



# Effect of decreased temperature on the tissue dissolution ability of sodium hypochlorite: An in vitro study

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**Purpose:** The present in vitro study aimed to compare the tissue dissolution ability of a 5% sodium hypochlorite solution at 3 different temperatures.

**Methods:** Thirty standardized fragments were prepared from bovine muscle tissue and randomly divided into 3 experimental groups (n = 10) according to the temperature of the sodium hypochlorite. The tissue was immersed in a 1.5-mL test tube containing 20 mL of sodium hypochlorite at the specific temperature and stored for 15 min. The solution was agitated with an ultrasonic tip working for 1 min. Then the solution was filtered, and the tissue sample was dried. The weight loss of the tissue was measured as dissolved tissue by the sodium hypochlorite. A one-way analysis of variance was used to compare the mean dissolved tissue weight between groups ( $p < 0.05$ ).

**Results:** The highest dissolution values were found in the 60°C sodium hypochlorite group, achieving significantly greater mass loss ( $p < 0.05$ ), while no significant difference was found between the solutions applied at 20°C and 2.5°C ( $p > 0.05$ ).

**Conclusion:** This in vitro study found that the application of sodium hypochlorite by cooling for cryotherapy did not alter its capacity for dissolving organic tissue compared to application at room temperature.

**Keywords:** Activation; cold; heat; intracanal cryotherapy; NaOCl; room temperature.

## Introduction

Cryotherapy is termed as the therapeutic use of general or local cold application on a target tissue with the goals of managing pain and swelling following an operation or injury by lowering the local temperature (1,2). Intraoral or extraoral application of cold after an oral and maxillofacial surgical procedure is routinely preferred due to its benefits regarding controlling hemorrhage and metabolic rate (3). Intracanal cryotherapy emerged as a postopera-

tive pain reduction measure to control endodontic pain, periapical oedema, and inflammation (4-6). In 2015, an in vitro study by Vera et al. (7) reported that application of 2.5°C cold saline as a final irrigant for 5 min decreased the surface temperature and maintained it low, allowing the effect to be described as cryotherapy. The procedure was termed intracanal cryotherapy and was tested in numerous clinical trials (5,6,8,9). The studies applied cryotherapy to different pulp and periapical conditions, populations, and tooth types, with different numbers of patients and differ-

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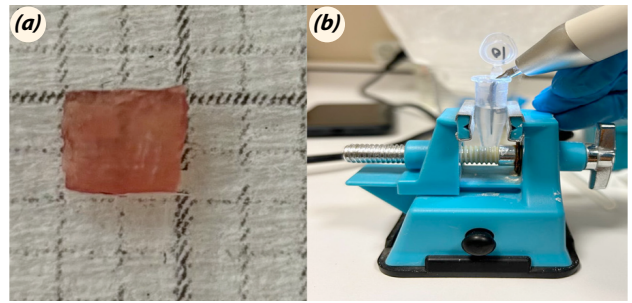


ent irrigation delivery devices (10). Since there is no study directly comparing needle irrigation with the negative pressure irrigation system, which was originally advocated by the original study, both needle irrigation and negative pressure irrigation were reported to decrease postoperative endodontic pain (8,9,11).

Recent systematic reviews and meta-analyses concluded that intracanal cryotherapy was effective in controlling short-term postoperative pain; however, more controlled randomized clinical trials are required to draw conclusions with a higher degree of evidence (12,13). Therefore, several clinical trials are continuing to test the efficacy of cryotherapy in terms of pain control, not only in an orthograde route but also with different applications with different purposes (10,14). Intraoral and extraoral application of cold have been compared with intracanal cryotherapy and seemed to be as effective as the latter (15,16). Cryotherapy has also been used to decrease pain during dental anesthesia and hemorrhage in vital pulp treatment (14,17). Moreover, intracanal cryotherapy applications also varied. Some authors used cold sodium hypochlorite during canal preparation to prolong the duration of the cold applied with the intention of preventing possible oedema and inflammation (10). However, changing the temperature of an irrigation solution might influence its physical and chemical properties (18,19). Lowering the temperature of such solutions might affect their abilities in terms of tissue dissolution, smear layer removal, and antimicrobial efficacy. Heating is a well-known activation method to increase the tissue dissolution ability of sodium hypochlorite (20); however, to the authors' knowledge, no study evaluated the tissue dissolution ability of cold sodium hypochlorite. The present *in vitro* study aimed to compare the tissue dissolution capabilities of sodium hypochlorite solutions heated to 60°C and cooled to 2.5°C with those at room temperature (20°C). The null hypothesis would be that temperature would not significantly alter the tissue dissolution ability of sodium hypochlorite.

## Materials and Methods

The present study is reported in accordance with preferred reporting items for laboratory studies in PRILE 2021. The sample size calculation was performed using the effect size of a previous study evaluating the dissolution potential of activated sodium hypochlorite (1.66) (22). The analysis of variance (ANOVA) test (fixed effects, omnibus, one-way) was chosen with an alpha-type error of 0.05 and a power beta of 0.90 at G\*Power 3.1.9.2 (Universität Düsseldorf, Germany). The sample size calculation indicated a minimum of nine samples per group would be required to achieve a similar effect size.



**Fig. 1.** (a) 2×2×1 mm cut bovine muscle tissue (b) Experimental setup with ultrasonic activation.

A total of 30 fragments of bovine muscle tissue were prepared by cutting into equal-sized pieces of 2 × 2 × 1 mm using a #15 stainless-steel blade (Broche Medical, Istanbul, Türkiye) (Fig. 1). The weight of the tissues was measured using a calibrated electronic balance (Precisa XB220 Dietikon, Switzerland) following blotting dry with paper towels. The mean weight of the samples was 3 ± 0.09 mg. The tissue fragments were divided into 3 groups (n = 10) according to the temperature of the 5% sodium hypochlorite (Werax, Izmir, Türkiye): heated (60°C), cooled (2.5°C), and room temperature (20°C). The heating was achieved with a bottle warmer, while the cooled solution was obtained by storing it in the refrigerator. The temperatures were monitored by a digital thermometer (Hanna HI98501, Leighton Buzzard, UK). Statistical comparisons ensured the pre-weight of the mean specimen mass was similar among the experimental groups ( $p > 0.05$ ).

The pulp tissue was transferred to glass tubes containing 20 mL of sodium hypochlorite, which was agitated with ultrasonic energy with an ED5 ultrasonic tip for 1 min (Woodpecker, Guilin, China). The tissue was stored in the tube for 15 min. The solution was filtered through absorbent filter paper, and the remaining tissue was dried. The weight of the remaining pulp tissue was measured, and the difference between the initial and final measurements was recorded as the dissolved tissue weight.

The normality of the data was tested with a one-way ANOVA and a post hoc Tukey test. All statistical analyses were performed by a blinded evaluator using SPSS (V.21, Chicago, IL, USA) with a significance threshold of 5%.

**Table 1.** The mean value and the percentage of weight loss of immersed samples in sodium hypochlorite solutions with different temperatures

Group	Weight loss (mg [%])
Room temperature NaOCl (20°C)	0.72 ± 0.3 (24) <sup>a</sup>
Cooled NaOCl (2.5°C)	0.78 ± 0.7 (26) <sup>a</sup>
Heated NaOCl (60°C)	2.05 ± 0.9 (68.3) <sup>b</sup>

## Results

The mean weight loss in the tissue samples is provided in Table 1. The NaOCl dissolved similar amounts of tissue in the 2.5°C and 20°C groups ( $p > 0.05$ ), while heated NaOCl at 60°C led to significantly greater weight loss ( $p < 0.05$ ).

## Discussion

Due to the complex anatomy and irregular morphological structure of the root canal system, it is very challenging to completely eliminate pulp tissue during endodontic treatment (23,24). The majority of the pulp tissue is removed prior to working length determination; however, some residue might be present in inaccessible areas of root canals such as the isthmus, fins, or long oval irregular canals. Effective root canal irrigation is important to compensate for the limits of mechanical preparation and aims to remove necrotic tissue residues along with intracanal debris and bacteria (25,26). Root canal irrigation is considered an indispensable step of root canal treatment in the field of endodontics, as it facilitates the removal of biofilms and these residual necrotic tissues from the root canal system (24-26).

The use of irrigation solutions at low temperatures first emerged as a technique to reduce the surface temperature of the outer root and maintain to exert a possible effect on periapical tissues (7). Then, final irrigation with cooled distilled water or saline solutions to control postoperative pain and decrease analgesic use was tested by clinical trials (9,13,15). Routine cooling of distilled water or saline in the final irrigation has been proven effective, with varying degrees of evidence following clinical studies reporting in favor of reducing postoperative pain and analgesic use (12,27-29). Since such application only occurred following the completion of biomechanical root canal preparation, clinical studies cooling sodium hypochlorite (10,11) used during mechanical preparation should be read with caution due to the lack of laboratory studies indicating possible alterations in the biological and physical properties of sodium hypochlorite, which is the most important irrigation solution used in endodontics for decades. In addition to its high antimicrobial properties, its organic tissue dissolving feature makes it unique among other irrigation solutions, with the additional benefit of being a relatively low-cost and easily available solution (22). Without knowing how cooling affects the effectiveness of irrigation solutions, their use may not be appropriate. In this study, sodium hypochlorite irrigation solutions at different temperatures were compared for the 1st time, and the routinely used room temperature value was used as a reference.

The majority of studies on endodontic tissue dissolution published in the literature were conducted using irrigant solutions at room temperature (30-35). This is due to the practical difficulties of maintaining controlled temperatures in *in vitro* environments. In routine clinical studies, irrigant sodium hypochlorite is used at room temperature, although theoretically this temperature may increase while in the root canal. However, an irrigant with a flow rate of 5 mL/min is renewed in approximately 0.12 s in an average-sized root canal (33). In this case, although the temperature of the irrigant in the root canal is not clear, it can be thought to be similar to or the same as room temperature due to the short contact time in the canal (22).

It is known that the organic tissue dissolving property of sodium hypochlorite increases when heated, but there is no data in the literature on how the organic tissue dissolving property of cooled sodium hypochlorite is affected. This laboratory study evaluated the organic tissue solubilization ability of sodium hypochlorite used at three different temperatures, and the null hypothesis was rejected because there were differences between the temperatures.

The use of bovine muscle tissue instead of real human dental pulp tissue during the tissue dissolution stage can be considered a potential weakness of this study. Bovine muscle tissue samples with the most similar size, shape, and initial mass to dental pulp due to their homogeneous composition were prepared and used in the study. The closest similarity to dental pulp was achieved with bovine muscle tissue samples measuring  $2 \times 2 \times 1$  mm. However, in order to most accurately test the tissue dissolving ability in endodontic treatment, real human pulp tissue must be used. Although there are histological differences in tissue dissolution measurements of sodium hypochlorite between bovine muscle tissue and human dental pulp, there are no studies showing conflicting results in the literature (34,35). In addition, it is not possible to standardize all tissue samples to be used in the study of human tooth pulp tissue in terms of size and shape. Providing sufficient amounts of human pulp tissue for the study is another limiting factor, which will undermine the standardization of working conditions. The use of bovine pulp tissue, which was frequently used in the past, is no longer preferred due to the risk of serious viral infection (22).

Another limitation of the study is that the sodium hypochlorite solution was tested using a test tube model that differs from the actual human root canal system in size and shape. However, this model enables the specimens in the study to be compared visibly, reproducibly, and quantitatively. Safe standardization of tissues could not be practically achieved in test tubes with optimal dimensions equivalent to the root canal system. For these reasons, further

studies using various models mimicking root canal configurations of different designs are warranted to evaluate the dissolving effectiveness of sodium hypochlorite with different temperature values.

## Conclusion

Within the limitations of this in vitro study, it has been shown that the tissue dissolving efficiency of sodium hypochlorite used at the temperature in intracanal cryotherapy is not affected by cold. However, further studies may be useful to investigate how other physicochemical properties of sodium hypochlorite are affected.

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