Effect of Irrigants and Cementum Injury on Diffusion of Hydroxyl Ions through the Dentinal Tubules

Sherma Saif, DMD, MS, Clifton M. Carey, PhD,† Patricia A. Tordik, DMD,* and Scott B. McClanahan, DDS, MS**

Abstract

This study measured hydroxyl ion diffusion through dentinal tubules into a bathing solution. Eighty single-canal, instrumented teeth were divided into 8 groups. Control groups 1 and 3 were irrigated with 10 mL 0.9% saline and 10 mL 6% sodium hypochlorite (NaOCl), respectively. Control groups 5 and 7 were irrigated with 3 mL and 1 mL 17% ethylenediaminetetraacetic acid (EDTA) and then 10 mL 6% NaOCl, respectively. Experimental groups 2, 4, 6, and 8 were irrigated as groups 1, 3, 5, and 7, followed by placement of calcium hydroxide ($Ca(OH)_2$) into canals. Bathing solution pH was recorded for 30 days, a cementum defect was made, and then pH was recorded for another 30 days. With a paired difference test, average pH during steady state was statistically different and higher after the defect ($P < .001$). With Tukey multiple comparisons, post-defect pH for group 6 was found to be significantly greater ($P < .01$) than in other groups. This study indicated final canal irrigation with 3 mL 17% EDTA and 10 mL 6% NaOCl before $Ca(OH)_2$ placement allowed the greatest hydroxyl ion diffusion to the root surface. *(J Endod 2008;34:50–52)*

Key Words

Calcium hydroxide, diffusion, ethylenediaminetetraacetic acid, hydroxyl ions, smear layer

From the *Naval Postgraduate Dental School, Bethesda, Maryland; and †Paffenbarger Research Center, American Dental Association Foundation, National Institute of Standards and Technology, Gaithersburg, Maryland.

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Address requests for reprints to Dr Patricia A. Tordik, 8617 Augusta Farm Lane, Laytonsville, MD 20882. E-mail address: patricia.tordik@med.navy.mil.

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The prognosis for avulsed or luxated teeth is unfavorably affected by surface resorption, inflammation, or replacement resorption after replantation, repositioning, and stabilization (1, 2). In external inflammatory root resorption, the cementum and outer layer of dentin are resorbed. Pulp extirpation and introduction of calcium hydroxide ($Ca(OH)_2$) as an intracanal medicament within 7–10 days has been recommended to prevent or stop resorption (3, 4). Tronstad et al. (3) suggested that $Ca(OH)_2$ can influence local root resorption by increasing pH. Diffusion of hydroxyl ions through dentinal tubules and production of an alkaline environment in areas of resorption induce necrosis of osteoclasts and can inhibit inflammatory root resorption in monkeys (5).

To prevent and treat external root resorption, $Ca(OH)_2$ must diffuse through dentin. Deardorf et al. (6) demonstrated the rate of $Ca(OH)_2$ diffusion differed according to tooth. Another study showed that hydroxyl ions did not significantly diffuse through an intact root surface (7). Foster et al. (8) observed that removal of smear layer with 10 mL of 17% ethylenediaminetetraacetic acid (EDTA) before $Ca(OH)_2$ placement might facilitate ion diffusion.

The smear layer is an amorphous structure consisting of dentin, odontoblast processes, pulp tissue, and bacteria (9). It is found on canal walls of instrumented teeth and was measured to be 1–2 μm thick, friable, loosely adherent, and packed up to 40 μm into dentinal tubules (10). An effective method of smear layer removal from canal walls used 10 mL of 17% EDTA followed by 10 mL of 5.25% sodium hypochlorite (NaOCl) (11). Recommendations of contact time and irrigation volume required to effectively remove the smear layer by a chelating agent vary. Goldberg and Spielberg (12) suggested 15 minutes as the optimal working time for smear layer removal with EDTA and 0.84 g of a quaternary ammonium bromide (EDTAC). Zaccaro et al. (13) reported 20 mL of EDTA irrigation for 3 minutes as effective. It was also reported that 10 mL of 17% EDTA irrigation for 1 minute was effective, whereas 17% EDTA for 10 minutes caused dentin erosion (14). Crumpton et al. (15) demonstrated effective smear layer removal with a final 1-minute rinse of 1 mL of 17% EDTA followed by 3 mL of 5.25% NaOCl.

To date, no study has reported a $Ca(OH)_2$ diffusion rate through radicular dentin when using reduced volumes of EDTA to remove the smear layer. The purpose of this study was to measure hydroxyl ion diffusion through dentinal tubules after root canal smear layer removal by using 1 or 3 mL 17% EDTA and then 10 mL 6% NaOCl before and after a cementum defect was made on a root surface.

Materials and Methods

Eighty anterior and premolar human, permanent teeth stored in 0.2 % sodium azide were decoronated at the cementoenamel junction with a #557 carbide bur (Henry Schein, Melville, NY) in a high-speed hand piece with water spray. Presence of a single root canal was verified with 2 digital radiographs (Schick Technologies, Long Island City, NY) in mesiodistal and buccolingual directions. A #10 FlexoFile (Dentsply Maillefer, Johnson City, TN) was placed in the canal until visible at the apical foramen and patency was verified. One millimeter was subtracted to establish working length. With a crown-down technique, all teeth were cleaned and shaped with ProFile 0.04 rotary instruments (Tulsa Dental, Tulsa, OK) to a master apical size #40. All were irrigated with 5 mL of 0.9% sodium chloride, USP (Baxter Healthcare Corp, Deerfield, IL) (saline) during canal instrumentation by using a 30-gauge Max-i-Probe (Dentsply Rinn, Elgin, IL), placing the irrigation tip 1 mm from working length. All canals were

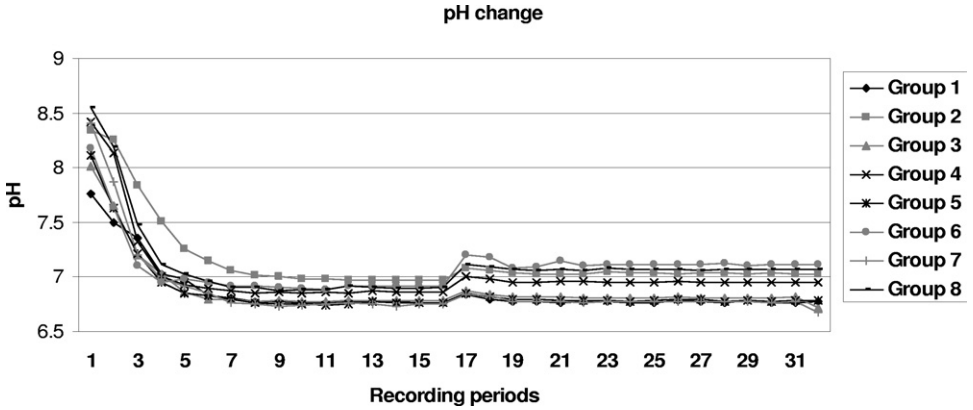

Figure 1. Average of pH change for all groups at each recording period. All groups containing $Ca(OH)_2$ had a higher pH at pre-defect and post-defect steady state. The cementum defect was placed immediately after the 16th recording period.

dried with paper points. When $Ca(OH)_2$ was used, 20 g of $Ca(OH)_2$ powder (Henry Schein, Melville, NY) was mixed with 20 mL of saline and then placed into canals by using a lentulo spiral size #35 (Henry Schein, Melville, NY). Teeth were randomly divided into 8 groups of 10 each: group 1 (S), 10 mL saline irrigation; group 2 (SC), canals irrigated as group 1, $Ca(OH)_2$ placed; group 3 (N), 10 mL 6% NaOCl (Chlorox Company, Oakland, CA) irrigation; group 4 (NC), canals irrigated as group 3, $Ca(OH)_2$ placed; group 5 (E3N), 3 mL 17% EDTA (Pulpdent Corporation, Watertown, MA) followed by 10 mL 6% NaOCl irrigation; group 6 (E3NC), canals irrigated as group 5, $Ca(OH)_2$ placed; group 7 (E1N), 1 mL of 17% EDTA followed by 10 mL of 6% NaOCl irrigation; and group 8 (E1NC), canals irrigated as group 7, $Ca(OH)_2$ placed.

Each tooth in groups 5 (E3N), 6 (E3NC), 7 (E1N), and 8 (E1NC) had a 1-minute contact time with 17% EDTA. Coronal access was sealed with a cotton pellet and 2 mm of Cavit (3M ESPE, St Paul, MN). The external coronal and apical 3 mm of each tooth was sealed with heated utility wax. Each tooth was rinsed with 10 mL of deionized water and placed in a covered glass vial containing 15 mL of pH near neutral, deionized water from the same 2-L container. The water pH was measured at 24 hours and every 48 hours subsequently for a total of 30 days. To simulate external resorption damage, a defect was made on the buccal or lingual, mid-root surface of each tooth with a #35 (1 mm long $\times$ 1 mm wide) bur (Henry Schein, Melville, NY) at a depth sufficient to remove cementum. Each defect was irrigated with 1 mL of 17% EDTA (pH 8.0), followed by 10 mL of deionized water to remove inorganic smear created by the bur. Each tooth was replaced in its vial without changing the solution. Again, pH was measured at 24 hours and every 48 hours for a total of 30 days. The pH changed slowly, and more frequent measurements were not needed. The 30-day observation times were chosen to ensure the solution had reached steady state. An Accumet AR15 pH meter (Fisher Scientific, Pittsburgh, PA) calibrated to pH values of 4, 7, and 10 at each session measured pH. Each vial was stirred for 10 seconds, an electrode was placed in the solution, and pH was recorded after it stabilized. The electrode was dried with Kimwipes (Kimberly-Clark Corp, Roswell, GA) between measurements. The observed standard deviation was assumed to be the standard uncertainty for the pH measurements. Statistical comparisons of pH were done by analysis of variance and Tukey multiple comparisons.

Results

The steady state of the pH for all groups was calculated from the 9th to the 16th recording period before introducing the defect and from the 19th to the 31st recording period after defect. Correlation analysis at steady state confirmed that pH of the bathing solution was not changing. With a paired difference test, the average pH during steady state before the defect was statistically different and lower than steady state pH after the defect ($P < .001$) for all experimental groups. The control group was not different ($P = .432$) (Fig. 1). The greatest increase in pH was for group 6, which was significantly greater than any other group ($P < .01$). With Tukey multiple comparisons ($P < .05$), the order of change was group $6 > 8 > 4 > 2 > 3 \sim 5 \sim 7 >> 1$ (Table 1). The $>$ indicates a significant difference at $P = .05$, $\sim$ indicates no difference at $P = .10$, and $>>$ indicates a significant difference at $P = .001$.

Discussion

Diffusion rate appears to be related to type and volume of irrigant. Different irrigants have different abilities to bond and react with organic and inorganic dentinal debris. The volume of irrigant affects gross debris removal (16). A faster flushing rate of EDTA rinse (3 mL/min) removes more chemically reacted smear layer from the canal than a slower rate (1 mL/min). The faster rate quickly refreshes the available EDTA solution in the canal, resulting in greater diffusion of hydroxyl ions and greater pH change. Crumpton et al. (15) found that higher volume flushes of root canals such as 10 mL/1 min were difficult to achieve clinically and demonstrated effective smear layer removal with reduced volumes of EDTA followed by NaOCl.

The average pH for samples at recording periods 1–7 ranged from 7.75 in group 1 to 8.6 in group 8. Variation can be explained by noting that samples were different teeth, from different de-identified sources, and stored for different time periods. All samples did not meet the same average pH during the experiment. However, more importantly, every

TABLE 1. pH [Mean (standard deviation), n = 10] of Each Group at Pre-defect and Post-defect and Change in pH for Each Group

Group	Identifier	Pre-defect pH	Post-defect pH	Change in pH*
1	S	6.77 (0.004)	6.77 (0.01)	0.002 (0.006)
2	SC	6.98 (0.01)	7.03 (0.01)	0.054 (0.008)
3	N	6.78 (0.003)	6.81 (0.004)	0.030 (0.004)
4	NC	6.86 (0.01)	6.95 (0.005)	0.092 (0.005)
5	E3N	6.76 (0.01)	6.79 (0.01)	0.029 (0.007)
6	E3NC	6.91 (0.1)	7.11 (0.01)	0.203 (0.013)
7	E1N	6.75 (0.01)	6.77 (0.004)	0.028 (0.006)
8	E1NC	6.89 (0.02)	7.07 (0.01)	0.173 (0.009)

*For all experimental groups, the average pH during steady state before the defect placement was statistically different and lower than the steady state pH after introducing the cementum defect. Paired difference test ($P < .001$).

sample did reach steady state before a defect was made. A small bur was large enough to remove cementum that has a reported mean mid-root width of 87.55 ± 3.58 μm in nondiabetic patients (17). It is unknown whether smear layer is present on external root resorption defects in vivo. Therefore, we chose a 17% EDTA rinse, pH 8.0, to remove any inorganic smear created after nicking the cementum with a bur. A slight pH rise was observed at recording period 17 in all groups. It is possible that the deionized water flush did not completely wash away the EDTA, but all groups did return to steady state, and the average pH after introducing the defect was statistically greater.

In a study by Vogel et al. (18), enamel and dentin were shown to have a positive membrane potential and cementum a negative membrane potential charge of −0.8 mV ± 8.2 mV. This chemical property can hinder negatively charged hydroxyl ion diffusion through dentin. When there is a defect on the root surface, a disruption in the negative membrane potential of cementum could allow faster ion diffusion. In another in vitro analysis, the mean pH of 40 mL of distilled water surrounding teeth packed with $Ca(OH)_2$ did not significantly change from the original solution pH (19). Unlike the present study, Fuss et al. (19) did not remove cementum and used a larger volume of water, which had a dilution effect on the pH.

The results of this study are in agreement with the findings of Foster et al. (8), who demonstrated that smear layer removal with 10 mL of 17% EDTA and 10 mL of 5.25% NaOCl facilitated hydroxyl ion diffusion through dentinal tubules. In our study, irrigating with reduced volumes of 17% EDTA provided a similar outcome of enhanced hydroxyl diffusion rate. All groups with EDTA as an irrigant allowed hydroxyl ion diffusion significantly better than other groups. Under the parameters of this study, facilitating the removal of smear layer with a final 1-minute irrigation of 3 mL 17% EDTA and 10 mL 6% NaOCl significantly enhanced the diffusion rate of hydroxyl ions when a cementum defect was present. Further investigation is needed to evaluate the effect of smear layer on ion diffusion through dentinal tubules.

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