



## Evaluation tissue dissolution property of 2.5 % Sodium Hypochlorite Prepared by Hydrochloric Acid and Sodium Bicarbonate: An in vitro

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### ABSTRACT

Successful endodontic treatment requires chemical preparation in addition to mechanical preparation. The most common material for chemical preparations is sodium hypochlorite. One way to reduce the effects of pH adjustment is the use of sodium hypochlorite. The present paper was conducted to examine the effect of dilution with hydrochloric acid and sodium bicarbonate and reduce pH on ability of tissue solubility of sodium hypochlorite. The present study was conducted in vitro on bovine muscle tissue. Ability of tissue solubility was conducted in four groups respectively with active ingredient including 1) sodium hypochlorite diluted with distilled water 2) sodium hypochlorite diluted with sodium bicarbonate 3) sodium hypochlorite diluted with hydrochloric acid and finally 4) distilled water (control group). Each sample was firstly weighed and then placed in contact with 10 m/L solution for 60 minutes (five 12 -minute intervals). The sample was weighted every five minutes and solution was renewed. The results were analyzed using SPSS-21 Software based on variance analysis, Tukey and T-test ( $\alpha=0.05$ ). The findings showed that there was significant difference between first, second and third groups in terms of ability of tissue solubility. However, the tissue solubility in second and third groups was lower than first group and it was similar in second and third groups ( $P$  Value  $<0.001$ ). Reduction of sodium bicarbonate PH using sodium hypochlorite and hydrochloric acid reduces ability of tissue solubility in sodium hypochlorite.

**Key words:** Dilution, sodium hypochlorite, solubility

### INTRODUCTION

Bacteria and necrotic materials have primary role in periapical lesions[1], so complete debridement and channel disinfection have significant role in the long term success of root canal treatment. It should be noted that it could not be achieved by mechanical preparation[2], so the use of a variety of detergents is recommended alongside mechanical preparation[3]. sodium hypochlorite and Chlorhexidine are such these materials[4].

Sodium hypochlorite is widely used and accepted in endodontics[5]. Sodium hypochlorite has bactericidal properties to dissolve necrotic tissues[6-8]. Several studies have been conducted on the effect of concentration on the antibacterial properties and tissue solubility of sodium hypochlorite[8-10]. The studies indicated that although 5.25 % hypochlorite has sufficient ability, but 0.5% concentration has limited effects. Moreover, there is no difference

between 2.6 % and 5.25 % concentrations in terms of ability to dissolve tissue. Since hypochlorite has cytotoxicity and color changes effects, use of the lowest effective concentration e.g. 2.5% is more logical [8, 9, 11-13].

There are different ways for dilution of sodium hypochlorite. Some authors believe that 1% solution of bicarbonate should be used instead of distill water for dilution so that pH could be reduced to neutral level[14, 15]. In contrast, some researchers believe that dilution with water is sufficient to obtain a suitable concentration[16, 17].

Several studies have been conducted on dilution with different materials and the effects of dilution. Camps et al. [18]used solution containing hydrochloric acid which enhances the antibacterial property. According to the study by Zehnder et al.[17], sodium bicarbonate buffer will not cause changes in anti-bacterial properties. According to Stojicic et al. [19]study, tissue solubility property of Sodiumhypochlorite increase in higher concentration of. In contrast, according to the study Trepagnier et al.[8], there was no difference in terms of ability of tissue dissolution between 5.25% and 2.6% which was consistent with the study by Baumgartner and Cuenin[20].

Although various methods of dilution are available with different philosophies, however a comprehensive study has not been conducted so far. The present paper examines the effect of hydrochloric acid and sodium bicarbonate solutions to neutralize and buffer the sodium hypochlorite as well as the effects on ability of tissue solubility of sodium hypochlorite on beef muscle tissue.

### MATERIALS AND METHODS

The present paper was conducted in vitro in Isfahan University of Medical Sciences in spring 2016. The study was conducted on muscle tissue of bovine. Based on statistic expert consultation, minimum sample size for significance was determined 15 samples in each group. In order to study the effects, four groups were definedincluding: two control groups and two intervention groups. The two control groups included one group exposed to sodium hypochlorite diluted with distilled water (positive control) and one group exposed to distilled water (negative control). Two intervention groups included one group exposed to sodium hypochlorite buffered with sodium bicarbonate and one group exposed to sodium hypochlorite neutralized with hydrochloric acid.

#### *Preparation of sodium hypochlorite diluted with distilled water:*

5.25% sodium hypochlorite (Arman Sina Co, Arak, Iran) was prepared. To prepare a concentration of 2.5%, two 500 -mL volumetric flask was provided and 250 ml of hypochlorite in each balloon. 250 ml of 0.3 M bicarbonate solution was added to first flask and 250 ml of 0.30 M hydrochloric acid solution was added to second flask. The final pH was measured using a pH meter (HANNA Instruments, Tanneries, France). The final product was 2.5% sodium hypochlorite with 7 PH.

In order to preparation of 2.25% hypochlorite dilluted with distilled water, the combination of 250 ml hypochlorite sodium and 250 ml of distilled water was used. The final pH was measured using a pH meter (Hanna Instruments, Tanneries, France). The final product was 2.5% sodium hypochlorite with 12 PH[17, 18].

#### *Tissue dissolution evaluation:*

Bovine muscle tissue was used as tissue sample. Therefore, 60 pieces weighing approximately 100 mg were prepared. Because of the importance of cross-section, parts were prepared quite the same size. To achieve the same cross-sectional area of the parts, all components were cut in a cube shape with dimensions of approximately 4 x 5 x 5 mm. Initial weight of each piece was defined using the specified scales(Mettler, Gerifensee, Switzerland) and were maintained at a temperature of 15 ° C until the research was implemented.

The parts reached the ambient temperature, dried and weighed again prior to the start of the trial. Parts were randomly divided into four 15-member groups:

- 1- The first group was exposed to sodium hypochlorite prepared by distilled water.
- 2- The first group was exposed to sodium hypochlorite prepared by sodium Bicarbonate.
- 3- The third group was exposed to sodium hypochlorite prepared by hydrochloric acid.
- 4- The fourth group was exposed to distilled water.

All parts were exposed to 10 ml solution for 5 minutes. Then they were removed, dried and weighed and parts' weight was recorded. For one hour, the solution was replaced every 5 minutes. The samples were renewed in the vicinity of the solution and the sample were weighed. This protocol was implemented on a constant basis for all 4 groups [21].

The data were inserted in SPSS-21 Software. The results were analyzed using SPSS-21 Software based on variance analysis, Tukey and T-test ( $\alpha=0.05$ ).

**RESULTS**

Data were gathered during 60 minutes (12 periods of 5 minutes) in 4 groups with 15 samples. Table (1) demonstrates the average weight changes over time in each of the 4 groups.

**Table 1: Mean weight of meat in each of the intervals**

60	55	50	45	40	35	30	25	20	15	10	5	Time per minutes	
0	0.87	1.6	18.60	39.0	52.07	65.2	75.40	84.33	93.47	100.60	104.87	Mean	Group 1
0	0.87	1.09	1.12	0.59	0.52	0.43	0.30	0.29	0.19	0.21	0.24	Standard Deviation	
0	2.47	19.07	35.27	47.93	57.8	66.96	76.13	85.33	94.47	99.73	104.93	Mean	Group 2
0	1.33	1.31	1.14	0.93	0.45	0.34	0.24	0.23	0.16	0.15	0.28	Standard Deviation	
0	1.4	17.93	33.67	47.2	58.2	67.73	76.87	86.53	94.93	99.93	105.0	Mean	Group 3
0	0.95	0.77	0.57	0.43	0.40	0.48	0.36	0.35	0.28	0.25	0.31	Standard Deviation	
105.13	105.27	105.2	105.27	105.27	105.2	105.07	105.07	105.2	105.2	105.2	105.2	Mean	Group 4
0.35	0.30	0.30	0.30	0.30	0.31	0.34	0.34	0.33	0.33	0.33	0.33	Standard Deviation	

The data were analyzed by analysis of variance for replicated data. According to the test results in Table 2, variables of time and solution have a significant effect on tissue weight (P value <0.01).

**Table 2: Variance Analysis**

P-value	F-Statistics	Mean of Squares	Degree of Freedom	Sum of Squares	Source of Changes
0.000	114.33	3356535.56	1	3356535.56	Model Constant
0.000	12374.13	49025.37	11	539279.04	Time
0.000	180.74	109599.75	3	328799.26	Solution
1.000	404.05	17.86	14	247.49	Replication
	0.06	271.25	691	187436.66	Error
			720	4412298.0	Total

On completion of the analysis, based on Tukey test with significance level of 5%, Group 2 and 3 have similar performances and group 1 has significant difference with other groups.

**Table 3: Tukey test to compare the mean weight of meat in 4 solvents**

Categorization	Number of Observation	Group
A	180	4
B	180	3
B	180	2
C	180	1

**DISCUSSION**

Based on the results, there was significant difference in terms of ability of texture solubility between first group (prepared by distilled water) and second group (prepared by sodium bicarbonate) and third group (prepared by hydrochloric acid). Fourth group (distilled water) lacked tissue solubility. Weight gain was also observed due to osmotic properties and water absorption by the tissue cells. In the field of weight loss in groups 1, 2 and 3 after 35 minutes, the weight loss process accelerated.

A number of articles have been conducted to examine the effects of pH reduction on sodium hypochlorite properties. Campas et al. [18] used hydrochloric acid to neutralize the sodium hypochlorite. According to results, neutralization with hydrochloric acid will decrease tissue dissolution ability compared to the solution with the same concentration. However the results showed increased antibacterial effect of neutralized solution. The study by Zehnder et al. [17] used sodium carbonate and sodium bicarbonate for neutralization of sodium hypochlorite. The results indicated that buffering with sodium bicarbonate and sodium carbonate reduced the effect of tissue dissolution. The study by Mercade et al. [21] used acetic acid for neutralization of sodium bicarbonate. The study has not measured the effect of tissue dissolution, but the study conducted on the antibacterial effects. The present paper used hydrochloric acid and sodium bicarbonate in order to reduce pH to neutral levels. According to findings, neutralization and buffering leads to reduced solubility effect of solution compared to non-buffered solution with the same concentration. It was consistent with the results of previous study.

Different studies have reported different results in terms of tissue dissolution ability. The study by Hasselgren et al. [22] conducted on pork muscle tissue indicated that use of 0.5% hypochlorite buffered with sodium Bicarbonate, in event of non-renewal of solution was not able to dissolve 20 mg of pig muscle tissue. While the renewal of the solution every 30 minutes caused this size of sample to be dissolved during 180 minutes. Okino et al. [23] studied bovine pulp using 0.5%, 1% and 2.5% sodium hypochlorite with pH=9. PH had been reduced by Boric acid and during contact with the sample solution, shaker with 1500 round was used. The average time required to solve the pulp tissue of bovine for 0.5%, 1% and 2.5% concentrations were respectively 100, 70 and 51 minutes. Hand et al. [7] conducted the study on dissolution of connective tissues of mouse in contact with sodium hypochlorite at various concentrations. According to results, sodium hypochlorite at concentrations of 0.5%, 1%, 2.5% and 5.25% resulted in reduced weight percentage equal to 0.01%, 4% and 72% during 10 minutes. The results of present study showed that ability of tissue dissolubility reduce with neutralization and buffering. To dissolve an amount of tissue, we need less non-buffered solution in less time than solutions buffered with sodium bicarbonate and neutralized with hydrochloric acid.

Clarkson et al. [24] implemented a study to examine the effect of concentrations and different brands of sodium hypochlorite on solubility of dental pig's pulp tissue. According to results, there was significant difference between similar commercial brands in terms of ability of tissue solubility, as though 1% solutions of White King, Forte and Milton brands in similar circumstances at the time of 28, 38 and 99 minutes could dissolve the tissue. This is one of the reasons for the current study results differences with other studies.

It seems that ability of tissue solubility is related to factors such as concentration, time, volume, pH, temperature, type of preparation, material brand and tissue-related factors such as type, amount and section area [7, 25-27]. Different studies used different tissues such as rabbit liver [26], connective tissues of rat [7], palatine tissue of pig [28], pig muscle [22, 25] and bovine muscle [27]. The large number of these variables creates difficulties in comparing different studies with each other [29].

Due to the lack of access to equipment for determining chlorite level, the study was conducted to examine the effect of one factor on ability of tissue dissolubility. It is recommended to implement further studies on the effect of more variables such as vibration and temperature. Studies in the field of antimicrobial capability are also recommended.

## CONCLUSION

According to the results obtained from present study and comparison with the results of other studies, it seems that use of materials to reduce pH reduces the performance of the hypochlorite solution. Also, according to the results of this study to reduce the pH, use of sodium bicarbonate and hydrochloric acid creates the same results in terms of tissue solubility.

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