Long Term Effect Of Calcium Hydroxide On The Microhardness Of Human Radicular Dentin – A Pilot Study”
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Citation

Abstract
Aim: The objective of this study was to evaluate the long term effect of calcium hydroxide-glycerin combination on the microhardness of human radicular dentin. Materials and methodology: One hundred and fifty freshly extracted single rooted human premolars were used in this study. All the teeth were decoronated at the cemento-enamel junction and the root canals were instrumented with protaper rotary files. The teeth were then randomly divided into three groups with 50 teeth in each group, where group I and II had teeth with calcium hydroxide application for 30 and 90 days respectively and group III had teeth with saline application for 90 days. The teeth in all the groups were sectioned longitudinally into two equal slabs and then subjected to Vickers microhardness testing. Statistical analysis: Statistical analysis was performed with the aid of SPSS v 12.0 software and the data were analyzed using One-Way ANOVA and Tukey’s HSD test. Results: There was a significant reduction (p<0.001) in the hardness values of specimens in Group-II (44.53 HV) as compared to Group-I – 57.87 HV and Group-III – 68.91 HV. Conclusion: The reduction in hardness can be attributed to the prolonged application of Calcium hydroxide, which can have a detrimental effect on the teeth making it more vulnerable to fracture.

INTRODUCTION
Endodontic treatment is primarily directed towards the prevention and control of pulpal and periradicular infections. It is a well established fact that microorganisms play an important role in the pathogenesis of periradicular lesions; and the outcome of the endodontic therapy depends on their reduction or elimination.1 Although thorough chemomechanical preparation may help in reducing the bacterial population, total elimination is difficult to accomplish.1,2,3 By remaining in the root canal between appointments, intracanal medicaments may help to eliminate the surviving bacteria.1,3

Since its introduction in 1920, calcium hydroxide has been widely used in endodontics as an inter-appointment intracanal medicament. It is a strong alkaline substance, which has a pH of approximately 12.5,4 and has various biological properties such as antimicrobial activity,1 tissue-dissolving ability,4,5 inhibition of tooth resorption,6 and induction of repair by hard tissue formation.1 Because of such effects, calcium hydroxide has been recommended for use in several clinical situations.1 Currently, this chemical substance is acknowledged as one of the most effective antimicrobial dressings during endodontic therapy.

When placed within the root canal system, calcium hydroxide dissociates into calcium and hydroxyl ions,8 and the hydroxyl ions diffuse through the dentinal tubules.9,10 The high pH and antimicrobial properties of calcium hydroxide,11 combined with the permeability of dentin,12,13 may account for its effectiveness as an intracanal inter-appointment medicament, an inhibitor of inflammatory root resorption, and an inducer of apical closure in nonvital immature teeth. However, when calcium hydroxide is used in these applications, therapy may extend from months to years before the desired effects are achieved.14,15 Furthermore, it has been observed that calcium hydroxide treated immature teeth show a high failure rate because of an unusual preponderance of root fracture and it has been suggested that changes in the physical properties of dentin might be caused by the calcium hydroxide medicament.16 Studies have shown that, a 5-wk exposure to calcium hydroxide resulted in a 32% decrease in the strength of bovine dentin.17 In sheep dentin treated with calcium hydroxide, a marked decrease in the fracture strength with
increasing storage time was observed.\textsuperscript{18} Most importantly, when immersed in a saturated solution of calcium hydroxide for 1 wk, a reduction in the flexural strength of human dentin was demonstrated.\textsuperscript{19}

Exposure of root dentin to the bioactive effects of calcium hydroxide may affect its physical characteristics and could have important clinical implications for the treatment of traumatized teeth and immature teeth with nonvital pulps.\textsuperscript{20} The purpose of the present study was to determine whether intracanal exposure to calcium hydroxide with glycerin as a vehicle for 30 and 90 days alters the microhardness of human dentin.

**MATERIALS AND METHODOLOGY**

One hundred and fifty freshly extracted human premolars were used in this study to evaluate the hardness of dentin. Premolars which were extracted for orthodontic and periodontal reasons were used. After extraction soft tissues, debris and calculus were removed using curette. Before using it for the study the teeth were stored in distilled water (G.M traders, Chennai, India) for one week and disinfected by immersing in 0.10 g/ml Thymol (Jayshree chemicals, Indore, India) at 5º C for 3 months. Storing the samples in Thymol was preferred over the other methods like sterilization of teeth by autoclaving, immersion in Formalin, Sodium Hypochlorite, as all these methods have been shown to alter the dentin permeability and the organic and inorganic content of teeth.\textsuperscript{21} Radiographs were taken to eliminate the teeth with any possible canal obliteration and internal or external resorption.

The teeth were decoronated at the cemento-enamel junction using a diamond saw mounted on a slow speed micromotor under water coolant. A fifteen-sized k-file was introduced in the canal and the stopper was set at the point where the file was seen at the apex. Coronal third of the root canal was flared using Gates-Glidden drills (#1, 2 and 3) (Mani. Inc, Japan) and the canals were instrumented with Protaper files (Dentsply, Maillefer, Switzerland). Instrumentation with Protaper was done using the following sequence (S1 until 2/3rds of the WL- Sx till the middle third- S1 until the working length- then F1, followed by F2 and F3 until the working length). Between each step the canals were thoroughly irrigated with Saline (Baxter India Pvt. Ltd, Tamilnadu, India). Sodium hypochlorite and EDTA were not used as it reduces the hardness and the fracture resistance of teeth.\textsuperscript{22}

All the teeth were randomly divided into three groups, wherein all the groups had 50 teeth each. Group-I and Group-II contained teeth with Calcium Hydroxide (Apexcal, Ivoclar Vivadent, Liechtenstein) application for 30 and 90 days respectively and Group-III (n=50) was taken as a control group with Saline application for 90 days. The root canals of the teeth in group I and group II were filled with calcium hydroxide and sealed apically with bonded composite resin and coronally with a cotton pellet and bonded composite resin (Restofill, Stedman pharma, India). The root canals of the teeth in group III were filled with saline. To ensure intimate contact with the canal walls and a dense fill of the canal space, excess calcium hydroxide was intentionally extruded past the apex using a 30 sized Lentulo spiral (Mani. Inc, Japan) having a 0.02 taper. As with groups I and II, the teeth in groups III were sealed apically with bonded composite and coronally with a cotton pellet and bonded composite. All the teeth were stored in three separate petridishes and covered with aluminium foil.\textsuperscript{23} The teeth were then mounted on a metal jig and then longitudinally sectioned into two equal slabs, thus obtaining a total of 100 slabs in each group.

**PREPARATION FOR VICKERS MICROHARDNESS TESTING:**

The teeth from each group were rinsed thoroughly with saline and mounted on acrylic cylinders (figure 1) with the calcium hydroxide treated surface facing up. The specimens were then introduced in a hard tissue microtome (figure 2) and sectioned horizontally (figure 3) to obtain a specimen of 2mm thickness (figure 4). The surface of the specimens were finished using Silicon carbide paper having 400, 800, 1200 grit.
Microhardness testing was carried out on a Vickers microhardness tester (figure 5) at 100gm load for a 15 sec dwell time. Three indentations were made in the mid root area 1mm away from the canal area and the microhardness values were calculated using the following formula [ \( VHN = \frac{P \times 1.8544}{d^2} \) (\(P=\text{load applied in kg}\) and \(d=\text{arithmetic mean of the diagonals } d_1 \text{ & } d_2 \text{ caused by the indentation} \)].
STATISTICAL ANALYSIS

The microhardness values for different groups were tabulated and the Statistical analysis was performed using the SPSS v 12.0 software. Data were analyzed using One-Way ANOVA and Tukey’s HSD test. The p value was set at 0.05.

RESULTS

There was a significant reduction (p<0.001) in the hardness values of specimens in Group-II (44.53 HV) (90 day treatment period) as compared to the other Groups [Group-I – 57.87 HV (30 day treatment period), Group-III – 68.91 HV (control group with saline application)]. The mean microhardness and standard deviation values of their corresponding groups are given in table 1. Graph 1 shows the bar diagram with mean values of all the groups.

DISCUSSION

Calcium hydroxide is a slow working antiseptic, which is often used over extended periods of time in the root canals as an intra-canal medicament. Although Calcium hydroxide cannot be categorised as a conventional antiseptic,
there is well documented evidence for its use as an inter-
appointment dressing over the past 40 years by clinicians.24 It has been shown to kill bacteria in the root canal space. In addition to its bactericidal effect, calcium hydroxide has the extraordinary ability to hydrolyze the lipid moiety of bacterial lipopolysaccharides, thereby inactivating the biologic activity of lipopolysaccharide and reducing its effect.24,25 This is a very desirable effect because, dead cell wall material remains after the bacteria has been killed which can stimulate an inflammatory response in the periradicular tissue. All these properties make calcium hydroxide as the material of choice for inter-appointment dressings in endodontics.

The action of calcium hydroxide in the root canal space mainly depends on its dissociation into calcium and hydroxyl ions; but at body temperature less than 0.2% calcium hydroxide is dissolved into Ca^- and OH^- ions.1,23,24 So generally, calcium hydroxide is used as slurry in a water base. Also, calcium hydroxide has been tried with various other vehicles like saline, distilled water, glycerin, ringer’s lactate solution, iodoform, cresatin, camphorated parachlorophenol. Mixing with cresatin results in the formation of calcium cresylate and acetic acid, whereas mixing with camphorated parachlorophenol results in calcium parachlorophenolate. In both cases hydrolysis is inhibited, and the advantageous pH is not reached.24 Glycerin has enjoyed its part as a vehicle since it renders a longer duration of action for calcium hydroxide and the hygroscopic properties of glycerin makes it a suitable vehicle as it can penetrate the tubules.

Previous studies have shown that the calcium hydroxide, along with vehicles like saline, distilled water and glycerin has reduced the fracture resistance and microhardness of human root dentin.23 Glen Doyon et al (2005), found that the mean fracture resistance of teeth treated with calcium hydroxide decreased by 10-20%.20 The purpose of the current research was to examine the possible deleterious effects of calcium hydroxide-glycerin combination on human root dentin. Apexcal (Ivoclar, Vivadent, Liechtenstein) was used in this study which has calcium hydroxide and glycerin as its components. The composition of Apexcal is given in table 2.

In the present study microhardness was used as a parameter to assess the strength of the teeth, as microhardness of a material is not a measure of a single property. It is influenced substantially by other fundamental properties of the material such as yield strength, tensile strength, modulus of elasticity and crystal structure stability. Thus, it can be used as an indicator of the overall strength or resistance to deformation when compared with baseline information.26 Currently two types of microhardness testing methodologies are present, viz; Knoops and Vickers, but vickers microhardness testing was employed as it is more sensitive to measurement errors, less sensitive to surface conditions and small specimens can be tested with good accuracy.

The results of the current study showed a significant reduction in the microhardness values Group I (57.86 HV) and Group II (44.53 HV) as compared to Group III (68.91 HV). Similar findings were seen in other studies suggestive that long-term exposure of the root dentin to calcium hydroxide could cause a reduction of microhardness values.17,18,20 Yoldas et al (2004) and Seyed Mohsen et al (2009) have shown that reduction in the dentin microhardness with application of calcium hydroxide could be caused due to the structural changes in root dentin associated with it.23

It has been shown that calcium hydroxide dissolves pulp tissue,1,4,5,21,24 a process that may occur by denaturation and hydrolysis. In addition, the pH increase observed after exposure to calcium hydroxide may also reduce the organic support of the dentin matrix. This alkalinity can cause breakdown of protein structure that could negatively influence the mechanical properties of dentin. Andreasen’s theory supports the fact that the proteolytic action of calcium hydroxide could weaken the tooth up to 50% in one year. He suggests that the reduction in the microhardness could be due to the disruption in links between the collagen fibers and

### Table 2

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight percent</th>
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<tbody>
<tr>
<td>Calcium hydroxide</td>
<td>29.0</td>
</tr>
<tr>
<td>Bismuth subcarbonate</td>
<td>22.0</td>
</tr>
<tr>
<td>Excipients (polyethylene glycol, glycerin, water)</td>
<td>49.0</td>
</tr>
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Figure 9
Alacam et al (1998),27 Osol and Hoover (1975) suggested that the reduction of the dentin microhardness associated with calcium hydroxide can be increased if glycerin is present as the vehicle.23 This reduction could be attributed to the hygroscopic properties of glycerin and with the slight dissociation of calcium hydroxide in glycerin. This renders calcium hydroxide to act for longer periods of time, confining it to the site of action.

CONCLUSION

The findings of this study appear to support the contention that long term exposure to calcium hydroxide-glycerin alters the physical properties of dentin, which may be a result of a change in the organic matrix. Further testing is required to test the clinical relevance of these findings.

References

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