

Rapid tissue dissolution efficiency of electrically-activated sodium hypochlorite on bovine muscle

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ABSTRACT

Objective: Sodium hypochlorite (NaOCl) is a common antimicrobial and tissue-dissolving irrigant. The aim of this in vitro study is to evaluate and compare dissolution capacities of sodium hypochlorite solutions after electrically activation (E-NaOCl) on bovine muscle specimens at various time periods and concentrations. **Materials and Methods:** Three sodium hypochlorite solutions of 1.25%, 2.5%, and 5% were tested at 3-min. and 5-min. with and without activation by electrically. Distilled water and NaOCl solutions without electrically activation were used as controls. Pieces of bovine muscle tissue (34 ± 2 mg) were placed in 10 mL of each solution at room temperature. In the group of E-NaOCl, electrically activation was performed through the potentiostat. The tissue specimens were weighed before and after treatment, and the percentage of weight loss was calculated. **Results:** Weight loss of the tissue increased with the concentration of E-NaOCl and NaOCl. Higher concentration and electrically activation considerably enhanced the efficacy of sodium hypochlorite. The effect of electrically activation on tissue dissolution was much greater than that of same concentrations in the groups of NaOCl ($P < 0.001$). Tissue weight loss was significantly higher in 2.5% and 5% E-NaOCl at 3 min. than in 2.5% and 5% NaOCl at 5 min. ($P < 0.05$). There were not any significant differences between the 2.5% E-NaOCl and 5% NaOCl at 5 min. ($P > 0.05$). **Conclusion:** Electrically activation can improve the tissue-dissolving effectiveness of sodium hypochlorite.

Key words: Electrically activation, sodium hypochlorite, tissue dissolution

INTRODUCTION

Chemomechanical preparation is the important part of the root canal treatment. Sodium hypochlorite (NaOCl) is the most typically used irrigating solution because of its tissue dissolution and antimicrobial activity.^[1,2]

The tissue dissolving ability of NaOCl depend on not only exposed time of the solution, concentration and

its volume, but also its depend on the tissue surface area^[3] The effects of NaOCl solutions about tissue dissolution can be increased by:

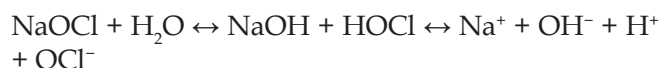
- Raising the solution pH^[4]
- Increasing the temperature of the NaOCl solutions^[4-6]
- Ultrasonic activation of the solution^[4-6]
- Elongated working time^[5]
- Continuous agitation of the irrigation solution.^[4-6]

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Pécora *et al.*^[7] showed the “dynamic balance” of NaOCl as followed by the reaction:



Those chemical reactions between NaOCl and organic tissue were confirmed. The dynamic balance of NaOCl resulted saponification reaction, amino acid neutralization reaction and chloramination reaction.^[7-9]

Electrochemically activated solution (ECA) are produced from water and low concentration solutions with salt.^[10-12] The terms of ECA is principally transferred liquids from the metastable membrane via to anode or cathode action using an elementary reactor. *In vitro* studies testing ECA as an irrigant, suggested an alternative to NaOCl solution endodontic therapy, but ECA show less antimicrobial effectiveness than NaOCl.^[13-15]

Electrolysis is a method of using direct electric current by a potentiostat/galvanostat to drive an otherwise nonspontaneous chemical reaction.^[16] As a rule, the activation liquid is performed in a diaphragm or nondiaphragm electrolysis device. During this activation, liquids directly contact with cathode or anode and turn into a metastable phase. Consequently, chemical structure of these liquids change and properties, as well as their microcluster structure, hydrogen concentration and oxidation reduction potential.^[12] It is conceivable that electrically-activated sodium hypochlorite (E-NaOCl) could enhance its effect by electrochemistry. However, there is no data available on the characteristics of electrolysis of NaOCl solutions relevant to the endodontist.

The purpose of this study was to compare the dissolution capacity achieved by solutions E-NaOCl on the dissolution of bovine muscle specimens at different concentrations and time periods.

MATERIALS AND METHODS

Solutions

Sodium hypochlorite solutions with concentrations of 1.25%, 2.5%, and 5% were tested. Stock solution of the 5% NaOCl (NaOCl 5%, Wizard™, RehberKimya, Istanbul, Turkey) was obtained from manufacturers. 1.25% and 2.5% solutions of the NaOCl were diluted from the stock solution using distilled tap water. The solutions were covered amber glass bottles for protecting from CO₂ and stored at 4°C and restored to room temperature before use.

Tissue collection

Bovine muscle tissue was used to test the dissolving capabilities of NaOCl at differing concentration, time and electrolysis activation. The tissues were kept refrigerated at -16°C and 100% humidity. The frozen bovine muscle tissues were cut into round pieces of 5 mm diameter and 2 mm thickness using sterilized tissue biopsy punch (Sterile Dermal Biopsy Punch, Kai Industries Ltd., Seki, Japan). Each tissue samples prepared with standard shape and size. The preweighed samples had 34 ± 2 mg weight. (*n* = 154), the tissue samples were preweighed using a digital scale (Presica 205a, Dietikon, Switzerland). After the weights were recorded, the samples were placed in 10 mL of the solution to be tested according to time periods.

Electrically activation of sodium hypochlorite

In the group of E-NaOCl were conducted using Autolab PGSTAT 302 Potentiostat/Galvanostat controlled by GPES 4.9 software (Ecochemie, The Netherlands). Three electrodes were used for all computations; a platinum electrode of 2 mm diameters as a working electrode, a Pt electrode and an Ag-AgCl reference electrode [Figure 1]. Then, the electrodes were performed by triangular potential cycling 3V for 3 min or 5 min according to the groups. The electrolyte solution consisted of 10 mL NaOCl solutions.



Figure 1: Schematic diagram of electrically activation of sodium hypochlorite; (a) working electrode (pyrolytic graphite electrode), (b) auxiliary electrode (platinum sheet), (c) reference electrode (Ag/AgCl)

All experiments were conducted at a room temperature and 10 mL liquids following experiment and control groups. The samples pieces were then removed, dried, and then weighed again. The percentage of tissues weight loss were calculated.

The groups were as follows:

Control groups;

Group 1: Distilled water, 3 min ($n = 11$)

Group 2: Distilled water, 5 min ($n = 11$)

Group 3: NaOCl 1.25%, 3 min ($n = 11$)

Group 4: NaOCl 1.25%, 5 min ($n = 11$)

Group 5: NaOCl 2.5%, 3 min ($n = 11$)

Group 6: NaOCl 2.5%, 5 min ($n = 11$)

Group 7: NaOCl 5%, 3 min ($n = 11$)

Group 8: NaOCl 5%, 5 min ($n = 11$)

Experimental groups;

Group 9: ECA 1.25%, 3 min ($n = 11$)

Group 10: ECA 1.25%, 5 min ($n = 11$)

Group 11: ECA 2.5%, 3 min ($n = 11$)

Group 12: ECA 2.5%, 5 min ($n = 11$)

Group 13: ECA 5%, 3 min ($n = 11$)

Group 14: ECA 5%, 5 min ($n = 11$).

Data analyses

The averages, standard deviation, minimum, maximum and median were calculated for each group. Resulting data were analyzed by statistically using the multi-way ANOVA and Tukey HSD tests. The level of the alpha-type error was set at < 0.05 .

RESULTS

Tissue weight loss with different concentrations of NaOCl and E-NaOCl for two different time periods is shown in Table 1.

Tissue dissolution increased in concentration of NaOCl and E-NaOCl. A significant weight loss was observed after exposure to 2.5% and 5% ($P < 0.001$) of NaOCl compared with distilled water and 1.25% NaOCl.

The concentrations of 2.5% and 5% E-NaOCl solutions greatly increased the tissue dissolution to same concentrations of NaOCl groups ($P < 0.001$).

When times were grouped for evaluation, it was shown that longer exposure times resulted in improved

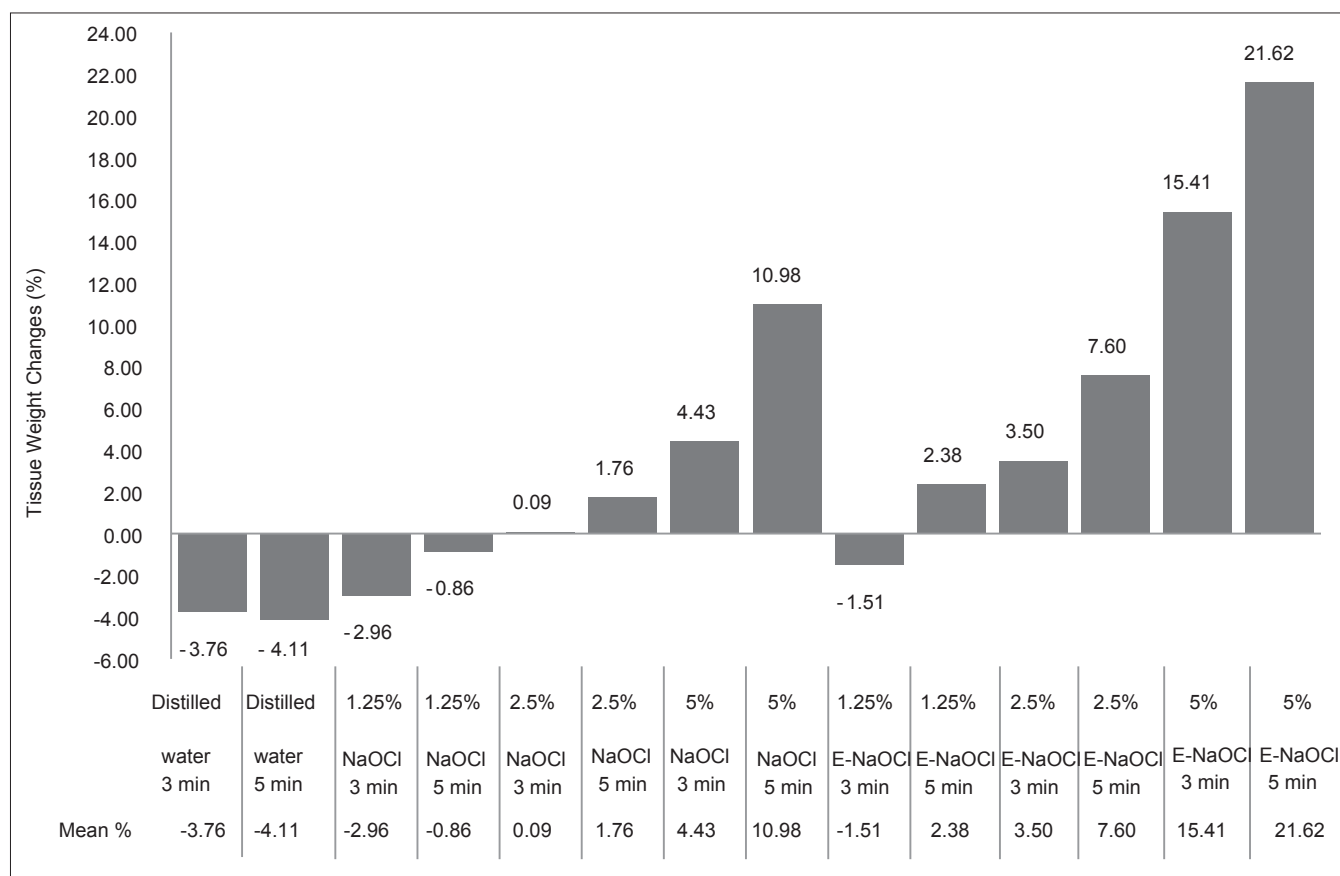


Figure 2: Bar chart depicting relative amounts (percent of original weight) of remaining bovine tissue, ($n = 11$ /group) after 10 mL of sodium hypochlorite

Table 1: Effect of electrically activation on tissue dissolution (percentage of tissue weight change loss \pm SD) by the solutions compared with distilled water

| Solutions | 3 min | 5 min |
|-----------------|--------------------------------|--------------------------------|
| Distilled water | 3.76 \pm 2.92 ^a | 4.11 \pm 2.11 ^a |
| 1.25% | | |
| NaOCl | 2.96 \pm 1.64 ^a | 0.86 \pm 2.76 ^a |
| E-NaOCl | 1.51 \pm 2.14 ^a | -2.38 \pm 1.92 ^{ab} |
| 2.5% | | |
| NaOCl | -0.09 \pm 2.17 ^b | -1.76 \pm 2.46 ^b |
| E-NaOCl | -3.50 \pm 0.63 ^c | -7.60 \pm 3.82 ^d |
| 5% | | |
| NaOCl | -4.43 \pm 3.29 ^c | -10.98 \pm 5.93 ^d |
| E-NaOCl | -15.41 \pm 3.50 ^e | -21.62 \pm 5.53 ^f |

The same superscript letters are demonstrate no significant differences ($P < 0.05$). SD: Standard deviation, NaOCl: Sodium hypochlorite, E-NaOCl: Electrically-activated sodium hypochlorite

tissue dissolution in control and experimental groups ($P < 0.05$). Comparing time periods within groups; tissue weight loss was significantly higher on the 2.5% and 5% E-NaOCl at 3 min than 2.5% and 5% NaOCl at 5 min ($P < 0.05$). There were no significant differences between the 2.5% E-NaOCl and 5% NaOCl at 5 min ($P = 0.087$).

These results showed that the E-NaOCl was used, the less time or less concentrations that were required for solution contact to get similar tissue dissolution [Figure 2].

DISCUSSION

The use and more effective of NaOCl irrigation solutions have been well published.^[16,17] This study focused on the effect of electrically activation of NaOCl at different concentration and time on the fresh bovine muscle tissue. Previous studies used different kinds of tissue. Bovine pulp,^[18] muscle tissue of porcine,^[19] rabbit liver,^[20] connective tissue of rat,^[3] and oral mucosa of pig^[21] have been studied as a model to achieve dissolution capability of irrigants. The main reason of using different type tissues except human dental pulp has been a possibility to obtained and the easier standardization of the surface area of each sample.^[22]

The importance of many activation methods on the tissue dissolving capability of NaOCl has been reported, but there are no studies about the effect of electrically activation on NaOCl solution. The present study showed that the electrically activation of NaOCl raised its capacity of dissolving organic material. For the same concentrations of NaOCl and E-NaOCl

within the same time intervals, the percent of tissue loss was greater in E-NaOCl ($P < 0.001$).

High concentration of NaOCl solutions has been toxic effect on living tissues. Therefore, this irrigation solution is supposed to use less concentration during the endodontic therapy preventing toxic effects. Previous studies have shown that if NaOCl as irrigation solutions is diluted, the tissue-dissolving ability decreases.^[21,23,24] Results from the present study showed that tissue weight loss after 5 min in 5% NaOCl was no significant difference to 2.5% E-NaOCl ($P > 0.05$). It should be emphasized that lower concentrations of E-NaOCl have a similar effect to more concentrated NaOCl solutions on tissue.

Tissue specimens immersed in 1.25% NaOCl, and distilled water increased in weight during the 5-min and 1.25% E-NaOCl at 3-min after exposure. Low-concentration hypochlorite solutions and distilled water can probably cause hydration of the bovine tissue. Previous studies have also reported that a similar effect on the organic tissue by these concentrations and distilled water.^[6,21]

The E-NaOCl experiments conducted 3V potential and current did not exceed 10 mA. The current value is tolerable for human beings according to the standard.^[25]

CONCLUSION

It should be recorded that the present study was performed in an ideal *in vitro* study model to test tissue dissolving ability of E-NaOCl. Results may not be directly estimated to the clinical applications. Furthermore, it still unclear whether E-NaOCl solutions would be more or less effective than agitation and ultrasonic-activation methods of NaOCl at different temperatures.

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