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RESEARCH ARTICLE

TISSUE DISSOLUTION CAPACITY OF SODIUM HYPOCHLORITE IN COMBINATION WITH DIFFERENT CHELATING AGENTS – AN INVITRO STUDY

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ABSTRACT

Background/Introduction: Irrigation of root canal system is one of the most important steps in endodontic treatment. Combining different irrigants may modify the efficiency of the irrigation in removing organic and inorganic debris during biomechanical preparation.
Aim/Objective: The aim of the study was to determine the chemical interaction that occurs during combination of various irrigating solutions and its effect on tissue dissolution.
Materials : Muscle tissue samples of equal dimensions with their initial weights were taken and the samples were immersed in saline, sodium hypochlorite, ethylene diamine tetra acetic acid(EDTA), etidronic acid/1-hydroxyethane 1,1-diphosphonic acid(HEDP), ethylene glycol bis –N N N N' – tetraacetic acid(EGTA)separately and in combinations of these agents for 5, 10 & 15 minutes.
Results: Maximum tissue dissolving capacity was shown by sodium hypochlorite followed by sodium hypochlorite and HEDP combination, sodium hypochlorite and EGTA combination, sodium hypochlorite and EDTA combination. HEDP, EGTA, EDTA, and saline solutions when used alone did not show any tissue dissolving property.
Conclusion: Sodium hypochlorite either alone or in combination with HEDP and EGTA maintains adequate available chlorine which could be the reason for its high efficiency.

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INTRODUCTION

Success of root canal therapy depends on effective chemo mechanical debridement of pulpal tissue, dentin debris and microorganisms (Irala *et al*., 2010). Because of complex anatomy of root canals 30-60% remain uninstrumented resulting in insufficient debridement, providing source of nutrition for surviving bacteria (Naenni *et al*., 2004). Irrigation allows for cleaning beyond, which cannot be achieved by instrumentation alone (Agrawal *et al*., 2014). Ideally, endodontic irrigant should be, effective as a germicide and fungicide with prolonged antimicrobial effect, should be non-irritating to the periapical tissues, should be stable in solution and active in the presence of blood, serum, and protein derivatives of tissue, having low surface tension to be able to completely remove the smear layer, able to disinfect the dentin/dentinal tubules without any adverse effect on physical properties of exposed dentin, preventing staining of the tooth structure, should be inexpensive, easy to use/apply with no adverse effect on the sealing ability of filling materials.

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Classification of the commonly used irrigating solutions

Chemical agents

- Tissue dissolving agents: NaOCl
- Antibacterial agents:
  - **Bacteriostatic:** CHX, some antibiotics
  - **Bactericidal:** some antibiotics, NaOCl
- Chelating agents:
  - Weak: HEDP, EGTA
  - Strong: EDTA
- Combination products (tissue dissolution & antibacterial effect):

MTAD, QMIX, SmearClear, Tetraclean

Natural agents

- Antibacterial agents: Green tea, Triphala

Two frequently used irrigants in endodontics are aqueous solutions of sodium hypochlorite and ethylene diamine tetraacetic acid.

Sodium hypochlorite is used at concentrations ranging from 0.5 to 5.25% and is known for its good tissue dissolution capacity and dentin disinfecting potential, but because of its limited effect on smear layer removal, it is recommended with chelating agents like EDTA (Grawehr, 2003). Chelating agents were introduced to endodontics by Nygaard – Ostby in 1957 and they react with Ca^{2+} ions in dentine and form soluble calcium chelates, thereby decalcifying the dentin. Alternate irrigation of 17% EDTA and NaOCl has become the recommended regimen for removal of both organic and inorganic components of smear layer (Agrawal *et al*., 2014). Recently introduced and possible alternatives to existing chelating agents are HEDP and EGTA. Etidronic acid or Hydroxy ethylidene bisphosphonate (HEDP) belongs to bisphosphonates family which has been widely used, in the treatment of osteoporosis, Paget's disease and hypercalcemia associated with malignancies. It was considered as a substitute for traditional chelators because of its fewer adverse effects on dentin (Tartari *et al*., 2013).

Figure 1. Armamentarium

EGTA or ethylene glycol bis –N N N N' – tetraacetic acid is also effective in removal of smear layer, because of its calcium ion specificity (Hegde *et al*., 2016). Endodontic irrigants with low systemic toxicity should allow optimal disinfection of root canal system. And it has been found that none of the irrigating solutions which are available, can be regarded as optimal. The aim of the study was to determine the tissue dissolution capacity of various irrigating solutions when used alone or in combinations at different time intervals.

MATERIALS AND METHODS

Solutions: 18% HEDP was prepared by dissolving 18 grams of pure chemical in 100 ml of distilled water. Similarly 17% EDTA was prepared by dissolving 17 grams of disodium EDTA in 100ml of distilled water with aid of sodium hydroxide and then the pH was adjusted to 7 by adding hydrochloric acid. 17% EGTA was prepared by dissolving 17 grams of EGTA in 100ml of distilled water with aid of NaOH and then the pH was adjusted to 7.5 by adding hydrochloric acid. Physiological saline was used as negative control and 2.5% & 5% NaOCl were taken as positive control. All chemical substances were prepared just prior to the usage as in Figure 2.

Tissue dissolution assay: Goat muscle tissue (*Capra aegagrus hircus)* was obtained from slaughter house shown in Figure 3 and was cut immediately into pieces of 8 × 6 × 4 mm using stainless steel blade as in the Figure 4 and were weighed to determine their initial weights in Figure 5.

Figure 2. Prepared solutions

Figure 3. Muscle tissue

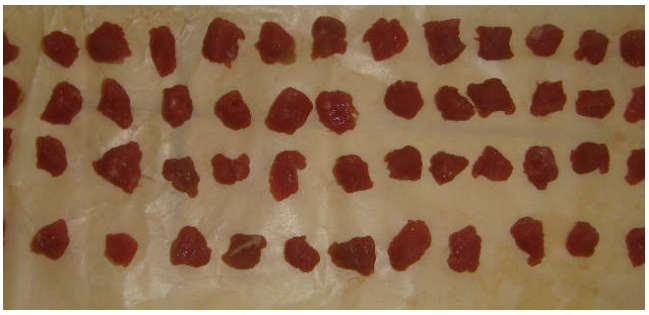

Figure 4. Tissue fragments

Figure 5. Electronic balance

Based on the irrigant used grouping was done as follows

Group 1 –0.9% physiological saline
Group 2 – 2.5% NaOCl
Group 3 – 17% EDTA
Group 4 – 17% EGTA
Group 5 – 18% HEDP
Group 6 – 2.5% NaOCl + 8.5% EDTA
Group 7 – 2.5% NaOCl + 8.5% EGTA
Group 8 - 2.5% NaOCl + 8.5% HEDP

After measuring the pH of test solution in each group with a pH meter, as shown in Figure 6, test tubes were filled with 15ml of test solutions, and the tissue fragments were submerged individually in the solutions and mechanical agitation was performed with CM 101 cyclomixer (REMI) for 15 s per minute for 5 minutes as in Figure 7.

At the end of agitation, the samples were taken out and submerged in distilled water for 30 s to remove the excess test solution. They were then blotted dry and reweighed for comparison with initial values as in Figure 8,9. Similarly, this procedure was repeated for 10 min and 15 min time period also, and initial weight and final weight after subjecting the sample to test solutions, were noted.

Figure 6. Measuring the pH of the solutions sample

Figure 7. Agitation of tissue immersed in test solution with the help of cyclomixer

Statistical analysis: The values were tabulated and subjected to statistical analysis using analysis of variance (ANOVA) followed by paired t test to assess if there is any difference between specimen weights before and after submersion for different time periods and between groups for same time periods.

Figure 8. Submersion in distilled water and blotting dry of sample

Figure 9. Reweighing the tissue sample

RESULTS

Significant decrease (P<0.01)in weight of tissue fragments for all the time periods of immersion was observed for group 2(NaOCl), group 8 (NaOCl+HEDP) and group 7(NaOCl+EGTA). Other groups were not associated with significant loss of weight at any period which were observed from the table no 1. Significant reduction in weight (P<0.01) occurred in the following order according to the table no 2. At the end of 5 min G2>G8>G7>G6>G3=G5=G4>G1, after 10 min G2>G8>G7>G6>G4>G5=G3>G1 and after 15 min G2>G8>G7>G6>G3=G4>G5>G1

DISCUSSION

Goal of cleaning and shaping regimen in endodontic therapy is to maximally reduce microbial load and necrotic tissue remnants in root canal system as remnants of organic debris inside root canal system, will serve as substrate for growth of microorganisms that survive the biomechanical preparation and contaminate the root canal after treatment (Love, 2001).

Table 1. Mean in weight (mg) before submersion (T0) and after 5(T5), 10(T10) & 15(T15) min of submersion into test solutions

Groups	pH	T0	T5	T10	T15	F value	P value
G1- SALINE	6.8	353.33	363.33	354.33	338.00	0.50	0.62
G2 – 2.5% NaOCl	11.8	353.33	231.00	232.33	188.00	27.57	<0.01
G3 – 17% EDTA	7.0	352.00	327.67	348.00	331.00	0.62	0.56
G4 – 17% EGTA	7.5	332.67	313.33	344.67	323.67	2.4	0.16
G5 – 18% HEDP	10.8	351.67	331.00	344.00	322.33	1.26	0.34
G6–2.5%NaOCl+ 8.5% EDTA	7.4	346.67	237.67	275.33	278.00	3.9	0.07
G7-2.5%NaOCl+ 8.5% EGTA	8.3	363.00	249.67	228.67	221.33	306.05	<0.01
G8-2.5%NaOCl+ 8.5% HEDP	11.2	376.67	258.00	225.67	198.33	254.82	<0.01

Table 2. Difference to baseline(T0) mean in weight (mg), and standard deviation(SD) after 5(T5),10(T10),15(T15) min of submersion into irrigant solutions

Time	Group	Difference to T0 mean	SD	t -value	P - value
	G1	-10.00	32.53		
	G2	122.33	1.00		
	G3	24.33	23.46		
	G4	19.34	6.11		
5min	G5	20.67	7.55	3.51	<0.01
	G6	109.00	1.53		
	G7	113.33	1.53		
	G8	118.67	10.00		
	G1	1.00	32.04		
	G2	141.67	1.15		
	G3	14.00	32.05		
	G4	19.66	10.02		
10min	G5	15.67	27.78	2.77	<0.01
	G6	71.34	11.68		
	G7	131.66	2.52		
	G8	132.33	11.02		
	G1	2.00	29.05		
	G2	166.33	7.55		
	G3	26.33	11.79		
	G4	24.33	28.36		
15min	G5	18.67	4.04	3.44	<0.01
	G6	82.00	5.57		
	G7	104.67	1.53		
	G8	162	2.52		

Hence biomechanical preparation in conjunction is always done with irrigation for optimal results. Sodium hypochlorite with concentration ranging from 0.5 to 5.25% is the most recommended for its antimicrobial, tissue dissolving and dentin disinfecting potential.

These properties are explained through the following reactions

Saponification reaction: NaOCl acts as an organic and fat solvent, degrading fatty acids, transforming them into salts (soap) and glycerol (alcohol), that reduces the surface tension of the remaining solution (saponification reaction).

Amino acid neutralization: NaOCl neutralizes amino acids forming water and salt

Chloramination reaction: Amino acid reacts with hypochlorous acid to form into chloramines and water. The free available chlorine comprises of hypochlorous acid (HOCl) and hypochlorite ion (OCl-), which exist in equilibrium depending on pH of the solution. Hypochlorite ions (OCl^-) exists in alkaline solutions (pH >7), because of its stronger oxidative effect shows higher tissue dissolving capacity. Hypochlorous acid exists in acidic solutions (3< pH < 7), shows powerful bactericidal effect. The disinfecting properties decrease with increase in pH of the solution, paralleling the concentration of disassociated hypochlorous acid.

Despite these properties, NaOCl only removes organic structure of smear layer produced during mechanical instrumentation and chelating agents like EDTA are required for effective removal of inorganic part of smear layer, which allows deeper penetration of NaOCl into the dentinal tubules (Grawehr *et al*., 2003). Chelating agents react with Ca^{2+} ions in dentine and form soluble calcium chelates, thereby decalcifying the dentin (Agrawal Vineet et al., 2014). Subsequently, use of 17% EDTA and NaOCl has become the recommended regimen for removal of both organic and inorganic components of smear layer. But, whenever EDTA comes in contact with NaOCl, it results in exothermic reaction with complete loss of chlorine gas immediately in the form of bubbles which effects the tissue dissolution and antibacterial activity of NaOCl (Prado *et al*., 2013). Hence, this study focuses on the use of alternative chelating agents like HEDP & EGTA to be used along with NaOCl. Etidronic acid or Hydroxy ethylidene bisphosphonate (HEDP) was considered as a substitute for traditional chelators as it requires 300 seconds to remove smear layer with fewer adverse effects on dentin, which makes it a weak chelator (Tartari, 2013). EGTA or ethylene glycol bis –N N N N' – tetraacetic acid is also effective in removal of smear layer, because it binds more specifically to calcium ions without causing dental erosion as seen with EDTA (Hegde *et al*., 2016). The available chlorine content upon combination of the irrigating solutions, can be assessed indirectly through the tissue dissolution capacity which can be assessed through various methods like the weighing method, total protein assay & hydroxyproline

determination (Koskinen *et al*., 1980). In this study, the weighing method is chosen to determine tissue dissolution as it is simple and reliable and also in other assays, irrigant solutions may interfere with the regeants used in those procedures (Irala *et al*., 2010). Previous studies (Irala *et al*., 2010; Grawehr *et al*., 2003; Moorer, 1982; Almeida, 2013) have used different kinds of tissue to determine the tissue dissolution capacity including, porcine muscle, pig palatal mucosa, rabbit liver, bovine muscle and bovine and Human dental pulp tissue. Goat muscle tissue was used in this study due to its easy availability and ease of standardization of surface area of sample. This has shown that, tissue dissolution was greater where NaOCl was used alone in all time periods, followed by, where NaOCl was mixed with HEDP and EGTA in equal parts, with the dissolution not so much seen in the combination of NaOCl and EDTA.

No tissue dissolution was seen when fragments were immersed in saline and chelating agents alone, which was observed in previous studies also (Irala, 2010; Naenni, 2004; Grawehr *et al*., 2003). Increase in weight of specimens was observed after 5min in saline and after 10min in EDTA, EGTA and in HEDP, which may be because of tissue hydration as a result of absorption of water by the tissue sample in the solutions. Previous studies have shown that tissue dissolution capacity of NaOCl is direct function of free available chlorine which consists of hypochlorous acid and hypochlorite ion (Grawehr *et al*., 2003). Hypochlorous acid has powerful bactericidal activity & hypochlorite ion shows greater tissue dissolving property (Macedo *et al*., 2010). Interaction of NaOCl with chelating agents results in chlorine gas evaporation and reduction of available chlorine (Zehnder, 2006). The present study confirms the earlier report on reduction of available chlorine in solution because of chlorine gas evaporation, when sodium hypochlorite interacts with acidic hydrogen of chelating agents. Hence there is least dissolution of the tissue upon combination of these irrigants. Greater tissue dissolution potential observed in mixture of NaOCl with HEDP or EGTA, compared to EDTA, might be due to the fact that being the weak chelators they did not actively interfere with chlorine of NaOCl, thereby maintaining the available chlorine in the solution which was required for dissolution (Tartari *et al*., 2013; Sayin *et al*., 2007). Also previous studies have shown that, weak chelators also remove smear layer efficiently without much erosion of peritubular and intertubular dentin maintaining sufficient root dentin hardness (Sayin *et al*., 2007).

Limitations of the study

The antibacterial efficacy of NaOCl when combined with various weak chelators as well as the pH potential of irrigants at various intervals could not be determined which are the limitations of this study.

Conclusion

Combining NaOCl with weak chelators like EGTA, HEDP gives the advantage of removing both organic and inorganic parts of the smear layer, making it a better irrigant than when used alone.

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Conflicts of interest: None

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