This study was designed to evaluate the effect on root dentin microhardness of 2.5% and 6% sodium hypochlorite solutions for various irrigation periods. Forty-two bovine roots were divided into seven groups. The control group was irrigated with saline. The experimental samples were continuously irrigated with 2.5% or 6% NaOCl for 5, 10, or 20 min. Microhardness was measured at depths of 500 μm, 1000 μm, and 1500 μm from the lumen. A decrease in microhardness was found at 500 μm between the control and samples irrigated with 6% NaOCl and 2.5% NaOCl (p = 0.352, p = 0.084 respectively) at all irrigation periods. There also was a significant difference in groups irrigated for 10 or 20 min (p < 0.001, p < 0.001 respectively). At all distances, the decrease in microhardness was more marked after irrigation with 6% NaOCl than 2.5% NaOCl.

Irrigation solutions and medications used during endodontic treatment may lead to changes in the chemical and physical properties of dentin (1–4). The most commonly used irrigation solution and intracanal dressing are 0.5% to 6% sodium hypochlorite and calcium hydroxide paste, respectively (5). These alkaline materials react with organic tissue, changing the chemical structure and affecting the mechanical properties of dentin (1, 6–9). In untreated dentin, tensile strength varies with location and orientation of dentinal tubules (10, 11).

Sedgley and Messer (12) did not find significant differences in punch shear strength, toughness, and load-to-fracture of vital compared with contralateral endodontically treated teeth, but dentin was 3.5% harder in the former than in the latter. By contrast, in an earlier study, Lewinstein and Grajower (13) did not find any difference in the Vickers microhardness of dentin in vital and extracted teeth at various times after endodontic treatment. However, in both studies the endodontic materials and techniques were not evident.

Saleh and Ettman (3) reported decreased dentin microhardness after irrigation with 3% H₂O₂ and 5% NaOCl or 17% EDTA for 60 s. In a similar experimental model, dentin microhardness, which was greater closer to the lumen than in the outer layers, decreased after instrumentation and irrigation with 2.5% NaOCl (4). This study was designed to evaluate the effect of various NaOCl concentrations and irrigations periods on dentin microhardness.

**MATERIALS AND METHODS**

Roots of young, bovine, lower central incisors, 20 to 22 mm in length, were used. All teeth were stored in sterile saline in 4°C. The pulps were removed using barbed broaches and #40 K-files, and the cementum covered with nail polish to prevent entry of the irrigation solution.

Forty-two roots were randomly divided into seven groups: six experimental groups irrigated with 2.5% or 6% NaOCl for 5, 10, or 20 min, and a control group irrigated with saline. The solutions were replenished every minute throughout the experimental period to simulate clinical conditions.

After the irrigation procedure, the roots were cut into two 10-mm segments and embedded in self-curing acrylic resin held in plastic rings. The coronal portion of each embedded segment was ground with carborundum paper discs (300, 600, and 1200 grade) under running water and then polished using diamond paste.

Microhardness measurements were performed on each section at 500 μm, 1000 μm, and 1500 μm from the pulp-dentin interface. At each depth, three indentations were made using a 300-g load oriented perpendicular to the indentation surface for 10 s. Measurements were taken with a Vickers Diamond Microhardness Tester (DMH-2 No 9157 Matsuzawa Seiki Co. LTD, Tokyo, Japan) in Vickers Hardness units (VHN). Statistical analysis was performed by the ANOVA test.

**RESULTS**

There was a difference in dentin microhardness between treated samples and controls in all groups tested. In the control group, the mean values of dentin microhardness at 500 μm, 1000 μm, and 1500 μm were 38.91, 45.74, and 48.73 VHN, respectively. Irrigation with 2.5% NaOCl decreased root-dentin microhardness (Figure 1) after all experimental periods compared with the control (not significant after 5 min (p = 0.084) but statistically significant (p = 0.001 and p < 0.001) after 10 and 20 min, respectively).

Irrigation with 6% NaOCl reduced dentin microhardness for all experimental periods compared with the control (not statis-
tically significant after 5 min (p = 0.352) and statistically significant after 10 and 20 min, respectively (p < 0.001 and p < 0.001; [Fig. 1]).

At 500 μm, the decrease in microhardness was statistically significantly more marked after irrigation with 6% NaOCl than 2.5% NaOCl (p = 0.031). At the same depth, after irrigation for 10 and 20 min, microhardness was lower than after 5 min (p < 0.001), and there was no significant difference between irrigation for 10 and 20 min.

At 1000 μm, statistically significant decreases in microhardness but the differences among groups were not statistically significant (p = 0.520). At the same depth, increasing NaOCl concentration from 2.5% to 6% did not have a significant effect (p = 0.130). At a depth of 1500 μm, there was statistically significant reduction in microhardness after 5, 10, and 20 min compared with the control group, but the differences for various irrigation periods were not statistically significant (p = 0.511). At the same depth, neither concentration had a statistically significant effect (p = 0.686; [Fig. 2]).

**DISCUSSION**

Bovine teeth are a good substitute for human dentin. Schilke et al. (14) did not observe by scanning electronic microscopy any significant differences in dentinal tubule diameters in human and bovine dentin. Tubular density found in bovine root was 23,738 ± 4,457 (number of tubules per mm²) compared with 21,343 ± 7,290 (number of tubules per mm²) in human dentin. The concentrations chosen for this experiment were 2.5% and 6% NaOCl; the former is routinely used for root canal treatment and the latter is the highest concentration used in endodontic irrigation (15).

According to Pashley, dentin microhardness, which depends on the amount of calcified matrix per mm², is inversely correlated to tubular density (16). Our observations were in keeping with the published data (17–19), because the microhardness found next to the lumen in which the tubuli are more dense was higher than in the periphery in which the tubuli are less crowded in both the control group and the test groups. Kinney et al. (20)
showed that the decrease in hardness was caused by a decrease in stiffness of intertubular dentin matrix caused by heterogeneous distribution of the mineral phase within the collagen matrix.

In a previous study, we suggested that irrigation period may have a crucial effect on dentin microhardness (4). This study showed that irrigation for 5 min did not lead to a significant change in dentin microhardness. However, a decrease was found after 10 min, with a further decrease, albeit statistically not significant, after 20 min. It should be emphasized that under clinical conditions, irrigation solutions are replenished frequently, because the antimicrobial effect is sustained only as long as the free chlorine is available in the solution, and the chlorine is consumed during tissue breakdown (5). Dentin contains 22% organic material, mainly collagen type I, which plays a major mechanical role (21). Depletion of the organic phase after NaOCl treatment may cause mechanical change (22). The lack of difference between the 10- and 20-min periods suggests maximal removal of the organic material during the first 10 min from the dentin walls.

Both concentrations caused a substantial decrease in microhardness at 500 μm compared with the control group. As expected, at 500 μm, 6% NaOCl gave a more significant decrease in microhardness than 2.5% NaOCl for all irrigation periods. At 1000 μm and 1500 μm, only a nonsignificant trend was found, possibly because of limited sodium hypochlorite penetration further away from the lumen.

A 6% sodium hypochlorite caused a more significant decrease in microhardness compared with a 2.5% concentration. Bearing in mind that there is only little antibacterial advantage to the use of higher concentration of sodium hypochlorite (23) and that higher concentration of NaOCl also may be cytotoxic (24), it may be prudent not to select higher concentrations of NaOCl to not hamper the physical properties of dentin.

CONCLUSIONS

Exposure of bovine dentin to sodium hypochlorite solution for more than 10 min decreases dentin microhardness. It may, therefore, be advantageous to limit the irrigation time to a period shorter than 10 min to not weaken the tooth or to use an alternative irrigation solution, such as chlorhexidine. Further research is required to find out if chlorhexidine has any advantage over NaOCl in retaining the physical properties of dentine.

At a depth of 500 μm from the lumen, 6% NaOCl has a greater effect on dentin microhardness than 2.5% NaOCl. Therefore, it is advisable not to use higher concentrations of NaOCl, so to not alter the physical properties of dentin—and jeopardize the tooth.

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