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In vitro Study of Temperature Changes on the Outer Root Surface of Extracted Human Teeth Under Different Parameters of Intracanal Cryotherapy

Marieta Valentinova Todorova[1] Silviya Dimitrova Dimitrova[1] Plamen Ivanov Zagorchev[2]

[1] Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University - Plovdiv, Plovdiv, Bulgaria
[2] Medical Physics and Biophysics, Medical University-Plovdiv, Plovdiv, Bulgaria

Address for correspondence Marieta Valentinova Todorova, PhD student, Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University - Plovdiv, Plovdiv, Bulgaria (e-mail: marieta.todorova@mu-plovdiv.bg).

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Abstract

Objectives The aim of the present study is to investigate the temperature changes on the outer root surface of extracted human teeth during irrigation with different volumes of solutions at different temperatures.

Materials and Methods Thirty premolars extracted for orthodontic or periodontal indications were used for the first experiment and 12 for the second experiment. Root canals were instrumented using WaveOne Gold. Thermosensors were attached to the apical 5 mm and to the middle 5 mm of the outer root surface. Control irrigations with 0.9% NaCl, 2.5% NaOCl, and 17% EDTA at room temperature and experimental irrigations at 2.5°C with 0.9% NaCl, 2.5°C with 2.5% NaOCl, and 2.5°C with 17% EDTA were conducted.

Results During the intracanal cryotherapy, a minimum temperature more than 10°C lower than the initial temperature value was reached. There was no significant difference between the lowest temperature values reached in the irrigations carried out with the three irrigation solutions studied. These results indicate that the effect of intracanal cryotherapy can be successfully achieved using all three types of irrigation solutions, i.e., NaOCl, 0.9% NaCl, EDTA. Intracanal cryotherapy needed to last more than 2 minutes to achieve more than 10°C difference with the initial temperature.

Conclusions A total of 5 mL of irrigation solution for 5 min is insufficient to achieve a cryotherapeutic effect. A temperature difference of 10°C between initial and lowest temperatures recorded was reached fastest at a volume of 20 mL.

Keywords
- postendodontic pain
- intracanal cryotherapy
- EndoVac
- *in vitro* study

Introduction

Postendodontic pain is a complex, multifactorial phenomenon.[1] In the recent years, new methods for assessing pain after endodontic treatment have been developed–pain scales[2,3] and measuring biomarkers' levels.[4] New strategies for controlling postendodontic pain such as preemptive analgesia, low-level laser therapy, and intracanal cryotherapy, have been introduced.

Pain control during and after endodontic treatment is an important aspect of good endodontic practice. In recent years, intracanal cryotherapy has been applied in the search for a reliable method to deal with this problem. Borrowed

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from physiotherapy, in endodontics, it is applied as a final irrigation of the root canal with cold solution. Cold application induces three main physiological tissue responses: reduction of blood flow, reduction of metabolic activity, inhibition of neural receptors in skin and subcutaneous tissues.[5,6]

In recent years, multiple studies have reported the use of intracanal cryotherapy to reduce post-endodontic pain (PEP). Vera et al, in an *in vitro* study, evaluated the effect of cold saline (2.5°C) as a final irrigant for 5 minutes. They concluded that its use caused a decrease in the temperature of more than 10°C on the outer root surface, which was maintained for 4 minutes. Presumably, this temperature reduction was sufficient to produce a local anti-inflammatory effect in the periradicular tissues.[7] The same authors, in an *in vivo* study, found a significant reduction in drug intake and PEP in the cryotherapy group.[8] In a clinical study, Keskin et al found that application of 2.5°C 0.9% NaCl as a final irrigant resulted in a significant reduction in PEP levels compared with the control group.[9] Al-Nahlawi et al evaluated the effect of intracanal cryotherapy administered using EndoVac on 75 vital single-rooted teeth in a single-visit endodontic treatment. The results showed that VAS pain scores at 6, 12, 24, and 48 hours in the group with final irrigation with cold 0.9% NaCl were lower than those in the other groups studied, and the differences were statistically significant.[10]

There is no precise protocol established in the world literature for the conduct of cryotherapy in endodontics. The temperature of solutions for the administration of intracanal cryotherapy varies between 1.5 and 4°C. In some studies, a refrigerator is used to obtain a cold solution and a thermometer to control the temperature.[6,10,11] Both the volume of irrigation solutions from 5 mL and[9,12] 10 mL[11,13] were varied up to 20 mL,[8,10,14] and the duration of exposure from 1 min[12,13] up to 5 min.[8–11,14] In several studies, cryotherapy was performed using an EndoVac system with negative pressure for 5 minutes.[8,10,13] Further studies are needed to provide conclusive evidence to confirm its therapeutic effect.[15]

Materials and Methods

Experiment 1. Investigation of the effect of temperature of irrigation solutions during intracanal cryotherapy with 0.9% NaCl, NaOCl, EDTA on two points on the outer root surface of extracted human teeth.

Thirty freshly extracted human permanent premolars extracted for orthodontic or periodontal indications were used for the study. Immediately after extraction, the teeth were placed in 5% sodium hypochlorite (NaOCl) for 30 minutes. The specimens were cleaned of calculus with ultrasound. The prepared samples were placed in distilled water to avoid dehydration until the time of the experiment. The specimens were standardized to 15 mm root length using a diamond burr and a turbine handpiece. Root canals were instrumented using WaveOne Gold (Dentsply Sirona, Ballaigues, Switzerland) and hand endodontic files (K-files, Dentsply Sirona, Ballaigues, Switzerland). Standard four-wall endodontic access was prepared using the Cavity Access Z Set (Dentsply Sirona, Ballaigues, Switzerland). The working length of all root canals was measured using a #10 K-file until it showed through the apical foramen. From this length, 0.5 mm was subtracted, and the resulting value was documented for each root canal. The teeth were fixed to a specially designed plastic stand. Thermosensors were attached to the apical 5 mm and to the middle 5 mm of the root surface by wax. To fix the thermosensors, small perforation holes were made using a contra-angle handpiece and a round metal burr. The holes were on the outer root surface and did not penetrate the root canal.

a) *Control irrigations* with 0.9% NaCl, 2.5% NaOCl, 17% EDTA at room temperature.
   The study was conducted on 30 specimens (10 for each irrigation solution). Root canal irrigation was performed with three solutions-0.9% NaCl, 2.5% NaOCl, 17% EDTA each with a volume of 20 mL for 5 min. All irrigation solutions were annealed at room temperature and drawn to full working length using the EndoVac system microcannula. The time was monitored with a stopwatch. The temperature change was recorded at 2 points on the outer root surface using two thermosensors. The room temperature at the beginning and throughout the experiment was recorded.
b) *Experimental irrigations* with 2.5°C 0.9% NaCl, 2.5°C 2.5% NaOCl, and 2.5°C 17% EDTA (►**Fig. 1**).

Fig. 1 Experimental irrigation with 2.5°C 0.9% NaCl, 2.5°C 2.5% NaOCl, 2.5°C 17% EDTA.

For the purpose of the study, 20 mL syringes of 0.9% NaCl, 2.5% NaOCl, 17% EDTA were kept refrigerated in a tray of ice. Root canal irrigation was performed 5 minutes after control irrigation on the same specimens. Using 20 mL of 2.5°C 0.9% NaCl, 2.5°C 2.5% NaOCl, 2.5°C 17% EDTA, the irrigation was continued for 5 minutes. The irrigants were brought to full working length using the EndoVac system. The irrigation solutions and microcannulas were stored in a refrigerator, in a tray with ice, until use. The temperature change was recorded at two points on the outer root surface using two thermosensors.

Statistical Analysis

Experimental data were processed using Statistica 4.5 (StatSoft, Inc. Microsoft) and SPSS 11.5.0. Student's *t*-test was used to determine the statistically significant difference in means between groups at a significance level of $p < 0.05$. Analysis of variance-one-way ANOVA was employed.

Data processing

Temperature changes during the process were visualized on a computer screen thanks to the developed two-channel interface system for collecting, recording, and processing laboratory data. The system had an amplifier unit (DC instrumentation amplifier with a gain factor of 800), a 10-bit analog-to-digital converter and a serial communication unit with a PC (BaudRate = 115200, 8, N,1). This ensures the tracking of the two temperatures with an accuracy of $\pm 0.05^{o}$C at a sampling interval of 125 ms, the serial port of the PC being fed for this interval with data obtained by collecting and averaging 100 measurements. A program had been developed to allow graphical tracking of the temperature differences and allow subsequent processing of the experimental data (►**Fig. 2**).

Fig. 2 Two-channel interface system.

Experiment 2. Investigation of the effect of irrigation solution volume during intracanal cryotherapy with 2.5°C 0.9% NaCl on two points on the outer root surface of extracted human teeth

For the purpose of this study, specimens were prepared to an apical foramen size of 35/.06 ($n = 12$). Two specimens were retained for positive and negative controls. The remaining 10 specimens were subjected to the following irrigation protocols:

1) Irrigation with cold 2.5°C 0.9% NaCl-5 mL
2) Irrigation with cold 2.5°C 0.9% NaCl-10 mL
3) Irrigation with cold 2.5°C 0.9% NaCl–20 mL

All syringes and the EndoVac system microcannulas were stored in a tray of ice in a refrigerator and removed one at a time immediately before use. When the syringe to be used for irrigation was removed, it was immediately wrapped in a gel-ice cuff.

Results on Experiment 1

The first irrigation performed was a control irrigation to determine whether there was a difference in temperature at the outer root surface at two points (middle and apical) when irrigated with solutions at room temperature of 24°C. From the study, it was found that there was no statistically significant variation in the temperature measured at both the middle and apical parts of the root when different substances were applied as irrigation solutions, at room temperature (►**Fig. 3**).

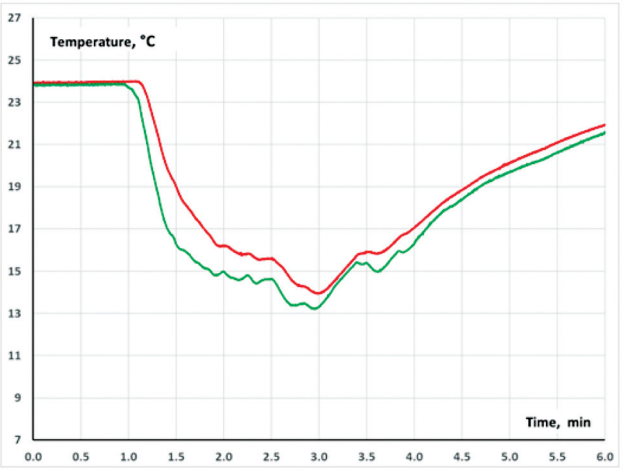

Fig. 3 Temperature changes in dynamics during the experimental irrigation with 20 mL 2.5°C 0.9% NaCl. The apical-third of the outer root surface is indicated in red and the middle-third in green.

In the experimental irrigation, a decrease in temperature was found in both zones studied (middle and apical). A minimum temperature of more than 10°C lower than the initial temperature was reached. The smooth decrease in temperature, the short retention of the lowest temperature (about 1.5 min) mainly in the middle-third and the rapid return of the temperature to the initial values were noteworthy, as shown in ►**Figure 3**. It was concluded that to retain the lowest temperature values for a longer period, the syringe was needed to be wrapped in a gel-ice cuff.

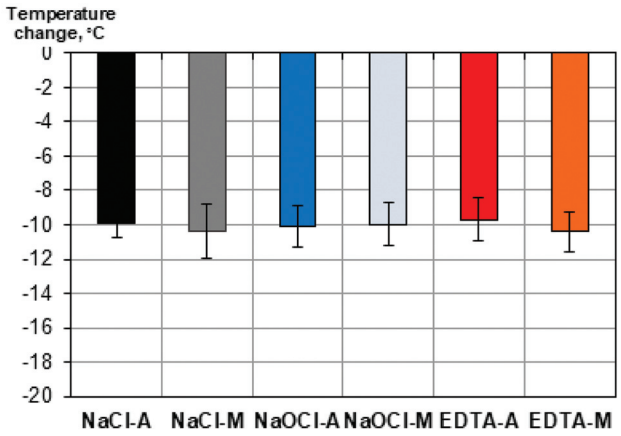

Fig. 4 Lowest temperatures reached during irrigation with the three types of irrigation solutions in the two zones (A-apical, M-middle).

►**Figure 4** demonstrates the lack of significant difference between the lowest temperature values reached in the irrigations performed with the three irrigation solutions used-NaCl, NaOCl, and EDTA. The results obtained indicate that the type of solution was not significant in the experiments conducted.

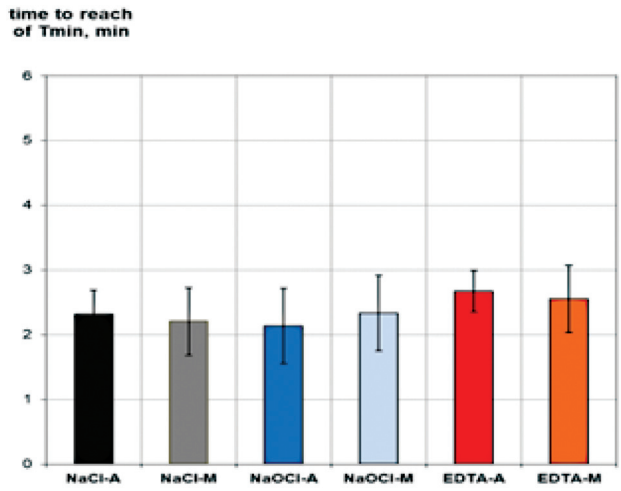

Fig. 5 Time of reaching the lowest temperature values for the three types of irrigation solutions in the two zones (A-apical, M-middle).

In the course of the study, the time required to reach the lowest temperature was also investigated. From ►**Fig. 5**, it can be concluded that the intracanal cryotherapy needed to last more than 2 min to reach more than 10°C difference with the initially measured temperature.

Discussion on Experiment 1

In 2015, Vera et al in their *in vitro* study investigated the effect of intracanal cryotherapy on temperature reduction at the outer root surface.[7] The authors achieved more than 10°C reduction in the temperature, which they think was sufficient to achieve a cryotherapeutic effect. The original methodology developed by the authors forms the basis of the present work. The results we obtained in the control irrigation fully support those of Vera et al. The use of room temperature irrigation solution did not lead to a significant decrease in temperature at the outer root surface. The same authors considered that the type of irrigation solution was not relevant to the effect of cryotherapy and used NaOCl for the control and 0.9% NaCl for the experimental irrigation. In the course of the present experiment, we also found that when irrigated with room temperature solution, the type of irrigation solution did not affect the results.

There is no validated and specific protocol in the available literature regarding the choice of irrigation solution for intracanal cryotherapy. Mandras et al[16] and Nandakumar et al[17] used NaOCl, Vieyra et al[13] chose EDTA, while most researchers preferred 0.9% NaCl.[6,8–12] This fact brings out the necessity of conducting an experiment with three of the most frequently used irrigation solutions-NaOCl, 0.9% NaCl, EDTA. In the present study, there was no statistically significant difference between the lowest temperatures recorded during experimental irrigation with the three types of solutions. These results indicate that the effect of intracanal cryotherapy can be successfully achieved using all three types of irrigation solutions-NaOCl, 0.9% NaCl, EDTA. In the course of the present study, it was found that to retain the lowest temperature values for a longer period of time, we need to modify the methodology by wrapping the syringe with a gel-ice cuff throughout the experiment. This new technique will ensure better cryotherapeutic effect. According to the analysis of the results, the time to reach a temperature difference of 10°C between the baseline and the lowest reached values was more than 2 min for both reported zones-middle and apical. It could be concluded that to achieve a cryotherapeutic effect, the duration of the conducted irrigation should exceed 2 min.

Results on Experiment 2

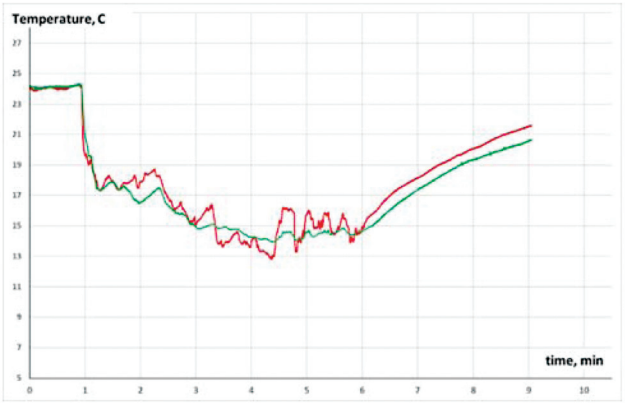

Fig. 6 Temperature changes in dynamics during irrigation with 5 mL 2.5°C 0.9% NaCl. The apical-third of the outer root surface is indicated in red and the middle-third in green.

The aim was to determine whether the use of different volumes of irrigation solution (5 mL, 10 mL, and 20 mL) had an impact on the effect of cryotherapy. The original

methodology was modified by wrapping the syringe with a gel-ice cuff throughout the experiment, as it was found that due to the rapid warming of the syringe, the lowest temperature values were not sustained long enough.

From ►**Fig. 6**, the rapid drop in temperature, the longer retention of the lowest temperature reached, and the gradual return to the original values are noticeable. These results are probably due to the modification of the methodology-wrapping the syringe with ice.

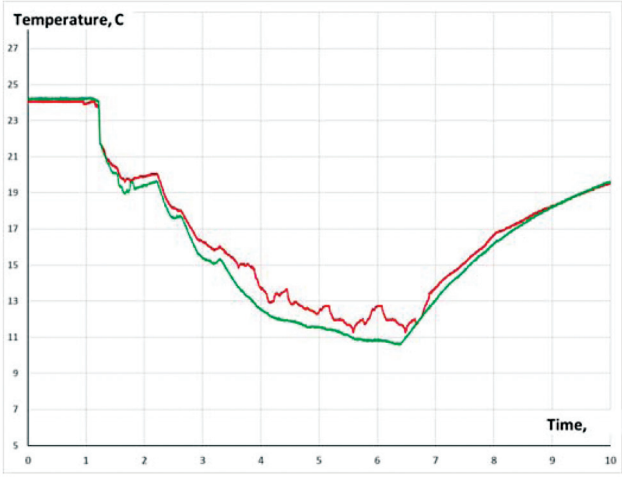

Fig. 7 Temperature changes in dynamics during irrigation with 10 mL 2.5°C 0.9% NaCl. The apical-third of the outer root surface is indicated in red and the middle-third in green.

It can be seen from ►**Fig. 7** that the larger volume of irrigation solution (10 mL) results in smaller fluctuations in the temperature curves, indicating that the amount of solution is distributed evenly over time. The lowest temperature is observed to be retained for a longer time, and the recovery of the initial temperature values is more gradual compared to the results with a volume of 5 mL.

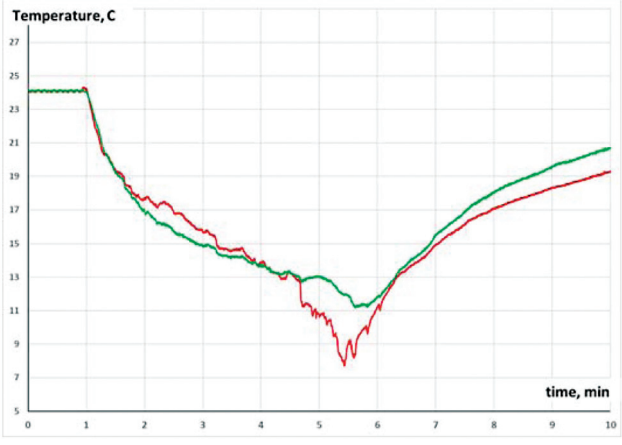

Fig. 8 Temperature changes in dynamics during irrigation with 20 mL 2.5°C 0.9% NaCl. The apical-third of the outer root surface is indicated in red and the middle-third in green.

►**Figure 8** visualizes the results of intracanal irrigation with 20 mL volume. In this experiment, the cryotherapeutic effect is most significantly established-lower temperatures are reached and maintained for the longest time, compared to the 5 mL and 10 mL experiments.

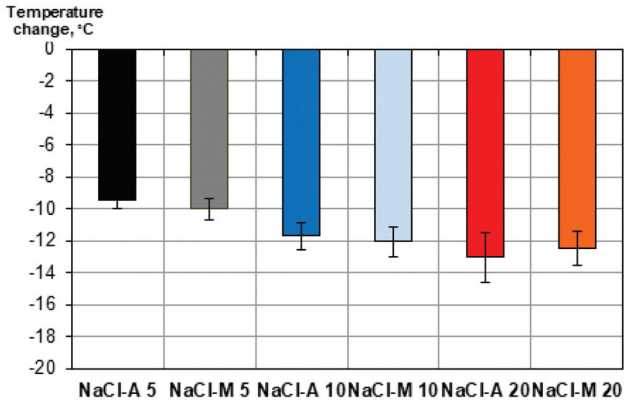

Fig. 9 Lowest temperatures reached during irrigation with 5 mL, 10 mL, 20 mL for the apical and middle root surfaces.

The recorded minimum values reflected in ►**Fig. 9** demonstrate reaching more than 10°C difference with the originally measured temperature at 10 mL and 20 mL volumes in both zones (middle and apical) and no such difference at 5 mL volume in the apical zone.

Analysis of the results demonstrated that the temperature difference was reached faster at the higher volume. A statistically significant difference was observed for irrigation with 5 mL and 10 mL and 5 mL and 20 mL in both the apical and middle zones. No statistical differences were observed between 10 mL and 20 mL for either zone.

Discussion on Experiment 2

There is still no consensus in the available literature on the volume of irrigation solution required to perform intracanal cryotherapy. Some authors used 5 mL[9,12] others preferred 10 mL[11] or 20 mL.[8,10,14] In the present study, specimens of the same apical foramen size 35/0.06 were selected and irrigation solutions of different volumes-5 mL, 10 mL, and 20 mL were applied. By unifying the preparation, the effect of volume factor of irrigation solution was analyzed. The modification of the methodology by wrapping the syringe with gel-ice led to a faster temperature drop, a longer retention of the lowest temperature recorded, and a more gradual return to the original values, compared to the results of the original methodology.

The analysis of the results shows that with a volume of 5 mL for a time of 5 min, it is very difficult to reach the desired difference of 10°C between the initially measured and the lowest temperature value reached. The statistical difference between the results at 5 mL, 10 mL, and 20 mL gives reason to conclude that 5 mL of irrigation solution is insufficient to achieve a cryotherapeutic effect. A temperature difference of 10°C between initial and lowest

temperatures recorded was reached the earliest at a volume of 20 mL. It is significant that no statistical difference was found between 10 mL and 20 mL for 5 minutes. This leads to the conclusion that dentists can choose between two different volumes of irrigation solutions-10 mL and 20 mL, adapting their decision to the individual clinical situation and irrigation technique.

The use of EndoVac in our study fully confirms the results of a number of authors demonstrating the effective performance of the system.[8,10,13,18,19] The close values of the recorded temperatures in the two measurement zones, as well as the sometimes-lower temperature values in the apical zone, prove that the negative pressure created by the system is very effective in the apical zone. Our results are in support of the conclusions of several authors. Topcuoglu et al, in their extensive study on 116 patients, compared the use of EndoVac and syringes with side-vented needles.[20] The authors found that at the 6th, 24th, and 48th hours after endodontic treatment, PEP was significantly more intense in the standard irrigation group. According to them, the use of a negative pressure system reduced PEP. According to Aydın et al,[21] EndoVac is a reliable irrigation system in terms of bacterial extrusion, and according to Buldur et al,[22] EndoVac has a better performance in removing the smear layer from the apical-thirds of root canals compared to conventional needle irrigation.

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Conflict of Interest
None declared.

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