



Comparison of the Effect of Two Sodium Hypochlorite Concentrations on Micro-hardness of Butterfly-shaped Sclerotic Dentin in Single-rooted Premolar Teeth

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ABSTRACT

Background and aim: Given the significance of the butterfly effect in premolars undergoing root canal treatment and their increased risk of vertical root fracture, it is important to investigate the impact of irrigation with different concentrations of sodium hypochlorite on premolar teeth with the butterfly effect.

Material and methods: This experimental study evaluated 30 single-rooted premolar teeth with butterfly-shaped sclerotic dentin. The teeth with circular root sections were selected to ensure an equal mesiodistal-to-buccolingual width ratio. The roots were sectioned into coronal, middle, and apical thirds. Microhardness measurements were conducted on the buccal and mesial sides at four points within the middle third of each root. After specimen preparation, the samples were allocated into three groups: 0.9% normal saline (control), 2.5%, and 5% concentration of sodium hypochlorite (intervention). Microhardness was assessed before and after the intervention; data were recorded in the checklist, and statistical analysis was performed at the 0.05 significance level using SPSS 26.

Results: The average microhardness of dentin with the butterfly effect in all groups, including 2.5% and 5% sodium hypochlorite and physiological saline, was less than before treatment. Sodium hypochlorite at the two investigated concentrations had a significantly greater effect than physiological saline ($p < 0.05$), but there was no statistically significant difference between the two concentrations ($p > 0.05$).

Conclusions: Sodium hypochlorite at both 2.5% and 5.25% concentrations reduces the microhardness of dentin with a butterfly effect. A 2.5% concentration is recommended to minimize potential side effects.

1. Introduction

Physiological sclerotic dentin is characterized by mineral deposits accumulating in the dentinal tubules, starting 3 to 4 years post-eruption, initially at the apical root, then advancing coronally and toward the pulp. This condition is more prevalent in mesial and distal root regions, resulting in a butterfly shape in horizontal sections and a jagged interface with natural dentin in vertical views. Electron microscopy reveals the absence of odontoblasts and predentin where sclerotic dentin is present, indicating that odontoblasts, though involved in peritubular dentin formation, do not contribute to sclerotic dentin development.^[1-5] The butterfly effect was first documented through photographs by Beust in 1931, revealing a distinctive shape in root sections associated with variations in tubular sclerosis of dentin. Sclerosed dentin, being more transparent than normal, leads to altered light transmission due to changes in dentinal tubule density. Root sections exhibit a significantly higher density of dentinal tubules compared to buccolingual sections, a pattern consistent across different age groups. This difference in tubule density may have important clinical implications, as teeth displaying the butterfly effect were found to be less stiff buccolingually, potentially impacting fracture patterns.^[6,7] Research by Von Arx et al. identified “opaque

dentin” prevalent in posterior teeth.^[8] At the same time, Rama et al. established a uniform occurrence of the butterfly effect in incisors, canines, and premolars without significant variations among these types, suggesting the phenomenon's clinical relevance is primarily observed in posterior teeth.^[9] Sodium hypochlorite has been the primary irrigant in root canal therapy for over 70 years. Known for its broad antimicrobial efficacy, a 2.5% solution can eliminate *Enterococcus faecalis* in 15 minutes, while a 1% solution kills *Candida albicans* in under an hour. Its benefits include the dissolution of organic matter, potent antimicrobial actions, lubrication, and cost-effectiveness. However, it has drawbacks, including toxicity, potential tissue destruction, an unpleasant taste, and limitations in completely removing microbes and the smear layer.^[10-12] The ideal concentration for use remains debated, but higher concentrations enhance its antibacterial power.^[11-13] Sodium hypochlorite acts through the formation of Na^+ and OCl^- ions, with hypochlorous acid being the key agent for antibacterial activity at acidic and neutral pH levels. While it effectively dissolves organic compounds in dentin and pulp residues, it requires complementary agents, such as EDTA or citric acid, for complete removal of the smear layer. Sodium hypochlorite is commercially available in various concentrations and buffering options, with

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studies indicating that buffering does not significantly alter its properties.^[12, 14-20] This study aimed to compare the effects of two commonly used concentrations of sodium hypochlorite (2.5% and 5.25%) on the microhardness of butterfly sclerotic dentin in single-rooted premolar teeth. The findings of this study will provide valuable insights into the concentration-dependent effects of NaOCl on this specific type of dentin, enabling clinicians to make informed decisions regarding irrigant selection and optimize endodontic treatment outcomes.

2. Material and Methods

Before starting the research, the researcher registered the title of the research at the university and obtained the necessary permission. The principle of trustworthiness in presenting research sources was observed. The ethical code for this thesis was obtained from the Ethics Committee of Islamic Azad University, Khorasgan Branch, with the ethics code IR.IAU.KHUISF.REC.1402.231. The ethical considerations and methodological rigor align with the ethical standards in clinical research.^[11, 13] This study investigated 30 maxillary premolars with single roots, which had prismatic enamel solely on the mesial surface. Teeth were inspected under 20x magnification by a blinded examiner, and teeth with developmental defects, decay, root resorption, cracks, or fractures were excluded. Teeth extracted for orthodontic purposes were chosen to minimize age-related dentinal variations, and efforts were made to match the extraction time of the teeth with the examination time.^[21, 22] Samples were preserved in a 1% thymol solution (Merck, Germany) and standardized by length (22 mm \pm 1 mm total, 14 mm \pm 1 mm root).^[11] Only teeth with circular root sections were selected to ensure an equal mesiodistal-to-buccolingual width ratio; two periapical and proximal radiographs were taken to assess the number of canals; teeth with more than one canal were excluded from the study.^[21] Subsequently, the crown of all teeth was separated from the root at the CEJ area using a low-speed diamond disc. All teeth were then immersed in a 2.5% sodium hypochlorite solution (NaOCl) (Sarke Borsod, Poland) for 2 hours, and the remaining tissue residues around the root were removed. Teeth were embedded in self-curing acrylic resin (Quick Resin, Shofu, Japan) and sectioned into apical, middle, and coronal thirds. Two 2-mm-thick cuts were made at the middle third's boundaries, with a 1-mm section retained for analysis (Figs.1 and 2).



Fig. 1. Sample teeth mounted in self-curing acrylic resin.

Microhardness was measured before and after immersion. In each stage, 4 points in the buccal and mesial areas were tested at a distance of 500 μ m

from the lumen, and the average numbers were recorded as microhardness. Samples were divided into three groups according to the desired sodium hypochlorite concentration:

1. Control: 0.9% normal saline
2. Experimental 1: 2.5% NaOCl, followed by 0.9% saline
3. Experimental 2: 5.25% NaOCl, followed by 0.9% saline

Microhardness was assessed before and after 15-minute immersion, with fresh solution added every 5 minutes. The container containing the samples was intermittently shaken to ensure they remained in contact with the effective substance in the solution. Second microhardness measurements were made at 1 mm intervals from the initial points. Vickers Hardness Number (VHN) was calculated under a 3-kg force for 15 seconds. Data were recorded and analyzed using SPSS 26 at a 5% significance level, employing Kolmogorov-Smirnov normality tests and covariance analysis.^[11, 24]

3. Results

The mean microhardness values of pre-test groups are 85.93, 93.67, and 85.64 for 2.5% sodium hypochlorite, 5.25% sodium hypochlorite, and 0.9% normal saline, respectively. The mean microhardness values of the post-test groups are 71.76, 76.94, and 84.30 for 2.5% sodium hypochlorite, 5.25% sodium hypochlorite, and 0.9% normal saline, respectively (Table 1 & Chart 1).

Table 1. Mean microhardness of single-rooted premolars with the butterfly effect.

Group	Pre-test		Post-test	
	Mean	Standard deviation	Mean	Standard deviation
2.5% Sodium Hypochlorite	85.93	21.9	71.76	19.17
5.25% Sodium Hypochlorite	93.67	14.42	76.94	11.89
0.9% Normal Saline	85.64	6.39	84.30	8.18
Total	88.41	15.5	77.66	14.36

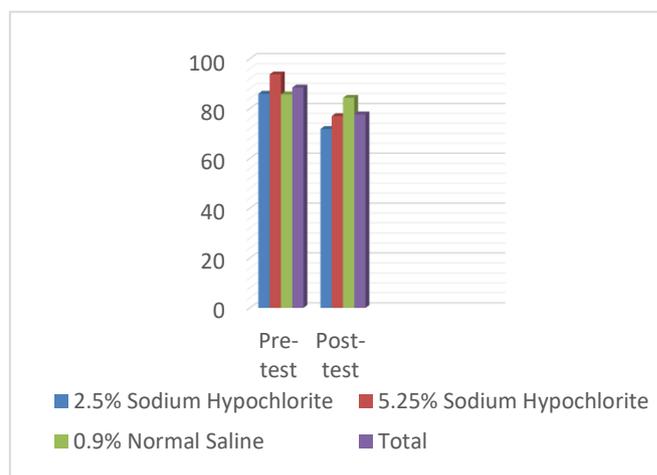


Chart 1. Mean microhardness of single-rooted premolars with the butterfly effect.

In Table 2, the F-value for the comparison of the three groups is 3.476, and the probability of its significance is 0.046, which is less than 0.05. Therefore, with a confidence level of 0.95, the null hypothesis, which suggests that there is no significant difference between the three groups (5.25% sodium hypochlorite, 2.5% sodium hypochlorite, and saline), is rejected. The LSD test was used to compare the pairs of groups, which is explained below. The LSD post hoc test results table shows a significant difference between the 5.25% sodium hypochlorite mean and the saline solution. Additionally, there is a notable difference between 2.5% sodium hypochlorite and a saline

solution. Therefore, the reduction in mean microhardness of the 2.5% sodium hypochlorite group compared to the saline solution group is statistically significant, and the reduction in the two groups, 2.5% sodium hypochlorite and 5.25% sodium hypochlorite, is significantly greater than that in the saline solution group. This reduction in microhardness is consistent with previous findings on the effects of sodium hypochlorite.^[11, 24, 25] However, there is no significant difference between the 2.5% sodium hypochlorite group and the 5.25% sodium hypochlorite group. (Table 3)

Table 2. Analysis of covariance test of premolars with the butterfly effect.

	Sum of Squares	Degree of Freedom	Average of Squares	F-value	Significance Value
Corrected model	2391.695	3	797.232	5.782	0.004
y-intercept	943.653	1	943.653	6.844	0.015
Pre-test	1597.989	1	1597.989	11.589	0.002
Groups	958.368	2	479.184	3.475	0.046
Error	3585.048	26	137.886	----	----
Total	186927.65	30	----	----	----
Corrected Total	5976.743	29	----	----	----

Table 3. LSD table for comparing pairs of mean values of dentin microhardness.

i Group	j Group	Difference in Mean Values (i-j)	Standard Deviation	Significance Value
	2.5% Sodium Hypochlorite	-1.354	5.37	0.803
5.25% Sodium Hypochlorite	0.9% Normal Saline	-12.68	5.252	0.023
2.5% Sodium Hypochlorite	0.9% Normal Saline	-11.326	5.379	0.045

4. Discussion

This study examined the impact of 2.5% and 5.25% sodium hypochlorite (NaOCl) on the microhardness of butterfly-shaped sclerotic dentin. The results demonstrated that both 2.5% and 5.25% NaOCl solutions significantly reduced dentin microhardness, with no statistically significant difference between them. This suggests that 2.5% NaOCl may be as effective as 5.25% while minimizing potential adverse cytotoxic effects.^[11, 24, 25] The observed decrease in microhardness can be attributed to the strong oxidizing properties of NaOCl, which dissolve organic components such as collagen and alter the mineral content, thereby weakening the dentin.

Limitations and Future Directions

Several limitations should be considered when interpreting the results of this study. First, the study was conducted on single-rooted premolar teeth, which limits the generalizability of the findings to multi-rooted teeth with complex canal anatomies. Secondly, the use of microhardness as the sole outcome measure may not fully capture the complex changes that occur in dentin structure and composition after NaOCl treatment. Future studies could employ additional techniques, such as scanning electron microscopy, to provide a more comprehensive understanding of the effects of NaOCl on dentin. The use of microhardness as the primary outcome measure also presents limitations. While Ceres et al. discuss microhardness mapping and

its relationship to yield strength, it is important to remember that microhardness is just one aspect of dentin integrity.^[26] Other factors, such as changes in dentin structure, composition, and permeability, may not be fully captured by microhardness measurements. A more comprehensive assessment of dentin properties would strengthen the study's conclusions. Based on Pascon's study, there is strong evidence showing that sodium hypochlorite adversely alters the mechanical properties of root dentin when used as an endodontic irrigant,^[25] which is consistent with the results of this study. Finally, the *in vitro* nature of the study may not fully replicate the complex conditions of the oral environment. *In vivo* studies are needed to confirm the findings and assess the clinical relevance of the observed effects of NaOCl on dentin. Future research could also explore the long-term effects of different NaOCl concentrations on dentin microhardness and other mechanical properties. Additionally, investigating the optimal irrigation protocols that balance disinfection efficacy and dentin preservation would be beneficial for clinical practice.^[27]

These findings have significant clinical implications for endodontic treatment. The choice of irrigant concentration should be carefully considered to strike a balance between effective disinfection and minimal damage to the tooth structure. While higher concentrations of NaOCl may offer enhanced antimicrobial activity, they may also increase the risk of tissue damage and dentin weakening. Our results suggest that a lower concentration, such as

2.5% NaOCl, may provide adequate disinfection while reducing the risk of structural damage.^[11, 13]

5. Conclusion

This study provides evidence that a lower NaOCl concentration may be sufficient to achieve effective disinfection while minimizing the risk of dentin damage in butterfly-shaped sclerotic dentin. However, further research is necessary to confirm these findings and to explore the long-term implications of NaOCl use on dentin health. Considering the limitations of this study and the findings of previous research, clinicians can make informed decisions regarding irrigant selection to enhance endodontic outcomes.

Conflict of Interest

The authors declared that there is no conflict of interest.

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