Comparative evaluation of pulp tissue dissolution ability of sodium hypochlorite by various activation techniques: An in vitro study

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Abstract

Background: The success in endodontic therapy is dependent largely on the absolute elimination of pulp tissue remnants and the maximum reduction of pathologic microbial load present within the root canal system.

Aim: The aim of this in vitro study was to compare and evaluate the ability of pulp tissue dissolution with and without activation of 5.25% sodium hypochlorite (NaOCl).

Materials and Methods: Pulp tissue samples collected from intact, noncarious extracted third molars were standardized to 8 mg in each group. These samples were placed in conical Eppendorf tubes of 15 ml with 10 ml 5.25% NaOCl for 4 min and were activated suitably according to the group specifications. All the procedures in this study were carried out at room temperature. The preweighed pulp tissue samples were further weighed again after the dissolution phase, and residual weight loss in each group along with its percentage was obtained. The results were then statistically analyzed using one-way analysis of variance and Tukey post hoc test.

Results: The maximum amount of pulp tissue dissolution was found in the laser-assisted irrigation group, i.e., NaOCl with Er, Cr: YSGG (P < 0.05) and showed a statistically significant result in comparison with other groups.

Conclusion: Within the limitations of the study, laser-assisted irrigation with a radial firing tip (NaOCl activated by Er, Cr: YSGG) was the most effective in dissolving the pulp tissue in comparison with other groups. The ultrasonic activation also showed a considerable amount of pulp tissue dissolution comparable to laser-activated irrigation in this study.

Keywords: Irrigant activation; laser-assisted irrigation; photodynamic therapy; pulp dissolution; sodium hypochlorite

INTRODUCTION

Success in endodontic therapy relies on rigorous debridement of pulpal remnants, dentinal debris, and infectious microbes. For favorable outcomes after endodontic therapy, mechanical instrumentation along with chemical irrigation must be given utmost importance.

Grossman and Meiman reported that 5% sodium hypochlorite (NaOCl) dissolves pulp tissue between the time period of 20 min and 2 h.[1] Dioguardi et al. reported that, for an ideal irrigation protocol, it is indispensable to employ 5.25% NaOCl solution for an appropriate duration of time.[2] Due to the presence of complicated anatomical variations in the root canal, complete debridement of the...
canals is not always achievable. Enhancing the activity of irrigant flow inside the inaccessible sites of the canal is considered crucial. Few methods of improving irrigant activity include flooding of the region with the irrigant to achieve “churning effect,” heating the irrigant, use of ultrasonics, and photoactivation methods.[3]

The prewarming of NaOCl is an easy, simple, and most commonly used method to increase the reaction rate and enhance the dissolution of organic residues. Generally, a temperature range of 50°C–60°C is recommended for warming the NaOCl.[4]

Ultrasonics, another simple and cost-effective method of irrigant activation, works by delivering an acoustic stream that creates shear stress sufficient enough to dislodge the debris of instrumented root canals.[5]

Photodynamic therapy, otherwise known as photo-activated disinfection (PAD) or light-activated therapy, is one of the novel methods of disinfection in treating dental caries and in endodontic therapy.[6] In this study, PAD was used to evaluate if it exerts any tissue dissolution properties when activated with NaOCl.

Laser-activated irrigation (LAI) which uses photoactivation methods can increase the reaction rate of irrigants and is beneficial in endodontics.[7] The laser activates the irrigants by creating cavitation and large elliptic gas bubbles which gets expanded by increasing the pressure and raising the fluid level in the canal. Whenever there is a constriction encountered, the pressure gets lowered, and the liquid returns back to the canal to produce a secondary cavitation effect. Hence, the laser often is referred to as a liquid pump.[7] Erbium laser increases the disinfection of the root canal system without causing thermal destruction to the adjacent tissues.[8,9]

This in vitro study aimed to compare and evaluate the amount of pulp tissue dissolution by various activation methods (which includes warming the NaOCl and agitating manually with gutta-percha, using ultrasonic activation, using photodynamic therapy – low power diode laser and LAI – Er, Cr: YSGG), and using the NaOCl and nonagitated NaOCl as control.

MATERIALS AND METHODS

**Human pulp tissue collection**

Fifty-three intact, noncarious third molars intended for extraction were procured and stored in the physiological solution until usage. Each extracted tooth was sectioned by placing the longitudinal grooves with airotor handpiece and were then split manually using the chisel and mallet without causing damage to the underlying pulp. Pulp tissue from the chamber was then carefully removed with the help of a tweezer and number 15 surgical blade. The samples were cleaned gently with distilled water to get rid of any debris and blotted dry. The collected samples were then mixed to form a random mix of the human pulp tissue.

**Group specification**

The control group for the study was:

- **Group I** – Pulp tissue in nonagitated 5.25% NaOCl.
- The four experimental groups for the study were:
  - **Group II** – Pulp tissue in warm 5.25% NaOCl – manually agitated with gutta-percha.
  - **Group III** – Pulp tissue in 5.25% NaOCl – ultrasonically activated.
  - **Group IV** – Pulp tissue in 5.25% NaOCl – photodynamically stimulated with a diode laser (PAD).
  - **Group V** – Pulp tissue in 5.25% NaOCl – Erbium laser-activated NaOCl.

**Preweighing and sample allocation**

The entire random mixture of pulp tissue collected weighed approximately 44 mg (TECHway weighing scale, TECHMART, India). The pulp sample was then randomly divided into 8 mg each for four experimental groups and one control group. Again, within each group, 8 mg of pulp tissue was further divided into four samples (n = 4) of 2 mg in each. In this way, all the samples in five groups were standardized to receive 2 mg of the pulp tissue among 20 samples (N = 20).

**Dissolution phase**

**Group I** – Pulp tissue in nonagitated 5.25% NaOCl:

In the conical Eppendorf tube (Hoverlabs, Haryana, India) containing 10 ml of 5.25% NaOCl solution (Chemident, Delhi, India), the preweighed pulp sample was placed at room temperature without any agitation.

**Group II** – Pulp tissue in warm 5.25% NaOCl – manually agitated with gutta-percha:

Ten milliliters of 5.25% NaOCl was warmed using an electric kettle heater (Prestige, Chennai, India) from room temperature to 42°C (temperature checked with digital thermometer Rossmann TG380 Flexi tip). The Eppendorf tube containing warm NaOCl and pulp sample was agitated manually with gutta-percha vigorously.

**Group III** – Pulp tissue in 5.25% NaOCl – Ultrasonically activated:

Ten milliliters of 5.25% NaOCl at room temperature taken in a conical Eppendorf tube with preweighed pulp sample was agitated with an ultrasonic endodontic tip (Satelac ultrasonic endodontic tip #K25).
Group IV – Pulp tissue in 5.25% NaOCl – photodynamic stimulated with a diode laser (PAD):

The preweighed pulp sample was stained with a photosensitizer of 0.02% toluidine blue for 30 s. The sample was then placed in a conical Eppendorf tube containing 5.25% NaOCl and was activated at room temperature with diode laser (ezlase Biolase technology, Mumbai, India) of wavelength 940 nm, 1 watt power, and 50 mJ energy using the endodontic tip of 200 µm in a continuous wave motion.

Group V – Pulp tissue in 5.25% NaOCl – erbium laser activated (LAI):

10 ml of 5.25% NaOCl at room temperature taken in conical Eppendorf tube was activated with Er, Cr:YSGG laser (Waterlase-Biolase, Inc.) of wavelength 2940 nm, 1 W power, 50 mJ energy, and 20 Hz of frequency using 200 µm RFT2 endodontic tip. The components such as air and water on the system were turned off.

**Activation protocol**

For the various activations used, the tips were inserted into conical tubes containing 10 ml of 5.25% NaOCl. All the samples were agitated for three cycles of 30 s, with a resting period of 45 s in between activation. During the resting period, 3 ml of 5.25% NaOCl was withdrawn from the Eppendorf tube, and a fresh 3 ml of solution was then replaced. Subsequently, the total exposure time of the pulp tissue in solution was approximately 4 min.

Following an approximate exposure period of 4 min, the remaining pulp samples were taken out, blot dried, and checked for its weight again. The variation in weight of pulp tissue in the predissolution period and postdissolution period was noted as residual weight. The residual weight obtained was further divided by the initial weight of the pulp tissue before the dissolution phase and then multiplied by 100 to attain the tissue weight loss in percentage.

**Statistical analysis**

The data were then analyzed statistically using IBM SPSS for Windows, version 21.0 (IBM corporation, Armonk, New York, United States). The statistical tests performed were one-way analysis of variance (ANOVA) and Tukey post hoc tests, and the significance level established was $P < 0.05$.

**RESULTS**

The comparative evaluation of mean residual weights of pulp tissue for various groups obtained from one-way ANOVA is summarized in Table 1. The results from one-way ANOVA showed a statistically significant difference ($P < 0.05$) in mean residual weight loss of pulp tissue between the groups. Tukey post hoc test from Table 2 revealed that Er, Cr: YSGG laser-activated NaOCl (Group V) showed a statistically significant difference from other groups. The percentage change of residual weight loss obtained in Er, Cr: YSGG laser-activated NaOCl (Group V) was 85%, followed by ultrasonically activated NaOCl (Group III), which showed a percentage change of 35%.

**DISCUSSION**

In this *in vitro* study, the pulp tissue dissolution ability of NaOCl irrigant activated by various activation methods was evaluated and compared using test tube models. Human pulp tissue samples were used in this study to simulate the oral environment rather than bovine pulp samples used in similar studies.[10,11] The current study employed pulp tissue of lesser weight as it is from human origin. In addition, the weighing scale used for this purpose aided in measuring the quantity precisely. Studies have shown that activation of irrigant enhances the irrigant delivery throughout the canal system, where the conventional instruments cannot reach.[12]

Most endodontists surveyed use NaOCl as their primary irrigant mainly for its tissue dissolution capability.[13,14] With a concentration that ranges between 0.5% and 6%, NaOCl as an irrigant remains to be the gold standard for antimicrobial efficacy.[15] However, studies from Cullen *et al.* revealed that dilution of NaOCl decreases its pulp dissolution capacity.[16] In this study, NaOCl of 5.25% concentration was used. The purpose behind the usage was mainly to evaluate pulp dissolution at the highest safe concentration intended to use clinically. An ideal resting period of 45 s between every activation was given, and the fact that the irrigant was replaced with fresh solution throughout this study resembles the clinical protocol of root canal irrigation.[10] The present study employed the filtration method to assess the amount of residual weight loss as it is a simple and inexpensive way of assessment.

Heating 4% NaOCl with the baby bottle warmer at various temperatures increases the efficacy of bovine
pulp tissue dissolution.\textsuperscript{[17]} Cunningham and Balekjian made a comparison between 2.6% and 5.2% NaOCl at 21°C and 37°C and established that, with an elevation in temperature, a reduced concentration would be adequate to produce collagen dissolution.\textsuperscript{[18]} Dutner et al. reported that endodontists prefer the use of full-strength NaOCl (\textgtr 5.0%) as their irrigant.\textsuperscript{[13]} Another in vitro study evaluating the pulp-dissolving capacity using 2.5% and 5.25% NaOCl at 30- and 60- min time intervals also concluded that NaOCl dissolved the pulp tissue efficiently at both the time intervals and concentrations.\textsuperscript{[19]} Since the concentration used in the study was higher, i.e., 5.25%, the temperature used for warming NaOCl was a little lower, i.e., 42°C as opposed to other studies. However, Abou-Rass and Oglesby recommended that, despite the concentration used, increasing the temperature of NaOCl by heating substantially improves its efficacy of tissue dissolution.\textsuperscript{[20]} Another recent in vitro study evaluating the level of pulp-dissolving ability through various irrigation protocols concluded that the combination of heating NaOCl within the canal and the use of ultrasonic activation was found to produce an average amount of pulp tissue dissolution than performing the irrigation alone with preheated NaOCl or ultrasonic activation.\textsuperscript{[21]} The result of this study in terms of pulpal dissolution between warm NaOCl and NaOCl at room temperature showed that the latter though not statistically significant experimentally performed better, which is in accordance with various other studies mentioned above.

The action of the alkaline solution of NaOCl can be increased remarkably when they are activated with ultrasonic energy or pulsed lasers. As the energy produced from these sources creates fluid motion within irrigants, there is an improved contact established between the irrigating fluid and root canal walls, which are usually inaccessible to the canal shaping instruments. This elevates the temperature of the irrigant that further improves its chemical interaction on hard and soft tissues.\textsuperscript{[18]}

Several studies on LAI have found that the absorption of water is strongest when the lasers operate in the mid-infrared spectrum, and the lasers included in such category are Er, Cr:YSGG laser (2780 nm wavelength) and the Er:YAG laser (2940 nm wavelength).\textsuperscript{[22]} The 2780 nm wavelength of Er, Cr:YSGG laser is highly absorbable by a water molecule. As a result of absorption, the air bubbles and steam are generated within the irrigating fluid through photoacoustic and photomechanical processes. Although the bubbles created burst inward the same way as that of ultrasonic tips, the tip position remains stable without movement in the case of lasers. Hence, there is no need for the operator to manually direct the laser tip to obtain the desired irrigant activation. Shockwaves produced due to the collision of bubbles result in rapid movement of irrigant.\textsuperscript{[23,24]} While performing activation with lasers in the current study, to avoid any dilution of NaOCl irrigant and in order to prevent any additive damage to the pulp tissue, air, and water on the laser apparatus was shut off. A major drawback of using laser for irrigant activation is irrigant extrusion due to enormous fluid motion and generation of pressure waves exerted on the root canals. This can be minimized using specialized honeycomb tips where the irrigant movement is directed on to the walls of the canal instead of moving apically when compared to the conventional plain-ended tips.\textsuperscript{[22]}

Antimicrobial photodynamic therapy is a two-step procedure which includes the introduction of a photosensitizer, i.e., photosensitization of infected tissue and then subjecting

### Table 2: Intergroup comparison among various groups with Tukey post hoc test and \( P \) value associated with each comparison

| Group | Groups | \( P \) |
|-------|--------|-----|
| NaOCl without agitation (Group I) | Manual agitation with warm NaOCl (Group II) | 1.000 |
| | Ultrasonic activated NaOCl (Group III) | 1.000 |
| | Photodynamically stimulated NaOCl (Group IV) | 1.000 |
| | Erbium laser-activated NaOCl (Group V) | 0.000* |
| Manual agitation with warm NaOCl (Group II) | NaOCl without agitation (Group I) | 1.000 |
| | Ultrasonic activated NaOCl (Group III) | 1.000 |
| | Photodynamically stimulated NaOCl (Group IV) | 1.000 |
| | Erbium laser-activated NaOCl (Group V) | 0.000* |
| Ultrasonic activated NaOCl (Group III) | NaOCl without agitation (Group I) | 1.000 |
| | Manual agitation with warm NaOCl (Group II) | 1.000 |
| | Photodynamically stimulated NaOCl (Group IV) | 1.000 |
| | Erbium laser-activated NaOCl (Group V) | 0.000* |
| Photodynamically stimulated NaOCl (Group IV) | NaOCl without agitation (Group I) | 1.000 |
| | Manual agitation with warm NaOCl (Group II) | 1.000 |
| | Ultrasonic activated NaOCl (Group III) | 1.000 |
| | Erbium laser-activated NaOCl (Group V) | 0.000* |
| | Manual agitation with warm NaOCl (Group II) | 0.000* |
| | Ultrasonic activated NaOCl (Group III) | 0.000* |
| | Photodynamically stimulated NaOCl (Group IV) | 0.000* |

*Statistically significant \( P < 0.05 \). SD: Standard deviation, NaOCl: Sodium hypochlorite
it to the light illumination of the sensitized tissue, i.e., irradiation of photosensitized tissue, all of which would lead to toxic photochemistry on the target cell, hence causing cell lysis. Although each of the individual elements used will not exert any action independently, together they have a synergistic effect to produce the desired action.[25]

This study attempts to assess if PAD has any pulp tissue dissolving capacity and it confirms that PAD is not found to exert any substantial pulp-dissolving capacity compared to other irrigant activation methods used. Furthermore, the statistically significant tissue dissolution ability of NaOCl activated with Er, Cr: YSGG laser in this research can be described due to the use of increased pulse energy (50 mJ) and decreased fiber diameter (200 μm) used.[10]

The major limitation of the study is that the entire pulp dissolution assays were carried out in a test tube model which does not mimic the clinical environment entirely. Nevertheless, the more extensive and long-lasting clinical trials are necessary to validate these observations. Since the results of this study showed a highly significant value, it could prove clinically significant as well.

CONCLUSION

Within the limitations of the study, the outcome of this in vitro study demonstrates that NaOCl activated with Group V, i.e., Er, Cr:YSGG laser with endodontic tip is the most successful in dissolving the pulp tissue. Furthermore, there was a considerable amount of pulp tissue dissolution in NaOCl activated by ultrasonic tips. Although photodynamic therapy has been highly successful in the disinfection of canals, its pulp-dissolving ability is still questionable.

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Conflicts of interest

There are no conflicts of interest.

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