# Effect of Heated Sodium Hypochlorite Irrigant on Structural Changes and Microhardness of Radicular Dentin: An *In Vitro* Study

Shruthi ST[1], Rajeswari Kalaiselvam[2], Lakshmi Balaji[3]

## ABSTRACT

**Aim:** This study is aimed to evaluate the combined effect of sodium hypochlorite at varied concentrations and temperatures on radicular dentin microhardness along with its surface structural changes using an FTIR spectrometer.

**Materials and methods:** Mandibular premolars were cleaned and shaped up to F3 Protaper gold rotary files, after which they were subjected to five experimental conditions – group I – neutral saline as negative control, group II – 3% NaOCl solution, group III – 5% NaOCl solution, group IV – 3% intracanal-heated NaOCl solution, and group V – 5% intracanal-heated NaOCl solution. Following this, the microhardness of radicular dentin at 100 μm and 300 μm from the canal lumen and Fourier-transform infrared (FTIR) spectroscopic analysis were performed.

**Results:** The results showed that intracanal-heated sodium hypochlorite group reduced root dentin microhardness at 300 μm than its nonheated counterpart. No difference in microhardness values was observed between 3% intracanal-heated and room-temperature sodium hypochlorite groups at 100 μm. Reduction in amide/phosphate ratio was noted in all the groups treated with sodium hypochlorite irrespective of temperature and concentration.

**Conclusion:** Thus, considering that the level of alteration in physical and structural changes of root dentin with or without heating is insignificant, intracanal-heated low-concentration sodium hypochlorite solutions could be used as an alternative to high-concentration sodium hypochlorite.

**Clinical significance:** Intracanal-heated low-concentration sodium hypochlorite enables the clinicians to achieve maximum disinfection while keeping the structural and physical properties of the dentin similar to room-temperature sodium hypochlorite.

**Keywords:** Fourier-transform infrared spectroscopy, Heated sodium hypochlorite, Intracanal heating, Root dentin microhardness, Root canal irrigant.

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## INTRODUCTION

Endodontic treatment success depends on the reduction and elimination of microorganisms from the root canal system preventing further recontamination after the treatment along with promising longevity of nonvital teeth.[1,2] Considering the complexity of tubular root canal anatomy, several studies have reported that chemical irrigants are indispensable for the eradication of pathogens.[3–6] To date, sodium hypochlorite solutions (NaOCl) are the most efficient root canal irrigants based on their antimicrobial and tissue-dissolving properties.[7,8] It is well-known that with the increase in the concentration of NaOCl solutions, faster and greater canal disinfection can be achieved.[9–11] But there is a serious concern regarding its toxicity to the surrounding tissues when extruded through the root apex.[12] With the focussed research to identify alternative techniques of enhancing irrigants' efficacy, use of heat-activated low-concentration sodium hypochlorite is growing in the field of endodontics. Temperature activation has been shown to improve their immediate tissue-dissolution capacity and, at the same time, amplify the antimicrobial effect, which is the primary goal of root canal therapy.[13,14] The use of proteolytic agents such as sodium hypochlorite causes significant changes in the physical and chemical properties of root canal dentin, which cause a detrimental effect on radicular dentin.[15–17] The extent of harm to dentin depends on the concentration, volume, and contact time of the solution.[18,19] However, the combined effect of heated sodium hypochlorite irrigant on root dentin characteristics remains unknown. Hence, the purpose of this study is to evaluate the effect of sodium hypochlorite at varied concentrations and temperatures on root dentin microhardness along with its structural changes using Fourier-transform infrared (FTIR) spectroscopic analysis.

[1–3]Department of Conservative Dentistry and Endodontics, Sri Ramachandra Institute of Higher Education & Research, Chennai, Tamil Nadu, India

**Corresponding Author:** Rajeswari Kalaiselvam, Department of Conservative Dentistry and Endodontics, Sri Ramachandra Dental College and Hospital, Chennai, Tamil Nadu, India, Phone: +91 9791109099, e-mail: rajeswarik@sriramachandra.edu.in

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## MATERIALS AND METHODS

The approval for the research was obtained from the committee for the student's proposal, a constituent of the Institutional Ethical Committee of Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai [CSP/22/JUN/111/336]. The study was conducted from August 1, 2022 to December 31, 2022.

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Fifty freshly extracted single-rooted, single-canal, intact human mandibular premolars extracted for orthodontic purposes were used in this study. Proximal view radiographs (mesial and distal) were taken to confirm the presence of a single-patent canal. Teeth with root caries, internal resorption, cracks, and curved and obliterated canals were excluded. Crowns of all teeth were cut off at cementoenamel junction using a high-speed handpiece with the help of a double-faced diamond-impregnated disc, and the length of roots was standardized to a 14 ± 1 mm length. Access cavity preparation was done, and a size 10 K file was used to determine its patency. The working length (WL) was determined 0.5 mm short of the radiographic apex. All the root canals were mechanically prepared using the Protaper gold Rotary System up to size F3 (30.09). All root canals were irrigated with distilled water during biomechanical preparation. The apical foramen was sealed using a composite resin-restorative material, and the external surfaces of the teeth were covered with a layer of varnish to provide a tight seal from liquid penetration. The 50 root specimens were randomly assigned to five groups with 10 samples in each group – group I – neutral saline as negative control, group II – 3% NaOCl solution, group III – 5% NaOCl solution, group IV – 3% intracanal-heated NaOCl solution, and group V – 5% intracanal-heated NaOCl solution. The following experimental irrigants were used for 5 minutes of contact time inside the root canal system.

For groups IV and V, two uniform holes were created on the cemental root surface at 5 and 10 mm above the apex by using a round carbide bur of 0.6 mm in diameter burr under abundant irrigation. Two K-type thermocouples for each tooth were applied as follows: two were inserted through the holes and positioned at the apical and middle third, which were represented as (T5) and (T10), respectively. To keep the thermocouples in position without protruding into the intracanal lumen, the last file (30.09) used for the preparation was placed into the root canal, and the thermocouples were placed to touch the file initially and were slightly withdrawn to finally attach flush with the root canal wall using a total-etching (37% phosphoric acid) gel and bond (G-PREMIO Universal Adhesive) and a flowable light-cured composite resin on the cementum surface.

To measure the temperatures, thermocouples were then connected to a digital recorder. The apex was closed with the same bond/composite system, creating a watertight seal. The samples were then mounted in addition to silicone putty material. The digital recording was initiated at least 3 seconds before the start of the experimental irrigation procedure with an accuracy of 0.1°C. The element-free device was set at a temperature of 150°C. NaOCl thermal activation was performed with a 25.06 heat plugger at 1 mm short of WL. Heat plugger was activated for 3–5 seconds once in every 60 seconds.

For the microhardness test, the treated roots were sectioned longitudinally by placing grooves on the lingual and buccal external surface of the roots to divide them into two halves using a double-faced diamond disc under water cooling with a low-speed handpiece. The split dentin samples were embedded in autopolymerized acrylic resin and held in a customized ring made of putty impression material. The embedded samples were then polished gradually with sandpaper, up to 1200 grade, and cleaned in an ultrasonic bath with distilled water for 2 minutes. Ten split samples from each group were taken for microhardness evaluation ($n = 10$). The radicular dentin surface hardness measurements were carried out using Vickers Diamond Microhardness Tester (Shimadzu Microhardness Tester). The microhardness measurements were taken at two different points, at depths of 100 μm and 300 μm from the lumen. Each measurement was carried out by using a “300-gram load for 10 seconds” oriented perpendicularly to the surface.

Five split-root dentin samples from each group were used for FTIR spectroscopic analysis ($n = 5$). For the ATR–FTIR spectra collection, three different spots on the surface of each treated specimen closer to the canal lumen were randomly chosen. The samples were placed directly on the diamond crystal carefully so that the pointed tip of the pressure tower would be just focused onto the center of the spot. The pressure arm was then positioned over the sample and standard pressure is applied. Spectra were collected in the range from 750 to 4000/cm$^{-1}$ at a 1 cm resolution throughout 15 scans following irrigation with the respective experimental irrigants, and the average was taken for final analysis. After baseline correction, smoothing, and normalization, the effect of NaOCl on collagen depletion was evaluated using the “collagen and apatite ratio (the ratio of absorbance of I amide peak to phosphate v3 peak)”. Amide, phosphate, and carbonate peak levels were determined (in cm$^{-1}$). Smaller the amide:phosphate ratio, the greater will be the extent of dentin deproteination.

Parametric methods were applied to analyze the data. To compare mean values between groups, ANOVA test was applied, followed by Tukey’s HSD *post hoc* test for multi pairwise comparisons. To compare values between subgroups, independent samples *t*-test was applied. To analyze the data, SPSS (IBM SPSS Statistics for Windows, version 26.0, Armonk, NY: IBM Corp. Released 2019) was used. Significance level was fixed at 5% ($\alpha = 0.05$).

## RESULTS

Statistical analysis by ANOVA test showed that the mean microhardness value decreased in sodium hypochlorite-treated groups (groups II–V) compared with the group treated with neutral saline (group I) ($p < 0.001$) (Table 1). The decreasing order of microhardness values was as follows: group I > group II > group III > group IV > group V (60.220 > 54.870 > 49.105 > 48.765 > 45.525) (Table 1). Overall, the mean microhardness value at 100 μm from the canal lumen was less compared with the values at 300 μm ($p < 0.05$). Statistical analysis using *post hoc* multiple comparison by Tukey HSD showed no significant difference in microhardness values at 300 μm from the canal lumen between groups treated with 3% NaOCl and neutral saline ($p = 0.048$) (Table 2). Intergroup comparisons reveal no statistically significant difference in the level of microhardness reduction between 3% sodium hypochlorite and 3% intracanal-heated sodium hypochlorite group at 100 μm from the canal lumen, ($p = 0.009$) whereas, at 300 μm, the specimen treated with 3% intracanal-heated NaOCl solution showed reduced microhardness values compared with the nonheated counterpart ($p < 0.001$) (Table 2). No significant difference in microhardness values among specimens treated with 5% room temperature and 5% intracanal-heated NaOCl groups at both 100 μm and 300 μm from the canal lumen (Table 2). FT-IR spectroscopic analysis showed decreased mean amide/phosphate ratio in all groups compared with the group treated with neutral saline. However, statistical analysis using *post hoc* multiple comparisons by Tukey HSD showed that there was no statistically significant difference in intergroup comparison ($p < 0.05$) (Figs 1 and 2).

From the results of the present study, it was inferred that sodium hypochlorite reduced root dentin microhardness, irrespective of concentration and temperature, with its effect more pronounced

**Table 1:** One-way ANOVA to compare mean microhardness between groups

| *Group* | *N (At 100 and 300 μm from canal lumen)* | *Mean microhardness* | *Std. dev.* | *p-value* |
|---|---|---|---|---|
| NS | 20 | 60.220 | 4.5946 | |
| 3% NaOCl | 20 | 54.870 | 5.9231 | |
| 5% NaOCl | 20 | 49.105 | 3.5057 | <0.001[a] |
| 3% IH NaOCl | 20 | 48.765 | 3.0716 | |
| 5% IH NaOCl | 20 | 45.525 | 3.4511 | |

[a]Statistically significant difference for comparisons within groups on combining the mean microhardness values at both 100 and 300 from the root canal lumen

**Table 2:** Tukey's HSD post hoc tests for multiple comparisons in subgroups

| *Subgroup* | *Group (pair)* | | *Mean difference* | *p-value* |
|---|---|---|---|---|
| 100 μm | NS | 3% NaOCl | 7.1500 | <0.001[b] |
| | | 5% NaOCl | 10.5800 | <0.001[b] |
| | | 3% IH NaOCl | 10.2000 | <0.001[b] |
| | | 5% IH NaOCl | 13.2000 | <0.001[b] |
| | 3% NaOCl | 5% NaOCl | 3.4300 | 0.002[c] |
| | | 3% IH NaOCl | 3.0500 | 0.009 |
| | | 5% IH NaOCl | 6.0500 | <0.001[c] |
| | 5% NaOCl | 3% IH NaOCl | 3800 | 0.992 |
| | | 5% IH NaOCl | 2.6200 | 0.033 |
| | 3% IH NaOCl | 5% IH NaOCl | 3.0000 | 0.010 |
| 300 μm | NS | 3% NaOCl | 3.5500 | 0.048 |
| | | 5% NaOCl | 11.6500 | <0.001[d] |
| | | 3% IH NaOCl | 12.7100 | <0.001[d] |
| | | 5% IH NaOCl | 16.1900 | <0.001[d] |
| | 3% NaOCl | 5% NaOCl | 8.1000 | <0.001[e] |
| | | 3% IH NaOCl | 9.1600 | <0.001[e] |
| | | 5% IH NaOCl | 12.6400 | <0.001[e] |
| | 5% NaOCl | 3% IH NaOCl | 1.0600 | 0.912 |
| | | 5% IH NaOCl | 4.5400 | 0.006 |
| | 3% IH NaOCl | 5% IH NaOCl | 3.4800 | 0.055 |

Lowercase letters indicate statistically significant differences for multiple comparisons within the subgroup

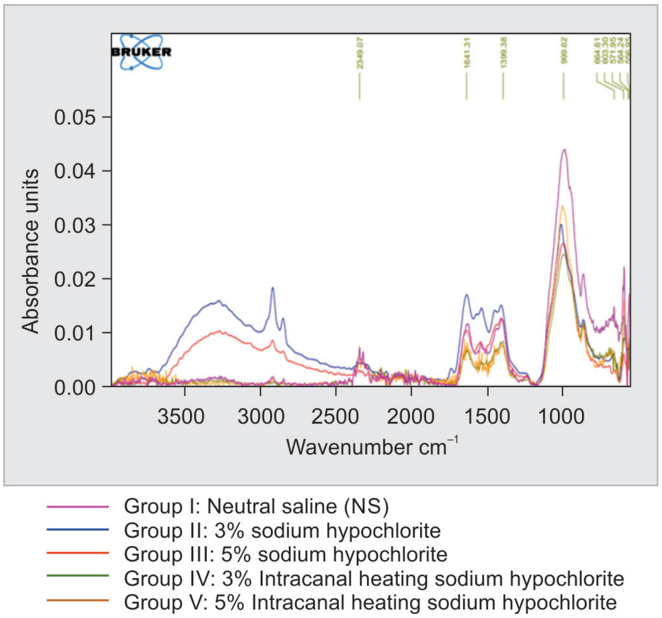

**Fig. 1:** FTIR spectroscopic analysis comparing five groups

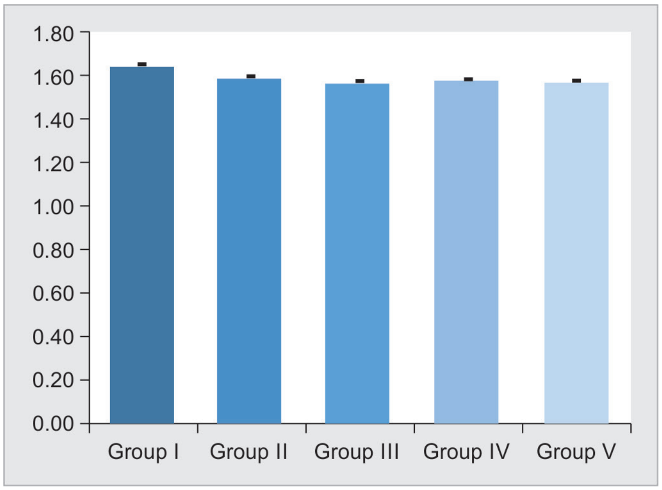

**Fig. 2:** Bar graphs depicting the mean amide/phosphate ratio values between the groups

closer to the canal lumen (100 μm) than far away (300 μm) (Table 2). About 3% sodium hypochlorite showed similar microhardness values at 100 μm at both tested temperatures (Table 2). Whereas, at 300 μm, intracanal-heated sodium hypochlorite groups (3% and 5%) exhibited reduced microhardness in contrast to the nonheated counterpart showing better tubular penetration depth (Table 2). From FTIR spectroscopic analysis, similar levels of dentin deproteination were noted in all the groups treated with sodium hypochlorite (Fig. 2).

## DISCUSSION

Dentin is an integral component of human teeth. Similar to most biomineralizing constituents, such as enamel and bone, dentin possesses a hierarchical organization of polymerized collagen fibers and apatite molecules cross-linking each other on a nanoscale forming tubular structures with different sizes and densities. These composites change with certain functional, chemical, and mechanical stresses.[20] Changes in dentin collagen cross-links with interrupted dentinal fluid distribution contribute to the "brittleness" of endodontically treated teeth.[21] Additionally, the use of alkaline-irrigating solutions like sodium hypochlorite causes undesirable effects on root dentin.[22] To ensure complete disinfection, endodontists vary not only the concentration but also the volume, duration, flow rate, delivery devices, activation systems, and temperature of the irrigant used. Heating NaOCl increases its dissolving and disinfecting properties and could be of the greatest advantage in nonsurgical root canal retreatment cases.[23,24] Different studies have used various methods to achieve thermal activation of the irrigants such as bottle warmer, preheated syringes, laser energy, and so on. Intracanal heating helps in constant contact of warm irrigants within the root canal system and is thus proven to be better than preheated solutions.[25] The fact that heated sodium hypochlorite solutions within the root canal enhance its potency, made us curious to study its effect on physical and chemical properties of radicular dentin.

Microhardness provides the initial step to determine the restoration/surface interface and failure sites.[26] Vickers microhardness test has been used in the current study for assessing the microhardness of root dentin, after the application of different temperatures and concentrations of sodium hypochlorite as it is considered as the one giving more accurate results on both hard and soft tissues. According to Pashley et al., dentin microhardness close to the lumen is lower than at its periphery.[27] The results of the present study are similar to these observations that the microhardness values closer to the lumen (at 100 µm) were less compared with the values away from the canal lumen (at 300 µm) (Table 2).

A previous study by Slutzky-Goldberg et al. in the year 2004 found decreased microhardness values after 10 minutes of contact with frequently replenished sodium hypochlorite solutions. Also, 6% NaOCl gave a more significant decrease in microhardness than 2.5% NaOCl for all irrigation periods,[18] which is in accordance with the results of the present study that 5% NaOCl showed a pronounced reduction in microhardness levels compared with 3% NaOCl. In the experimental setup, Zou et al. deduced that the depth of sodium hypochlorite penetration usually varied between 77 and 300 µm,[28] thus, investigations were limited within 300 µm in the current study. Collaborating the results of the present and previous studies, it is evident that both temperature and concentration all play a role in determining the depth of sodium hypochlorite penetration into dentinal tubules, and the deepest penetration had been obtained when these factors were present simultaneously, suggesting a synergistic effect, thus, affecting the microhardness of root dentin at 300 µm in intracanal-heated sodium hypochlorite groups. This action is most desirable in single-visit endodontic retreatment procedures that help to achieve enhanced disinfection with better dentinal tubular penetration.

Sodium hypochlorite is a potent organic tissue solvent and does not affect mineral salts. Collagen fibers are polymerized polypeptides,[29] encapsulated within hydroxyapatite crystals. Thus, if the collagen fibers are protected by hydroxyapatite, they are not directly affected by the proteolytic agent. But the penetration of the low molecular size hypochlorite anion (Mw = 52.5) may still contribute to collagen degradation. Therefore, the effect of NaOCl on mineralized collagen fibers is time and concentration-dependent.[30] Thus, 5% sodium hypochlorite shows more damage to the radicular dentin compared with the low-concentrated solutions.[18] The use of NaOCl alone has demonstrated surface areas with high concentrations of the organic matrix of dentin, and such deproteination is a nonuniform slow process, creating vertical channels, termed deproteinization channels.[31] It is well-known that the hydroxyapatite mineral component in hard connective tissues contributes to elastic modulus and strength, whereas the collagen fibers are responsible for the toughness of the dental tissues.[32] Destruction of the collagen matrix in mineralized tissues makes the tooth more susceptible to crack propagation. The difference in concentrations and temperatures did not change the carbonate:phosphate ratios on dentin surfaces, indicating that sodium hypochlorite did not alter the inorganic components of dentin. The carbonate peaks remained almost unchanged in the present study. Thus, the results of amide/phosphate ratios were only interpreted. A constant, although not a statistically significant decrease in the amide/phosphate ratio, was noted with an increase in the concentration of sodium hypochlorite. An increase in temperature showed additive action along with higher concentration sodium hypochlorite by causing deproteination of radicular dentin but not very significant.

Collagen shrinkage due to heating is reversed on rehydration. The phenomenon of thermal stabilization of collagen molecules is explained as a "polymer in a box mechanism".[33] Intertubular dentin is more prone to degradation by heating to 140°C, but peritubular dentin remains unaffected. The presence of interpeptide hydrogen bonds stabilizes collagen fibers against thermal challenges, but in the presence of water, these interpeptide hydrogen bonds get depleted, and spaces between the collagen fibers become maximum affecting the stability of collagen peptides. In a dry state upon heating, the absence of solvent to transmit the thermal energy to disrupt its architecture makes the collagen peptide bonds more stable.[34] The results of the effect of temperature alone remain uncertain as the specimen was tested immediately post intervention, and the rebound effect of collagen molecules and its restabilization with time remains unknown.

There are concerns regarding the effect of heat on the surrounding dentoalveolar tissues. Heat transfer may occur during rinsing of the root canal system with heated solutions through the dentin and jeopardize adjacent supporting tissues. Eriksson and Albrektsson found that the application of 47°C for 1 minute resulted in the resorption of bone without subsequent regeneration. The results of these studies have suggested that the critical temperature for the bone to be susceptible to irreversible injury can be as low as 47°C (10°C rise from normal body temperature). Moreover, the damage to the vital tissues is also dependent on the duration for which the vital tissues are subjected to high temperatures.[35] On monitoring temperature evolution during the experimental procedures, it was observed that the temperature reaches equilibrium within 60 seconds. An intact vascularity in the surrounding area and the thermal conductivity of the periodontal membrane and the alveolar bone may also help to dissipate the heat rise on the root surface. Also, the degree of protection offered by dentin depends on its thickness, which plays an important role in heat transfer to the external root surface.

Thus, attempts were made in the present study to collaborate the following parameters to study surface changes in radicular dentin and observed that the effect of heat accelerates the potential of low-concentration sodium hypochlorite, enhancing its tubular penetration without causing destructive damage to the surface characteristics and physical properties of radicular dentin compared with higher concentration solutions.

The limitations of this study are that the experiments were performed in extracted human mandibular premolars. Thus, the results may not be directly applicable to clinical situations. However, the cytotoxic levels of heated sodium hypochlorite and their effect on periradicular tissues remain unknown.

Microhardness and FTIR spectroscopic analysis were carried out immediately post treatment in the present study, future studies should be conducted to analyze the changes in physical and chemical properties of root dentin after a predetermined waiting period following the treatment with an increase in temperature of sodium hypochlorite irrigant. The effect of saliva, supporting dentoalveolar tissues, the thermal conductivity of dentin, and other buffers might slightly alter the results. Thus, future clinical trials with long-term follow-ups have to be conducted to derive at conclusive evidence.

## CONCLUSION

Within the limitations of the present study, it can be concluded that intracanal heating of low-concentration sodium hypochlorite irrigant could be used as a potential alternative technique to enhance root canal disinfection and achieve better dentin tubular penetration without causing an undesirable effect on dentoalveolar tissues, unlike higher concentration analogs.

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