# Can the sodium hypochlorite tissue dissolution ability during endodontic treatment really be trusted? An in vitro and ex vivo study

Alexandre Augusto **ZAIA**[1]
Emmanuel João Nogueira Leal da **SILVA**[2]
Aline Cristine **GOMES**[3]
Danna Mota **MOREIRA**[4]

## ABSTRACT

**Objective:** The aim of this study was to (1) evaluate the tissue dissolution effect of sodium hypochlorite (NaOCl) at different concentrations on the apical portion of mesial root of human mandibular molars with isthmuses and (2) evaluate the dissolution time of bovine pulp tissue in direct contact at different concentrations and volumes of NaOCl. **Methods:** Histologic investigation was performed in thirty mesial roots of human mandibular molars that were instrumented using the Mtwo system and irrigated with 2.5% NaOCl or 5.25% NaOCl. Saline solution was used as control. Each sample was submitted to histologic processing and the images were analyzed using the ImageJ software. The percentage of area occupied by tissue was calculated by dividing the area of tissue by the canals area. Data were analyzed by means of the analysis of variance with Tukey test ($P < 0.05$). Dissolution time was analyzed by immersing bovine pulp tissue in different volumes of 2.5% and 5.25% NaOCl solution. **Results:** No significant difference was found between the NaOCl concentrations in the histological investigation. No substance was able to completely clean the isthmuses. Moreover, a higher dissolution rate for the bovine pulp tissue was found in NaOCl with a concentration of 5.25%, in addition to a shorter dissolution time for larger volumes. **Conclusion:** The NaOCl is effective for tissue dissolution when in direct contact, however, NaOCl solution, even in high concentrations, was not competent to dissolve remnants of pulp tissue in root isthmuses during endodontic treatment.

**Keywords:** Sodium hypochlorite. Dissolution. Anatomy.

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[1]Full professor and adjunct professor of Orthodontics at State University of Campinas (UNICAMP).
[2]Postdoc in Endodontics, UNICAMP. Adjunct Professor at UNIGRANRIO.
[3]Masters student in Dental Clinic,UNICAMP.
[4]Postdoc in Biomaterials, Univeristy of Texas.

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Contact address: Emmanuel João Nogueira Leal da Silva
Rua Herotides de Oliveira, 61 – Icaraí – Niterói/RJ — Brazil
CEP: 24.230-230 – Email: nogueiraemmanuel@hotmail.com

## Introduction

One of the goals of endodontic therapy is the removal of all vital or necrotic tissues, microorganisms and microbial by-products from the root canal system.[1,2,3] Complete debridement of the root canal system is complicated due to the presence of a complex system of isthmuses, accessory canals and deltas that can provide ideal locations for bacteria and harboring debris.[1,4,5,6]

An isthmus is defined as a narrow, ribbon-shaped communication canal between two root canals that contains pulp tissue.[7,8] The prevalence of isthmuses in the mesial root of mandibular first molars has been reported in previous studies in which observations were carried out using different methods and at varying distances from the apex.[8,9,10] These areas have proved to be inaccessible to conventional manual and rotary instrumentation.[4,6,11,12,13] To aid in the removal of debris and the disinfection of these areas, the use of various irrigating solutions has been advocated.[14,15] Sodium hypochlorite (NaOCl) is the most widely recommended irrigating solution used during the chemomechanical preparation of the root canal system. This endodontic irrigant has the ability to destroy a broad spectrum of microbes, and its antimicrobial property has been widely reported.[16,17,18] Furthermore, the NaOCl is a non-specific proteolytic agent with excellent tissue dissolution ability.[19]

Tissue dissolution depends on 3 factors: frequency of agitation, amount of organic matter in relation to the amount of irrigant in the system and amount of available tissue surface area.[20] Many studies have examined the tissue dissolution ability of NaOCl, and have presented some conflicting results.[1,2,21,22] Apparent inconsistencies among the results could be explained by the great variety of methods used for assessing tissue solubility in those studies. Several in vitro studies have showed the NaOCl ability to dissolve the pulp tissue by direct contact[16,23]. However, studies in which the real goal was not to show the NaOCl dissolution ability demonstrated that this irrigating solution was not able to dissolve pulp tissue in anatomical complexity areas, especially in isthmus.[1,2,22,24]

Thus, the purpose of this in vitro study was to evaluate (1) the tissue dissolution effect of NaOCl at different concentrations (2.5% and 5.25%) on the apical portion of mesial root of human mandibular molars with isthmuses and (2) the dissolution time of bovine pulp tissue in direct contact at different concentrations and volumes of NaOCl (2.5% and 5.25%).

## Material and Methods

### Histological evaluation

Thirty mesial canals of vital freshly extracted human mandibular molars were selected for this study. Pulp vitality of the 30 test teeth was initially established with Endo-Ice refrigerant spray (Hygenic Corp., Akron, OH). The teeth were referred to extraction due to nonrestorability or patient's refusal of endodontic treatment. After extraction, the teeth were stored in 0.1% thymol solution and maintained at 4°C before use. Conventional access preparations were made and #10 K-type file was introduced into each canal until it reached the apical foramen. The working length (WL) was established at this distance. Canals were randomly divided into three groups of ten teeth each according to the irrigation protocol.

The root canals were prepared by the crown-down technique using the Mtwo system (VDW, Munich, Germany) with up and down movements, as recommended by the manufacturer. After an initial enlargement with a stainless #10 file, sequential Mtwo instrumentation (10/.04; 15/.05; 20/.06; 25/.06) was performed to the working length. The root canal was irrigated with a 27-gauge needle syringe. A volume of 10 mL of each irrigating solution (2.5% sodium hypochlorite; 5.25% sodium hypochlorite and 0.9% saline solution) was used in each tooth after each file, and then 10 mL of the same solution was used for final irrigation. A total volume of 50 mL of solution was used in each tooth. Recapitulation was performed with a #10 file at the WL. Afterwards, the same procedure was carried out with larger files. The solution was kept in the root canal system for a period of 45 minutes.

Apical 4 mm pieces of each root was sectioned and removed for histological processing. Canals were flooded with 10% neutral buffered formalin and stored in this same solution until histological processing was carried out. Specimens were then washed and decalcified in an aqueous solution containing equal parts of 50% formic acid and 50% sodium citrate for 20 days, and embedded in paraffin wax. Serial cross-sections were cut at 6 μm and alternately stained with hematoxylin and eosin. The images taken were analyzed by means of the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The outline of the canals were traced to determine the surface area of the region. Areas occupied by stained tissue in the region were also determined.

The percentage of area occupied by tissue was calculated by dividing the tissue area by the canal area, for each canal. Data were analyzed by means of analysis of variance with Tukey post hoc tests (significance level, $P < 0.05$).

### Bovine pulp tissue dissolution

The pulp tissue was collected from extracted bovine teeth stored in 0.1% thymol solution and maintained at 4°C. Two longitudinal grooves were cut in the proximal surfaces of the teeth with diamond burs. The teeth were split in halves. The bovine pulp tissue samples were weighed and standardized at 0.20 g. Pulp tissues with lower weight were discarded and those, in which the weight was greater, had some parts removed to achieve the proposed weight. Pulp tissues that were fragmented during removal were discarded.

Each bovine pulp tissue sample was placed separately into amber vials with NaOCl solution at different concentrations (2.5% and 5.25%). The pH of the solutions was 11. At first, the samples were immersed in 1 mL of solution and kept under constant agitation, at 37°C, during 30 minutes, until all the pulp tissue was dissolved. Tissue dissolution was timed, and the average of dissolution time was calculated among the three tissue samples in observation. When the pulp tissue was not dissolved within 30 minutes of observation, the sample was collected and weighed. Afterwards, pulp tissue samples were immersed in NaOCl solutions increased by 1 mL, until the volume used did not alter the time of tissue dissolution.

All the experiments were done in triplicate. The mean tissue dissolution times were compared using the One Way ANOVA statistical test for comparison between the different volumes used for each concentration as well as the comparison of the ability to dissolve tissue between the different NaOCl concentrations (2.5% and 5.25%).

## Results

The in vitro bovine pulp tissue dissolution results are presented in Table 1. The One-way ANOVA test indicated statistically significant differences between the 5.25% and 2.5% NaOCl solution, in which the former had a higher tissue dissolution rate. Conversely, no statistically significant difference was observed between different volumes of 5.25%, which did not happen for 2.5% NaOCl solution. No pulp dissolution was found with less than 13 mL of 2.5% NaOCl and 4 mL of 5.25% NaOCl. The 5.25% NaOCl showed a stable dissolution time as from 8 mL of solution.

The histological evaluation results of pulp tissue dissolution in isthmuses are presented in Table 2. There were no differences among the different sodium hypochlorite solutions used for irrigation in endodontic treatment for tissue dissolution in root isthmus. Although the three substances used were able to remove parts of the pulpal tissue in the isthmus, none of them was able to completely clean it (Figs 1A to F).

**Table 1.** Comparison between the mean bovine pulp tissue dissolution times, in minutes, according to the volume in mL, and the NaOCl concentration (Mean ± standard deviation).

| Volume (ml) | Dissolution time | |
|---|---|---|
| | 5.25% NaOCl | 2.5% NaOCl |
| 1 | * | * |
| 2 | * | * |
| 3 | * | * |
| 4 | $29.00 \pm 1.01^{A}$ | * |
| 5 | $27.15 \pm 1.62^{AB}$ | * |
| 6 | $23.56 \pm 1.02^{BC}$ | * |
| 7 | $24.43 \pm 0.75^{BC}$ | * |
| 8 | $22.50 \pm 1.83^{C}$ | * |
| 9 | $22.63 \pm 0.25^{C}$ | * |
| 10 | $22.30 \pm 0.81^{C}$ | * |
| 11 | $21.30 \pm 0.81^{C}$ | * |
| 12 | $21.06 \pm 1.67^{C}$ | * |
| 13 | $21.64 \pm 1.92^{Cb}$ | $28.00 \pm 0.34^{Aa}$ |
| 14 | $21.56 \pm 0.92^{Cb}$ | $28.06 \pm 0.40^{Aa}$ |
| 15 | $21.03 \pm 1.92^{Cb}$ | $28.01 \pm 0.26^{Aa}$ |

Different lowercase letters used horizontally indicate statistically significant differences between concentrations ($p < 0.05$). Different uppercase letters in column indicate statistically significant differences between the volumes at the same concentration ($p < 0.05$) *Tissue dissolution not observed after 30 minutes.

**Table 2.** Mean ± standard deviation of percentage of canal cleanliness.

| Group | Canal cleanliness |
|---|---|
| 0.9% saline solution | $62.5 \pm 14.6^{A}$ |
| 2.5% sodium hypochlorite | $68.6 \pm 09.7^{A}$ |
| 5.25% sodium hypochlorite | $71.1 \pm 10.2^{A}$ |

Different letters show statistically significant differences among the groups ($p < 0.05$).

## Discussion

Sodium hypochlorite, at different concentrations, has been used as an irrigant of the root canal for a long period. Its use is supported not only by its good physical and chemical properties, but also by other properties such as antimicrobial and tissue dissolution.[25,26,27] The ability to dissolve tissues can be considered one of the most important properties of the NaOCl solution.[28] However, many studies have shown that variables such as concentration, contact time and volume affect the solution tissue dissolution capacity, questioning the NaOCl ability to dissolve pulp tissue.[1,2,16,20,25,28,29] One of the objectives of the present study was to assess the irrigant solution volume needed to dissolve pulp tissue; varying the volume throughout the experiment and using pulp samples of same weight.

It was noted that the higher the concentration, the greater the ability to dissolve pulp tissue when different concentrations of NaOCl were evaluated. These results are in accordance with previous studies, which stated that stronger concentrations of NaOCl result in greater tissue dissolution.[23,25]

It was also observed that in the first minutes of contact with the solution, the tissue dissolution occurred rapidly and the ability to dissolve decreased with time. This fact indicates that the potential for tissue dissolution will be reduced over time and it may be related to the chemical reactions (saponification) that occur between NaOCl and the pulp tissue. In this reaction, NaOCl in contact with organic material hydrolyzes proteins transforming them into amino acids and lipids that are converted into free fatty acids.[30] As result, the NaOCl is dissociated and

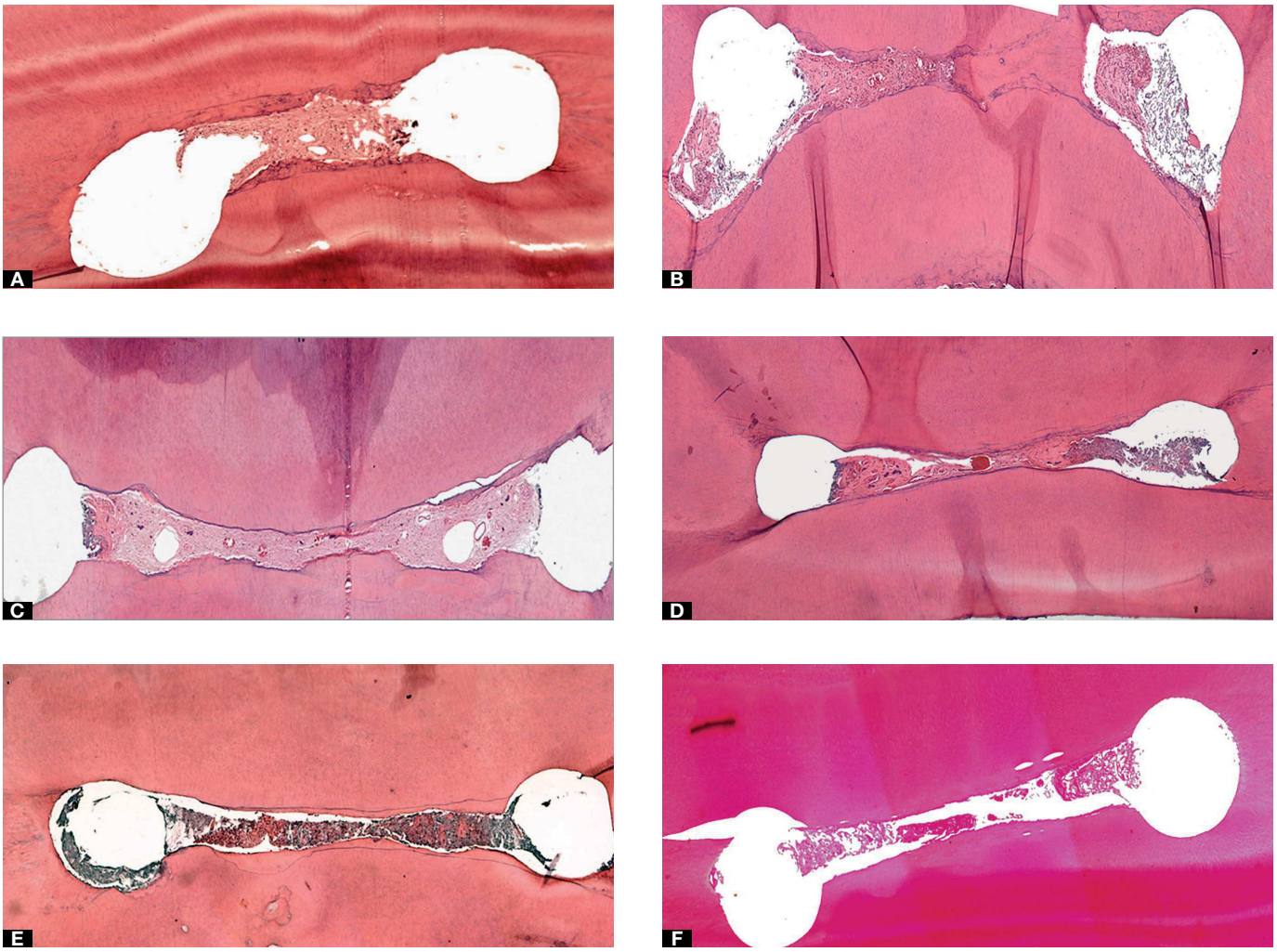


**Figure 1.** Light microscopy images of hematoxylin and eosin stained cross-sections of root specimens that were irrigated with (**A**, **B**) 0.9% saline solution; (**C**, **D**) 2.5% sodium hypochlorite; and (**E**, **F**) 5.25% sodium hypochlorite (Original magnifications, 40X).

the activity of tissue dissolution is reduced.[31] Due to the consumption of reagents and their influence on the speed of chemical reactions, it is suggested that constantly renewing the irrigating solution during endodontic procedures would increase both speed and potential for tissue dissolution when compared to simple contact between NaOCl and pulp tissue.

Moreover, the volume used directly affects the ability of the solution to dissolve tissues. A minimum volume of 4 mL of 5.25 % NaOCl was requested to completely dissolve 0.2 g of bovine pulp tissue. On the other hand, a minimum volume of 14 mL of 2.5% NaOCl was necessary to reach the total tissue dissolution of a similar sample.

Although tissue dissolution was observed upon direct contact with NaOCl solution, some studies evaluating different instrumentation techniques show that when the canal anatomy is not favorable, the NaOCl becomes less effective in dissolving organic tissues and, in some cases, no tissue dissolution is observed in difficult anatomic areas.[1,2,24,32,33] The results of the present study confirm these findings and show that despite being a tissue solvent ,the NaOCl is not competent to dissolve remnants of pulp tissue in root isthmus during endodontic treatment. Even when it was used at 5.25%, the NaOCl was not able to completely clean any isthmus. The presence of pulp tissue was observed in areas where endodontic files were not able to reach and carry out the physical cleaning.

In an attempt to remove pulp tissue not removed by tissue dissolution, recent studies have indicated the use of different irrigating devices, such as the passive ultrasonic irrigation device, due to the fact that the ultrasound technique shows improvements in pulp tissue removal, including areas with isthmus.[1,2,12,22] However, no study has showed canal and isthmus cleanliness values of 100%.

This fact leads us to believe that the endodontist should use increasingly higher concentrations of NaOCl to achieve total tissue dissolution in areas of difficult access. However, this practice is inadvisable because the increase in tissue toxicity is proportional to the increase in solution concentration. High concentrations can lead to risks of harm to patients and delay regeneration of periodontal tissues.[34,35] Despite considering that, in clinical practice, the NaOCl solution at higher concentrations should be used in areas of difficult access for mechanical instrumentation, such as the isthmus ones, doubts arise when the small volume of solution that reaches those areas is taken into account. Thus, the actual ability to dissolve pulp tissue by direct contact is questionable.

Understanding that the NaOCl is unable to dissolve tissue in irregular areas such as isthmus on root canal, and that it may cause injury during the endodontic treatment, allows endodontists to think about other auxiliary chemical substances which have good antimicrobial properties and offer lower risks to patients during the course of endodontic treatment. Furthermore, if we accept that a large number of endodontic treatments keep tissue inside the root canal without interfering in the success of treatment, we can reconsider the importance of completely cleaning the root canal in order to achieve a successful endodontic treatment. Such success is evaluated by means of assessing the health of the periodontal ligament in the apical region and the periodontal ligament associated with openings in the lateral canals. Even when the root canal is all contaminated, parts of the periodontal ligament surrounding the root of the tooth will only make inflammatory changes when there is a communication canal, such as a lateral canal or an apical foramen. Thus, it seems more likely that the success of the endodontic treatment depends more on blocking the communication between root canal and periodontal ligament than on the complete removal of pulp tissue from root canal systems.

## Conclusions

In conclusion, the present study demonstrated that depending on the concentration, a minimum volume of the NaOCl solution is necessary to dissolve pulp tissue in direct contact. However, NaOCl solution at different concentrations was not competent to dissolve remnants of pulp tissue in the root isthmus during endodontic treatment.

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