








Antimicrobial action, pH, and tissue dissolution capacity of 2.5% sodium hypochlorite gel and solution

Ação antimicrobiana, pH, e capacidade de dissolução tecidual de gel e solução de hipoclorito de sódio 2,5%

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Abstract

Objective: to evaluate antimicrobial action, pH, and tissue dissolution capacity of 2.5% sodium hypochlorite (NaOCl) gel and solution. **Methods:** The 2.5% NaOCl gel was produced from a colloidal base. The test groups included 2.5% NaOCl gel and solution and the control groups included gel base and distilled water. The antimicrobial activity was evaluated by the broth dilution technique against *Enterococcus faecalis* (ATCC 29212) at 15 and 30 seconds and at 1, 5, and 10 minutes. To evaluate tissue dissolution capacity, 30 pulp fragments of bovine incisors were weighed, 10 for each test group and 5 for each control group before and after exposure to the chemical auxiliaries. The final mass percentage of each fragment was calculated. The pH of the substances was measured in triplicate through a digital pH meter. **Results:** pH levels of 13.08 and 9.75 were observed for 2.5% NaOCl solution and gel, respectively. The antimicrobial action of 2.5% NaOCl was the same for both solution and gel, for all tested times. The 2.5% NaOCl solution group showed higher tissue dissolution capacity (Kruskal-Wallis and Student-Newman-Keuls tests $P < 0.0001$). **Conclusions:** The type of medium, either solution or gel, containing 2.5% NaOCl did not influence the antimicrobial action at any of the tested times. However, 2.5% NaOCl gel did not present tissue dissolution capacity.

Keywords: Dental pulp. *Enterococcus faecalis*. Hydrogen-ion concentration. Sodium hypochlorite. Dissolution. Bacterial Sensitivity Tests

Resumo

Objetivo: avaliar a ação antimicrobiana, pH e capacidade de dissolução tecidual promovida por hipoclorito de sódio (NaOCl) 2,5%, nas formas líquida e gel. **Métodos:** O gel de NaOCl 2,5% foi produzido a partir de base coloidal. Os grupos testes foram NaOCl 2,5% gel e solução e os grupos controle incluíram a base gel e água destilada. A ação antimicrobiana foi avaliada por meio de método de diluição em caldo, frente a *Enterococcus faecalis* (ATCC 29212) após 15 e 30 segundos, e também a 1, 5 e 10 minutos. Para o teste de diluição tecidual, 35 fragmentos de polpa bovina (sendo 10 para cada grupo teste e 5 para o grupo controle) foram pesadas antes e após a exposição aos auxiliares químicos. O percentual de massa final de cada fragmento foi calculada. O pH dos auxiliares químicos foi medido em pHmetro digital, em triplicata. **Resultados:** Valores de pH iguais a 13,08 e 9,75 foram observados para solução e para o gel de NaOCl, respectivamente. A ação antimicrobiana do NaOCl foi a mesma para o gel e a solução, em todos os períodos testados. Maior capacidade de dissolução tecidual foi obtida no grupo onde se utilizou a solução de NaOCl 2,5% (Testes de Kruskal-Wallis e Student-Newman-Keuls, $P < 0,0001$). **Conclusões:** A apresentação na forma de gel ou de líquido do NaOCl 2,5% não modificou a ação antimicrobiana em qualquer um dos períodos testados. Porém, o gel de NaOCl 2,5% não demonstrou capacidade de dissolução tecidual.

Palavras-chave: Polpa dental. *Enterococcus faecalis*. Concentração de Íons de Hidrogênio. Hipoclorito de sódio. Dissolução. Testes de Sensibilidade Microbiana.

INTRODUCTION

Sodium hypochlorite (NaOCl) is the chemical auxiliary most widely used during chemomechanical root canal preparation because it associates antimicrobial activity and tissue dissolution capacity¹. The chemical efficacy and/or the pulp tissue dissolution capacity of NaOCl are significantly influenced by several parameters such as concentration, exposure time, activation method (ultrasound or laser), temperature, and pH². NaOCl concentration is directly proportional to its antimicrobial action and inversely proportional to its biological compatibility, increasing deleterious effects on periapical tissues^{3,4}.

Nearly half of endodontists from the American Board of Endodontics have reported the occurrence of at least one accident with NaOCl during endodontic treatment⁵. The accidents reported in the literature were: extrusion into the maxillary sinus, facial bruising and edema⁶, limitation of mouth opening, mucosal ulceration⁶, severe pain^{3,6}, ocular chemical burn³, and bleeding and rhinorrhea⁷. Extrusion of NaOCl into periapical tissues during endodontic treatment causes immediate, potentially serious, and speculative acute symptoms^{7,8}.

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Chemical auxiliaries in gel form for endodontics have been studied because they prevent accidental extrusion of irrigation solution during endodontic treatment, due to their high viscosity⁹. Chlorhexidine gel has essential properties, such as viscosity and low toxicity to periapical tissues, and keeps the active agent in greater contact with the root canal walls and dentinal tubules¹⁰. However, chlorhexidine has no ability to dissolve organic tissues. The literature presents studies on the antimicrobial action of NaOCl gel¹⁴; however, there are no reports of its tissue dissolution properties.

Some instrument manufacturers, particularly of nickel-titanium instruments, have been recommending the use of endodontic gels due to their higher lubrication capacity⁴. NaOCl gel can be a safe alternative as a chemical auxiliary in endodontic treatment because of its proven tissue dissolution capacity and maintenance of its antimicrobial activity. Thus, the present study aimed to evaluate the antimicrobial activity, pH, and pulp tissue dissolution capacity of 2.5% NaOCl gel.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Dental School from the Federal University of Rio Grande do Sul (Porto Alegre, RS, Brazil). The research protocol was also registered in the SISGEN (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado), process number AEA118B.

Preparation of 2.5% NaOCl gel

The gel base was produced from a 10% gel matrix, with a viscosity of 450 centipoises, similar to that of 2% chlorhexidine gel (Essential Pharma, Itapetininga, São Paulo, Brazil), at 25.9 °C and 10 revolutions per minute (rpm). The base used is a water-soluble, biocompatible, biologically inert, non-mutagenic, and non-cytotoxic synthetic polymer with excellent transparency and without carcinogenic or antigenic activity.

2.5% NaOCl gel or solution was produced using the formula: $C1.V1 = C2.V2$ (Concentration1 X Volume1 = Concentration2 X Volume2), starting from a concentrated solution of NaOCl, previously titrated (Mediquímica Indústria Ltda, Porto Alegre, Rio Grande do Sul, Brazil). The groups are shown on Table 1.

Table 1: Experimental and control groups according to substance and to presentation (gel or solution).

Group	Substance	Presentation
Control 1	Distilled water	Solution
Control 2	Gel base	Gel
Group 1	2.5% Sodium hypochlorite	Solution
Group 2	2.5% Sodium hypochlorite	Gel

Antimicrobial activity

For the evaluation of antimicrobial activity, the broth dilution technique was employed in triplicate against *Enterococcus faecalis* (ATCC 29212). This methodology was adapted from Vianna et al. (2004) (11). *Enterococcus faecalis* (ATCC 29212) was subcultured on BHI agar plates and incubated at 37° C for 18-24 hours. After growth in the solid medium, the isolated colonies were suspended in BHI broth. The suspension was adjusted on a spectrophotometer at 600 nm and at an absorbance of 0.036 to reach the concentration equivalent to the 0.5 McFarland standards (1.5 x 10⁸ bacteria/mL). In the laminar flow chamber, 1 mL of BHI broth containing the viable microorganism was deposited into the well of a culture dish (TTP, Trasadingen Switzerland), in addition to 1 mL of 2.5% NaOCl gel and solution (test groups), gel base, or distilled water (control groups). In the other five wells, there was BHI broth containing the neutralizing agent (0.5% sodium thiosulfate, Synth, Diadema, São Paulo, Brazil) and they represented each of the five evaluation times, except the first well. Aliquots of 200 µL were withdrawn from the wells with chemical auxiliaries and controls after 15 seconds, 30 seconds, 1 minute, 5 minutes, and 10 minutes, respectively, in the wells containing the neutralizing agent. All plates were incubated in a microbiological oven at 37 °C with 10% CO₂ for 24 hours. The results were analyzed according to the presence or absence of turbidity in the wells. To verify the presence of viable microorganisms, aliquots of 25 µL of each well (turbid or not) were plated in BHI agar medium. The plates were incubated in a microbiological oven at 37 °C with 10% CO₂ for 24 hours. After the incubation period, antimicrobial activity was confirmed when there was no bacterial growth in the BHI agar.

pH evaluation

The pH of the NaOCl solution and gel (test groups) and distilled water and gel base (control groups) was evaluated in triplicate using Digital pH meter (Digimed DM 21, São Paulo, SP, Brazil).

Pulp tissue dissolution capacity

To evaluate pulp tissue dissolution, a technique adapted from Cobankara et al. (2010) was used (12). Thirty bovine pulp fragments (standardized from 0.00439 g to 0.03487 g measuring approximately 10 mm in length) were used, 10 samples per test group (2.5% NaOCl gel and solution) and 5 samples per control group (water and gel base). The number of samples was based on similar studies (12,13). The pulp fragments were in contact with the auxiliary substance for 3 minutes, with 20 seconds of vortexing at each minute. To calculate the final mass percentage of the samples, the pulp fragments were weighed before and after exposure to 2.5% NaOCl solutions and gels and to controls (gel base and distilled water).

Statistical analysis

The statistical analysis was performed by Statistical Package for

the Social Sciences – SPSS version 22.0 (SPSS Inc, Chicago, IL). The median values for the pH of the tested chemical auxiliaries were calculated (Excel, Ref). The normality of the data was assessed by the Shapiro-Wilk test ($\alpha=5\%$). The null hypotheses were:

a) There is no statistical difference in antimicrobial effect among the tested chemical auxiliaries (Kruskal Wallis test and Student-Newman-Keuls post-hoc test);

b) There is no statistical difference between the initial and the final mass of the samples exposed to a specifically chemical auxiliary (T-test for paired samples);

c) There is no statistical difference in the percentage of pulp tissue dissolution promoted by the tested chemical auxiliaries (Kruskal Wallis test and Student-Newman-Keuls post-hoc test);

RESULTS

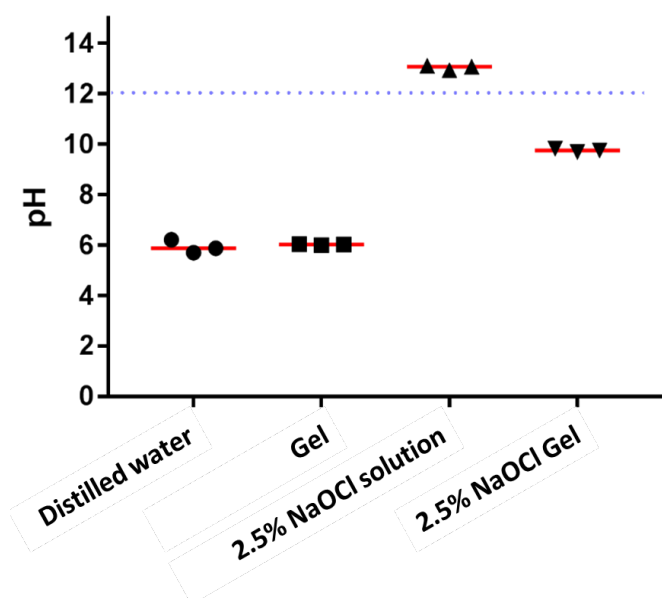
Antimicrobial activity

Both NaOCl samples (solution and gel) showed antimicrobial activity at all tested times. The control groups (distilled water and gel base) did not show antimicrobial activity. When evaluating the viability of bacterial cells, there was growth only in the samples obtained from aliquots of the wells that presented turbidity, confirming the bactericidal effect of 2.5% NaOCl solution and gel.

pH evaluation

The median pH values of NaOCl gel and solution were 9.75 and 13.08, respectively. The median values for test control substances were 5.87 for distilled water and 6.02 for the gel base, as shown in Figure 1.

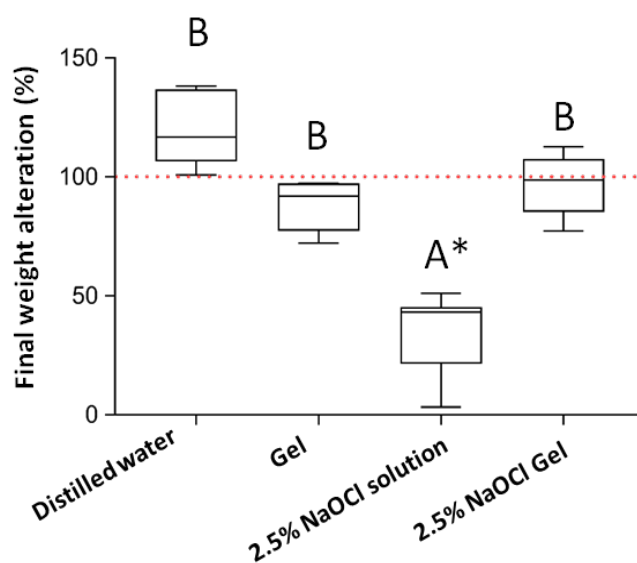
Figure 1. Median pH values obtained for test and control substances. Dashed line indicates extremely basic pH.



Pulp tissue dissolution capacity

The median of the final mass change in the samples as compared to the initial mass was: 98.63% for 2.5% NaOCl gel; 43.18% for 2.5% NaOCl solution; 116.80% for distilled water; and 92.06% for the gel base. 2.5% NaOCl solution had a higher dissolution capacity than that of the other tested groups (Kruskal Wallis test and Student-Newman-Keuls post-hoc test; $p < 0.05$). The results are also shown in Figure 2.

Figure 2. Median of the final mass percentage of samples after exposure to the test and control substances when compared to the initial mass. The dashed line indicates the initial mass of the sample. The asterisk (*) indicates no statistically significant difference between the initial and final moments in the same group (Student's t test, $P < 0.05$). Different letters show statistically significant differences between the groups (Kruskal-Wallis and Student-Newman-Keuls post-hoc tests, $P < 0.05$).



DISCUSSION

The literature presents studies on the antimicrobial activity of NaOCl gel (14); however, there are no reports of its tissue dissolution properties. NaOCl solutions with higher pH significantly dissolve more organic tissue than solutions at the same concentration, but with a lower pH (15). However, NaOCl acidification increases antimicrobial activity but decreases the ability to dissolve tissue (16, 17). Therefore, the pH of the solution determines the balance between free chlorine, hypochlorite ion (OCl^-), and hypochlorous acid (HClO). The balance influences the biological effect of NaOCl, which can be defined as antimicrobial capacity and tissue dissolution (18). Considering the scarcity of studies in the literature and the need to study irrigants that are safer for clinical use, it is essential to evaluate the antimicrobial properties, pH, and tissue dissolution capacity of NaOCl gel.

In the present study, both 2.5% NaOCl gel and solution presented

antimicrobial activity in all the time periods tested, and there was no difference between them. Conversely, Zand et al. (2016) reported that the antimicrobial activity of NaOCl gel was lower than that of the solution¹⁴. A similar behavior was observed by Vianna et al. (2004), who showed that chlorhexidine gel also took a longer period of time to exert antimicrobial activity than did the chlorhexidine solution¹¹.

The samples exposed to 2.5% NaOCl solution had a large difference between the initial and final masses, in agreement with the literature findings^{13,19}. Conversely, NaOCl gel showed no tissue dissolution capacity, and there was no difference between the initial and final mass values (98.63%). NaOCl gel (pH 9.75) showed lower pH than the solution (pH 13.08) at the same concentration, although both had alkaline values. In a study with swine muscle tissue, pH influenced the tissue dissolution capacity of NaOCl. In solutions with a pH lower than 9, there was a decrease in tissue dissolution capacity²⁰.

In this experiment, for the evaluation of tissue dissolution capacity, the total exposure time of the specimens to the substances was 3 minutes, which may have been insufficient for the action of NaOCl gel at pH 9.75. The slightly alkaline pH of NaOCl gel may have influenced the tissue dissolution capacity when compared to NaOCl solution at the same concentration.

One hypothesis for NaOCl gel not presenting tissue dissolution capacity in the experimental period was attributed to the fact that it presented a median pH of 9.75, while NaOCl solution had a median pH of 13.08 at the same concentration. Jungbluth et al. (2011) stated that NaOCl solutions with higher pH dissolve significantly more organic tissue than do NaOCl solutions at the same concentration, but with a lower pH¹⁵. According to Siqueira et al. (2005), in a study with bovine dental pulps, pH influences the tissue dissolution capacity of NaOCl. NaOCl with pH values below 9 exhibits decreased tissue dissolution capacity. Also in Siqueira's study, total exposure time to the substances did not exceed 120 minutes (21). In the present study, exposure time to the chemical solutions was 3 minutes, which may have been insufficient for the action of NaOCl gel with a pH of 9.75.

Tartari et al. (2016) reported, in a study on tissue dissolution by different concentrations of NaOCl using bovine muscle tissue; that tissue dissolution was directly dependent on the time of immersion of the samples in auxiliary chemical substances, with longer immersion time leading to greater tissue dissolution²². These findings reinforce the hypothesis that the time of exposure of the samples to the auxiliary chemicals in the present study may have been insufficient for NaOCl gel to have tissue dissolution capacity.

In the test groups, the gel base showed no difference in mass values before and after the experiment and the median of the final mass percentage was 92.06%. For distilled water, there was no difference between the initial and final mass values, with a median of final mass of 116.80%. The specimens used in the present study were frozen and unfrozen prior to the experiment. According to Colla and Prentice-Hernandez (2003)²³, while a

food is being unfrozen, strong exudation occurs, with loss of nutrients, especially in the intracellular environment. Exudation and loss of nutrients after the tissue is unfrozen tend to cause a decrease in tissue mass. When the tissue is completely unfrozen and in contact with distilled water, rehydration and incorporation of liquid occurs, increasing mass²³. Care was taken to standardize the drying procedures of the pulp fragments both before and after the experiment. However, such a procedure does not prevent rehydration, incorporation of liquid, and the increase in mass that an unfrozen tissue goes through after coming in contact with distilled water. Cobankara et al. (2010) also observed a mass increase in bovine pulp tissue in contact with saline solution in a study on the dissolution capacity of organic tissue¹².

NaOCl is the only chemical auxiliary with the ability to dissolve tissue. The literature cites different models to test this property, such as porcine palatal mucosa used by Jungbluth et al. (2011) (15), porcine pulps by Clarkson et al. (2006)²⁴ and Clarkson et al. (2012)²⁵, bovine muscle tissue by Tartari et al. (2015)²², and human tooth pulps by Slutzky-Goldberg et al. (2013)¹³. Bovine pulp tissue was chosen in the present study because it presents characteristics similar to that of human pulp tissue and because it has a considerably larger volume than that of the human dental pulp²⁶.

The viscosity of NaOCl gel prepared in the present study is directly proportional to the initial chlorine concentration of NaOCl stock solution. The lower the amount of active chlorine, the lower the viscosity of the gel, as it will require a higher amount of NaOCl solution and a lower amount of gel to reach the desired concentration. According to the findings of Romolu et al. (2015), there is little control among some manufacturers regarding the active chlorine concentration of commercially available NaOCl solutions, with large variations in both industrial and manipulated sources²⁷. The concentration of NaOCl stock solution used in this experiment was acquired by the manufacturer at 12%; however, after titration, there was 4.3% of active chlorine. For the present study, the discrepancy between the NaOCl concentration reported by the manufacturer and its actual concentration identified by the titration was relevant because it allowed the formulation of a less viscous gel.

CONCLUSION

Under the conditions of the present experiment, there was no influence of the presentation form of 2.5% NaOCl on antimicrobial activity in any of the time periods tested. However, NaOCl gel did not show tissue dissolution capacity. Additional studies should be performed by modifying the pH of NaOCl gel and increasing the exposure time of the sample to the chemical auxiliary.

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