ORIGINAL RESEARCH

Efficacy of Cryotreated NaOCl on Soft Tissue Dissolution: An *In Vitro* Study

Sameer Makkar[1], Iflah Javed[2], Rishika Thakur[3], Shabnam Negi[4], Vivek Aggarwal[5], Avijit Awasthi[6]

Received on: 16 April 2023; *Accepted on:* 21 May 2023; *Published on:* 31 August 2023

Abstract

Objective: The most frequent endodontic irrigant is sodium hypochlorite due to its antibacterial and tissue-dissolving properties. Recently, in endodontics, cryotherapy has been suggested to be an effective technique for controlling postoperative pain. The purpose of the study was to assess the effectiveness of cryotreated NaOCl for tissue dissolution.

Materials and methods: Palatal mucosal tissue of goat was collected to determine a benchmark of tissue dissolution properties of NaOCl at different temperatures. Fifteen pieces of palatal mucosa of goat of similar size were prepared and weighed. The sample was then randomly divided into three experimental groups, depending on the temperature of 5.25% NaOCl irrigant. Group I—tissue treated with warm 5.25% NaOCl, Group II—tissue treated with 5.25% normal NaOCl, Group III—tissue treated with 5.25% cryotreated NaOCl. Following an approximate exposure period of 2 minutes, the remaining tissue samples were taken out, blot dried, and checked for their weight gain. The variation in the weight of tissue in the pre-dissolution period and post-dissolution period was noted as residual weight.

Results: Kruskal–Wallis test showed that the time taken for complete dissolution was statistically significantly different among different groups ($p < 0.001$), with a mean rank tissue dissolution readings of 3.50 for warm NaoCl, 9.50 for normal NaOCl and 15.50 for cryotreated NaOCl.

Conclusion: Tissue dissolution with cryotreated NaOCl was less compared with normal and warm NaOCl. Cryotreated NaOCl did dissolve the tissue, but dissolution was slower when compared with normal and warm NaOCl.

Key message: To evaluate the efficacy of cryotreated NaOcl on soft tissue.

Keywords: Cryotreated NaOCl, Tissue dissolution, Temperature, Warm NaOCl, Soft tissue.

Dental Journal of Advance Studies (2023): 10.5005/djas-11014-0014

Introduction

Sodium hypochlorite (NaOCl) solutions are the most favored root canal irrigants because of their antibacterial, tissue-dissolving, and lubricating properties.[1,2] It is widely used as an endodontic irrigant, at concentrations ranging from 0.5 to 6%. Regardless of concentration, heated NaOCl was shown to be more effective as a tissue solvent, according to Abou-Rass and Oglesby.[3] Bashetty and Hegde, in 2010, in their study observed that the 5.25% NaOCl group presented with postoperative pain.[4]

The word "cryotherapy" comes from the Greek word "cryos," which means cold. It has been used often in medicine, primarily to treat pain associated with hernia operations, abdominal pain, gynecological pain, and sports injuries.[5–7] Cryotherapy has been used in dentistry for implant placement, periodontal surgery, and intraoral excisional surgical procedures. It is efficient at reducing inflammation, pain, and arthritis induced around by temporomandibular joint diseases.[8] Nandkumar et al. conducted a randomized controlled clinical trial and concluded that the use of intracanal cryotreated NaOCl reduced the incidence of post-endodontic pain.[9]

As cryotreated NaOCl has been shown to reduce postoperative pain but how does it perform with tissue dissolution when compared with warm NaOCl? As a result, the goal of this study was to assess and compare the effects of normal and heated NaOCl and cryotreated NaOCl on tissue dissolution.

[1–4]Department of Conservative Dentistry & Endodontics, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India

[5]Department of Conservative Dentistry and Endodontics, Jamia Millia Islamia, New Delhi, India

[6]Department of Public Health Dentistry, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India

Corresponding Author: Iflah Javed, Department of Conservative Dentistry & Endodontics, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India, Phone: +91 7006135692, e-mail: iflahjavaid@gmail.com

How to cite this article: Makkar S, Javed I, Thakur R, *et al*. Efficacy of Cryotreated NaOCl on Soft Tissue Dissolution: An *In Vitro* Study. Dent J Adv Stud 2023;11(2):62–65.

Source of support: Nil

Conflict of interest: None

Materials and Methods

Tissue Specimen

Fifteen fragments obtained from the palatal mucosa were dissected from goat. Immediately after dissection, small pieces of mucosa of similar sizes and equal weight of approximately 0.6 mg were cut for subsequent analysis to determine a benchmark of tissue dissolution properties. The tissue was stored at –15°C. The sample was then randomly divided into three experimental groups. The samples were gently cleaned with distilled water to remove any dirt, and then they were blotted dry. The samples were apportioned into three groups: group I—tissue was treated in warm 5.25% NaOCl (42°C), group II—tissue was treated in 5.25% normal NaOCl (room temperature), Group III—tissue was treated in 5.25% cryotreated NaOCl (2–4°C).

The sodium hypochlorite concentration was 5.25%. Three 2-ml syringes were filled with NaOCl solutions. For group I—an electric kettle heater was used to reheat 2 mL of 5.25% NaOCl to 42°C (temperature checked with a digital thermometer). Group II—2 mL of sodium hypochlorite at room temperature. Group III—consisting of 2 mL of cryotreated sodium hypochlorite maintained between 2 and 4°C.

Each specimen from each group was placed in their respective NaOCl solutions for 2 minutes. The remaining tissue samples were removed after an approximate 2-minute exposure period, blot dried, and their weight was again measured. Remaining weight was defined as the difference in tissue weight between the pre- and post-dissolution periods. The residual weight that was acquired was then added to the appropriate solutions. Frequent replenishment was done until complete tissue dissolution took place. Dissolution was considered complete when no visible tissue fragments were present in the beaker.

Statistical Analysis

Statistical analysis was performed using a software package of statistical analysis (SPSS for Windows, version 19, Armonk, NY: IBM Corp). Mean and standard deviation were calculated for descriptive statistics. Comparison of the time taken for complete dissolution among the study groups was assessed using Kruskal–Wallis test followed by Mann–Whitney test for multiple comparisons. A *p*-value of less than 0.05 was determined as the level of significance for the current study.

Results and Observations

Kruskal–Wallis test was conducted to determine if the time taken (in minutes) for the complete dissolution of samples and it was different for various study groups. The study samples were classified into three groups. The data for the time taken for complete dissolution are presented as mean, standard deviations, and mean ranks. Descriptive statistics showed that the time taken for complete dissolution was highest in cryotreated NaOCl group, followed by the normal NaOCl group, and least in warm NaOCl and was statistically significant (Fig. 1, Table 1).

Multiple pairwise comparisons using Mann–Whitney test showed that (as shown in Fig. 1, Table 2):

- Time taken for dissolution in the cryotreated NaOCl group was significantly higher compared with that in the normal NaOCl group and warm NaOCl.
- Also, the time taken for dissolution in the normal NaOCl group was significantly higher compared with that in the warm NaOCl group (mean difference = 2.000, $p = 0.001$).

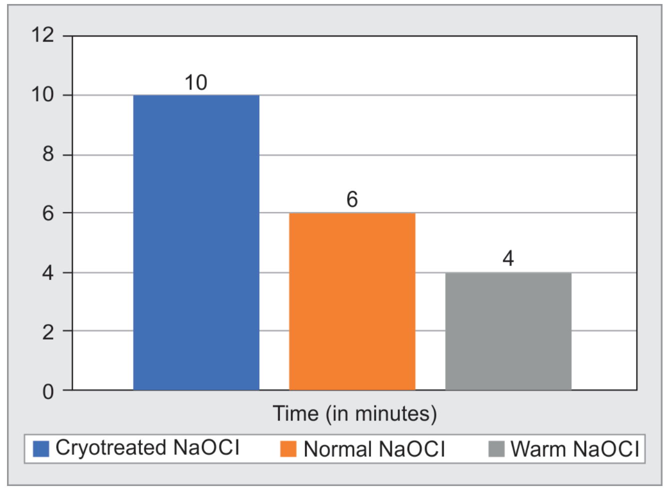

Fig. 1: Comparison of time taken for complete dissolution

Discussion

Over the years, NaOCl is still one of the best irrigants for root canal procedure. It is antibacterial, dissolves tissue, and acts as a lubricant as well. In fact, the role of warm NaOCl in tissue dissolution is an established fact as studied by Rossi-Fedele et al. At the same time, postoperative pain is of concern to the patient. Patients are more likely to acquire chronic or persistent postoperative pain as a result of the severity and length of their pain, which has a longer term negative psychological, social, and financial impact.[10] Both from a humanitarian standpoint and for effective delivery of healthcare services, postoperative pain prevention and alleviation must be optimized.

Bashetty and Hegde in 2010 found that only at the sixth hour postoperatively (*p*-value 0.05) the 5.25% NaOCl group exhibited more pain than the 2% CHX group. It is well known that the pain peak that occurs in the first few hours following endodontic treatment is specifically related to the presence of inflammation.[4]

The recent advent of cryotherapy is medium and dentistry has shown several benefits in response to relieving pain. Intracanal cryotherapy has been used, according to Al Nahlawi et al., to decrease postendodontic pain.[11] The initial overview of cryotherapy's application in endodontics was made by Vera et al. In their *in vitro* study, the researchers studied the effects of using a cold saline solution (2.5°C) as a final irrigant for 5 minutes. They found that doing so caused the external root surface temperature to drop by more than 10°C and remain at that level for 4 minutes. It was hypothesized that this temperature drop would be sufficient to have a local anti-inflammatory effect in the periradicular tissues.[12]

Additionally, it reduces postoperative pain by vasoconstriction with an antiedematous effect, decreases blood flow and supply of oxygen to that area, and limits tissue damage by slowing down cellular metabolism. Additionally, cold reduces the number of leukocytes that migrate to the site of injury, which lessens endothelial dysfunction and the inflammatory response. It affects peripheral nerve endings by reducing the intensity needed to activate tissue nociceptors and increasing the rate at which painful nerve impulses propagate through the body.

In the recent study by Nandakumar M et al. it was seen that cryotreated NaOCl reduced the postoperative pain. At the measured time intervals of 6 hours, 24 hours, and 48 hours after surgery, it demonstrated a significant decrease in postoperative pain levels, as well as a decrease in analgesic consumption. This may be attributable to the cold-treated irrigant's synergistic effect, which

Table 1: Kruskal–Wallis test

			95% Confidence interval for mean		
	Mean ± SD	*Mean rank*	*Lower bound*	*Upper bound*	*p-value*
Cryotreated NaOCl	10.00 ± 0	15.50	10.0000	10.0000	<0.001*
Normal NaOCl	6.00 ± 0	9.50	6.0000	6.0000	
Warm NaOCl	4.00 ± 0	3.50	4.0000	4.0000	

*Statistically significant ($p < 0.05$, Kruskal–Wallis test)

Table 2: Mann–Whitney test

					95% Confidence interval	
Dependent variable	*Group*	*Group*	*Mean difference*	*p-value*	*Lower bound*	*Upper bound*
Time taken for complete dissolution	Cryotreated NaOCl	Normal NaOCl	4.00	0.001*	–0.68	2.52
	Cryotreated NaOCl	Warm NaOCl	6.00	0.001*	–4.00	–0.79
	Normal NaOCl	Warm NaOCl	2.00	0.001*	–4.92	–1.71

*Statistically significant ($p < 0.05$, Mann–Whitney *U* test)

lowers the temperature of the external root surface and causes the periradicular tissues to become less inflammatory.[9]

Thus, arises the question that cryotreated NaOCl reduces postoperative pain but does it dissolve the tissue sufficiently and efficiently when compared with warm NaOCl. Therefore, this study was undertaken to carry out the efficacy of tissue dissolution by cryotreated NaOCl (2.5–4) and to compare it with normal and warm NaOCl.

In this study, 15 pieces of palatal mucosa from goat were used to evaluate the tissue-dissolving property of cryotreated, warm, and normal NaOCl. A rectangular-shaped tissue specimen with a standardized weight of 0.6 mg was prepared from goat palatal mucosa for use in the current study. Pilot tests showed that the significant number of bubbles (a byproduct of the saponification reaction) made it impossible to estimate whether the tissue had completely dissolved. As a result, a fixed period was employed, and the samples were weighed before and after exposure. The beaker containing cryotreated, warm, normal NaOCl and the sample were placed in the beaker containing 2 mL of 5.25% NaOCl for 2 minutes.

In our study, following an approximate exposure period of 2 minutes, the remaining tissue samples were taken out, blot dried, and checked for their weight again. Remaining weight was defined as the difference in tissue weight between the pre- and post-dissolution periods. The resulting residual weight was then added to the appropriate solutions. Moorer WR et al. conducted a study and stated that frequent replenishment of the solution is therefore essential to ensure the availability of irrigant for dissolution, therefore, in this study, the solution was replenished of active molecules for further reaction and to remove remnants of dissolved tissue.[13] For optimum activity of an irrigant, the volume of the solution should be in excess compared with the amount of tissue to be dissolved. Excess organic matter depletes the available chlorine in NaOCl and causes the pH to drop, thereby decreasing its activity. Therefore, continual replenishment with fresh irrigant is essential.

NaOCl was shown to be more effective as a tissue solvent when heated, as reported by Abou-Rass and Oglesby. The concentration of the solution also had an impact on this action.[3] Sirtes et al. observed that after 60 minutes of heating at 45 and 60°C, 1%, 2.62%, and 5.25% solutions of NaOCl solution were still stable, showing their stability for a clinically significant period of time.[14]

The present study showed that the cryotherapy group results in tissue dissolution in a slower rate when compared with normal and warm NaOCl. Thus, it was observed in group I – complete tissue dissolution took place in 4 minutes, while in group II and group III, it was 6 minutes and 10 minutes, respectively. Statistical analysis was performed using Krushal–wallis test followed by Mann–Whitney test for multiple comparisons. The level of significance was set at the *p*-value of less than 0.05. Descriptive statistics showed that the tissue taken for complete dissolution was highest in cryotreated NaOCl followed by normal NaOCl and least in warm NaOCl.

So, while tissue dissolution with warm NaOCl is definitely better but cryotreated NaOCl dissolved the organic tissue completely. Therefore, in order to achieve a balance between less postoperative pain and complete tissue dissolution of tissue, more solution of cryotreated NaOCl may be required. On one hand, this will increase the time for tissue dissolution but on the other hand, will be comfortable for patient as well. Although more studies are recommended to establish the use of cryotreated NaOCl as an irrigant in endodontics.

Further research should be directed toward understanding the effect of cryotreated NaOCl and the various methods which would enhance pulp dissolution.

Conclusion

From the current study, it was determined that:

- Cryotreated NaOCl dissolves tissue but at a slower rate.
- In 10 minutes, the tissue in the cryotreated NaOCl group was significantly reduced statistically.
- Dissolution capacity of the warm NaOCl is more when compared with the normal and cryotreated NaOCl.
  - Warm NaOCl > normal NaOCl > cryotreated NaOCl

Data Availability

"No datasets were generated or analyzed during the current study"

Author Contributions

Dr Iflah Javed was involved in Concepts, literature search, clinical studies, manuscript preparation. Dr. Sameer Makkar's contribution includes Design, the definition of intellectual content, experimental studies, final approval of the version to be published. Dr Avijit Awasthi was involved in Data analysis, statistical analysis. Dr Shabnam Negi and Dr Vivek Aggarwal were involved in manuscript editing, and manuscript review. Dr Arpit Sikri was involved in drafting the article or revising it critically for important intellectual content.

References

1. Güçlüer Ö, Akarsu E, Yavuz E, et al. Human pulp tissue dissolution ability of different extracts of Sapindus mukorossi: an in vitro study. Chin Herb Med 2020;12(2):178–182. DOI: 10.1016/j.chmed.2019.11.005.
2. Rosenfeld EF, James GA, Burch BS. Vital pulp tissue response to sodium hypochlorite. J Endod 1978;4(5):140–146. DOI: 10.1016/S0099-2399(78)80129-0.
3. Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. J Endod 1981;7(8):376–377. DOI: 10.1016/s0099-2399(81)80059-3.
4. Bashetty K, Hegde J. Comparison of 2% chlorhexidine and 5.25% sodium hypochlorite irrigating solutions on postoperative pain: a randomized clinical trial. Indian J Dent Res [Internet]. 2010;21(4): 523–527. DOI: 10.4103/0970-9290.74225.
5. Bleakley C, McDonough S, MacAuley D. The use of ice in the treatment of acute soft-tissue injury: A systematic review of randomized controlled trials: A systematic review of randomized controlled trials. Am J Sports Med [Internet]. 2004;32(1):251–261. DOI: 10.1177/0363546503260757.
6. Gundogdu EC, Arslan H. Effects of various cryotherapy applications on postoperative pain in molar teeth with symptomatic apical periodontitis: A preliminary randomized prospective clinical trial. J Endod [Internet]. 2018;44(3):349–354. DOI: 10.1016/j.joen.2017.11.002.
7. Higgins TR, Heazlewood IT, Climstein M. A random control trial of contrast baths and ice baths for recovery during competition in U/20 rugby union. J Strength Cond Res [Internet]. 2011;25(4):1046–1051. DOI: 10.1519/JSC.0b013e3181cc269f.
8. Filho JRL, Silva E De Oliveira, Batista CI, et al. The influence of cryotherapy on reduction of swelling, pain and trismus after third molar extraction. J Am Dent Assoc 2005;136(6):774–778. DOI: 10.14219/jada.archive.2005.0261.
9. Nandakumar M, Nasim I. Effect of intracanal cryotreated sodium hypochlorite on postoperative pain after root canal treatment – A randomized controlled clinical trial. J Conserv Dent [Internet]. 2020;23(2):131–136. DOI: 10.4103/JCD.JCD_65_20.
10. Rossi-Fedele G, De Figueiredo JAP. Use of a bottle warmer to increase 4% sodium hypochlorite tissue dissolution ability on bovine pulp. Aust Endod J [Internet]. 2008;34(1):39–42. DOI: 10.1111/j.1747-4477.2007.00110.x.
11. Nahlawi A, Hatab T, Alrazak TA, et al. Effect of intracanal cryotherapy and negative irrigation technique on postendodontic pain. J Contemp Dent Pract. 2016;17:990–996. PMID: 27965485.
12. Vera J, Ochoa-Rivera J, Vazquez-Carcaño M, et al. Effect of intracanal cryotherapy on reducing root surface temperature. J Endod 2015;41(11):1884–1887. DOI: 10.1016/j.joen.2015.08.009.
13. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J 1982;15(4):187–196. DOI: 10.1111/j.1365-2591.1982.tb01277.x.
14. Sirtes G, Waltimo T, Schaetzle M, et al. The effects of temperature on sodium hypochlorite shortterm stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod 2005;31:669–671.