International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Quantitative Assessment of Pulp Tissue Dissolution after Exposure to 5.25% Sodium Hypochlorite at 1min, 5min, and 60min at 37 °C and 60 °C.

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Abstract: ***Context*: The success of endodontic treatment mainly depends on effective irrigation methods to dissolve pulpal tissue, remove debris, and clean the complex root canal system. Sodium hypochlorite (NaOCI) is a widely used endodontic irrigant due to its tissue - dissolving and antibacterial properties. *Aim*: Quantitative evaluation of human pulp tissue dissolution at different temperatures of sodium hypochlorite. *Materials and Methods*: Twenty - four samples of human pulp tissue were collected from freshly extracted premolars. The samples were divided into two groups: Group I with normal saline and Group II with 5.25% NaOCI. Each group was further divided into two subgroups according to temperature (37°C and 60°C) and three divisions based on time intervals of tissue dissolution (1 minute, 5 minutes, and 60 minutes). *Result*: The results showed that normal saline did not show any dissolution of pulp tissue. In contrast, 5.25% NaOCl demonstrated a significantly higher tissue dissolution capacity compared to normal saline at both temperatures and all - time intervals. Less pulp dissolution was seen when kept in contact for 1 minute but increased subsequently when kept in contact for 5 minutes to 60 minutes at 60°C. *Conclusion*: According to the findings of the current study, it can be concluded that 5.25% NaOCl exhibits maximum pulp tissue dissolution at 60°C temperature when kept in contact with pulpal tissue for a minimum of 5 minutes and a maximum of 60 minutes.***

Keywords: endodontic irrigation, sodium hypochlorite, pulp tissue dissolution, temperature effects, time intervals

1. Introduction

Achieving successful results in the field of endodontic therapy depends on a triad of the accuracy of biomechanical preparation, the potency of chemical disinfection, and the efficient closure of all apical. The challenging and complex internal anatomy of the root canal system makes it difficult to achieve thorough disinfection of the root canal system. Therefore, irrigation is necessary for the elimination of dentinal debris, the dissolving of remaining pulpal tissue, and the cleaning of the anatomical complexity of the root canal system [1].

Acids (citric and phosphoric), chelating agents (EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative agents (hydrogen peroxide and GlyOxide), local - anesthetic solutions, and regular saline have all been used as root canal irrigants [2]. The irrigant most frequently employed in endodontics is sodium hypochlorite [3].

Sodium hypochlorite, earlier known as Dakins' solution, was first used to disinfect infected wounds during World War I between 1916 and 1918. Dakin's original recommendation for concentration was 0.5%. It has a broad spectrum of antibacterial and tissue - dissolving qualities. After considering the various advantages of the Dakin solution, Coolidge et al introduced the use of NaOCl as an irrigating solution in endodontics in 1919, because of its capacity to liquefy organic tissue, low viscosity, availability, and affordability [4].

Sodium hypochlorite acts through several steps to achieve its antimicrobial effects and break down pulpal tissue. First, Saponification: Reacts with fatty acids to form soap and glycerol, reducing surface tension. Then, Amino Acid Neutralization: Neutralizes amino acids to form water and salt, releasing hydroxyl ions and forming chloramines that interfere with cell metabolism. And lastly, Chlorination: Chlorine, a strong oxidant, inhibits bacterial enzymes by oxidizing sulfhydryl groups, leading to antimicrobial action [5].

Sodium hypochlorite was discovered to be the most effective at dissolving pulp tissue among the irrigants examined by Grossman and Meiman et al. in 1941 [6]. The only substance known to dissolve pulp tissue is sodium hypochlorite [7]. Hence preferred by clinicians. Research has shown that the optimal concentration of sodium hypochlorite is between 0.5 and 5.25% [8]. In accordance with this, dilute levels of sodium hypochlorite are ineffective at dissolving any residual tissue, thus maximum concentration i. e.5.25% is advised [9]. Senia ES et al. (1971) also proved that 5% sodium hypochlorite works effectively as a tissue solvent [10].

On the contrary, Abou - Rass and Oglesby confirmed that the sodium hypochlorite solutions heated to 140° F (60° C) have superior capacity in tissue dissolving, regardless of concentration (2.6% or 5.25%) [11 - 12].

The antibacterial and tissue - dissolving properties of hypochlorite increase with concentration, but so does its toxicity. According to Stojicic et al. in 2010, a higher concentration of hypochlorite solution, duration spent in contact with the surface, the pH of the solution, agitation technique, and temperature can all improve the effectiveness of root canal disinfection [13 - 14].

2. Literature Gap

Although concentrations of NaOCl have been studied extensively, there is a lack of quantitative evidence investigating the impact of increased temperature and duration on NaOCl's ability to dissolve human pulp tissue specifically. In literature, very few studies have evaluated the quantitative variations in tissue dissolving at various temperatures. Therefore, the present study was designed to evaluate the human pulp - dissolving ability of 5.25% sodium hypochlorite at different temperatures and time durations.

3. Materials and Methods

In this study, approval was obtained from the Institutional Ethics Committee (IEC/VSPMDCRC/22/2022) before commencing the research. Freshly extracted human permanent premolars were acquired from the Department of Oral and Maxillofacial Surgery, specifically selected for orthodontic reasons (Fig *1*).

Figure 1: Intact Premolar

From these 24 samples fulfilling inclusion criteria were randomly selected and stored according to CDC guidelines. The teeth were thoroughly cleaned to remove any debris and soft tissues adhering to their surfaces. They were kept moist in saline, and placed in a container with a secure lid to prevent leaking during transport or storage until they were needed for the study.

Inclusion Criteria and Exclusion Criteria

Inclusion criteria specified that only human permanent premolars extracted for orthodontic reasons would be included in the study. This criterion ensured that the samples were consistent in terms of tooth type and extraction purpose. Conversely, exclusion criteria were defined to eliminate teeth with any anomalies that could potentially affect the study outcomes. These anomalies included caries, fractures, root calcification, root resorption, and developmental anomalies.

Sample Preparation

Initially, a diamond disc in a micromotor handpiece was used to make longitudinal grooves on the mesial and distal surfaces of the premolars. This step allowed for the controlled splitting of the teeth, ensuring uniform exposure of the pulpal tissue. Subsequently, the teeth were split using a chisel and mallet, following established protocols for tooth sectioning. Careful attention was paid to avoid damaging the pulpal tissue during this process. Once the teeth were split, the pulpal tissue was meticulously removed using a number 15 blade (Fig: 2).

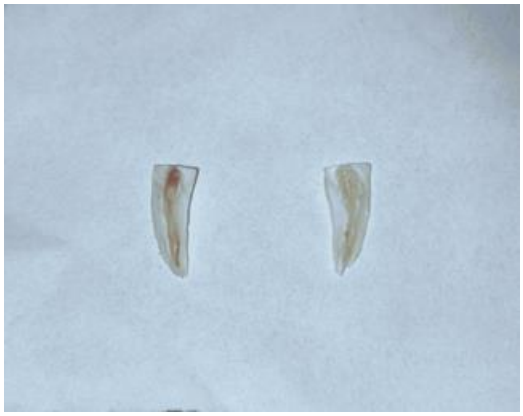

Figure 2: Sectioned tooth with vital pulp sample

This procedure was repeated until a standard volume of 2mg of pulpal tissue was obtained for each sample. This standardized volume ensured uniformity in the amount of tissue tested across all experimental conditions. Following tissue extraction, the pulpal tissue samples were placed in glass test tubes for storage and subsequent experimentation (Fig: 3).

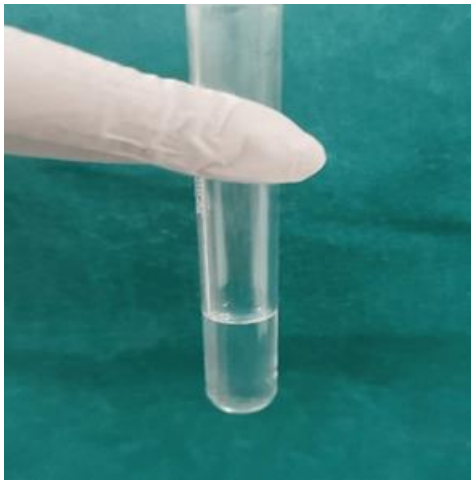

Figure 3: Sodium hypochlorite with pulp

4. Method

The 24 pulpal specimens obtained were randomly divided into two main groups: Group I (n=12) and Group II (n=12). Group I served as the control group, with pulpal tissue submerged in normal saline, while Group II consisted of pulpal tissue submerged in 5.25% sodium hypochlorite. Each group was further subdivided into two subgroups based on temperature: Subgroup A (solution at 37° C) and Subgroup B (solution at 60° C) (Fig: 4). By varying the temperature of the solutions, the researchers aimed to evaluate the impact of temperature on the efficacy of tissue dissolution.

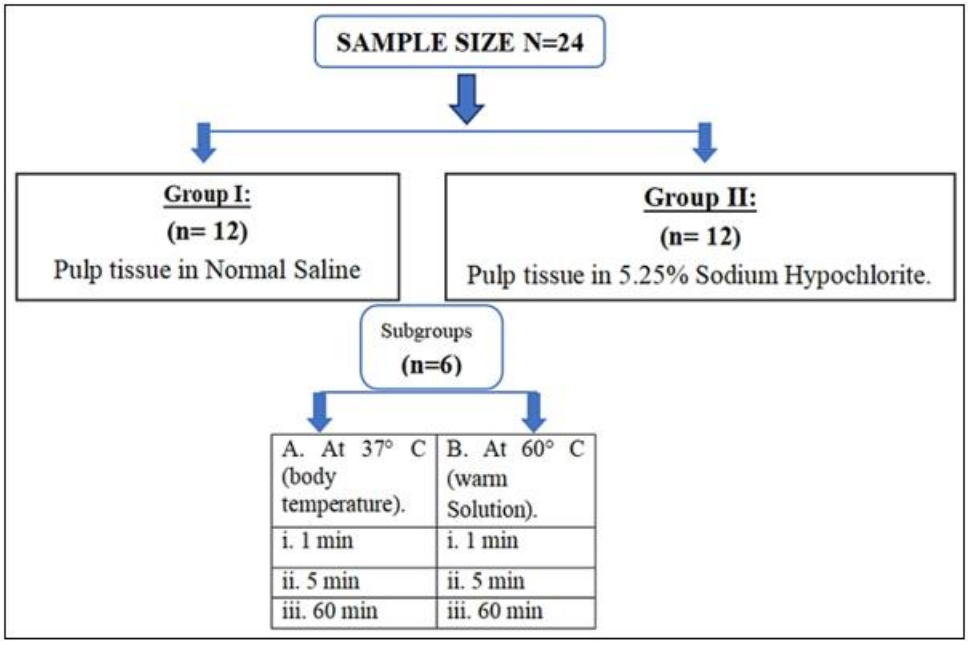

Figure 4: Flow Chart - Distribution of experimental groups and control groups with respective sample sizes

5. Observation and Measurement

Following the subdivision into temperature subgroups, the samples were subjected to observation at specific time intervals: 1 minute, 5 minutes, and 60 minutes. This observation period allowed us to monitor the rate and extent of tissue dissolution over time. Samples in Subgroup A were placed in an incubator set at 37° C, while those in Subgroup B were placed in a water bath maintained at 60° C. After the specified observation period, the samples were filtered on pre - weighed Whatman filter paper. Filtration facilitated the separation of dissolved tissue from the solution, enabling accurate measurement of tissue dissolution. The filter paper containing dissolved tissue was then dried overnight to remove any residual moisture (Fig: 5).

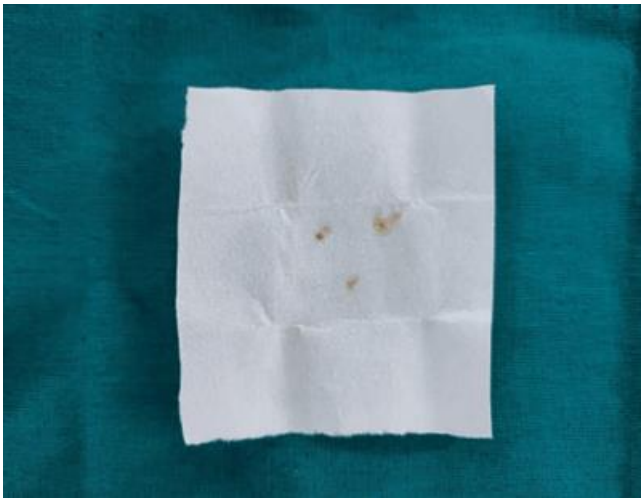

Figure 5: Overnight dried filter paper

Method of Measurement:
Finally, the weight difference between the initial pulp tissue with filter paper (before dissolution) and the dried pulp with filter paper was measured on a precision balance machine. Precision balances are precise balances with readability to 0.0001g making them ideal for high - precision applications (Fig: 6). This difference in weight served as a quantitative measure of tissue dissolution, allowing for objective comparison between experimental conditions.

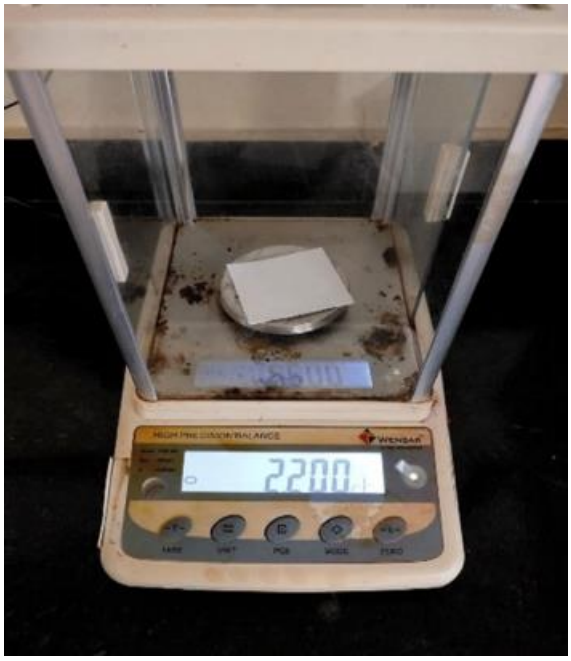

Figure 6: Precision Balance

6. Results

The data obtained from the following test were subjected to Statistical Product and Service Solution (SPSS) Statistics for Windows, Version 28.0, IBM Corp. (2021), Armonk, NY. An unpaired t test was used to compare the efficacy between saline and sodium hypochlorite at different temperatures. Paired t test was used to compare efficacy at two different temperatures in each group. Anova F test was used to compare time - dependent tissue dissolution.

Table 1: Intra - group comparison of the efficacy of normal saline and 5.25% sodium hypochlorite in dissolving human pulp at two different temperatures - 37° C vs 60° C in terms of weight of tissue dissolved using paired t test

Groups	Time	Subgroup A (37° C) Mean (SD)	Subgroup B (60° C) Mean (SD)	Paired t test	P value, Significance
Group I (Normal Saline)	1 min	0.0 (0.0)	0.0 (0.0)	t = 0.0	p = 1.000 (NS)
	5 min	0.0 (0.0)	0.0 (0.0)	t = 0.0	p = 1.000 (NS)
	60 min	0.0 (0.0)	0.0 (0.0)	t = 0.0	p = 1.000 (NS)
Group II (5.25% Sodium Hypochlorite)	1 min	0.025 (0.007)	0.075 (0.021)	t = - 3.162	p = 0.087 (NS)
	5 min	0.065 (0.007)	0.31 (0.028)	t = - 11.884	p = 0.007*
	60 min	0.12 (0.014)	0.57 (0.09)	t = - 6.364	p = 0.024*

p>0.05 – no significant difference (NS) *p<0.05 – statistical significant

The weight of tissue dissolved in normal saline at 37° C vs 60° C at all time intervals was compared, there was no statistically significant difference found (p>0.05) i. e. there was no pulp dissolution found in normal saline.

No significant difference was observed in the weight of pulpal tissue dissolved at 1 minute for both Group II A and II B. However, greater dissolution of pulp was observed when 5.25% NaOCl was heated at 60° C for 5 min (p - value=0.007) and 60 min (p - value= 0.024), and these results were statistically significant.

Table 2: Inter - group comparison of the efficacy of 5.25% sodium hypochlorite as compared to normal saline in dissolving human pulp at 37° C and 60° C in terms of weight of tissue dissolved using unpaired t - test.

Subgroups	Time	Group I (Normal Saline) Mean (SD)	Group II (5.25% NaOCl) Mean (SD)	Unpaired t - test	P value, Significance
Subgroup A (37° C)	1 min	0.0 (0.0)	0.025 (0.007)	t = - 5.0	p = 0.038*
	5 min	0.0 (0.0)	0.065 (0.007)	t = - 13.0	p = 0.006*
	60 min	0.0 (0.0)	0.12 (0.014)	t = - 12.0	p = 0.007*
Subgroup B (60° C)	1 min	0.0 (0.0)	0.075 (0.021)	t = - 5.0	p = 0.038*
	5 min	0.0 (0.0)	0.31 (0.028)	t = - 15.5	p = 0.004*
	60 min	0.0 (0.0)	0.57 (0.09)	t = - 8.143	p = 0.015*

*p<0.05 – statistically significant

A significant difference was observed in the weight of pulpal tissue dissolved at 1 min, 5 min, and 60 min for Group I A and II A, and greater dissolution of pulp was observed in Group II when 5.25% NaOCl was kept at 37° C for 5 min (p - value=0.006) and 60 min (p - value= 0.007).

Significant difference was observed in the weight of pulpal tissue dissolved at 1 min, 5 min, and 60 min for Group I B and II B and greater dissolution of pulp was observed in Group II when 5.25% NaOCl was kept at 60° C for 5 min (p - value=0.004) and 60 min (p - value= 0.015).

Table 3: Intra - group comparative efficacy of 5.25% NaOCl (Group II) in dissolving human pulp at two different temperatures - 37° C and 60° C in terms of weight of tissue dissolution at three different time intervals.

Group II (5.25% Sodium Hypochlorite)	Subgroup A (37° C) Mean (SD)	Subgroup B (60° C) Mean (SD)
1 min	0.025 (0.007)	0.075 (0.021)
5 min	0.065 (0.007)	0.31 (0.028)
60 min	0.12 (0.014)	0.57 (0.09)
F test	F = 45.5	F = 33.29
p - value, Significance	p = 0.006*	p = 0.009*

*p<0.05 – statistically significant

The pulpal tissue dissolution increased significantly in both Group II A and II B from 1min to 5 min and then to 60 min. Thus, the pulp tissue dissolution was found to be maximum at 60° C at 60 min in Group II B. (Table *3*)

Results also show that increasing the temperature of NaOCl solution at both 37° C and 60° C up to 1min did not significantly improve the pulp dissolution capacity. However, a significant difference was seen as better tissue dissolution occurred when the temperature was elevated from 5 min to 60 min for both subgroups II A and II B.

7. Discussion

Sodium hypochlorite (NaOCl) is one of the most popular endodontic irrigants due to its excellent nonspecific proteolytic and antibacterial action [1]. Callahan and Grossman et al 1941 [6] provided evidence of the significance of a solvent capacity in endodontic irrigants. According to Strindberg et al in 1956, the presence of pulpal remnants after appropriate root canal therapy may lead to post - operative discomfort and can have the potential of developing a periapical lesion [15 - 16].

It activates through a number of chemical processes, including the neutralization of amino acids, chloramination, and saponification, which cause tissue breakdown and have an antibacterial impact. In 1978, Rosenfeld et al. reported using 5% NaOCl as an efficient solvent for human pulp tissue. This claim supports the findings of Baumgartner and Cuenin's study in 1992, which showed that tissue dissolution rose with concentration. Furthermore, according to Hand et al (1978), 2.5% NaOCl was not as effective as 5.25% NaOCl as a solvent. As numerous researchers have found that this percentage of sodium hypochlorite solution effectively dissolves pulpal tissue, we chose to use it as the preferred concentration in the current investigation (5.25%) [17 - 19]

In 2005, Sirtes et al. reported that 1%, 2.62%, and 5.25% sodium hypochlorite solutions, respectively, had an unaltered amount of chlorine accessible for an hour at 45° C and 60° C [20]. According to research by Giampiero Rossi - Fedele et al. (2008), heating 4% sodium hypochlorite using a baby bottle warmer speeds up the dissolution of bovine pulp, but it reaches a plateau at 60° C (Fedele, Giampiero Rossi). There are two possible explanations for this: either the temperature rise has a limit to how much of its dissolving power it can exert i. e increases in temperature may increase the availability of chlorine till it reaches a plateau at 60° C, or over time the sodium hypochlorite in the tube exhausted and no more additional chlorine was available [21]. The current study's goal was to ascertain the effectiveness of pulpal tissue dissolution at 1 minute, 5 minutes, and 60 minutes at 37° C and 60° C.

Fresh human pulp tissue was chosen for the current study in order to acquire more precise results since it offers a more accurate model for examining particular processes, reactions, and potential outcomes in humans. In contrast, there were limitations if bovine pulp tissue was used instead of human pulp in earlier investigations. The distinctions might affect experimental outcomes and limit the application of the findings to human situations. In earlier studies by Koskinen et al, Gordon et al in 1980 used bovine pulp to simulate human pulp, which may not give accurate results [22].

The use of the filtration method in the current study, which involved subtracting the initial weight of the pulp and filter paper from the weight of the overnight - dried pulp and filter paper in order to calculate the precise dissolution of the pulp tissue, is a unique feature. This unique filtration procedure overcame the drawbacks of earlier studies, which prevented an accurate calculation of pulp tissue dissolving due to residual levels of the irrigant [7]. So that it provides us with quantitative outcomes.
Normal saline shows no significant difference in the pulp tissue dissolution at room temperature or at 60° C at any periods. [23].

In the investigation by Bukiet et al. (2013), NaOCl viscosity decreased as the temperature was raised from 22 to 37° C. Because molecules move faster at 37° C than at 22° C, thermal agitation of the molecules may be the cause of this well - known phenomena (Guyon et al.2001). Because of the molecules increased kinetic energy or speed, surface tension likewise reduces as temperature rises. The decrease in surface tension is thus caused by the weakening of intermolecular forces [24].5.25% Sodium Hypochlorite at 37° C considerably increased tissue dissolving for all time intervals in the current study when compared to normal saline, and the findings were in accordance with those of Stojicic et al in 2010 [13].

The pulp dissolution in the current study quantitatively increased with increasing time duration, and enhanced dissolution showed up from 5min to 60min at 60° C, when we compared time intervals. According to research by D'Arcangelo et al., pulp samples exposed to 2.5% NaOCl exhibited solubility values of 0.98 in one minute, 29.1 in five minutes, and 57 in ten minutes [25]. In another study, to simulate potential in vivo contact durations, human pulp tissue cells were obtained from extracted third molars, plated, cultured, and exposed to several concentrations of NaOCl (0.33%, 0.16%, 0.08%, and 0.04%) at intervals of 5, 10, and 15 minutes. The quantity of viable cells remaining in the culture after treatment was ascertained. The findings show that the viability of the cells was unaffected by the lowest dose of NaOCl examined [26]. By irrigating the specimens with 1.5% and 2.5% NaOCl at pH values of 5, 7, and 12 for 20 minutes, Del Carpio - Perochena et al (2015). investigated whether changing the pH of sodium hypochlorite (NaOCl) improved its antibacterial and dissolving capacity on in situ - generated polymicrobial biofilms. The study found that although acidifying NaOCl increases its antibacterial activity, it also lessens the irrigant's capacity to dissolve [27].

The study's limitations include the fact that the heated 5.25% NaOCl solution cannot be left in a patient's oral environment for 60 minutes; instead, the purpose of the study was to determine how well sodium hypochlorite solution dissolved pulp tissue.

8. Conclusions

The results of this study indicate that sodium hypochlorite has an efficient tissue - dissolving property. The efficacy of dissolution is maximum with a 5.25% concentration of sodium hypochlorite at 60° C and when kept in contact with pulp tissue for 60 minutes. Normal saline has no tissue dissolving property at any temperature and exposure time. Therefore, clinicians should make an informed decision while employing various irrigants during endodontic therapy.

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