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A Comparative Evaluation of Pulp Tissue Dissolving Ability of Three Different Pulp Dissolving Agents With 5.25% Sodium Hypochlorite: An In-Vitro Study

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Abstract

Introduction: The dissolution of pulp tissue is an essential aspect of root canal treatment, as it aids in the removal of necrotic or infected tissue from the root canal; thereby reducing the microbial load. **Aim:** This in-vitro study aimed to compare the pulp tissue dissolving ability of three different pulp-dissolving agents a with 5.25% sodium hypochlorite (NaOCl) solution. **Materials and Methods:** 128 intact, non-carious extracted human single-rooted premolar teeth were selected and sectioned to harvest intact pulp tissue. Pulp tissue samples were randomly divided into four experimental groups (n=32) based on the pulp dissolving agent used: Group I (5.25% NaOCl solution), Group II (5% Calcium hypochlorite solution), Group III (5.25% NaOCl gel) and Group IV (8% Papain gel). The initial weight of each sample was recorded and samples were exposed to specific pulp-dissolving agents for a specified time intervals. The time required for complete dissolution of the pulp tissue was recorded for all samples till 120 minutes. **Results:** The study analyzed the mean weight of pulp tissue in various groups, including Group I, Group II, Group III, and Group IV. After 30 minutes, complete dissolution of pulp tissue was evident in Group I, while Group II showed 0.0058 grams of remaining tissue after 30 minutes, 0.0040 grams after 60 minutes, and 0.0022 grams after 120 minutes. Group III showed 0.0072 grams of remaining tissue after 30 minutes, and Group IV showed 0.0075 grams of remaining tissue after 30 minutes, 60 minutes, 90 minutes, and 120 minutes. **Conclusion:** 5.25% NaOCl has the highest pulp tissue dissolution efficacy compared to other dissolving agents. Further clinical studies are warranted to validate these findings and assess the feasibility of these agents in clinical practice.

KEYWORDS

Pulp tissue; Pulp dissolution; Sodium hypochlorite; Pulp dissolving agents; Endodontic treatment; Irrigating solutions

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1 | INTRODUCTION

Successful endodontic treatment requires knowledge of root canal anatomy, proper access cavity preparation, thorough cleaning-shaping, irrigation, and three-dimensional (3D) obturation of the root canal space.[1,2] Cleaning and shaping are considered as one of the most important steps in Endodontic therapy. Cleaning refers to the removal of inflamed or diseased pulp tissue, microorganisms, their by-products, and dentinal shavings from the root canal system. Shaping on the other hand entails the establishment of a specific cavity form that provides access into the root canal and creates an apical preparation to achieve three-dimensional obturation of the root canal system.[3]

A tooth having a straight root with a single canal is not a rule but rather an exception. The root canal system is highly variable and possesses anatomical complexities such as lateral canals, accessory canals, isthmus, etc. Due to the presence of these micro-complexities, the operator cannot reach and clean all intricacies of the root canal system, even with available modern hand and rotary endodontic instruments.[4,5] Studies have shown that these complex areas harbor pulp tissue debris, microbes, and their by-products; which may interfere with a close adaptation of the obturating material with the canal wall and may result in persistent periradicular inflammation and subsequent Endodontic failure.[6]

Irrigants play a vital role in the dissolution of pulp tissue and disinfection of the root canal.[7] It also lubricates the canal walls and helps in the dislodgment of the loosened and suspended debris from the root canal, thereby preventing the packing of the dentinal shavings and pulp tissue in the apical portion of the root canal. They also help in dissolving organic and inorganic components of the smear layer to clean the dentine surface and thus, improve the bonding ability of the root canal sealer. Irrigants also possess antimicrobial properties when introduced in direct contact with microorganisms.[8,9] Thus, irrigants have become an essential part of root canal debridement protocol.

Taft et al. in 1859, was the first to use root canal irrigant and emphasized the need for frequent irrigation during root canal therapy. He recommended the use of a 'deodorizing agent' like chloride of sodium as an Endodontic irrigant.[10] Schreier (1893) introduced potassium and sodium ions for the dissolution of pulp and necrotic tissues in the root canal. Studies conducted by Grossman and Meiman in 1941 led to the introduction of the combined use of Sodium hypochlorite (NaOCl) and hydrogen peroxide to wash out remnants of pulp tissue and dentinal shavings by its mechanical flushing action(effervescent action).[11]

Among all irrigants, NaOCl is considered as a gold standard irrigating solution due to its excellent pulp tissue dissolving ability and antimicrobial properties.[12] It usually dissolves the pulp in 20-30 minutes. It is also an antiseptic and lubricant and has been used in various dilutions ranging from 0.5% to 5.25%.[13] It has a strong proteolytic and antimicrobial effect lasting as long as free chlorine is available in the solution. It is also inexpensive, readily available, and possesses a long shelf life.[14,15] NaOCl dissolves tissue by three different mechanisms namely saponification, amino acid neutralization, and, chloramination reaction.[16] Although; it has excellent tissue-dissolving properties it has some inherent drawbacks such as, acute inflammation followed by necrosis when it comes in contact with living tissue. It also has an unpleasant taste and odour, lack of substantivity, and inability to remove the smear layer. Rarely, inadvertent extrusion of NaOCl beyond the apical foramen may result in intense pain, edema, hyperemia, and necrosis of the tissues.[17] To overcome these demerits of NaOCl liquid, some other alternatives in Endodontic irrigants have been used.

Dutta et al. in 2012 used Calcium hypochlorite solution as an endodontic irrigant for the first time. Primarily it was used for industrial sterilization, bleaching and water purification treatment.[18] It is a white-colored powder that is to be dissolved in distilled water and used as a freshly prepared solution for root canal irrigation. It is more stable and potent than NaOCl solution due to the amount of readily available chlorine content (up to 65%).[19] Calcium hypochlorite also dissolves tissue by the same mechanism as NaOCl.

Recently, to control flow and to prevent the caustic effect of NaOCl solution, NaOCl gel has been proposed in Endodontics. Manufacturers claim that this gel has better control over a flow and it has a greater duration of contact with pulp tissue than its solution form. Gel has more shelf life than the solution and also it is more biocompatible, biodegradable and nontoxic.[20]

With evolution, the trend of materials used in dentistry is shifting from synthetic chemicals to herbal or natural products. Among these, the Papain gel has been used as irrigating solution in Endodontics. Duarte et al. in the study concluded that 8% Papain gel can also be used as irrigating solution.[21] Papain is a natural enzyme extracted from the pulp plant 'carica papaya', Caricaceae family, better known as 'Papaya' fruit. This enzyme has ability to break down organic molecules of amino acid, known as polypeptides. The optimum pH for the activity of Papain is in the range of 3 to 9.[22] Couto De Oliveira et al. indicated that papain is antioxidant agent against (hydrogen peroxide) H_2O_2-induced damage and has ability of pulp dissolution due to its proteolytic enzyme, in addition to its bactericidal action.[23,24]

Currently, there is very insufficient documented scientific data about the various pulp dissolving agents, to either prescribed or refute their use as a better alternative to NaOCl solution. Hence, on this background this study was planned to examine and compare the tissue dissolving ability of 5.25% NaOCl solution, 5% calcium hypochlorite solution, 5.25% NaOCl gel and 8% papain gel at different time intervals. The aim of the study was to compare the human pulp dissolving ability of 8% Papain gel, 5% calcium hypochlorite solution, 5.25% NaOCl gel with 5.25% NaOCl solution.

2 | METHOD

After obtaining approval from the Institutional Ethical Committee, the collection of teeth samples was determined.

2.1 | Collection of sample teeth

128 freshly extracted premolars were collected from the Department of Oral and Maxillofacial Surgery of the institute. The premolar teeth which are sound, noncarious having a single root, with mature apex were selected for study. Teeth with any visible cracks or fracture, developmental anomalies, root resorption or periodontally compromised teeth were exluded.

2.2 | Preparation and Harvesting of pulp tissues

Freshly extracted teeth were cleaned of all debris and placed in normal saline. Teeth were used immediately to avoid changes in the tissue properties. With due care, the tooth was split into two sections using a vertical groove on the proximal surfaces without touching the pulp. The pulp tissue was removed in toto with a spoon excavator and sterile forceps.

2.3 | Preoperative weighing of pulp tissue

Pulp tissue was placed in a petri dish and gently washed to remove debris and coagulated blood. The pulp tissue sample was blotted using filter paper (sterile absorbent paper) placed on either side of the tissue. Then, the pulp tissue was kept on a pre-weighted whatman filter paper. The weight of the pulp tissue sample was determined by using a precision balance machine that was precalibrated for pre-weighted Whatman filter paper. (dry method of weighing) in an airtight container, and this weight was referred to as 'W1'.
Assignment of pulp tissue samples for various groups:

- Group I: 5.25% NaOCl solution
- Group II: 5% Calcium hypochlorite solution
- Group III: 5.25% NaOCl gel
- Group IV: 8% Papain gel

2.4 | Pulp Dissolution Test

After initial weighing pulp tissue (W1), it was immersed in 5 ml of a 5.25% NaOCl solution for 30, 60, 90, and 120 minutes. The weight of undissolved pulp tissue was measured after 30 minutes (W2), 60 minutes (W3), 90 minutes (W4), and 120 minutes (W5) using the dry method. For the removal of excess gel in Group III and IV, thorough washing with 5 ml of distilled water was done in a petri-dish. The same procedure was repeated for all groups.

2.5 | Statistical analysis

The study collected data using a coding system and compiled it on an MS Office excelsheet. The statistical analysis was carried out with SPSS. Weight loss in human dental pulp tissue in four study groups was measured, and the results were shown as mean and standard deviation at intervals of 30, 60, 90, and 120 minutes. Intra-group and inter-group comparisons in each group were done using one-way ANOVA tests.

3 | RESULTS

The study analyzed the mean weight of pulp tissue in various groups, including Group I (5.25% NaOCl solution), Group II (5% Calcium hypochlorite solution), Group III (5.25% NaOCl gel), and Group IV (8% Papain gel). After 30 minutes, complete dissolution of pulp tissue was evident in Group I while Group II showed 0.0058 grams of remaining tissue after 30 minutes, 0.0040 grams after 60 minutes, and 0.0022 grams after 120 minutes (Table 1). Group III showed 0.0072 grams of remaining tissue after 30 minutes, and Group IV showed 0.0075 grams of remaining tissue after 30 minutes, 60 minutes, 90 minutes, and 120 minutes.

4 | DISCUSSION

The objectives of irrigating solutions in endodontics include flushing out the debris, lubricating the canal, dissolving organic and inorganic tissue, preventing the formation of a smear layer during instrumentation, or dissolving it once it has formed.[1] The chemical effectiveness depends on the concentration of the irrigant, the area of contact, and the duration of the interaction between the irrigant and the infected material. The biological function of irrigants is related to their antimicrobial effects. Dissolution of the pulp tissue is the most desirable requisite of any endodontic irrigating solution. The solution dissolves both necrotic and vital pulp tissue. Its effectiveness can be increased by increasing the time of exposure and the temperature of the solution. The mechanism of action of NaOCl for tissue dissolution takes place in various steps like saponification, amino acid neutralization, and chloramination reactions.[16] NaOCl acts as an organic and fat solvent that degrades fatty acids and transforms them into fatty acid salts (soap) and glycerol (alcohol), reducing the surface tension of the remaining solution, suggesting a saponification reaction. It neutralizes the amino acids by forming water and salt simultaneously with the exit of hydroxyl ions; thus, pH is reduced. Dutta et al. used Calcium hypochlorite ($Ca(OCl)_2$) solution as an endodontic irrigant for the first time.[18] $Ca(OCl)_2$ also dissolves pulp tissue by the same mechanism as NaOCl. Some studies showed that an accidental periapical extrusion of $Ca(OCl)_2$ may cause less tissue irritation as compared to NaOCl. $Ca(OCl)_2$ was also found to be effective in eliminating *E. faecalis* in a planktonic state and exhibited acceptable cytocompatibility. De Almeida et al. showed that $Ca(OCl)_2$ used in conjunction with ultrasonic activation is efficient in dissolving pulpal tissue and reducing root canal infection.[24] Though the $Ca(OCl)_2$ releases more free chlorine; due to the formation of the calcium hydroxide layer around the pulp tissue, it takes 120 minutes for the dissolution of pulp tissue completely which may pose a challenge clinically.

Papain is another endodontic irrigant which is a proteolytic enzyme extracted from the latex of Carica Papaya. Papain acts by breaking the polypeptide chain of the proteins apart. A proteolytic enzyme of papain directly attaches the active site i.e., carbonyl carbon in the backbone of the peptide chain, which leads to degradation of the protein. Studies conducted by Couto De Oliveira et al.[23] and Pithon et al.[24] evaluated the effect of different combinations of papain-based gels on dissolving pulp tissue and found that all the gels that contained papain and the 0.5% chloramine gel promoted pulp tissue dissolution.[23]

NaOCl gel (5.2%) has been recently introduced in endodontics as an irrigant. Its gel form formulation is desired to control the flow of the material and increase its duration of tissue contact.[20] Contact time is one of the factors that affect the dissolution property of the pulp. Though, gel form has shown promising results on pulp tissue dissolution in this study; it has inherent limitations to flow into the intricacies of natural teeth. Thus, additional means of irrigation should be recommended that will facilitate the gel to reach these anatomical complexities. NaOCl gel can be used in open apex cases, where extrusion of NaOCl liquid is likely expected. It is a potent pulp tissue solvent that dissolves the tissue completely at 60 minutes.

Various methodologies have been used to evaluate the pulp dissolution abilities of the different irrigating solutions over a period of time. In a study by Moorer et al.[26] measured the amount of available chlorine in the solution after complete pulp tissue dissolution. Stojicic et al.[27] in their study, stated that determining the end point of complete pulp tissue dissolution is difficult due to the presence of a number of bubbles as a result of saponification reaction and varied as per the eye perception of the examiner. Therefore, to overcome this bias in the present study, the contact time of irrigants with human dental pulp tissue samples was standardized (i.e., 30, 60, 90, and 120 minutes) and the samples were weighed before and after exposure to the irrigating solution. The majority of the irrigants presently used in endodontics require 30 minutes to 120 minutes (maximum) for the complete dissolution of pulp tissue including the cleaning and shaping procedure. Thus, for the study maximum time for tissue dissolution had kept at 120 minutes.

4.1 | Limitations of the study:

This study was performed on the pulp of healthy human vital teeth i.e. non inflammed pulp, which may not replicate a true clinical scenario for dissolution. Though due care was taken to remove pulp tissue in toto, some amount of damage to the pulp tissue is expected with procedural protocol. This study was performed on teeth with straight root canals. Therefore, the results of this study may not correlate clinically with the teeth having curved roots or root canals where irrigating solutions may not reach easily without agitation or adequate canal enlarging. Other factors that affect the pulp tissue dissolving capacity of endodontic irrigant such as concentration and pH, frequency and intensity of agitation, the temperature of irrigant, and rate of replenishment of irrigant have not been taken into consideration in this study.

5 | CONCLUSION

Among various agents used as intra-canal irrigants, NaOCl liquid (5.25%) is a potent pulp tissue dissolving solvent; when used for 20-30 minutes. It should be used cautiously considering its caustic effect, odour, taste, and hypochlorite accidents. Other irrigants though showing pulp dissolving abilities, the time required for complete pulp dissolution is more and not feasible clinically. To enhance their action various modes of agitation should be employed along with different concentrations, increased temperature, and volume of replenished irrigant. Thus, the search for an ideal endodontic irrigant is still continued which possesses all the desirable properties.

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Conflict of interest

The authors have no conflicts of interest to declare.

Supporting Information

Additional supporting information may be found at the journal's website.

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TABLE 1 Intra and Intergroup comparison of pulp tissue weights at different time intervals

Group	W1 (T0- 0 mins)	W2 (T1-30 mins)	W3 (T2-60 mins)	W4 (T3- 90 mins)	W5 (T4- 120 mins)
Group I	0.0071590 ± 0.0009840	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Group II	0.007297 ± 0.0009167	0.005822 ± 0.0006529	0.004025 ± 0.0007996	0.002253 ± 0.0003455	0.000 ± 0.000
Group III	0.007231 ± 0.0010209	0.001544 ± 0.0003999	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Group IV	0.007503 ± 0.0009533	0.005509 ± 0.0006397	0.003997 ± 0.0004410	0.003009 ± 0.0005012	0.0021075 ± 0.0004912

Values are in grams; mean ± standard deviation