

Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA

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Abstract: This *in vitro* study evaluated (1) the dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA; and (2) the pH of these solutions before and after the experiment. The independent variables were the concentration and the volume of the solution. Thirty bovine pulps were divided in equal fragments, resulting in 90 fragments of pulp tissue. Each fragment was immersed in one of the following solutions: 1% NaOCl (4 ml), 2.5% NaOCl (4 ml), 1% NaOCl + 17% EDTA (2 ml : 2 ml), 1% NaOCl + 17% EDTA (1 ml : 3 ml), 2.5% NaOCl + 17% EDTA (2 ml : 2 ml), and 2.5% NaOCl + 17% EDTA (1 ml : 3 ml). The test solutions were dichotomized as either *able* or *not able to dissolve the tissue*, the latter being attributed when the dissolution of the pulp tissue was not complete within 48 hours. When the samples were able to dissolve the tissue, the time required for complete tissue dissolution was submitted to statistical analysis. The pH of the solutions was measured before and after the experiment. The pH variable was dichotomized as either *changed* or *unchanged*. The results demonstrated that the NaOCl solutions combined with 17% EDTA were not able to dissolve the tissue. The *t*-test revealed that the 2.5% NaOCl solution presented a lower mean dissolution time than the 1% NaOCl solution ($p < 0.001$). The pH of the solutions with equal volumes of NaOCl and EDTA decreased in 48 hours.

Descriptors: Hydrogen-ion concentration; Sodium hypochlorite; Dissolution; Dental pulp.

Introduction

Successful root canal therapy depends on thorough chemomechanical debridement of pulp tissue, dentin debris, and infective microorganisms.^{1,2} Mechanical instrumentation alone is not able to reduce the microbial population and leave dentinal surfaces free of a smear layer.^{3,4} Even in well-shaped canals, there are remnants of pulp tissue and inorganic debris, especially in areas where instruments cannot reach.⁴

The use of a combination of sodium hypochlorite (NaOCl) and EDTA is effective in the removal of organic and inorganic debris.^{5,6,7,8} NaOCl is known for its antibacterial properties and its ability to dissolve organic components.^{9,10} EDTA is a Ca⁺² chelating agent and therefore capable of removing the smear layer.^{3,5,11,12} EDTA may be used as a final flush to open up dentinal tubules, thus allowing a greater number of lateral ca-

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nals to be filled,¹³ or may also be used alternately with sodium hypochlorite during the whole preparation of the root canal.^{5,14}

Many authors have reported the significant influence of a variety of factors, like concentration, time, temperature, tissue-irrigating contact area, canal preparation size, volume, tissue type, and mechanical action, on the ability of NaOCl to dissolve both necrotic and vital tissues.^{10,15,16,17,18,19} Christensen *et al.*²⁰ reported that concentration, time, and pH all play important roles in determining the amount of tissue dissolution. Higher concentrations and greater time periods all lead to greater amounts of tissue dissolution. Although antibacterial power may increase, tissue dissolution decreases as pH decreases.

However, the related literature lacks more consistent information on the tissue dissolution results obtained when NaOCl is combined with EDTA.

Thus, the purpose of this *in vitro* study was to evaluate (1) the dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA; and (2) the pH of the solutions before and after the experiment. The independent variables of this study were the concentration and the volume of the solution.

Materials and Methods

This study was submitted to the local Ethical Committee for evaluation and was approved. Bovine pulp tissue was used to test the dissolving ability of different NaOCl concentrations, and of NaOCl combined with EDTA.

Pulp tissue collection

The pulp tissue of the animals used was obtained from a meat packing plant in the city of Montenegro, RS, Brazil. The bone tissue containing the upper incisors was collected immediately after their death. The tissue was kept refrigerated in 100% humidity until the teeth were removed with the help of a number 7 carver (Golgran, São Paulo, SP, Brazil) and a Lecron carver (Golgran, São Paulo, SP, Brazil). Subsequently, pilot grooves were prepared on the buccal and oral aspects of the crowns using a diamond-coated fissure bur (Dentsply Maillefer, Ballaigues, Switzerland) without entering the pulp

chamber. The teeth were then carefully split into two fragments using a number 2 Ochsenbein micro-chisel (Golgran, São Paulo, SP, Brazil), and the entire pulp tissue was removed from the pulp chamber and the root canals using a number 17 spoon excavator (Golgran, São Paulo, SP, Brazil). Thirty pulp specimens were immersed in a 0.9% saline solution (Pharmacy-School of the Lutheran University of Brazil/ULBRA, Canoas, RS, Brazil) at room temperature for 30 min. Next, the 30 pulps were divided into equal 5-mm-long fragments by a number 15 scalpel blade (Swann-Morton, Sheffield, England), resulting in 90 fragments of pulp tissue.

Three fragments from each pulp were removed from the first 15 mm of the coronal region. The tissue remainder (apical third) was discarded. The sizes of 90 fragments were very similar. Length, as well as buccolingual and mesiodistal diameters were measured with a digital caliper (799 Starret, Itu, SP, Brazil). Uniform volumes of all fragments were prepared to prevent any interference with the dissolution time.

pH of the solutions

The solutions of NaOCl and EDTA were obtained from the Pharmacy-School of the University where the experiment was conducted, the Lutheran University of Brazil/ULBRA, Canoas, RS, Brazil. The pH of the solutions was measured before and after the experiment was performed, by pH measuring tapes (Merck KgaA, Darmstadt, Germany). The variable pH was dichotomized as either *changed* or *unchanged*. In samples where there was change, the final and initial pH values were recorded.

Tissue dissolution

Each fragment of bovine pulp tissue was immersed in 4 ml of solution. The solutions of NaOCl combined with EDTA were mixed with a plastic spatula (Golgran, São Paulo, SP, Brazil) before immersion of the fragment. Next, the solution was left to rest. The fragments were immersed in the test solutions through a randomized process conducted by someone who did not belong to the research group.

The solutions containing the fragments of pulp tissue were stored in glass containers with a rub-

ber stopper (Vidraria Sul Brasil S/A, Canoas, RS, Brazil). All glass containers were kept on a shelf at room temperature and protected from light. Fifteen samples were prepared for each type of solution (Table 1).

The room temperature measured by thermometer (Cotergavi, São Paulo, SP, Brazil) on the experiment days was 22°C. The variable dissolution ability was dichotomized as either *able* or *not able to dissolve the tissue*. A chronometer (Casio Computer Co. Ltd., Shibuyaku, Japan) measured the time from pulp tissue immersion to its complete dissolution. Complete dissolution meant that the tissue was not visible through a 4X magnification loupe (Pró Vista Imp Com Materiais Óticos Ltda, São Paulo, SP, Brazil).

The maximum time set for the experiment was 48 hours. The dissolution process was followed during this time by two researches (A.Z.M. and J.S.P.) in 6-hour shifts. If the pulp tissue was not completely dissolved after 48 hours, the solution sample was considered as *not able to dissolve the tissue*. For samples that were able to dissolve the tissue, the time required for complete dissolution was submitted to statistical analysis.

Results

Table 2 shows the mean times (in minutes) of the

Table 2 - Mean times (in minutes) of bovine pulp tissue dissolution promoted by the solutions analyzed.

Group	Solution		Number of samples	Mean dissolution time (min)
	Concentration	Volume (ml)		
G1	1% NaOCl	4	15	76.44
G2	2.5% NaOCl	4	15	34.95
G3	1% NaOCl + 17% EDTA	2 : 2	15	Not able to dissolve the tissue
G4	1% NaOCl + 17% EDTA	1 : 3	15	Not able to dissolve the tissue
G5	2.5% NaOCl + 17% EDTA	2 : 2	15	Not able to dissolve the tissue
G6	2.5% NaOCl + 17% EDTA	1 : 3	15	Not able to dissolve the tissue

Table 3 - Application of the *t*-test to the mean dissolution time (in minutes) of bovine pulp tissue dissolution promoted by the 1% NaOCl and 2.5% NaOCl solutions.

Group		n	Dissolution time (min)		p
			Mean	Standard deviation	
G1	1% NaOCl	15	76.44	10.18	< 0.001
G2	2.5% NaOCl	15	34.95	4.89	

bovine pulp tissue dissolution for each solution.

Since the solutions of NaOCl combined with 17% EDTA were unable to dissolve the pulp tissue, the statistical analysis was applied only to the pure solutions of NaOCl in order to assess if there was any statistically significant difference in dissolution ability between the 1% NaOCl and the 2.5% NaOCl solutions. The *t*-test revealed that the 2.5% NaOCl (4 ml) solution presented a significantly lower mean dissolution time ($p < 0.001$) than the 1% NaOCl (4 ml) solution (Table 3).

Only the pH values that changed after the experiment are reported in Table 4. The pH in all other solutions remained the same at the end of the experiment as in the beginning.

Table 1 - Concentration and volume of the solutions analyzed.

Group	Solution		Number of samples
	Concentration	Volume (ml)	
G1	1% NaOCl	4	15
G2	2.5% NaOCl	4	15
G3	1% NaOCl + 17% EDTA	2 : 2	15
G4	1% NaOCl + 17% EDTA	1 : 3	15
G5	2.5% NaOCl + 17% EDTA	2 : 2	15
G6	2.5% NaOCl + 17% EDTA	1 : 3	15

Table 4 - pH variation of the solutions measured before and after the experiment (48 hours).

Group	Solution		Number of samples	Initial pH	Final pH
	Concentration	Volume (ml)			
G3	1% NaOCl + 17% EDTA	2 : 2	15	10	8
G5	2.5% NaOCl + 17% EDTA	2 : 2	15	10	8

Discussion

Many authors have reported the significant influence of a variety of factors, like concentration, time, temperature, tissue-irrigating contact area, canal preparation size, volume, tissue type, and mechanical action, on the ability of NaOCl to dissolve both necrotic and vital tissues.^{10,15,16,17,18,19} Our study focused on the effect of varying NaOCl concentrations, on the effect of varying NaOCl concentrations combined with EDTA, and on measuring possible pH variations during the experiment.

The validity of the *in vitro* experimental outline is confirmed by the possibility of standardizing not only the solutions but also the fragments of pulp tissue in terms of their origin, surface size and storage conditions. In so doing, an evaluation can be made of the dissolution ability of NaOCl solutions and possible variations in pH for each solution without the interference of other factors. *In vitro* studies are also extremely valid because of their ability to generate hypotheses that may be tested later in other studies.

The results of the present study demonstrated that when NaOCl, in any of the concentrations and volumes tested, was combined with 17% EDTA, the solution was unable to dissolve the bovine pulp tissue in 48 hours. EDTA strongly reduces the available chlorine in the NaOCl solutions, possibly rendering them ineffective.^{21,22} Zehnder *et al.*²³ confirmed that EDTA solutions caused an almost complete loss of free available chlorine immediately upon mixing with NaOCl.

Because of its elevated alkalinity, the sodium hydroxide resulting from the dissociation of NaOCl has the ability of dissolving organic matter through the saponification of fats.^{8,9} Another explanation for the non-dissolution of the organic matter in samples where hypochlorite was combined with EDTA is that the sodium hydroxide provided ions to the

EDTA, which is originally disodium, turning it into trissodium, tetrassodium, and pentassodium. Some authors have already demonstrated that, in the presence of NaOCl, the chemical structure of EDTA has increased sodium.²⁴

Although it is difficult to make clinical practice inferences from the results of *in vitro* studies because of the different variables that may be present in studies with patients, the authors suggest that the ability to dissolve organic tissue in an alternating irrigating regimen is damaged because of the reactivity of NaOCl with EDTA. This fact has prompted us to indicate the use of EDTA as a final flush, instead of using it alternately with sodium hypochlorite during canal preparation.

Although possible changes in the antimicrobial ability of solutions combining NaOCl and EDTA were not evaluated in this study, other authors have already demonstrated that the reduction in the available chlorine content possibly damages the formation of chloramines, impairing the power of the solution to inhibit bacterial enzymes, ultimately leading to reduced antiseptic action.^{18,23}

Studies have shown that both the antibacterial properties and the tissue-dissolving properties of 5.25% NaOCl decrease when diluted. Based on the results of the present study, the ability to dissolve organic matter is directly proportional to the NaOCl concentration in the solution.^{9,10,17,19,25,26,27,28} However, it is important to remember that serious incidents have been reported when concentrated hypochlorite solutions were inadvertently forced into periodontal tissues,²⁹ or when such a solution leaked through the rubber dam onto the patient's skin.³⁰

As regards the volume of the solutions, since our study used the same volume (4 ml) for all pure solutions of NaOCl, no dissolution ability variation was observed owing to this factor. Further studies are recommended to analyze the effect of different vol-

umes of pure solutions of NaOCl, while maintaining the same concentration.

Regarding pH change, the pH of the solution decreased in all the samples where NaOCl was combined with EDTA in an equal volume proportion. An interesting fact was that the pH did not change in the solutions where the volume of EDTA was greater than that of NaOCl. We believe that EDTA induces an increased availability of negative ions by removing Na⁺ from NaOCl, leading to a reduction in pH. When a greater volume of EDTA was used, the EDTA also removed sodium ions, but the lower concentration of NaOCl led to a reduced availability of negative ions, thus leaving the pH unchanged. Further studies to elucidate this finding should be conducted. The fact that the samples presented a decrease in pH also may have contributed to the non-

dissolution of the bovine pulp tissue. Cristhensen *et al.*²⁰ and Zehnder²³ demonstrated that higher pH levels resulted in greater tissue dissolution.

Conclusion

In conclusion, the concentration of NaOCl and its association with EDTA had an important effect on the ability to dissolve bovine pulp tissue. Only the samples with 1% and 2.5% NaOCl not combined with EDTA and at equal volumes (4 ml) were able to dissolve the tissue in the time set for the experiment. A greater dissolution ability (measured by the mean time necessary for dissolution) was observed in the samples with greater concentration (2.5%). The pH of the solutions with equal volumes of NaOCl and EDTA decreased after the test time of 48 hours.

References

- Abbott PV. The periapical space: a dynamic interface. *Aust Endod J.* 2002 Dec;28(3):96-107.
- Takehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol.* 1965 Sep;20(3):340-9.
- McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod.* 1975 Jul;1(7):238-42.
- Davis SR, Brayton SM, Goldman M. The morphology of the prepared root canal: a study utilizing injectable silicone. *Oral Surg Oral Med Oral Pathol.* 1972 Oct;34(4):642-8.
- Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. *J Endod.* 1987 Apr;13(4):147-57.
- Cengiz T, Aktener BO, Piskin B. The effect of dentinal tubule orientation on the removal of smear layer by root canal irrigants. A scanning electron microscopic study. *Int Endod J.* 1990 May;23(3):163-71.
- Medici MC, Fröner IC. A scanning electron microscopic evaluation of different root canal irrigation regimens. *Braz Oral Res.* 2006 Jul-Sep;20(3):235-40.
- Liolios E, Economides N, Parissis-Messimeris S, Boutsioukis A. The effectiveness of three irrigating solutions on root canal cleaning after hand and mechanical preparation. *Int Endod J.* 1997 Jan;30(1):51-7.
- Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *J Endod.* 1978 Feb;4(2):60-4.
- Clarkson RM, Moule AJ, Podlich H, Kellaway R, Macfarlane R, Lewis D, *et al.* Dissolution of porcine incisor pulps in sodium hypochlorite solution of varying compositions and concentrations. *Aust Dent J.* 2006 Sep; 51(3):245-51.
- Torabinejad M, Handysides R, Ali Khademi A, Bakland LK. Clinical implications of the smear layer in endodontics: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002 Dec;94(6):658-66.
- Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indication for their use. *Int Endod J.* 2003 Dec;36(12):810-30.
- Villegas JC, Yoshioka T, Kobayashi C, Suda H. Obturation of accessory canals after four different final irrigation regimes. *J Endod.* 2002 Jul;28(7):534-6.
- Peters LB, Van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *Int Endod J.* 2002 Jan;35(1):13-21.
- Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigation solutions. *J Endod.* 1975 Apr;1(4):127-35.
- Louis I, Grossman, Benjamin W, Meiman. Solution of pulp tissue by chemical agents. *J Endod.* 1982 Jan; 8:10-12.
- Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. *J Endod.* 1981 Aug;7(8):376-7.
- Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endod.* 1992 Dec;18(12):605-12.

19. Rossi-Fedele G, De Figueiredo JA. Use of a bottle warmer to increase 4% sodium hypochlorite tissue dissolution ability on bovine pulp. *Aust Endod J* 2008 Apr;34(1):39-42.
20. Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue *in vitro*. *J Endod*. 2008 Apr;34(4):449-52. Epub 2008 Feb 7.
21. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J*. 1997 Sep;30(5):297-306.
22. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J*. 1998 Jan;31(1):1-7.
23. Zehnder M. Root Canal Irrigants. *J Endod*. 2006 May;32(5):389-98.
24. Basset J, Mendham J. Vogel - Análise química quantitativa. 6th ed. Rio de Janeiro: LTC; 2002. 462 p.
25. Radcliffe CE, Potouridou L, Qureshi R, Hababbeh N, Qualtrough A, Worthington H *et al*. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. *Int Endod J*. 2004 Jul;37(7):438-46.
26. Zehnder M, Grawehr M, Hasselgren G, Waltimo T. Tissue-dissolution capacity and dentin disinfecting potential of calcium hydroxide mixed with irrigating solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003 Nov;96(5):608-13.
27. Spångberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surg Oral Med Oral Pathol*. 1973 Dec;36(6):856-71.
28. Okino L, Siqueira EL, Santos M, Bombana AC, Figueiredo JAP. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate gel. *Int Endod J*. 2004 Jan;37(1):38-41.
29. Hulsmann M, Hahn W. Complications during root canal irrigation—literature review and case reports. *Int Endod J*. 2000 May;33(3):186-93.
30. Serper A, Ozbek M, Calt S. Accidental sodium hypochlorite-induced skin injury during endodontic treatment. *J Endod*. 2004 Mar;30(3):180-1.