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Fracture resistance of human root dentin exposed to calcium hydroxide intervisit medication at various time periods: an *in vitro* study

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Abstract – The aim of this study was to evaluate the effect of calcium hydroxide on the fracture resistance of human root dentin at different time intervals. After root canal preparation, one hundred single-rooted premolar human teeth were randomly divided into two equal groups. After placement of calcium hydroxide paste within the canal, one group of teeth was divided into 5 subgroups and incubated for a period of 1 week or 1, 3, 6, or 12 months at 37°C and 100% humidity. The second group of teeth was also divided into 5 subgroups and incubated under the same conditions without placement of calcium hydroxide paste. After mounting the teeth in a Zwick test machine, the force required to break each tooth was determined. Data analysis was performed using the one- and two-way analysis of variance tests. The results demonstrated that the mean force needed to cause fracture differed significantly between the two groups during the first, third and sixth months of incubation ($P = 0.001$, $P < 0.001$ and $P = 0.035$, respectively), and the amount of force necessary for fracture was greater in the control group. Therefore, it would appear from this study that using calcium hydroxide as a long-term intracanal dressing showed a significant decrease in peak load at fracture when compared with the control groups at the end of the first, third and sixth months of treatment and would suggest that using $Ca(OH)_2$ for periods longer than 1 month should be used with caution.

The proposed mechanism of action of calcium hydroxide ($Ca[OH]_2$) on tissue and bacteria is because of the dissociation of calcium and hydroxyl ions, as it is soluble in water, saline, local anaesthesia and Ringer's solution. However; it has a low solubility in oil-based vehicles, such as silicone oil, camphor, eugenol and metacresylacetate (1, 2). In addition, $Ca(OH)_2$ has a high pH, which can be affected when combined with other materials (3, 4). Calcium hydroxide as used is a commercially available pure powder for the treatment of necrotic pulp tissue associated with an open apex and can be combined with different vehicles. The powder contains pure $Ca(OH)_2$, and its liquid form contains methylcellulose or physiological serum (2, 5). An advantage of using the pure powder form is that it can be made into any viscosity that is suitable for the particular application and it does not contain any impure components that may be present in the commercial form (6).

The physical and chemical characteristics of pure $Ca(OH)_2$ present some disadvantages. For example, the material is radiolucent, relatively permeable to liquids, highly soluble in the periapical region and not viscous. Moreover, the placement of $Ca(OH)_2$ into canals is difficult (7). The long-term application of $Ca(OH)_2$ is commonly used when treating teeth with large periapical radiolucencies or in immature traumatized teeth. Changes in the dentin fracture resistance after root canal therapy has been reported (8), and Gher et al. (9) found that 71% of root fractures occurred following endodontic treatment.

Several factors are known to be associated with root fractures in endodontically treated teeth, including (i) the loss of tooth structure because of damage from tooth decay; (ii) the effect of endodontic cavity access preparation; and (iii) the effect of different endodontic materials such as using different irrigants, intracanal medicaments and filling materials on the physical properties of dentin (9–11). It has been suggested that changes in the physical properties of dentin because of the use of $Ca(OH)_2$ medicament may be also responsible for root fracture (12).

Andersen et al. (13) showed that $Ca(OH)_2$ dissolves pulp tissue within 2 h and the remaining tissue within 1 week. Moreover, it has been indicated that $Ca(OH)_2$ has an effect on collagen tissue. In 2007, Rosenberg et al. (14) conducted a study on 40 human teeth containing $Ca(OH)_2$ for periods of 7, 28 and 84 days and showed that the effects of $Ca(OH)_2$ increased with the duration of contact.

In this study, effect of using $Ca(OH)_2$ for varying lengths of time on tooth fracture resistance was examined. The maximum amount of time that $Ca(OH)_2$ may be used without affecting tooth fracture resistance was determined.

Material and methods

In this experimental study, 100 human single-rooted mandibular premolars with similar root diameter and fully developed roots were used. The teeth were collected during a period of several months from adult patients (mean age 23.3 years; range 20–25 years), who had required extractions for orthodontic or periodontal reasons. The teeth were examined for any cracks, fractures, or other defects using 6.0× magnifying loupes (ErgonoptiX, Almere, the Netherlands).

The teeth were placed in 0.5% sodium hypochlorite (NaOCl) for 5 min and stored in saline until they were used for the experiment. The clinical crowns were then cut perpendicular to the longitudinal axis of the roots with steel discs (Brasseler USA, Savannah, GA, USA) at a distance of 17 mm from the teeth apices. The working length was measured by carefully passing a #10 flexofile (Dentsply-Maillefer, Instruments SA, Ballaigues, Switzerland) along the canal, under the 6.0× magnifying loupes, until the tip was just visible at the apical foramen, and then 1 mm was subtracted. Rotary nickel–titanium instruments (RaCe; FKG Dentaire, La Chaux-de-Fonds, Switzerland) were used to prepare the canal (size 25/0.04 taper, size 30/0.04 taper, size 35/0.04 taper, size 40/0.04 taper), using the crown-down technique (MAF # 40). The smear layer was removed at the completion of instrumentation by flushing the root canals with 10 ml of 17% ethylenediaminetetraacetic acid (Ariadent, Tehran, Iran) followed by 10 ml of 5.25% NaOCl (Shamin, Tehran, Iran). The canals were finally rinsed with 10 ml of saline.

The teeth were randomly divided into two groups of 50; being control and experimental groups. Teeth in both groups were then divided into five further subgroups of ten, each based on the following time periods: 1 week, 1, 3, 6 and 12 months. The canals of the teeth in the control group were left empty. In the experimental group, $Ca(OH)_2$ paste was prepared by mixing $Ca(OH)_2$ powder (Golchadnet, Tehran, Iran) with normal saline in a 3:1 ratio.

A Handy Lentulo Paste Carrier (Handy Lentulo, Dentsply-Maillefer Instruments SA, Ballaigues, Switzerland) was used to place the paste into the canal and then $Ca(OH)_2$ was condensed in the canal. $Ca(OH)_2$ was replaced in the teeth at the indicated time points for each respective subgroup. The $Ca(OH)_2$ was removed from the canal with a #40 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) at the working length in a circumferential filing motion, and the canal was irrigated with 20 ml of saline. All canals were sealed with Cavite (Cavisol, Golchay, Tehran, Iran). Teeth in both groups were then incubated for the allocated time periods at 37°C in 100% humidity. After incubation, the teeth were mounted in self-curing acrylic resin (Acropars 200, Marlik, Tehran, Iran) with a diameter of approximately 1.5 cm.

Before mounting, to simulate periodontal ligament (PDL) width, teeth roots were first covered with a uniform layer of 'Wax Up' (Shokouh, Tehran, Iran). The thickness of the wax layer was measured by using callipers (Yates-Motloid, Chicago, Illinois, USA) to ensure an adequate thickness surrounding the tooth. The measurements were performed at 3 points along the length of the teeth (apical, mid-root and cervical) to ensure a 0.2-mm thickness of wax.

The roots were placed in a mould so that a 1-mm space was left between the top of the acrylic resin mould and the facial and lingual root surfaces. The teeth were then removed from the mould before the resin was completely cured. To eliminate the wax, the teeth were heated to 70°C for 10 s and then dried. With this simulated tooth/root model, a putty/wash system was used to simulate the PDL. After eliminating the wax coating, all tooth outer surfaces were impregnated with the prepared wash (Spidex, Coltene AG, Altstatten, Switzerland) between the tooth and self-cured acrylic resin. The teeth were positioned in the acrylic resin so that 1 mm of each tooth protruded above the resin. With this method, a uniform 0.2-mm layer of wash surrounded the root. Finally, the samples were placed in a fully computerized Zwick Machine (model Z250; Zwick, Ulm, Germany) and placed under force with a cross-head speed of 1 mm/min until the tooth samples failed. The analysis of data was performed using one- and two-way analysis of variance tests (ANOVA). The SPSS v11.5 (IBM, Chicago, USA) software was used for analysis where $P < 0.05$ was considered significant.

Results

The mean compressive force, in relation to the amount of time, required to break the samples in the experimental and control groups is shown in Fig. 1. The results showed that the average force required to cause breakage in the study groups was significantly different from the control group ($P < 0.001$). In addition, the mean force needed to cause breakage at different times was significant ($P < 0.001$). However, because of the existence of an interactive effect between the study groups and the time needed for the required force for breakage, the groups were compared based on the individual time of their test ($P = 0.003$).

Table 1 shows that the mean force needed to cause a fracture was significantly different between the two groups on the first, third and sixth months ($P = 0.001$, $P < 0.001$, $P = 0.035$, respectively), and the amount of force necessary for a fracture was higher in the control group. However, in the 1-week and the twelve-month groups, this difference was not significant ($P = 0.075$ and $P = 0.095$, respectively).

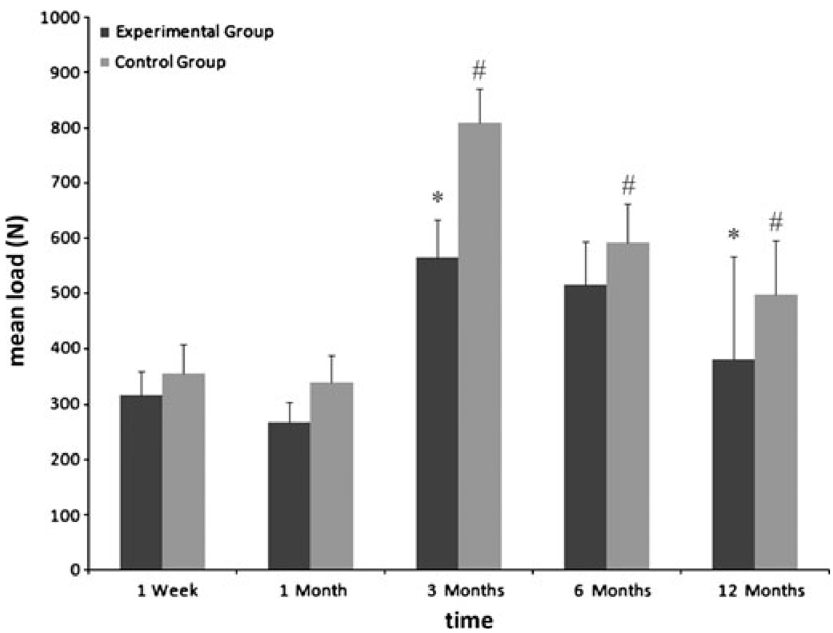

Fig. 1. The mean compressive force required to fracture the experimental and control group at various time periods. *above the bars in experimental group and #above the bars in the control group indicate that mean compressive force required to fracture was significantly different from the force required in comparison with the previous period of time.

Table 1. Comparison between the groups based on time

Time	Control group Standard deviation ± Mean (*N*)	Experimental group Standard deviation ± Mean (*N*)	*P* value
1 Week	356.91 ± 51.16	316.63 ± 43.90	0.075
1 Month	340.23 ± 48.04	267.34 ± 35.79	0.001
3 Months	808.81 ± 61.98	565.08 ± 68.87	<0.001
6 Months	591.92 ± 70.53	516.06 ± 78.31	0.035
12 Months	497.66 ± 97.84	380.98 ± 185.159	0.095
P value	<0.001, *F* = 79.33	<0.001, *F* = 16.85	

Discussion

The aim of this study was to examine the possible adverse effect of long-term $Ca(OH)_2$ therapy on human root dentin fracture strength.

In this study model, to reduce the influence of varieties between different tooth samples, single-canal human premolar teeth were used, in which all canals had a similar canal width, as assessed radiographically, and were sectioned at a distance of 17 mm from the teeth apices.

A 0.2-mm thickness of putty-based wash was used to surround the teeth, in a similar manner to that of Coolidge (15) study, who found the approximate thickness of PDL to be 0.21 mm during adolescence. This rubber-based material was used to simulate the physiological spacing found clinically between bone and root and to imitate the cushioning effect of the natural PDL.

Torres et al. (16) examined three methods for placing $Ca(OH)_2$: an Ultradent (South Jordan, UT, USA) tip, a Lentulo #35 spiral (Henry Schein, Melville, NY, USA) after injection with an Ultradent tip or Lentulo #35 alone methods, and concluded that at 1 mm from the canal terminus, the Lentulo was significantly better than the other two techniques. Also at three millimetre, the Lentulo technique and the combined technique were more effective than the Ultradent tip alone for $Ca(OH)_2$ paste placement. Therefore, a Lentulo instrument was used to place the $Ca(OH)_2$ in this study.

In this experiment, the $Ca(OH)_2$ was replaced at each time period. This model may duplicate more closely to the clinical application of $Ca(OH)_2$ and correspond to the periods recommended for replacing the dressing in clinic and also to ensure that fresh $Ca(OH)_2$ is always present in the canal. In addition, Abbott (17) found that radiographs are unable to reliably detect the amount of calcium hydroxide remaining in the canal and showed several advantages of regular replacement of $Ca(OH)_2$ such as clinical assessment of barrier formation and increasing the speed of bridge formation and apexification.

The results of this study suggest that exposure to $Ca(OH)_2$ for an extended time weakens root structure. Several studies have shown that long-term exposure to $Ca(OH)_2$ will change the organic matrix of tooth structure, and therefore alters the mechanical properties of dentin (12, 18). It has been shown that $Ca(OH)_2$ tends to dissolve soft tissue, and this action takes place by denaturation and hydrolysis of the pulp tissue (12). In another study, Nerwich et al. (19) have concluded that the interaction of collagen fibres and hydroxyapatite crystals is disrupted by the exposure to $Ca(OH)_2$ and so alters physical properties of dentin.

In 2002, Andreasen et al. (18) found that the use of $Ca(OH)_2$ over 2 weeks or at 1, 2, 3, 9 or 12 months increased the incidence of root fracture. Therefore, in this study, these time periods were selected because they represent the typical durations between $Ca(OH)_2$ replacements in the clinical situations.

When the mean and standard deviation were compared for the fracture load at the desired time period between the control and experimental groups, the fracture load for the control group was significantly higher than in the experimental group at months 1, 3 and 6

($P < 0.001$). These results indicate that $Ca(OH)_2$ generally increased tooth fragility regardless of the time duration between replacements. In addition, Rosenberg et al. (14) showed that $Ca(OH)_2$ caused a reduction in the resistance to breakage and reduced the load to fracture of teeth. They found a significant difference between the fracture strength of the calcium hydroxide-filled treated group after 84 days and the gutta percha-filled group as a control, but this difference was not significant after 7 days. The same results were obtained from this study.

The results of this study indicate that increased fragility in the tooth containing $Ca(OH)_2$ in the short-term group (1 week) was numerically higher but statistically insignificant; however, in longer treatment durations (1, 3 and 6 months), the fragility was numerically higher and statistically significant. These results indicate that using $Ca(OH)_2$ for more than 1 month increases the fragility of teeth. Andreasen et al. (18) hypothesized that the proteolytic activity of $Ca(OH)_2$ may weaken the root walls by up to 50%, and this effect can cause the tooth root to be susceptible to fracture. However, the study by Andreasen used immature mandibular sheep incisors (18), whereas this study has used mature human premolar teeth. Moreover, the control group in the Andreasen study was comprised of teeth that had been stored in saline for only 2 months. In contrast, this study had control groups that matched the same duration of time between replacements as the respective experimental groups.

The study by Andreasen et al. (18) indicated that time is a critical factor for the effects of $Ca(OH)_2$ and that if used for 1 year, will reduce tooth fracture resistance by 50%. The general effect of time as a factor in the current study is comparable to the results of the Andreasen study, and the results shown here indicate that time is a critical factor for the effects of $Ca(OH)_2$. However, considering the existence of differences between each group, it cannot be unequivocally concluded that $Ca(OH)_2$ caused increased fracture commensurate with increased time. In the Andreasen et al. study (18), the difference between control group and 1-year $Ca(OH)_2$-treated group was significant. This result was not duplicated in the present study. The different results may be attributed to methodological factors, such as repeated replacement of fresh $Ca(OH)_2$ at each period in this study, while Andreasen et al. left the dressing in place for 1 year. Also in their study, the experimental and control samples did not match compared with the duration of incubation before exposure to the force.

It is important to note that the observed increase in fracture resistance in both the control and experimental groups at the third month compared to the first month is inexplicable in the light of the current hypothesis of this study. Although it was found that the amount of time between replacement of $Ca(OH)_2$ was a significant factor in the effects of $Ca(OH)_2$, further studies are needed utilizing shorter time periods between $Ca(OH)_2$ replacement to demonstrate the importance of time with certainty. Further, other studies with *in vivo* models are required to validate these results in the clinical situation.

Conclusions

This investigation highlighted the potential disadvantage of $Ca(OH)_2$ as a medicament for intracanal treatment as it would seem reduce tooth fracture resistance. This study has shown that under *in vitro* conditions, using $Ca(OH)_2$ for periods longer than 1 month may reduce tooth fracture resistance.

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