

Assessment of Sodium Thiosulfate Neutralizing Effect on Micro-hardness of Dentin Treated With Sodium Hypochlorite

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Research article

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Abstract

Background

The aim of this study is to evaluate the ability of sodium thiosulfate (STS) to reverse the adverse effect of sodium hypochlorite (NaOCl) on dentin microhardness.

Methods

Fifty single-rooted teeth were decoronated and longitudinally sectioned. The samples were divided into a control and four sample groups (n = 20): Normal saline for 15 min as the control group, G1: 2.5% NaOCl for 15 min without an incubation period, G2: 2.5% NaOCl for 15 min, G3: 2.5% NaOCl for 15 min irrigated with normal saline followed by 5% Na₂S₂O₃ for 10 min, G4: Normal saline for 15 min followed by 5% Na₂S₂O₃ for 10 min. All groups except G1 were incubated for 1 week. The microhardness measurements were determined using the Vickers micro-hardness test. Data were analyzed using the Kruskal-Wallis test for pairwise comparisons. A p-value < 0.05 was considered significant.

Results

All groups showed a significant decrease in the micro-hardness value compared with the control group. NaOCl for 1 week (G2) reduced the micro-hardness of dentine compared with samples, tested immediately after immersion in NaOCl (G1) (p < 0.05). NaOCl alone (G2) or treated with Na₂S₂O₃ (G3) resulted in a significant decrease in microhardness compared with the Na₂S₂O₃ group (G4) (p < 0.05).

Conclusions

Sodium thiosulfate as a neutralizing agent could not prevent the microhardness downturn caused by sodium hypochlorite

Background

Irrigants and intracanal medicaments such as calcium hydroxide may affect the physical and mechanical characteristics of dentin and result in reduced flexural strength, micro-hardness, and modulus of elasticity(1–5). Sodium hypochlorite (NaOCl) is undeniably the most widely accepted irrigant in endodontics and will probably remain the primary choice in the future because of its efficacy against microorganisms and its ability to dissolve tissue (6, 7). Beside its superiority to other irrigation solutions, the dissolving capability of this irrigant leads to the depletion of dentinal tissue components, particularly the organic parts (8). The proteolytic effect of different NaOCl solutions used for an average contact time of 26 min causes 30% root weakening (9). A recent study showed that the increase in volume and or time of contact of 5.25% alkalized-NaOCl reduces the fracture strength of bovine teeth (10). Zhang et al.

suggested that the effect of NaOCl on mineralized dentin is both concentrations- and time-dependent (11, 12). Hu et al. reported that different time exposures with the same concentration of NaOCl, do not influence deproteinization (13).

Structural changes caused by NaOCl as a root canal irrigant could have an adverse effect on resin-dentin bond strength for tooth reconstruction. Previous studies have reported an adverse effect of this irrigant on bond strength (14–20). Studies have shown that the adverse effect of NaOCl is due to an oxygen-rich layer forming along the dentin surface followed by the breakdown of NaOCl into chlorine and oxygen. The remnants of the oxidative by-products interfere with the polymerization of adhesive cement and resin-based sealers (16–18). Moghaddas et al. showed that the oxidizing effect of NaOCl could remain even two weeks after its application on dentin (21). It has been suggested that applying an antioxidant solution such as STS or sodium ascorbate or long delay should be considered before the adhesive procedure to reverse this compromised bond strength (18, 22–24).

STS 5% is an antioxidant agent that is recommended to neutralize the effect of NaOCl on dentin and improve the resin bonding properties(25). It also reacts with oxidants, which were produced by NaOCl to reduce unpaired electrons to form a stable product (26).

The effect of this neutralizing agent on the physical properties of dentin is unknown. Thus, this *in-vitro* study evaluated the effect of STS on the micro-hardness of dentin treated with and without NaOCl at different time intervals. This study hypothesizes that STS will reduce the effect of NaOCl on dentin micro-hardness by neutralizing its remnants on the dentin surface after 1 week.

Methods:

Preparation of tooth specimens

Fifty straight single-rooted teeth with relatively similar dimensions and morphology and closed apices were extracted for orthodontic or periodontal reasons collected with the patients' informed consent. This study design was approved by the Ethics in Human Research Committee of Shiraz University of Medical Sciences (Ethics ID no. IR.SUMS.DENTAL.REC. 1398.138). Proximal view radiographs were taken to confirm the presence of a single patent canal. Teeth with root caries, cracks, curved canals, endodontic treatment, internal resorption, or calcification were excluded. Teeth were thoroughly cleaned of any soft tissue or calculus deposits and stored in isotonic saline solution at room temperature until the time of use. The crowns of all specimens were cut transversally at the coronal level of the roots with a double-faced diamond disc (Microdont, LDA, Brazil) at low speed with water coolant to ensure a uniform sample length of 14 mm (\pm 1 mm root length).

Specimen Preparation For The Micro-hardness Evaluation

Specimens were longitudinally sectioned in the buccolingual direction using a double-faced diamond disk at low speed, without passing through the canal space. A mallet and chisel were used to split the root. The root segments were horizontally embedded in auto polymerizing acrylic resin (Acrostone, Dent Product, Egypt), leaving their dentin surface exposed. The dentin surface of the mounted specimens was ground flat and smooth with a series of ascending grades of carbide abrasive papers (500, 800, 1,000, and 1,200 grit) (Bigo, Dent Product, Germany) under distilled water to remove any surface scratches and finally polished with a 0.1-Mm alumina suspension on a rotary felt disc (Microdont, LDA, Brazil) to obtain a smooth glassy mirror-like surface.

The samples were divided randomly into one control and four experimental groups based on the immersion solution and incubation time:

Control group: Normal saline for 15 min

G1: 2.5% NaOCl (Chloraxid, Cerkamed, Poland) for 15 min without an incubation period

G2: 2.5% NaOCl for 15 min

G3: 2.5% NaOCl for 15 min irrigated with normal saline followed by 5% STS (Merck, Darmstadt, Germany) for 10 min

G4: Normal saline for 15 min followed by 5% STS for 10 min

All groups except group 1 were incubated for 1 week in an incubator (37 °C with 100% humidity) before the micro-hardness test. The group 1 samples were tested immediately after immersion in NaOCl.

Dentin Micro-hardness Measurements

The microhardness measurements were taken either on the buccal or lingual side of each root. The sectioned root was divided equally into three-thirds representing the coronal, middle and apical thirds, and each area was tested separately. An indentation was made in the dentin surface approximately 200 µm from the canal-dentin interface for standardization. The Vickers hardness value was obtained by dividing the test force by the area of the sloping faces of the indentation. The resulting impression of the two diagonals was observed with an optical microscope and the average length of the two diagonals was measured with the built-in scaled micrometer and converted into the Vickers hardness number (VHN) with the following equation:

$$\text{VHN (HV)} = 1,854(F/D^2).$$

The constant value of the equation was calculated from the specific geometry of the indenter, F is the applied load in grams and D is the diagonal of the indentation in µm (27).

Specimen Preparation For Sem Evaluation:

One specimen of each group was dehydrated, mounted and gold-sputtered, for evaluation under a scanning electron microscope (Nova NanoSEM 450, FEI, Eindhoven, Netherlands) operated at 20KV. Photographs were taken from 3 points of each sample at 1000 × magnifications (Fig. 1).

Data were analyzed using the Kruskal-Wallis test for pairwise comparisons. A p-value < 0.05 was considered significant, and all analyses were carried out using SPSS software (SPSS version 16, SPSS INC., Chicago, IL, USA).

Results

The micro-hardness medians (means ± standard deviations) are shown in Table 1. The micro-hardness values of all of the groups decreased significantly compared with the control group ($p < 0.05$). As shown in Fig. 2, normal saline (control group) showed the highest micro-hardness while 2.5% NaOCl showed the lowest micro-hardness after 1 week (G2). A significant decrease in the micro-hardness value was observed between the samples tested immediately after NaOCl application (G1) and the samples incubated for 1 week (G2). Using NaOCl with STS together (G3) resulted in significantly lower micro-hardness than the control group and the STS alone group (G4) ($p < 0.05$).

Table 1
Median (Means \pm Standard deviation) of microhardness in all experimental groups

Group	Median (mean \pm SD)
0.9% NaCl for 15 minutes (control group) + 1-week incubation period	a 59.44(59.40 \pm 6.81)
1) 2.5% NaOCL for 15 minutes + without incubation period	bc 38.87(39.44 \pm 4.37)
2) 2.5% NaOCL for 15 minutes + 1-week incubation period	d 26.80(28.02 \pm 7.22)
3) 2.5% NaOCL for 15 minutes irrigated with NaCl followed by 5% TS for 10 min + 1-week incubation period	cd 33.12(31.41 \pm 4.80)
4) 0.9% NaCl for 15 minutes followed by 5% TS for 10 min + 1-week incubation period	b 41.83(40.45 \pm 6.70)
The same superscript letters in the column are not statistically significant ($p > 0.05$).	

SEM micrographs showed longitudinal dentinal tubes with the patches of some smear layers in the control group. In the other specimens, a unique pattern of smear layer, which is totally covered the dentin was obvious, although this layer seems thicker and more homogenous in the samples, which were in contact with sodium thiosulfate.

Discussion

The results of the present study show that both 2.5% NaOCl and STS decreased the micro-hardness of dentin compared with the control group. The remnants of NaOCl after 1 week significantly reduced the micro-hardness of dentin compared with the samples treated with NaOCl for 15 min.

Samples that were irrigated with NaOCl and then neutralized with STS, had significantly lower micro-hardness values than the samples irrigated with normal saline but were not different from the group immersed in NaOCl alone. Thus, STS not only reduced the effect of NaOCl on dentin micro-hardness but also may have had a synergistic effect on its weakening. Therefore, the hypothesis of this study was rejected.

Different studies have shown that using NaOCl as an irrigating solution significantly reduces the micro-hardness value (28–30). Most studies used short exposure times of 5–15 min and revealed a reduction in the dentin micro-hardness value compared with irrigating with normal saline (29, 30). Souza et al. reported that an increase in the volume and/or time of exposure to 5.25% NaOCl causes a significant reduction in root toughness. Even the increase in contact time without increasing the volume also negatively affects root toughness by about 37% (10).

Garcia et al. reported that 2.5% NaOCl, Chlor-XTRA, and 5.5% NaOCl gel all reduce the micro-hardness of dentin (31). Slutzky-Goldberg et al. discovered that a 5 min exposure to 2.5% and 6% NaOCl does not reduce dentin micro-hardness but exposure for more than 10 min causes a significant reduction in micro-hardness. They also showed that 6% of NaOCl causes a more significant decrease in micro-hardness than the 2.5% concentration (32). Thus, they suggested reducing the irrigation time to less than 10 min and using a lower NaOCl concentration.

Cochrane et al. exposed human dentine bars to 0.5% and 1% NaOCl gels and 1% and 4% NaOCl solutions for 7 days and then immediately subjected them to the Vickers micro-hardness test. Their results showed that the 0.5% NaOCl gel caused a significant decrease in the microhardness of the dentin bars (28). The results of the present study agree with these studies, as the micro-hardness value decreased in the presence of NaOCl.

The interesting finding from this study is that the samples that were immersed for 15 min in NaOCl and irrigated with normal saline showed a significant reduction in micro-hardness after the one-week incubation. This finding indicates that the remnants of NaOCl and its oxidative products may remain active during this period leading to additional decreases in dentin micro-hardness.

In contrast to our hypothesis, STS alone or with NaOCl significantly decreased the micro-hardness value compared with the control group. It seems that neutralizing the effect of NaOCl with STS did not improve the micro-hardness of the dentin bars. SEM micrographs also showed the same pattern of smear layers accumulation above the samples of NaOCl and STS (Fig. 1). This resemblance may explain the same effect of these materials on dentin microhardness.

A recent study on the effect of 5% STS for 10 min showed recovery of bond strength to dentin after treatment with NaOCl (25). To the best of our knowledge, no study has evaluated the micro-hardness of dentin in the presence of STS or assessed the reversing effect of STS on the micro-hardness of dentin.

STS is used for medical conditions, such as calciphylaxis secondary to chronic renal failure, because of its chelating effect on calcium salts (33). STS prevents calcification by chelating calcium and its acidosis-inducing properties (34). STS also significantly reduces calcium-containing crystal formation in cultured murine chondrocytes. Administering STS decreases the volume and crystalline content of the new calcific deposits formed in the joint (35).

According to these medical studies, it seems that the chelating action of STS on dentin bars decreases micro-hardness when used alone or even for neutralizing the effect of NaOCl.

Our results suggest that STS is not a suitable material for neutralizing NaOCl oxidant agents and may weaken dentin hardness. More studies are needed to confirm this rational or to test other materials, such as sodium ascorbate, for this purpose.

Conclusion

The reduction in dentine micro-hardness of all groups continued for 1 week even after irrigation the tested materials with normal saline. Neutralizing the remnants of sodium hypochlorite with sodium thiosulfate did not prevent this adverse effect.

Abbreviations

Sodium thiosulfate (STS), Sodium hypochlorite (NaOCl), Nano fast Cement (NFC), Scanning Electron Microscope (SEM)

Declaration

No potential competing interests

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Figures

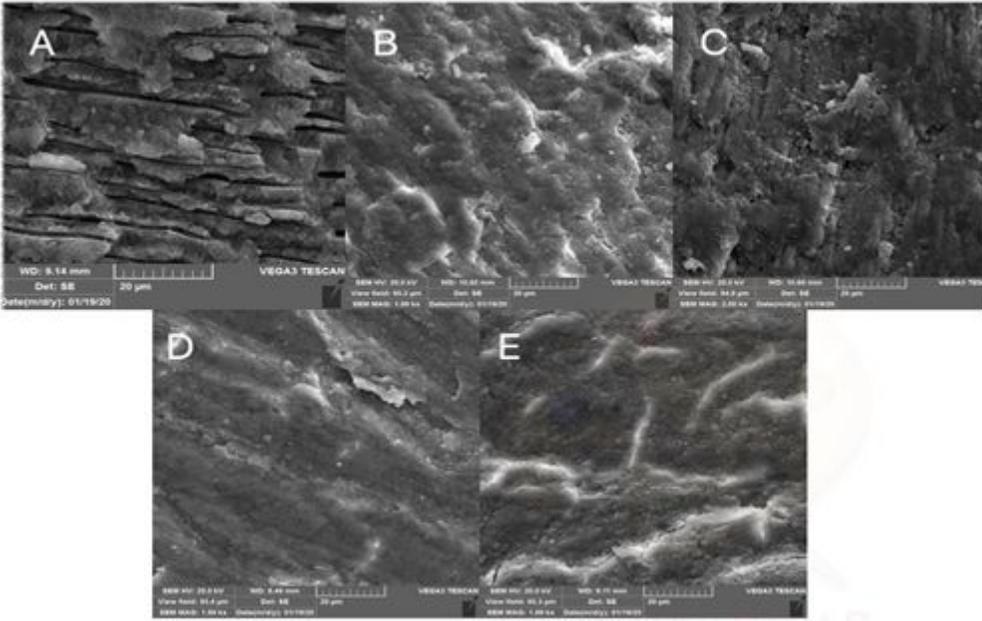


Figure 1

SEM microphotographs of the control sample at 1000× magnification. (A) Shows patent longitudinal dentinal tubule with patches of smear layers. Samples of NaOCl without incubation period and after one-week incubation (B and C) show dentin covered by smear layers with a few open dentinal tubes. The combination use of sodium thiosulfate and NaOCl or sodium thiosulfate alone (D and E) illustrated thicker and homogenous smear layers upon the dentin surface that covered all the dentinal tubules.

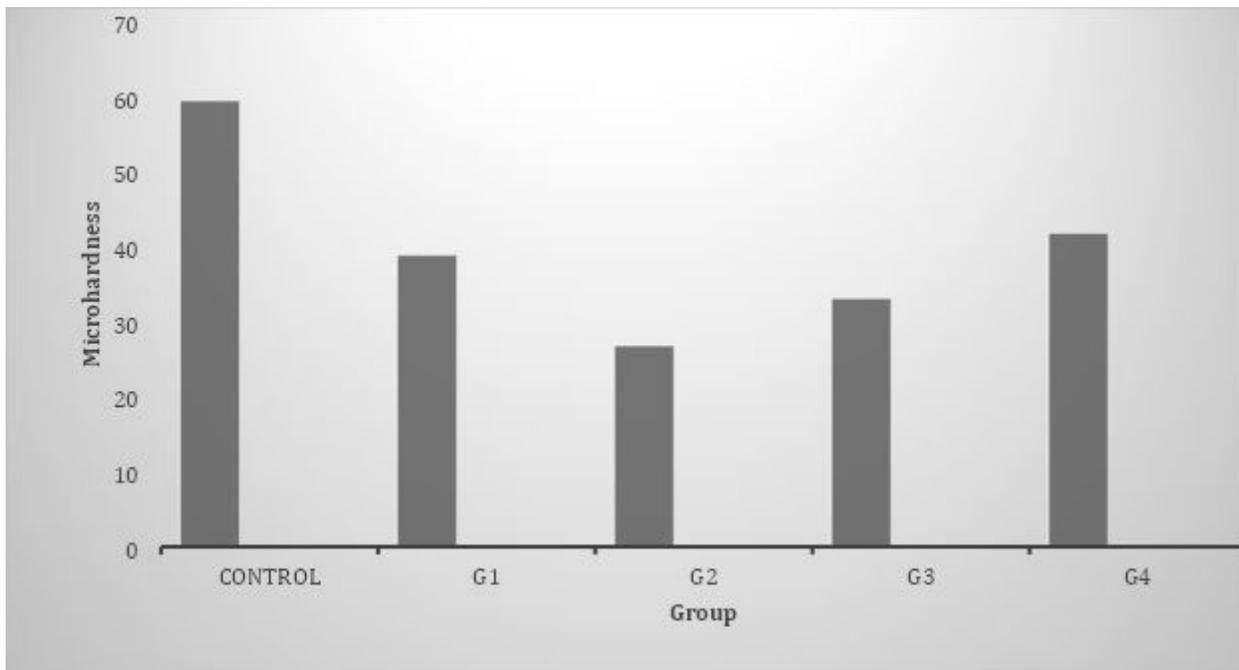


Figure 2

Microhardness of all experimental groups