

Effect of Microelectric Current and Other Activation Techniques on Dissolution Abilities of Sodium Hypochlorite at Different Temperatures in Bovine Tissues: An *in vitro* Study

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ABSTRACT

Aim: To study the effects of microelectric current and other methods at different temperatures, on the dissolution of sodium hypochlorite (NaOCl).

Materials and methods: Bovine muscle tissues (n = 165) were prepared and grouped into three temperature groups: (1) Room temperature, (2) 45°C, and (3) 60°C. Each temperature group was divided into five subgroups: (i) Control group: 5.25% NaOCl; (ii) 5.25% NaOCl with pipetting; (iii) 5.25% NaOCl with sonic activation; (iv) 5.25% NaOCl with ultrasonic activation; and (v) E-NaOCl = 5.25% NaOCl with microelectric current. Weight of specimens before and after treatment was done. In each group average, standard deviation and median were found out. The data were analyzed using multiway analysis of variance (ANOVA) test and Tukey honestly significant difference (HSD) tests. The alpha-type error was set at <0.05.

Results: Tissue dissolution was highest with the ultrasonic group (p < 0.05), and the tissue dissolution ability of other groups was significantly higher than the positive control (p < 0.05) at room temperature. E-NaOCl group dissolved greater quantity of tissue (p < 0.05) at 45°C and at 60°C.

Conclusion: NaOCl used along with microelectric current produced a higher tissue-dissolving ability. Combining with other techniques provides a synergistic effect on tissue dissolution.

Keywords: Microelectric current, Sodium hypochlorite, Temperature, Tissue dissolution.

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INTRODUCTION

Microorganisms and their products cause the development of pulp and periradicular pathology.¹ Sodium hypochlorite solution is the mostly used irrigant due to its tissue-dissolving and antimicrobial abilities.²

The tissue-dissolving capacity depends on time of exposure of the solution, concentration, volume, and the surface area of the tissue it acts upon.³ Methods to increase the extent of tissue dissolution are: Increasing the solution pH, and by increasing the temperature, ultrasonic activation, elongated working time, and by continuous agitation of the solution.⁴⁻⁶ Pécora et al⁷ studied the “dynamic balance” of NaOCl: $\text{NaOCl} + \text{H}_2\text{O} = \text{NaOH} + \text{HOCl} = \text{Na}^+ + \text{OH}^- + \text{H}^+ + \text{OCl}^-$. The results of dynamic balance of NaOCl were amino acid neutralization, saponification, and chloramination reaction.^{8,9}

Safe and effective irrigations, such as EndoActivator (EA) and ultrasound were helped by Irrigation devices and techniques. The EA has a noncutting polymer tip. It is vibrated using sonic energy to achieve vigorous agitation of irrigation solutions.^{10,11} Passive ultrasonic irrigation (PUI) produces ultrasonic waves to an irrigant.

Growing bubbles produced by ultrasonic energy collapse and the pressure-vacuum effect created contribute to the bactericidal effect. Passive ultrasonic irrigation instrument through its oscillation creates a resonance that agitates the irrigant what is called stable cavitation. An acoustic streaming¹² produced by the combination of these effects enhances the cleaning and decontamination effect of the irrigant.¹³ When NaOCl is used along with PUI, due to the ultrasound effect^{3,14} its organic tissue-dissolving and antibacterial efficacy is enhanced. In electrolysis, a direct electric current by a potentiostat/galvanostat is used.¹⁵ During activation, liquids turn into a metastable state. As a result, chemical structure, the hydrogen concentration, and oxidation reduction potential of these liquids change.¹⁶ Ertugrul et al¹⁷ showed that NaOCl which is microelectrically activated increases the tissue dissolution capacity.

This was an *in vitro* study conducted in bovine tissues to compare microelectric current activation and other methods, on dissolution capabilities of NaOCl at different temperatures.

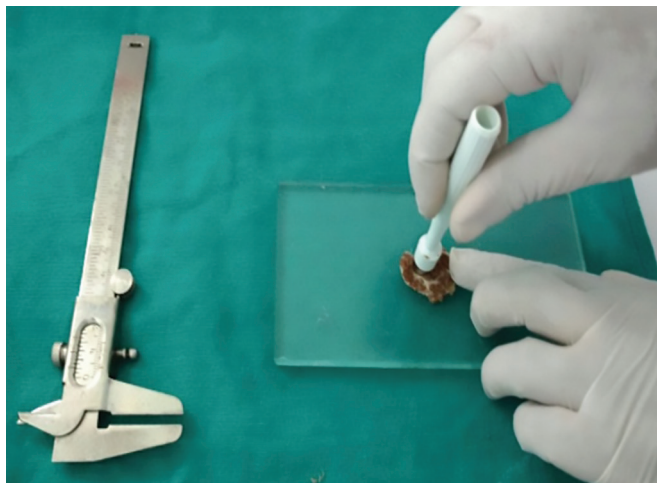


Fig. 1: Specimen collection



Fig. 2: Microelectric balance

MATERIALS AND METHODS

The specimen in the study was bovine muscle tissue got from a local butcher shop. NaOCl solution, (5.25% chlorine) was used and stored (+4°C). Bovine muscle tissue stored at 16°C in a 100% humid medium. Standardization of size and weight was performed by using a biopsy punch with a 6-mm diameter to collect the samples from a 2-mm tissue (Fig. 1), and weighing of samples was done with an electronic microbalance prior to the testing (Fig. 2).

Mean weight of tissue samples before treatment with NaOCl, was 58 mg. Similar to a study by Stojicic et al,⁶ the experiments were performed at 3 different temperatures: room temperature (28°C), 45°C, and 60°C. Containers in an acclimatized room is used to do experiments at room temperature.

For those experiments conducted at 45°C and at 60°C, a temperature-controlled water bath was used and an external thermometer was used to confirm the temperature. Each temperature group was then sub divided into five subgroups by different activation methods.

Table 1: Groups of bovine muscle tissues studied

<i>n</i>	<i>Room temperature group</i>	<i>45°C group</i>	<i>60°C group</i>
11	NaOCl	NaOCl	NaOCl
11	Pipetting	Pipetting	Pipetting
11	Sonic activation	Sonic activation	Sonic activation
11	Ultrasonic activation	Ultrasonic activation	Ultrasonic activation
11	E-NaOCl	E- NaOCl	E-NaOCl

Total sample contained 165 tissue samples, 11 in each group (Table 1). For each sample, the duration of the experiment conducted was 5 minutes.

The control group was 5.25% NaOCl solution without any activation. For the ultrasonic activation, stainless steel size of #25 ultrasonic tip was used which was operated at a moderate speed in the solution (Fig. 3).

The sonic activation was done with EA (polymer tip no. 25/04) which was run at about 10,000 cycles per minute (Fig. 4).



Fig. 3: Ultrasonic activation

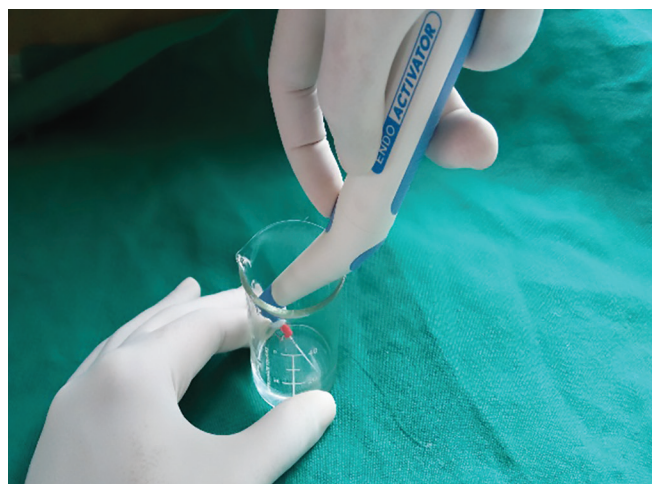


Fig. 4: Sonic activation

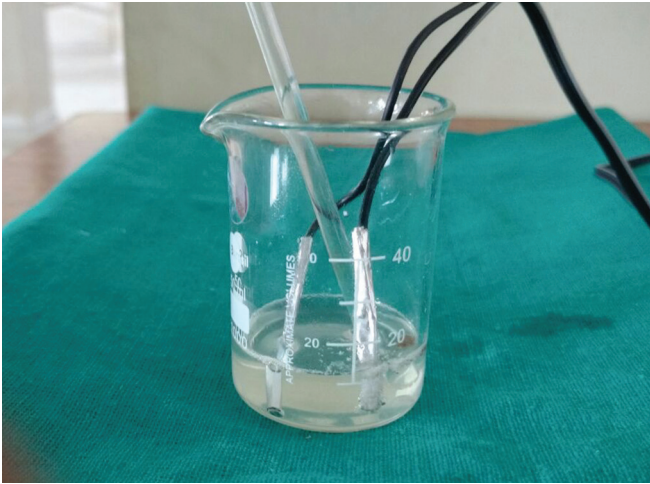


Fig. 5: Microelectric current activation

For the ultrasonic and sonic activation, the tips were activated 6 mm from the tissue after submerging up to 10 mm in the 5.25% NaOCl solution.

In conforming to a study done by Stojicic et al,⁶ pipetting was done with a glass rod which was mechanically activated 6 mm from the tissue. In the microelectric methods, a potentiometer was calibrated to provide 10 mA and 3 V to the NaOCl. Current activation was done for 15 seconds each minute for the 5 minute period (Fig. 5).

After 5 minutes, each sample was taken out, gently dried, and again weighed. Weight lost was calculated in percentage.

Statistical Analysis

Data were statistically analyzed using multi-way ANOVA and Tukey HSD test. The alpha-type error was at <0.05.

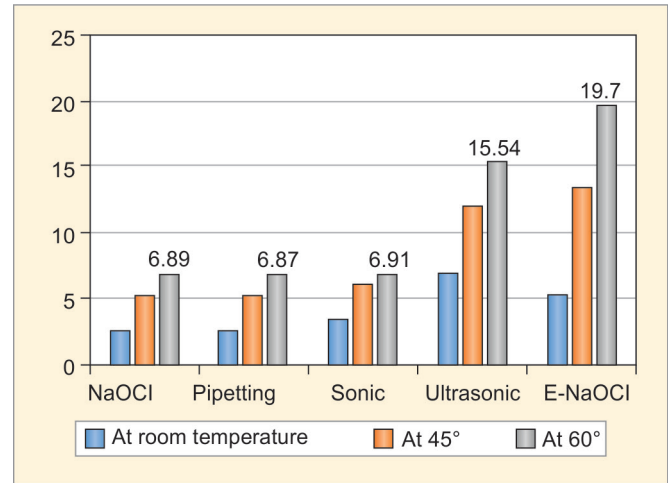
RESULTS

For each of the group, standard deviation and mean were calculated. All activation groups were dissolved a higher amount of tissue than the control groups ($p < 0.05$). Table 2 shows that at the room temperature, ultrasonic group dissolved a higher amounts of tissue followed by

Table 2: The effect of various methods of activation on tissue dissolution (percentage of tissue weight change loss \pm SD) at various temperatures

Activation techniques	Room temperature (mean % loss of mass \pm SD)	At 45°C (mean % loss of mass \pm SD)	At 60°C (mean % loss of mass \pm SD)
NaOCl	2.6 \pm 0.133 ^a	5.26 \pm 0.11 ^d	6.89 \pm 0.18 ^c
Pipetting	2.59 \pm 0.162 ^a	5.27 \pm 0.10 ^d	6.87 \pm 0.16 ^c
Sonic	3.46 \pm 0.07 ^b	6.14 \pm 0.03 ^e	6.91 \pm 0.19 ^c
Ultrasonic	6.88 \pm 0.162 ^c	12.04 \pm 0.01 ^f	15.54 \pm 0.10 ^h
E-NaOCl	5.28 \pm 0.11 ^d	13.6 \pm 0.18 ^g	19.7 \pm 0.27 ^t

SD: Standard deviation



Graph 1: Bar chart showing the relative amounts of loss of mass (n = 11 in each group) after using various activation methods

E-NaOCl and sonic activation ($p < 0.05$). There was not much difference in the mean percentage loss between the control and pipetting groups (Table 2).

At 45°C, there was not much difference in the mean percentage loss between control and pipetting group and at 60°C, there was not much difference in the mean percentage loss between the control, pipetting, and sonic groups ($p < 0.05$).

At 45°C and at 60°C, the E-NaOCl group showed superior dissolution ability than any other groups ($p < 0.05$). As the bar diagram shows, subgroups at 60°C showed a much higher dissolution ability followed by subgroups at 45°C and at room temperature ($p < 0.05$) (Graph 1).

DISCUSSION

Several studies have been demonstrated on the dissolution property of NaOCl. This study emphasized the effect of activation of NaOCl at different temperatures on the bovine muscle tissue. The agitation method increases the NaOCl's dissolution effect.^{3,6,18,19} Various tissues have been used by previous tissue dissolution studies including bovine pulp,²⁰ muscle tissue of porcine,²¹ rabbit liver,²² connective tissue of rat,³ and the oral mucosa of pig.¹⁸ Instead of pulp tissue, bovine muscle tissues were chosen as a specimen so as to standardize both weight and surface area.

Although there are many literatures supporting the importance of activation methods on increasing the tissue dissolution property of NaOCl, there are only few studies on the microelectric activation of NaOCl solution. The present study proved that the microelectric activation of NaOCl has superior results when compared with other activation methods. Pronounced improvement in the dissolution ability was seen when heated above room temperature.

In the current study, NaOCl was heated at 45°C and 60°C and the results proved that the dissolution ability of warmed NaOCl was found to be superior on increasing the temperature irrespective of any activation method, which was similar to the results obtained by other previous studies.²³⁻²⁵

Conventional agitation method like ultrasonic was tested in the group. At room temperature, ultrasonic activation produced a faster dissolution ability ($p < 0.05$), which was inconsistent with the results obtained in another study.²⁷ Microelectric activation of NaOCl produced an improved tissue dissolution ability when compared with sonic activation and pipetting ($p < 0.05$), contradicting the previous study.²⁶ Some previous studies have shown that ultrasonic-activated NaOCl cleaned root canals successfully.²⁵

However, other researchers found no difference in the syringe irrigation of the root canal between ultrasonic and conventional methods.²⁷⁻²⁹ The difference in results may be linked to the concentration of NaOCl, volume, power setting, and the duration of treatment with activation.

A positive combination results on NaOCl activated with heat, microelectric current and agitation methods on NaOCl's tissue-dissolving ability were obtained. The present study found not much difference between the control groups, sonic activation, and pipetting, which confirms those found by Stojicic et al.⁶

In previous studies, electrolyzed water was used as a canal washing solution.^{30,31} In the present study, a potentiometer was set to supply 10 mA and 3V to the NaOCl solution to change the dynamic structure of NaOCl, and the direct current was passed through the NaOCl solution at a micro level. The current value used here is tolerable for human beings according to the standard.³² However, the methodology differed from previous studies which used electrolyzed water. The results obtained may not be directly implicated to the clinical conditions; however, a direct current applied with a suitable potentiometer provides a faster tissue-dissolving capacity with the performance similar to a preheated NaOCl.

This activation method can be used in combination with other conventional activation systems, such as EA or ultrasonics during the final stage of irrigation.

CONCLUSION

Within the limitations of the study, the dissolution ability of the NaOCl can be increased effectively by the combined use of microelectric energy, heat, and other techniques like ultrasonic, sonic, and pipetting.

CLINICAL SIGNIFICANCE

It has to be noted that the current study was performed in an *in vitro* study model and is not directly estimated

with clinical conditions. However, further studies should be conducted on the activation of NaOCl with microelectric energy to better understand this technique in practice, and its the effect on the functioning of pace makers should also be well studied. Since the current value used in the study is tolerable for human beings according to the standard and the results obtained is far much desirable, it paves the way for a promising future in the field of irrigation.

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