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ORIGINAL ARTICLE

Effect of Calcium Hydroxide Intracanal Medicament on Root Dentine Microhardness: an In Vitro Study

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Abstract

Objective: **To evaluate root dentine microhardness after using calcium hydroxide ($Ca(OH)_2$) intracanal medicament.**

Materials and methods: **Forty single-rooted teeth were sectioned at the cementoenamel junction level, and the root canals were prepared till an apical size of #30. The prepared teeth were divided into two groups ($n = 20$): the no medicament (control) group and the $Ca(OH)_2$ group. After 7 days, medicaments were removed using a standardized volume of irrigation. All teeth were sectioned in a buccolingual plane and embedded in acrylic resin horizontally with the dentine surfaces exposed. Using Vickers microhardness tester with magnification of × 100 and a load of 25 g for 10 s, a microhardness assessment was performed. Independent *t* test was used for intergroup comparison. One-way analysis of variance and post-hoc Tukey's tests were used for comparisons ($P \leq 0.05$).**

Results: **Microhardness in the control group was significantly greater than in the $Ca(OH)_2$ group at the coronal ($P < 0.001$) and middle ($P = 0.006$) sections. At the apical third, no significant difference was detected ($P = 0.779$). In the control group, the coronal section was significantly greater than at the apical section ($P = 0.002$), while no significant difference was detected between the middle sections with both coronal ($P = 0.212$) and apical ($P = 140$) sections. In the $Ca(OH)_2$ group, no significant effects were noted between different sections ($P = 0.385$).**

Conclusions: **The $Ca(OH)_2$ reduced root dentine microhardness at both coronal and middle sections, but not the apical.**

Keywords: Calcium hydroxide, In vitro, Intracanal medicaments, Vickers test

Introduction

The ultimate objective of root canal therapy is to get rid of bacteria along with their byproducts inside root canals and avoid recurrent infection.[1] Despite a proper cleaning and shaping protocol, not all species of bacteria get cleared from the root canal system. Bacteria may proliferate in empty root canals left without dressing in between appointments.[2] Bacterial proliferation in-between endodontic visits can be decreased through using suitable root canal dressing.[3]

An ideal intracanal dressing material should have efficient germicidal and fungicidal properties with an extended antimicrobial activity. It should not irritate the periapical surrounding tissues in the event of extrusion, have a little surface tension, not discoloring the tooth, and be effective in the presence of any protein derivatives of tissues. Furthermore, it should not affect the renovation of periapical surrounding tissues and induce recovery besides hard tissue barrier formation. Also, intracanal medicament should limit root resorption caused by inflammatory processes, reduce pain, and abolish periapical exudates without altering the normal physiological activities of the surrounding tissues.[4]

In 1920, calcium hydroxide ($Ca(OH)_2$) was introduced as Calxyl, which was used for repairing perforations, fractured roots, resorptions, and traumatic

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harms. It is an odorless white powder and has the formula of Ca $(OH)_2$. It has a highly alkaline nature with a pH of 12–12.8. The antimicrobial action is achieved through releasing hydroxyl groups that destroy the cytoplasmic membrane of bacteria causing damage to the phospholipid structure in the cell membrane. It also causes protein denaturation and accumulates carbon dioxide, which disturbs the complex root canal system.[2,5] Dentine mechanical properties such as flexural strength, elasticity, and root resistance to fracture were found to be reduced by the use of Ca $(OH)_2$.[6–11]

A hardness test is not generally applied to forecast root fracture. However, the value of the performance of a well-standardized indentation test on dentine is to assess other mechanical criteria such as elasticity, tensile, and compressive strength.[12]

Vickers microhardness test is often termed the diamond-pyramid microhardness test as it uses a square-base diamond pyramidal indenter. The pyramid has an angle of 136° between its opposite faces.[13] This test is widely preferred because the diamond tip does not become distorted over time, it completely sticks to both soft and hard surfaces. It can measure the hardness of fragile specimens with accurate measurements.[14,15]

There were many reports on the impacts of Ca $(OH)_2$ on root canal dentine microhardness. However, no sufficient information, and there has been a conflict in many studies regarding the impacts of Ca $(OH)_2$ on the microhardness of root dentine, specifically along the root length (coronal, middle, and apical) whether affects or not or decreases the microhardness of root dentine. Therefore, the current study aimed to determine the effect of Ca $(OH)_2$ on the root dentine microhardness at the coronal, middle, and apical sections. The null hypothesis supposed that there would be no significant difference in dentine microhardness with or without Ca $(OH)_2$ intracanal medication.

Materials and methods

The current study was conducted after approval of the Local Ethics Committee (M04050422). The sample size was determined by using the G*Power software program (v3.1.9.7). Based on a previous study,[16] effect sizes (Cohen's d) were calculated for microhardness comparisons between both control and Ca $(OH)_2$ groups at the coronal site ($d = 3.497917$), middle site (Cohen's $d = 4.316581$), and at the apical site (Cohen's $d = 4.154726$). To be more conservative, we hypothesized a large effect size (Cohen's $d = 1.2$ at any site). Group sample sizes of 20 teeth were used.

Forty freshly extracted single-rooted human central incisors were assembled from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mansoura University. The teeth were extracted due to orthodontic and periodontal issues. All patients signed a consent form about using their teeth for scientific research according to the guidelines of the Local Ethics Committee.

The teeth were cleansed using sodium hypochlorite solution in addition to an ultrasonic cleaner to remove tissue debris. They were finally kept in distilled water. Teeth were selected according to certain inclusion criteria, which were single root canal, mature apex with patent foramina, absence of calcification through the canal system, and finally no clue of internal or external resorption. However, teeth having indications of root caries, cracks, calcifications, or blocked canals were ruled out.

Sample preparation

The teeth at the cementoenamel junction level were decoronated by the operator (R.I.T.) using a diamond saw mounted on a low-speed straight handpiece at 40 000 rpm (H4682; NSK, Tochigi, Japan) to a standardized length of 16 mm. After access opening, they were mechanically prepared by a nickel–titanium (NiTi) rotary system (ProTaper Gold; Dentsply Sirona, Ballaigues, Switzerland) by a single operator (R.I.T.) according to the instructions of manufacturer the file was used till an apical size of #30. After each rotary file cleaning, irrigation with 2 ml of 2.5% sodium hypochlorite was done, followed by 2 ml of saline to remove all dentine debris.[17] Each NiTi rotary file was used to prepare five teeth. Then the teeth were allocated into two groups ($n = 20$): the control group (treated with saline application only) and the Ca $(OH)_2$ group (in which the Ca $(OH)_2$ (Metapaste; Meta Biomed, Chung Buk, Korea) was placed by the tip of the ready-made injectable paste) by the operator (A.Y. B.). To make sure that the medicament filled all the canal and got in contact with all walls, excess medicament was extruded beyond the apex. Finally, an intermediate restorative material (Orafil-G; Prevest DenPro, Delhi, India) was used to seal the access cavity.[17] All the samples were successfully prepared for the test with no failure.

Incubation of prepared teeth after intracanal medicament placement

All specimens were placed inside the incubator (Binder; Binder GmbH, Tuttlingen, Germany) for 1 week at 37 °C and 100% humidity at Mansoura

Experimental Research Centre (MERC).[18] After the incubation period, the transient filling was dislodged and a K-file of size #25 (M.access; Dentsply Sirona, Switzerland) was used with an adequate saline irrigant to wash the intracanal medicament paste from all samples. Standard irrigation was used for all study groups.[19]

The prepared teeth were sectioned lengthwise in a buccolingual plane after the removal of medicaments. Sectioning was done by a low-speed precision cutter (IsoMet 4000; Buehler, Germany) as shown in Fig. 1. After sectioning into two halves, they were embedded horizontally in self-curing resin (Acrostone; Acrostone Dental, El Salam city cairo, Egypt) with their dentine surface upward as shown in Fig. 2. Then, the dentine surface of the fixed samples was flattened and smoothed using the sequence of carbide finishing sandpapers with ascending grades under flushing distilled water to eliminate any surface scrapes. Finally, to get a smooth polished mirror-like surface, samples were polished using 0.1 mm alumina suspension with a rotatory felt polishing disc (Polidont; Microdont, Sao paulo, Brazil).[20]

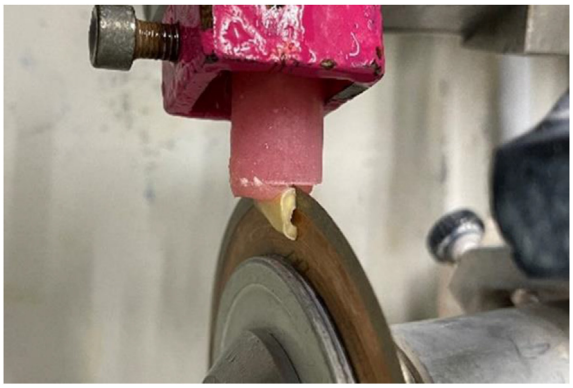

Fig. 1. Sectioning using the isomet into two halves in a buccolingual plane.

Fig. 2. Sectioned half of the root was embedded in self-curing resin with their dentine surface upward.

Dentine microhardness assessment (Vickers test)

Using Wilson hardness tester (Model Tukon 1102; Buehler, Germany), a load of 25 g was put on gently, devoid of heavily imposing the indenter on the samples. The indenter was held in position for 10 s as shown in Fig. 3.[19] Microhardness measurements were taken by a single operator (N.A.W.) at 3, 6, and 9 mm from the root apex, which correspond to the coronal, middle, and apical root sections at a distance of 0.5 mm from the canal lumen.[16] For accurate measurements, the physical quality of the indenter must be checked and the applied load must be under control. After removal of the load, the produced indentation was focused using a magnifying eyepiece as shown in Fig. 4. Then, two impression diagonals were calibrated to the nearest 0.1 μm using a micrometer and averaged.

Statistical analysis

The data were statistically analyzed by IBM-SPSS software (SPSS Statistics for Windows, v25; IBM Corp., Chicago, Illinois, USA). The normality of data was verified using the Shapiro–Wilk test. Independent *t* test was used for intergroup comparison at each site (coronal, middle, and apical). One-way analysis of variance followed by the post-hoc Tukey test was used for intragroup comparison between different sections. In terms of the previously used tests, *P* is considered significant when its value is less than 0.05.

Results

Intergroup comparison

At coronal and middle sections, in the control group the microhardness was significantly greater than that in the Ca $(OH)_2$ group ($P < 0.001$ and $P = 0.006$, respectively). No significant difference

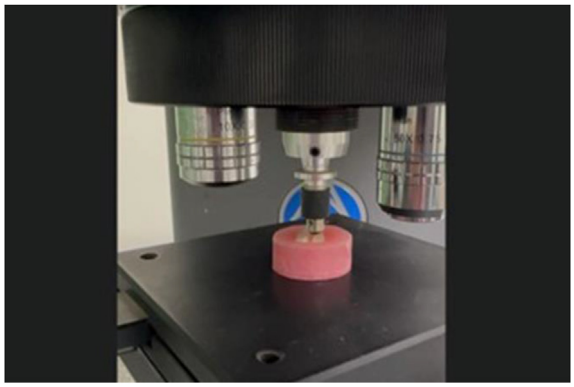

Fig. 3. Microhardness test of root dentine.

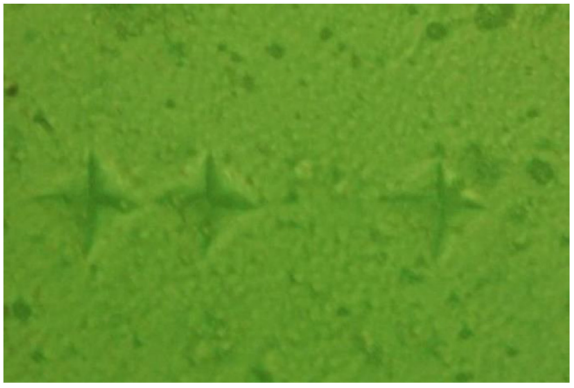

Fig. 4. An example of the magnified indentation under the eyepiece.

was noticed between both groups in the apical section ($P = 0.779$) (Table 1).

Intragroup comparison

In the control group: the microhardness at the coronal section was significantly greater than that at the apical section ($P = 0.002$). There was no significant difference in between the middle section with both coronal and apical sections ($P = 0.212$ and 140). In the Ca $(OH)_2$ group: no significant differences were noted between different sections ($P = 0.385$) (Table 2).

Discussion

Successful endodontic therapy relies on comprehensive disinfection plus complete sealing of the whole canal system. Moreover, the prognosis of root canal therapy relies on the complete eradication of microorganisms along with their byproducts.[21] Intracanal medications play an important contribution in disinfecting the root canal complex system in cases where conventional cleaning and shaping are inadequate to irradicate remaining bacteria, which may proliferate in-between endodontic visits.[22] Ideally, both physical and mechanical criteria of root dentine like hardness, elasticity, and bend strength should not be affected by intracanal medicaments. Also, it must be non-harmful to the periapical surrounding tissues. However, some root canal dressings are found to adversely affect vital physical and mechanical criteria of the root dentine, like microhardness.[23]

Microhardness could provide an indirect clue about mineral changes in dental mineralized tissue because it relies on the calcified matrix volume within a square millimeter.[24] For this specific reason, this parameter is selected for testing in this study. The research was designed to evaluate the impacts of using Ca $(OH)_2$ as root canal medication on root dentine microhardness.

In the current study, single-rooted teeth are selected for standardization because this presents a vital role in improving the reliability of hardness testing.[25] Each NiTi rotary file was used to prepare five teeth because of the high cyclic fatigue resistance of ProTaper Gold files.[26] In addition, mechanical preparation was done minimally till an apical size of #30 to reduce changes in dentine

Table 1. Intergroup comparison of microhardness at each section (coronal, middle, and apical).

Site	Groups				Test of significance		
	Control		Calcium hydroxide		t [38]	Significance	Cohen's d
	Mean	SD	Mean	SD			
Coronal	49.8^{A}	4.3	42.5^{B}	5.3	4.778	<.001	1.511
Middle	47.5^{A}	3.7	42.7^{B}	6.3	2.931	0.006	0.927
Apical	44.9^{A}	4.6	44.5^{A}	3.4	0.283	0.779	0.089

Different superscript upper case alphabetical letters indicate significant intergroups at different sites in the same row.
Cohen's d = effect size measure (effect size is small, medium, or large in cases when Cohen's d = 0.2, 0.5, or 0.8, respectively).
t [38] = t statistic at 38 degrees of freedom.
P: significance at less than or equal to 0.05.

Table 2. Intragroup comparison of microhardness between the three sections.

Group	Site						Test of significance			
	Coronal		Middle		Apical					
	Mean	SD	Mean	SD	Mean	SD	F [2, 57]	Significance	η^2	Cohen's f
Control	49.8^{A}	4.3	47.5^{AB}	3.7	44.9^{B}	4.6	6.609	0.003	0.188	0.481
Calcium hydroxide	42.5^{A}	5.3	42.7^{A}	6.3	44.5^{A}	3.4	0.972	0.385	0.033	0.185

Different superscript upper case alphabetical letters indicate significance intragroups at different sites in the same row.
F [2, 57] = F statistic at 2, 57 degrees of freedom.
η^2 and Cohen's d = effect size measures (effect size is small, medium, or large in cases when Cohen's f = 0.1, 0.25, or 0.4, respectively).
Significance P value less than or equal to 0.05.

collagen composition and thus reducing microhardness changes resulting from mechanical preparation.[27] Teeth sectioning was done longitudinally in a buccolingual direction because of the higher thickness of root dentine at the later direction, which is suitable for accurate measurements and simulation of clinical situations.[28]

Vickers microhardness test has been used in the current study because it is an acceptable and easy method to evaluate the surface changes in deeper hard dental tissues.[29] Also, it is extremely precise in measuring errors, minimally affected by surface conditions and furthermore, it can be applied on small samples.[30] Indentations were done usually to the nearest 0.1 μm from the root dentine surface for standardization and to provide precise assessment of the root dentine, which is in contact with the medicament.[31,32]

In the current study, the null hypothesis was partially accepted as the Ca $(OH)_2$ did not affect microhardness at the apical section, but conversely, it caused a significant decrease of dentine microhardness at both coronal and middle sections. This study showed that the Ca $(OH)_2$ group had a significantly minimal microhardness value at both coronal and middle sections than the controls, but no significant difference was found at the apical section.

This finding is in agreement with several authors,[19,33–35] who attributed the decrease of microhardness of root dentine to the destruction that occurred by the dentine inorganic structure breakdown or by means of denaturation of the organic matrix because of the high alkalinity of Ca $(OH)_2$.[6] Another reason may be due to the penetration of Ca $(OH)_2$ with their minute molecular size through the intrafibrillar assembly of mineralized collagen fibrils, causing variations in the three-dimensional configuration of tropocollagen, resulting in minimized microhardness of the dentine.[36]

While comparing the mean microhardness values at changed sites, it was revealed that no significant difference was noted in the Ca $(OH)_2$ group between the three sites. In the control group, a significant difference was detected in-between both the coronal and apical sites, while the middle site was not significantly different between both coronal and apical sites.

These findings are in agreement with other studies,[16,37,38] which revealed that there was a gradual decline in microhardness mean values of root dentine along the root length. This may be attributed to the higher volume of minerals in the coronal one-third, which may account for the increased microhardness at the baseline. This results in dentine with higher resilience and able to counter local deformation.[39]

However, Carrigan *et al.*[40] found that the microhardness of root dentine decreased from apical to coronal and that was attributed to the decreasing tubular density from the cervical to apical dentine and the inverse association between the tubular density and root dentine microhardness as reported by Pashley *et al.*[41] Several authors[42–45] have described that anatomical variations of the number, size, and direction of dentinal tubules plus the volume and mineral load of the intertubular dentine had an impact on microhardness values at different root sites.

One limitation of this study is that it was conducted in vitro, and therefore the results of this study do not accurately reveal what is happening in the oral cavity as it differs from the clinical circumstances, such as the oral temperature, presence of exudates, and the surrounding periapical tissues. However, these results provide an initial impression of the impact of intracanal medicament on the microhardness of root dentine. Additional studies are needed to prove the impact of Ca $(OH)_2$ on the microhardness of root dentine through other specific types of tests as the mineral volume assessment and the penetration ability of Ca $(OH)_2$ within dentinal tubules. In addition, Ca (OH) is recommended to be compared with other recent intracanal medicament.

Conclusions

Under the circumstances of the current study, the following conclusion was obtained:

(1) It was found that Ca $(OH)_2$ reduced root dentine microhardness at both coronal and middle sections, but not the apical.

Conflicts of interest

There are no conflicts of interest.

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