original article

An In Vitro Comparison of Quantitative Dissolution of Human Pulp in Different Irrigating Solutions

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ABSTRACT

Pulp tissue from freshly extracted, intact vital premolars was removed in toto *and was cut to get an approximate weight of 8.2 mg for each sample. Eighty samples thus obtained were divided into 4 groups of 20 samples each according to the irrigating solution used (5.25% NaOCl, 17% EDTA, BioPure MTAD and Distilled water (control group). Pulp tissue of specified equal weight (8.2 mg) was placed into each test tube of all groups carrying irrigants of measured volume (5ml each) at 37°C according to their specified subgroups time interval i.e. 10 min, 15 min, 20 min and 30 min respectively. The solution from each sample test tube was filtered and was left for overnight drying. The residual weight was calculated by filtration method. Results showed that the maximum amount of pulp was dissolved by 5.25% NaOCl at all time intervals. MTAD and 17% EDTA showed almost similar dissolution at all the time intervals.*

KEY WORDS

Irrigation, Filtration, Pulp

J Oral Health Comm Dent 2010;**4**(2):28-33

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INTRODUCTION

Advancements in techniques and materials have revolutionized the practice of endodontics. This has led to an increase in the success rate of endodontic treatment over the last few decades. Chemical preparation/mechanical preparation binomial forms the key requisite for the success of root canal instrumentation. The objective of these two interdependent factors consists of the cleaning of the canal and its eventual ramifications and removal of organic and bacterial debris in order to establish ideal conditions, which allow a functional recuperation of the dental organ and a regeneration of tissues eventually injured by infection.

Irrigation is an indispensable aid in achieving thorough debridement and in preparing and disinfecting the canal(1). In addition to the debriding action, irrigation serves the purpose of facilitating instrumentation by lubricating the canal walls and flushing out dentinal filings (2). The tissue-dissolving capability of any irrigating solution is important, because it potentially enhances root canal cleansing by removing pulpal remnants from the canal, particularly from inaccessible areas never contacted by endodontic instruments. The contact time of an irrigant is limited and the vascularity of vital tissue resists the action of certain irrigants (3). Therefore the speed/time of dissolution of vital tissue by different irrigants is an important factor which needs to be considered.

Sodium hypochlorite (NaOCl) has been the favored endodontic irrigant due to its antimicrobial action and its ability to dissolve organic tissue debris. But sodium hypochlorite has many limitations like significant toxicity when injected into periradicular tissues, disagreeable smell and taste, corrosion of metal instruments, inability to disinfect the root canal system consistently and it does not completely remove the smear layer from the dentin walls. Because of these limitations, the search for a better root canal irrigant continues.

Ethylenediaminetetraacetic acid (EDTA) is an insoluble, odourless, crystalline white powder. Ostby found that it has certain dentin dissolving effects desirable in all kinds of root canal therapy (4). This reduces the time necessary for debridement and aids in enlarging narrow or obstructed canals. It has been shown that EDTA has a little capacity to dissolve soft tissue (5).

Recently, a new irrigant available as BioPure MTAD has been introduced. This irrigant contains 3% doxycycline, 4.25% citric acid and Tween 80 detergent (6). It is used as the final rinse after initial rinsing with 1.3% NaOCl as recommended by manufacturers. Based on extensive well-conducted studies, MTAD has been shown to be a clinically effective, biocompatible and less erosive endodontic irrigant with sustained antibacterial activity (7, 8).

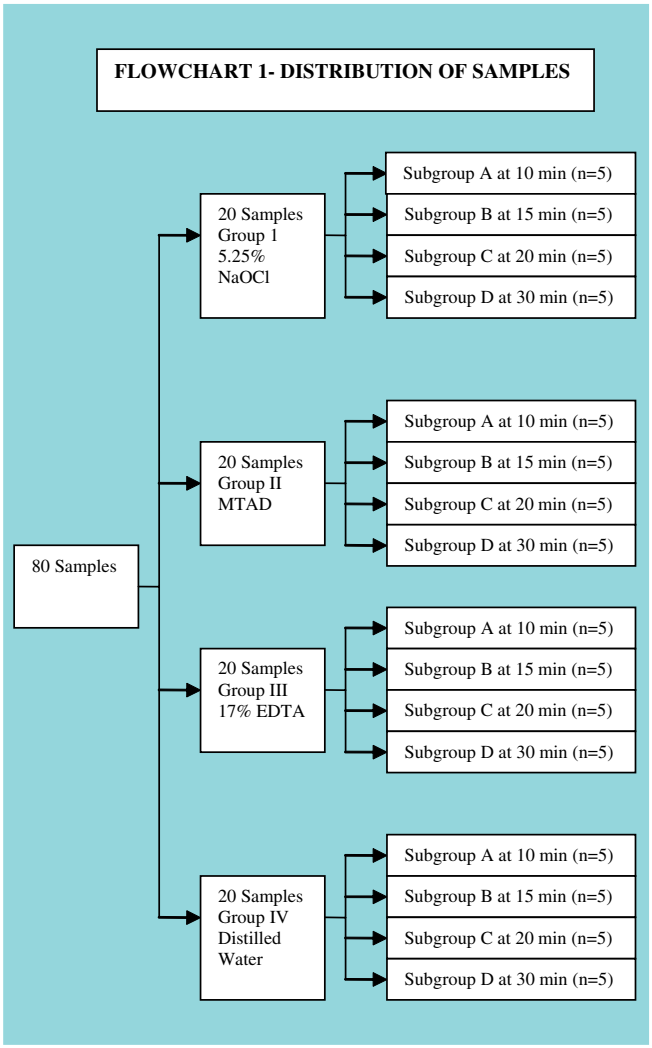
Most of the previous studies which have been done on pulp tissue dissolution have assessed dissolution of one of the component of pulp like hydroyproline, total phosphate extraction or loss of tissue collagen (9, 10). But very few studies have quantitatively assessed the total human pulp dissolution in toto by the irrigants. This in vitro study was conducted to evaluate the ability of three irrigating solutions to dissolve human pulp quantitatively by filtration method.

MATERIALS AND METHOD

Freshly extracted, intact vital premolars, extracted for orthodontic reasons were collected from the Department of Oral and Maxillofacial Surgery and freezed until required. Any carious or fractured teeth were discarded. The teeth were thawed to room temperature. Two longitudinal grooves on the proximal surfaces of the teeth were made with round bur. The teeth were split into two halves with chisel and mallet. Pulp tissue was removed in *toto*. It was cut with the 15 no. BP blade and placed on a preweighed filter paper. The weight of freshly extracted pulp was standardized to 8.2 mg. Eighty samples of standardized weight (8.2 mg) were taken. The samples were divided into different groups and subgroups as shown in Flowchart 1.

Eighty test tubes were taken in total, twenty for each group:

- **In Group I** - 20 test tubes were filled with measured volume (5 ml each) of 5.25% NaOCl.
- **In Group II** - 20 test tubes were filled with measured volume (5 ml each) of MTAD.
- **In Group III** - 20 test tubes were filled with measured volume (5 ml each) of 17% EDTA.



- **In Group IV** - 20 test tubes were filled with measured volume (5 ml each) of Distilled water.

Each group had 4 subgroups having 5 test tubes each according to different time period for which the sample is immersed in the irrigant. Pulp tissue of specified equal weight (8.2 mg) was placed into each test tube of all groups carrying irrigants of measured volume (5ml each). These were then kept in an incubator at 37°C according to their specified subgroups time interval that is 10 min, 15 min, 20 min and 30 min respectively. After the passage of specified time, the solution from each sample test tube was filtered through a Wattman filter paper. This was followed by overnight drying of the filter paper. After overnight drying, the weight of the dried filter paper was measured. The difference in the weight of the dried filter paper (with residue) and initial filter paper (before filtration) gave us the weight of residue left subsequent to filtration which is the sum of the weight of pulpal residue and irrigating solution residue. The similar procedure was carried out for all the samples.

Weight of Wattman filter paper was measured in an analytical balance. Irrigating solution (5 ml) was filtered through a preweighed filter paper. This was followed by overnight drying of the filter paper. Weight of the dried filter paper was taken. The difference in the weight of the dried filter paper and initial filter paper (before filtration) gave the dry weight of residue of that irrigating solution. This procedure was repeated for irrigants of all groups.

Weight of the residual pulp left on the filter paper of each sample was calculated by subtracting the weight of residue of respective irrigating solution from the weight of the total residue on filtration paper, measured earlier. Difference in the weight of pulp tissue before immersion and the weight of residual pulp left on the filter paper gave the amount of pulp dissolved by the respective irrigant of that group. Similarly readings for all samples of each subgroup were taken. Thus, by filtration method, the amount of pulp dissolved by various irrigating solution at different time intervals was measured quantitatively.

Statistics

The mean dissolution of pulp tissue by different groups at different time intervals was statistically analyzed by Kruskal Wallis test. The intergroup comparison at different time intervals was statistically analyzed by adjusted Mann-whitney test with Bonferroni correction and overall Kruskal Wallis test. All the groups were compared at 10 min, 15 min, 20 min & 30 min respectively.

RESULTS

The mean pulp dissolution was found to increase with passage of time for group I, II & III. Group IV did not show any pulp dissolution at all. It was found that there was statistically significant difference between Group I and Group II, Group I and Group III, Group I and Group IV, Group II and Group IV, Group III and Group IV with $p < 0.05$ at all time intervals. There was no significant difference between the amount of pulp dissolution in group II and group III at all time intervals. (Table 1)

Table 1 : Mean±SD and median values comparison at all time interval

TIME ! Type of Solution !	10 min	15 min	20 min	30 min
5.25% NaOCl(Group I)	2.72 ±0.16 mg(2.8 mg)	2.98 ± 0.14 mg(3 mg)	4.12 ± 0.08 mg(4.1 mg)	6.14 ± 0.11 mg(6.1 mg)
MTAD(Group II)	1.56 ± 0.11 mg(1.6 mg)	1.76 ± 0.11 mg(1.8 mg)	2.06 ± 0.08 mg(2 mg)	3 ± 0.15 mg(3 mg)
17%EDTA(Group III)	1.58 ± 0.13 mg(1.5 mg)	1.86 ± 0.05 mg(1.9 mg)	2.1 ± 0.07 mg(2.1 mg)	3.06 ± 0.05 mg(3.1 mg)
Distilled water(Group IV)	0	0	0	0

DISCUSSION

Chemomechanical preparation forms the key requisite for the success of root canal instrumentation. Complete debridement and disinfection of complex root canal system is highly dependent on the mechanical and chemical effects of the irrigants. Callahan (1894) and Grossman (1941) demonstrated the importance of the solvent ability of endodontic irrigant and emphasized the elimination of pulp tissue from the root canal (11, 12).

Therefore in this in vitro study, we evaluated the pulp dissolution capability of four irrigating solutions i.e. 5.25% sodium hypochlorite, 17% EDTA and distilled water using filtration method. Freshly prepared sodium hypochlorite solution (5.25%) was used in this study as at high concentrations, NaOCl is unstable. Different methods have been used during the past years for assessing the pulp dissolution like analyzing the hydroxyproline content (13), measuring the amount of total phosphate extracted, loss of tissue collagen (14) and dissolution by agitation method(15, 16). Hydroxyproline content is 13% of the collagen content of pulp. Therefore analyzing the tissue dissolution based on any single content was one of the drawbacks of most of the methods used in previous studies as they did not reflect the dissolution of the whole tissue. Moreover, irrigating solutions interfere with total protein assays and with hydroxyproline determination. Agitation can also affect the properties of the tissue (15) and the irrigant as used in other methods. Therefore in our study a simple, yet reliable, dry weight method was used to quantify specimen dissolution.

Bovine pulp, Human umbilical cord, porcine muscle, rat dermal connective tissue and rabbit liver have been used in previous studies to determine the efficacy of various root canal irrigant but the results of these studies cannot be accurately compared or related directly to the clinical conditions. Very few studies have been done on human pulp tissue dissolution in the literature. So we selected vital human pulp tissue and its dissolution by irrigants was checked at 37°C to simulate oral conditions. We selected analytical balance in our study for the measurement of weight because of its high degree of precision.

For standardization, equal amount of pulp tissue was obtained and was immersed in test tube containing specific irrigant. To simulate clinical conditions these test tubes were kept in the incubator at 37°C for respective time periods. The solution from all sample test tubes was filtered through a preweighed filter paper and was left for overnight drying to obtain residue left after filtration. One step in our study which has not been taken into consideration in previous studies is the weight of the residue of irrigating solution. In the pilot study we found that irrigating solution itself leaves considerable residue, which needs to be reduced from the total residue obtained in order to get accurate reading of the residual pulp. Readings were taken at each step of our methodology and weight of the residual pulp was obtained after subtracting weight of residue of irrigant from the total residue.

Mean percentage of pulp dissolution by Group I (5.25% NaOCl), Group II (MTAD), Group III (EDTA) was about 33%,19%, 19% respectively after 10 min, 36%, 22%, 22% after 15 min, 50%, 25%, 25% respectively after 20 min, 75%, 37%, 37% of the pulp respectively after 30 min (Table 1). The amount of pulp dissolved increased with the passage of time and was maximum at 30 min time interval. This is in accordance with the studies of other authors who have studied the dissolution capacity of NaOCl. (9, 12, 16, 17, 18, 20). **Beltz et al** did quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite and EDTA on bovine pulp & dentin (15). They found that 5.25% & 2.6% sodium hypochlorite were

the most efficient pulp solubilizers with~90% dissolution at 2 hrs while 55% dissolution of pulp tissue was observed by MTAD or EDTA in 2 hrs. The increased dissolution of pulp by NaOCl in their study as compared to this study is because of longer time period used and also because of agitation employed in their method. Moreover they had pulverized the sample tissue. Grawehr et al evaluated the interactions of EDTA alone and with sodium hypochlorite(5). They concluded that EDTA has little capacity to dissolve soft tissue. They found that at 30 min EDTA was able to dissolve just ~15% of the soft tissue as compared to ~37% in this study. This difference was due to the fact that they used palatal mucosa and the total amount of sample tissue was quantatively more (80 mg) as compared to 8.2 mg in our study. The relevance of the amount of organic matter, surface area has been shown by Moorer and Wesselink (19). Group IV (Distilled water) showed no dissolution of the pulp tissue at all time intervals. This is in agreement with the study by **Gordon et al** who confirmed that distilled water is an ineffective solvent of vital tooth pulp (20).

When Group I (5.25% NaOCl) and Group II (MTAD) were compared at all time intervals, 5.25% NaOCl showed better pulp dissolution with significance value $p<0.05$. Our results concur with the results shown by Beltz *et al*(15). They found that 5.25% sodium hypochlorite was efficient pulp solubilizer than MTAD. The dissolution property of NaOCl is due to the fact that when sodium hypochlorite comes in contact with organic material, several chemical reactions take place, i.e. fatty acids react with sodium hydroxide creating soap and glycerol (saponification reaction), amino acids react with sodium hydroxide creating salt and water (neutralization reaction) and also it react with hypochlorous acid creating chloramine and water. These reactions occur simultaneously and synergistically leading to liquefaction of organic tissue. Pulp dissolution by MTAD has been attributed to its acidic pH (2.15) and also may be due to its ability to dissolve the calcium and phosphate content present in the pulp tissue. When Group I (5.25% NaOCl) and Group III (17% EDTA) were compared at all time intervals, 5.25% NaOCl showed better pulp dissolution with significance value $p<0.05$. Our results are in accordance to the study done by Grawehr. He evaluated the interactions of EDTA alone and with sodium hypochlorite and found that NaOCl is a better tissue solvent than EDTA. They concluded that EDTA has little capacity to dissolve soft tissue. Beltz *et al* also found that 5.25% sodium hypochlorite was more efficient pulp solubilizer than EDTA (15). The better dissolution of NaOCl is because of organic solvent action as described earlier. Pulp dissolution by EDTA could be due to to its ability to dissolve the calcium and phosphate content present in the pulp tissue.

When Group II (MTAD) and Group III (17% EDTA) were compared at all time intervals, insignificant difference in pulp dissolution with $p>0.05$ was found. This is in accordance with the study by **Beltz et al** who had compared pulp dissolution between MTAD and 17% EDTA (15). They found that the solubilizing effects of MTAD on pulp were somewhat similar to those of EDTA.

Based on the results of our study, 5.25% NaOCl is the most effective pulp tissue solvent whose action increases with time. Further clinical studies are required to substantiate the findings of this study.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn-:

- The most effective pulp solubilizer was 5.25% NaOCl; followed by MTAD and 17% EDTA.MTAD and 17% EDTA had almost similar dissolution. Distilled water was an ineffective solvent of pulp tissue.
- The amount of pulp tissue dissolved increased with the passage of time in all the groups. Minimum pulp tissue dissolution was seen at 10 min and maximum at 30min time interval.
- Prolonged irrigation with 5.25% NaOCl is recommended for better tissue solvent action.
- EDTA or MTAD alone are incapable of solubilizing pulp tissue. Therefore, they should always be used in conjunction with NaOCl.

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