The In-Vitro Effect of Two Different Concentrations of Sodium Hypochlorite on Dentine Hardness

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ABSTRACT

Objective: To investigate the effect of two different concentrations of irrigant solutions of sodium hypochlorite (NaOCl) 2.25% and 5.25%, on dentine hardness.

Methods: Forty-eight extracted anterior and posterior teeth were decoronated and the roots were endodontically cleaned and shaped. Each root was sectioned into cervical, middle and apical sections of 3mm thickness and mounted in an acrylic resin disc-shape mould. After polishing the coronal surface of each section, microhardness was measured using a Wallace microhardness indentation tester at four locations 1mm away from the root canal lumen. Specimens were allocated into two groups and then irrigated with either 5.25% or 2.25% sodium hypochlorite for one hour and microhardness was measured again.

Results: A statistically significant decrease (p<0.05) in dentine hardness in the root dentine as a whole and in every root section after application of the two different concentrations of sodium hypochlorite was observed. There was no statistically significant (p>0.05) difference in the decrease of dentine hardness between the 5.25% and the 2.25% concentrations for the root as whole and in every root section.

Conclusion: Irrigation with 2.25% or 5.25% sodium hypochlorite significantly decreased root dentine microhardness.

Key words: Dentine, Hardness, Sodium hypochlorite, Wallace Hardness Tester

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dentine is the first step towards predicting the behaviour of the dentine/restoration interface\(^{(15)}\) and for evaluation of dental materials.\(^{(14)}\) Hardness is an indication of the resistance a material offers to scratching, indentation, elastic impact, cutting and permanent deformation.\(^{(15)}\) Hardness testing involves application of a predetermined load after which any residual impression left on the surface of the material is measured.\(^{(16)}\) Measurements of hardness are frequently used to obtain an indication of differences in mechanical properties, such as strength and abrasion resistance between materials of similar structure and composition\(^{(17)}\) and can be related to other dentine mechanical properties such as Young’s modulus and yield stress.\(^{(18-20)}\)

The universal use of sodium hypochlorite as a root canal irrigating solution is due mainly to its efficacy in pulpal dissolution properties.\(^{(21-24)}\) Sodium hypochlorite is an extremely effective solvent of necrotic tissue. It was found to dissolve seven times as much organic tissue as citric acid, chelating agents, oxidizing agents, normal saline and distilled water.\(^{(21)}\)

The effect of different irrigating solutions on root dentine hardness was studied by many investigators.\(^{(18, 25-27)}\) Saleh and Ettman\(^{(18)}\) found that root canal irrigation with various solutions led to structural changes represented by a reduction in dentine hardness. Also the microhardness of root canal dentine significantly decreased after instrumentation with stainless steel or rotary NiTi files using 2.5% sodium hypochlorite.\(^{(25)}\) Sodium hypochlorite at 2.5% as well as 6% resulted in significant decrease in microhardness after 10 and 20 minutes\(^{(26)}\) and 1% sodium hypochlorite significantly reduced the microhardness of root dentine at 0.5mm or 1mm from root canal lumen.\(^{(27)}\) Although these studies investigated the effect of irrigants on microhardness of root dentine, they did not produce clear conclusions related to the effect of two different concentrations of sodium hypochlorite (NaOCl) 2.25% and 5.25%, on the microhardness root dentine in a clinically relevant time.

**Methods**

Forty-eight maxillary and mandibular, single and multirooted recently extracted human teeth were selected, and stored in sterile water. Teeth were examined with the assistance of x4 magnifying loupes (Keeler Ltd., Berks, UK) and those with root cracks, caries, defects, restorations, previous root canal treatment or of root length less than 10mm were excluded. In multirooted teeth the palatal roots of the maxillary molars and the distal roots of the mandibular molars were chosen. The crowns of teeth used were removed at the cementoenamel junction using a beaver carbide bur® (Alston, Dental Rotary Instruments, UK) in a high speed hand-piece. A size 10 or 15 k-file was introduced into the canal until it exited the root at the apex. The working length was determined by measuring the length of the file from point of entry into the canal to the point of exit from the canal and subtracting 1mm from that length. Conventional root canal treatment was undertaken, pulp extirpation, cleaning and shaping of the canal using k-files, size 10 or 15 and enlarging to size 50 k-file and the remainder of the canal was shaped by stepping back at 1mm increments to size 70. Coronal flaring was achieved by using Gates Glidden burs sizes two, three and four used sequentially. The canal was irrigated with one ml of sterile water after each file used.

Following cleaning and shaping of the roots, each root was placed in the brass holder of the diamond wheel saw and transversely cut into three discs, each of 3mm in thickness. Each root section had a coronal surface and an apical surface. After sectioning the roots, the apical surface of the sections was covered with adhesive tape to seal the canal entrance and the sections were then placed upside down (i.e. on the coronal surface) on a sticky sheet in the polypropylene ring frame. Then self cure acrylic was mixed to a thin consistency and poured into the polypropylene ring frame until the acrylic totally covered the root sections but without entering into the canal lumen due to the presence of adhesive tape. After the self cure acrylic had set, the acrylic discs in which the root segments were embedded, were removed from the polypropylene ring frame and excess acrylic trimmed away. The coronal surface of each embedded section was smoothed using a series of increasingly fine wet silicon-carbide papers (Buckfast Tools Ltd. Manchester, UK). Each disc was given a number for identification purposes and the samples were ready to be tested. Hardness testing was accomplished by using a Wallace Hardness Tester® (Fig. 1). The test surfaces of the root sample embedded in the acrylic discs were oriented perpendicular to the indenter axis of the tester and each root segment had a series of four indentations made around the pulp space, i.e. collectively each root had 12 readings, four each for the coronal, middle and apical segments. Markings were made in the acrylic to show the site of the indentations.
The root section was divided into four quarters, and each quarter had one indentation one millimetre away from the pulp lumen (Fig. 2). The mean for each segment was calculated. Sodium hypochlorite (NaOCl) was prepared by diluting 12% Sodium Hypochlorite Solution (BDH, VWR International Ltd. UK.) to concentrations of (5.25% and 2.25%) using sterile water. After measuring the hardness, the acrylic discs in which the root segments were embedded were randomly allocated into two groups, (Group One and Two) of 24 roots each. The dentine surfaces of the coronal cut stump of each root section were masked with a non-porous, water-resistant adhesive tape before irrigation. In Group One, the canal portion of the root segments was irrigated with 5.25% sodium hypochlorite (NaOCl), using an irrigating syringe with a 27 gauge needle to fill the root canal lumen. After one hour, the irrigant was washed away with sterile water and the samples dried. Hardness was measured again in the same manner as discussed above at four different sites than those measured before irrigation (Fig. 2). Group Two was treated in the same way but a 2.25% NaOCl was used instead. Throughout the experiment and to avoid excessive drying of the dentine, care was taken to keep the specimens hydrated; they were placed into a plastic container in plastic bags containing sterile water.

### Results

The hardness data obtained were analyzed using SPSS software (11.5). For each root section four hardness measurements were taken before the introduction of the irrigant, and four hardness measurements after the introduction of the irrigant, leading to an overall 1,152 hardness readings.

### Measuring the Hardness of the Whole Root

For measuring the hardness of the whole root twelve hardness readings were added together, divided by 12 to calculate their mean. This was carried out for the data obtained before and after the...

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**Table 1:** A summarized table showing means and standard deviations of VHN for the whole root and for different root sections before and after introduction of the irrigants.

<table>
<thead>
<tr>
<th></th>
<th>Before Mean (SD)</th>
<th>After Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole root</td>
<td>39.01 (8.50)</td>
<td>30.45 (6.76)</td>
</tr>
<tr>
<td>Coronal</td>
<td>36.70 (10.60)</td>
<td>28.50 (9.78)</td>
</tr>
<tr>
<td>Middle</td>
<td>43.80 (10.50)</td>
<td>34.70 (10.00)</td>
</tr>
<tr>
<td>Apical</td>
<td>41.70 (12.70)</td>
<td>33.10 (9.40)</td>
</tr>
<tr>
<td>2.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole root</td>
<td>40.43 (6.50)</td>
<td>33.17 (6.78)</td>
</tr>
<tr>
<td>Coronal</td>
<td>41.42 (10.70)</td>
<td>31.81 (10.45)</td>
</tr>
<tr>
<td>Middle</td>
<td>44.60 (8.50)</td>
<td>38.20 (8.00)</td>
</tr>
<tr>
<td>Apical</td>
<td>39.20 (8.30)</td>
<td>33.50 (6.60)</td>
</tr>
</tbody>
</table>

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**Fig 1:** Wallace hardness instrument

**Fig 2:** Root sections embedded in the acrylic disc with marking to identify locations for hardness measurement, sample no.48.
Fig 3: Hardness (VHN) for the coronal, middle and apical sections of the teeth at 5.25 % and 2.25 % of NaOCl

introduction of the different concentrations of sodium hypochlorite (Table I). Independent t-test, used to compare means for the two samples either before or after the introduction of the irrigants, showed no statistically significant difference between them. Therefore at the baseline or after irrigation the teeth in both samples had no statistical difference in dentine hardness (p > 0.05). The paired t-test, done to compare means of either 5.25% or 2.25% samples before and after the introduction of 5.25% sodium hypochlorite, showed statistical significant difference (p < 0.05).

Hardness of Different Root Sections
To measure the hardness of the each section, the four hardness readings were added together, divided by four and the mean was calculated. This was carried out for the data obtained before and after the introduction of the two different concentrations of sodium hypochlorite (Fig. 3).

For all root sections; coronal, middle and apical when the independent t-test was used to compare means for the two samples before irrigation (baseline measurement) there was no statistically significant difference. Also no statistically significant difference was found when independent t-test was used to compare means for the two samples after irrigation P>0.05. On the other hand, when paired t test was used to compare means before and after irrigation for each section using either concentration there were statistically significant differences (P<0.05).

Discussion
Dentine is the most abundant mineralized tissue in the human tooth and evaluating the effect of irrigants on dentine properties is essential as detrimental effect may jeopardize the overall treatment success. Many studies have investigated the influence of sodium hypochlorite on the mechanical properties of dentine. However in some of these studies dentine specimens used were in the form of bars or cylinders which were sectioned from different levels of tooth and from which the cementum layer had been removed and the specimens were immersed in solutions or materials under investigation for a range of time periods. Such techniques of specimen preparation might not indicate the effect of the materials or irrigating solutions, as the case might be, in the clinical scenario. The in vitro model for preparation of dentine test specimens used in this study was originally described by Saleh and Ettman and used by Slutzky-Goldberg et al. It was chosen because it offered a simple and effective method for measuring hardness changes. The teeth were cleaned and shaped in a manner similar to the in vivo situation. A mixture of single and multirooted teeth of different sizes was used. This was to test the effect of sodium hypochlorite on a more diverse group of teeth. In multirooted teeth, the palatal root of the upper molars and the distal root of the lower molars were chosen to provide enough bulk of tooth structure to measure hardness. Three sections of each root were examined to assure that sodium hypochlorite could affect certain level than other levels. In this study, each specimen was examined before and after irrigation. In this way, each specimen was used as its own reference. This had the advantage of minimizing the structural variation of
different teeth, establish a reasonable baseline for later evaluation, compare changes within one specimen which is an important advantage when working with dental tissues sections. Moreover, sodium hypochlorite effect on dentine hardness would be reflected in the findings irrespective of the variability of specimens. Because microhardness calculations are based on the induced permanent surface deformation, which remains after removal of the load, extra care was taken that the indentations after the irrigation phase were made in different sites from the sites indented before the irrigation phase, to exclude the possibility of re-indentation. Before the irrigation phase, the coronal surface of each specimen was covered with a non-porous adhesive tape which was perforated to allow for irrigation of the root canal. The tape was useful in preventing direct contact of the irrigant to the coronal surface of the specimen and prevented scratching by the irrigating needle on the occlusal surface that might have occurred during root canal irrigation. The adhesive discs provide satisfactory sealing and leakage control when tested using Indian ink dye tracer applied into canal spaces and incubated at 37°C for 5h. Sodium hypochlorite has unique features that have made it the most popular root canal irrigant used worldwide. However, there is no consensus regarding the ideal concentration to be used. The concentrations selected (2.25% and 5.25%) represented concentrations commonly used clinically as reported in the literature. Site for hardness testing was an important issue. Numerous investigators have measured the hardness of dentine and reported wide variations along different regions of the root and showed that hardness of dentine is a function of location and decreases nearer the pulp. In this study, microhardness was measured only at 1000 μm (1mm) from the root canal lumen because it was thought that a statistically significant difference in root dentine microhardness at this distance might be of clinical significance especially after the reduction in root dentine thickness associated with root canal preparation. In this study, the irrigating solution was left for one hour in the root canal to compensate for the factors usually associated with endodontic preparation, such as mechanical instrumentation, multiple visits treatment, using a heated solution or exchange of irrigating solutions. In addition, some researchers recommend high concentration and long exposure time to eradicate certain strains of bacteria. Results obtained in the study were well within the range of dentine hardness figures reported within the literature, but different from values of Oliveira et al. Treatment of dentine with 2.25% and 5.25% sodium hypochlorite solutions caused a significant decrease in dentine hardness in all sections. The organic-dissolving properties of NaOCl on the collagen component of dentine could explain how it affected the hardness of dentine. Sodium hypochlorite is capable of degrading organic material (and possible minerals) from dentine tissues. When dentine proteins are removed, little support will remain to the very small dentine crystals which weakens the dentine even more than EDTA used alone. The work by Marending et al. linked the concentration of sodium hypochlorite to the dissolution of organic dentine components. When mineralized dentine powder was exposed to different concentrations of sodium hypochlorite Zhang et al. found that removal of the “superficial subsurface” organic phase from the powder to be both concentration and time dependent. In this study 5.25% concentration caused more of a decrease in hardness than the 2.25% concentration, which might be clinically important, however, statistical analysis of the data did not reveal this difference to be significant (Table I).

It was expected that the higher concentrations of sodium hypochlorite would have a greater effect on the properties of dentine than the lower concentrations however these results were in agreement with many studies which showed that both concentrations of sodium hypochlorite could decrease the modulus of elasticity, flexural strength or surface strain of dentine, with no significant difference between these concentration. A possible explanation could be due to similar tissue dissolving abilities of concentrations, ability to extract small amount of calcium from root dentine and similar dentine penetration. The decrease in microhardness can provide a potential effect of the solution on the dentine mineral components and could adversely affect the sealing ability and adhesion of dental materials. Hu et al. advised a concentration of 0.5% sodium hypochlorite during routine root canal treatment to minimize any NaOCl-induced dentine deproteination and suggested that 5.25% concentration should only be used in cases of persistent infection.

Conclusions

Irrigation with 2.25% or 5.25% sodium hypochlorite significantly decreased root dentine microhardness.
References


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