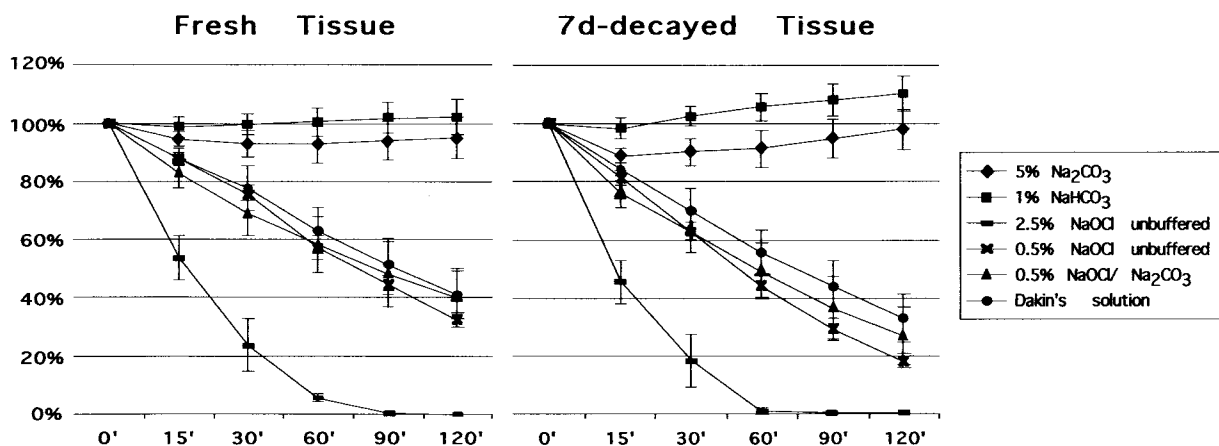


Fig 2. Fresh and 7-day-old decayed porcine palatal tissue was exposed to buffered and unbuffered hypochlorite solutions of different concentrations, in addition to the buffer solutions proper. Percent of remaining tissue weight is plotted as a function of time (minutes).

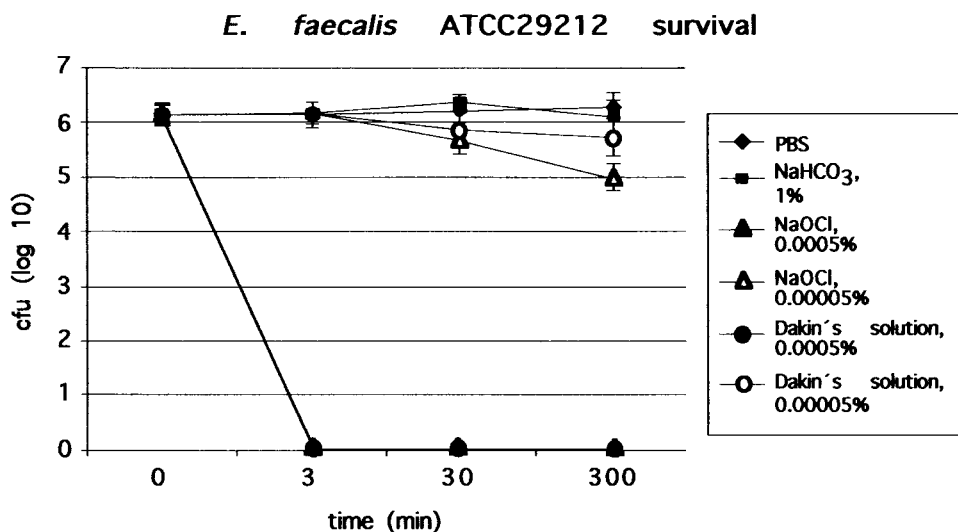


Fig 3. Bacteria-containing filter paper disks were directly exposed to the test and control solutions for 3, 30, and 300 minutes at 37°C. Logarithmic values of the colony-forming units (cfu) of remaining bacteria are shown at different time points (minutes).

sible for the tissue-dissolving properties of hypochlorite solutions. Although the antibacterial effects were maintained, lowering the pH from 12 to 9 according to Dakin's method did not render the solution less aggressive on fresh tissue.

Before the dissolution experiments, the degree of tissue decay in vitro was verified histologically. This analysis suggested that incubation at 37°C and 100% humidity for 1 to 2 days, as used in previous investigations,^{12,13} was hardly sufficient to produce tissue alterations commonly observed in necrotic root canals. To quantify specimen dissolution, a simple, yet reliable, weighing method was used because irrigation

solutions interfere with total protein assays and with hydroxyproline determination.¹⁴ The obtained results are in agreement with earlier findings showing that sodium hypochlorite dissolves decayed tissue more quickly than fresh tissue.¹⁵ Other previous results confirmed in this study concern the influence of pH of (unbuffered) hypochlorite solutions on tissue dissolution.¹⁶

Two tests were chosen to detect the antimicrobial activity of unbuffered sodium hypochlorite and Dakin's solution.^{10,11} The filter paper test is a simple and accurate method to obtain quantitative data, whereas the dentin block method is semiquantitative but provides a

Table. Growth of *Enterococcus faecalis* ATCC29212 cultivated from various depths of dentin blocks incubated for 15 minutes in PBS, bicarbonate buffer, sodium hypochlorite, and Dakin's solution

Compound	Concentration	Depth*	Block 1	Block 2	Block 3
PBS		033	+++	+++	+++
		035	+++	+++	+++
		037	+++	+++	+++
		040	+++	+++	+++
Sodium bicarbonate	1%	033	+++	+++	+++
		035	+++	+++	+++
		037	+++	+++	+++
		040	+++	+++	+++
Sodium hypochlorite	0.5%	033	-	-	-
		035	-	-	-
		037	-	-	-
		040	-	-	-
	0.25%	033	-	-	-
		035	-	-	-
		037	-	-	-
		040	-	-	-
	0.05%	033	++	++	+
		035	+++	+++	+++
		037	+++	+++	+++
		040	+++	+++	+++
Dakin's solution	0.5%	033	-	-	-
		035	-	-	-
		037	-	-	-
		040	-	-	-
	0.25%	033	-	-	-
		035	-	-	-
		037	-	-	-
		040	-	-	++
	0.05%	033	+++	++	++
		035	+++	++	+++
		037	+++	+++	+++
		040	+++	+++	+++

+, ++, +++, Degree of growth; -, no growth; PBS, phosphate-buffered saline.
*Round bur (International Standards Organization size) used for sampling.

means to examine the activity of the studied irrigants to eliminate biofilms on dentin and disinfect dentinal tubules. *E. faecalis* was chosen as a test organism because it is commonly associated with persistent apical peri-odontitis.¹⁷ Surprisingly low concentrations of both the unbuffered sodium hypochlorite and Dakin's solution showed complete efficacy against *E. faecalis* ATCC 29212 in the filter paper test. This may be partly because of the reduced environmental circumstances, because higher concentrations were needed to kill the bacteria in the dentin block test. This is in agreement with recent studies in the literature, which suggest that dentin acts as an inhibitory factor against several local medicaments commonly used in endodontics.¹⁸ Furthermore, the increased resistance of well-established biofilms in comparison with that of planktonic microorganisms, which is a recently reported phenomenon, may explain the higher concentrations needed to kill the bacteria in the dentin block test.¹⁹ However, both of the

tests in this study indicated the strong antimicrobial activity of both unbuffered sodium hypochlorite and Dakin's solution. This finding is in agreement with previously reported results.^{5,10} In the present study, neither test showed clear differences among the tested solutions. Furthermore, sodium bicarbonate did not show any effect on *E. faecalis*. In conclusion, there does not seem to be any clinically relevant difference in the antimicrobial activity between unbuffered sodium hypochlorite and Dakin's solution.

NaOCl dissociates in water to Na⁺ and OCl⁻/HOCl (hypochloric acid). The pKa value of hypochlorite is 7.6.²⁰ This means that at the pH of unbuffered hypochlorite and Dakin's solution, free available chlorine (OCl⁻ + HOCl) exists almost entirely as OCl⁻. However, it has been clearly demonstrated that HOCl is the more bactericidal form of this molecule.²¹ Therefore, buffering of a hypochlorite solution may be beneficial only at a slightly acidic pH. Experiments are currently

being performed to further delineate the influence of pH on endodontic irrigation solutions. In contrast to earlier statements, the results of this study do not suggest any benefit from buffering sodium hypochlorite with sodium bicarbonate according to Dakin's method. An irrigation solution with less dissolving potential may be obtained by simply diluting stock solutions of NaOCl with water.

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