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ORIGINAL ARTICLE

In Vitro Evaluation of Bovine Pulp Dissolution Using Dual Rinse HEDP at Different Temperatures

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ABSTRACT

Objective: To evaluate the degree of pulp tissue dissolution using a novel irrigant, Dual Rinse HEDP, at different temperatures.

Methods: Fifty standardized pulp tissue samples (1×1×1 mm) were divided into five groups (n=10): Group A, 0.1 mL of NaOCl (5.25%) at 36.8°C (body temperature); Group B, 0.1 mL of NaOCl (5.25%) at 80°C; Group C: 0.1 mL of NaOCl (5.25%) mixed with Dual Rinse HEDP at 36.8°C; Group D, 0.1 mL of NaOCl (5.25%) mixed with Dual Rinse HEDP at 80°C; Control group: 0.1 mL of saline solution at 36.8°C (n=5) and at 80°C (n=5). The dissolution time of the pulp tissue was recorded in seconds and minutes using a high-resolution digital microscope (20x magnification) and a stopwatch. Results were statistically analysed using one-way ANOVA and Tukey's HSD post hoc test with significant differences among the groups set at $p<0.05$.

Results: Statistical analysis indicated that NaOCl at 80°C (Group B: 0.369±0.034 min) and Dual Rinse HEDP at 80°C (Group D: 0.377±0.037 min) demonstrated the fastest dissolution time, without any significant difference between them ($p>0.05$). Samples treated at body temperature showed significantly longer dissolution times (Group A: 6.252±0.277 min; Group C: 6.389±0.410 min), without any significant difference between them ($p>0.05$). The control group (Group E) exhibited no pulp dissolution, which significantly differed from all other groups ($p<0.05$). Heating the irrigants to 80°C resulted in a statistically faster dissolution time compared to the groups tested at body temperature ($p<0.05$), with no significant differences among the groups tested at the same temperature ($p>0.05$).

Conclusion: The study highlights the critical role of temperature for the dissolution efficacy of both NaOCl and Dual Rinse HEDP and supports the potential use of Dual Rinse HEDP mixed with NaOCl for continuous chelation.

Keywords: Endodontics, HEDP, root canal irrigants, sodium hypochlorite, temperature

HIGHLIGHTS

- NaOCl and Dual Rinse HEDP at 80°C significantly accelerate pulp tissue dissolution compared to body temperature.
- At all tested temperatures, Dual Rinse HEDP demonstrated pulp dissolution efficacy comparable to NaOCl alone.
- Heating irrigants to 80°C enhances their effectiveness, supporting the use of continuous chelation in endodontics.

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INTRODUCTION

The primary objective of endodontic treatment is to reduce and/or eliminate damaged tissues and bacterial biofilm within the complex endodontic space, thereby promoting the healing of periapical tissues or preventing apical periodontitis (1–3). During the shaping phase, the anatomical complexity of root canals prevents manual and mechanical files from achieving sufficient bacterial and tissue eradication (4). It is crucial to utilise an antibacterial irrigant solution to achieve the disinfection effect. Sodium hypochlorite (NaOCl), used at concentrations ranging from 0.5% to 6%, remains the most effective irrigating solution in root canal therapy (1, 5).

Since its introduction into dental practice in 1920 (6), NaOCl became a cornerstone in chemo-mechanical root canal treatment, due to its unique biological properties, which include the dissolution of necrotic tissue, strong antibacterial effect and the ability to disrupt biofilms (3–7).

A recurring discussion in clinical endodontics is whether the efficacy and safety of NaOCl solutions can be enhanced through activation and modification (8).

Regarding activation, various techniques exist, one of which is internal heating, a method involving controlled internal heating. This technique has proven to be effective, simple to perform, and does not require expensive equipment.

The benefits of this approach are numerous: Enhanced dissolution of pulp tissue, improved antibacterial activity, more effective debris removal and better penetration of the irrigant into the intricate microanatomy of the root canal system (9).

Beyond simple pH adjustments, some manufacturers have begun incorporating additives such as surfactants to lower surface tension or antifoaming agents to optimise solution behaviour. Promoted advantages of such additives include enhanced penetration into complex root canal anatomies (e.g., lateral canals, isthmuses, deltas, loops, and dentinal tubules), improved stability, and synergistic effects when used with ultrasonic activation (8).

However, the exact composition of these additives is often not disclosed in the safety data sheets provided by the manufacturers and their clinical relevance remain unclear (10, 11). Manufacturers of enhanced NaOCl solutions, assert that these products offer benefits such as reduced surface tension, increased oxidising capacity and superior tissue dissolution compared to plain NaOCl in aqueous solutions (8). Despite these claims, the concept of reduced surface tension improving the clinical performance of root canal irrigants lacks substantial support from clinical studies (12).

Another innovation in modifying NaOCl solutions is incorporating chelating agents to achieve a dual action of disinfection and mild decalcification, a strategy referred to as "continuous chelation." Dual Rinse HEDP (Medcem, Weinfelden, Switzerland), which contains 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) (8, 13), is supplied as a capsule with 0.9 g of etidronate powder, intended to be mixed with 10 mL of NaOCl solution directly before use (14). Research indicates that HEDP reduces the active chlorine content of NaOCl solutions over time, necessitating its immediate preparation before clinical application (14). Although the addition of HEDP has been shown to increase the surface tension of the irrigant, it enhances NaOCl cleaning and disinfection capabilities within the root canal system (15). However, the impact of Dual Rinse HEDP on pulp tissue dissolution at various temperatures has not yet been investigated.

The aim of the present study was to assess the degree of pulp tissue dissolution achieved with Dual Rinse HEDP at different temperatures and compare its performance to conventional NaOCl. The null hypothesis tested was that the novel Dual Rinse HEDP irrigant would be less effective than traditional NaOCl at varying temperatures.

MATERIALS AND METHODS

Pulp Specimen Preparation

Bovine pulp tissue was obtained from mandibular incisors of four bovine jaws (aged 30–50 months), collected post-slaughter from animals designated for food production. This study was not classified as an animal experiment, as it neither influenced the conditions of the animals prior to slaughter nor altered the standard slaughtering procedures. Radiographic examinations of the teeth were performed to confirm the absence of pulp calcifications. If calcifications were present, the tooth was excluded from the study. The study was conducted in accordance with the Declaration of Helsinki.

Within 36 hours after slaughter, five fresh and intact mandibular anterior teeth were extracted and stored in glass vials containing a 0.1% thymol solution. The crowns were separated at the cementoenamel junction using a high-speed diamond bur (FG 879, 57 Ubi Crescent, #08–02 Excalibur Centre, Singapore 408596, Singapore) under continuous water irrigation. The pulp tissue was gently extracted using a periodontal probe and cotton pliers, then rinsed thoroughly with distilled water to remove any debris or residual blood. Each specimen was stored individually in a 1.5 mL Eppendorf tube (Merck KGaA, 64293 Darmstadt, Allemagne) containing 1 mL of distilled water at -20°C until further processing. Before experimental use, the samples were thawed by first keeping the tubes at room temperature for 30 minutes, followed by immersion in a water bath set at 37°C (Thermo Fisher Scientific, 2157 North Northlake Way,Suite 240 Seattle, Washington 98103) for 15 minutes to replicate body temperature.

Pulp specimens were standardized to 1×1×1 mm under 10x magnification (SOM 32, Karl Kaps GmbH & Co. K, 35614 Asslar/Wetzlar), using millimetre graph paper as a reference and a surgical scalpel blade No. 11 (Swann-Morton Limited Owlerton Green Sheffield S6 2BJ, England).

All experiments were conducted in a laboratory at a constant temperature of 24°C.

Sample Size Calculation

The sample size was determined using G*Power software (version X.X), with parameters including an effect size (f) of 0.4, a significance level (α) of 0.05 and a desired power (1-β) of

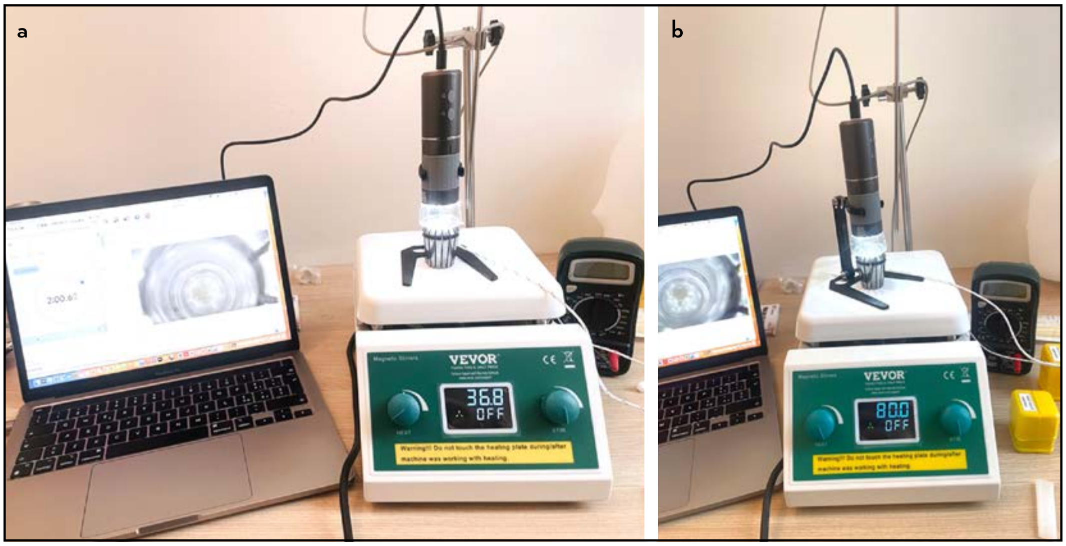

Figure 1. Representative images of the experiments. (a) Experiment conducted at 36.8°C; (b) experiment conducted at 80°C

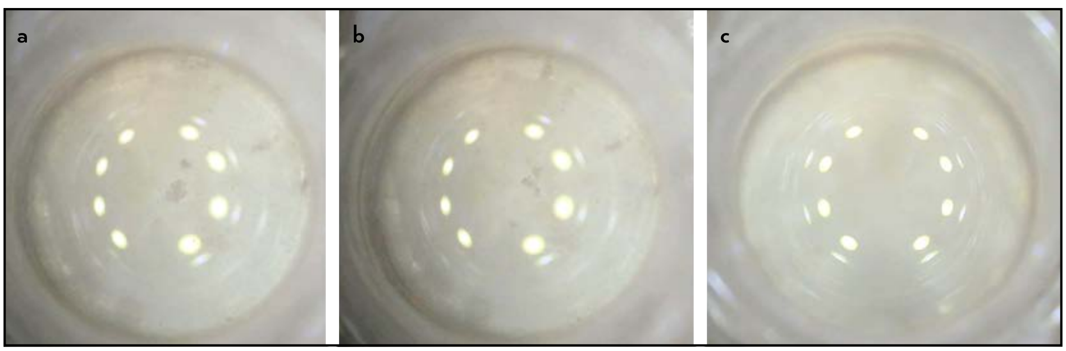

Figure 2. Representative images of pulp dissolution; (a) partially dissolved pulp, (b) almost completely dissolved pulp, (c) completely dissolved pulp

80%. Based on these calculations, a total of 50 samples were included in the study to ensure robust statistical analysis and account for potential variability.

Experimental Design

The samples were equally divided into five groups (10 samples per group), based on the type and temperature of the irrigant, resulting in a total of 50 samples:

- Group A: 0.1 mL of 5.25% NaOCl (CanalPro, Coltene/Whaledent Inc., Cuyahoga Falls, OH, USA) at 36.8°C (Body Temperature).
- Group B: 0.1 mL of 5.25% NaOCl at 80°C.
- Group C: 0.1 mL of Dual Rinse HEDP (Medcem GmbH, Vienna, Austria) at 36.8°C, prepared by mixing 10 mL of 5.25% NaOCl with one Dual Rinse HEDP capsule.
- Group D: 0.1 mL of Dual Rinse HEDP at 80°C.
- Group E: 0.1 mL of saline solution at 36.8°C (n=5) and at 80°C (n=5).

Temperature Control

During the experiment, the temperature of the irrigants was maintained and monitored using a thermostat with real-time display and control capabilities. The thermostat was equipped with a LED display with an accuracy of ±0.1°C (Vevor, SH-3ABEII, Shanghai, China). Additionally, a thermocouple was utilized to verify the liquid temperature (Fig. 1).

Testing Procedure

Each pulp sample was placed into a small glass container (3 mm in diameter; Thermo Fisher Scientific, Seattle, WA, USA) containing 0.1 mL of the irrigant. After each test, the used irrigant was discarded and fresh irrigant was introduced into the container. Once the irrigant reached the required experimental temperature, the pulp sample was introduced into the container. The container placed on the thermostat was continuously monitored under 20x magnification using a high-resolution digital microscope (5 MP, 8 LEDs; AM7515MZT, ZAC des Bruyères, 69760 Limonest, France).

After introducing the pulp sample into the liquid, a stopwatch was activated simultaneously. When complete dissolution of the pulp was achieved, the stopwatch was stopped and the time required for complete dissolution was recorded (Fig. 2).

Statistical Analysis

The one-way ANOVA test was performed to compare the mean dissolution times among the five groups and a Tukey's Honestly Significant Difference (HSD) test was conducted to evaluate

TABLE 1. Mean dissolution time and standard deviation

Group	Mean dissolution time (minutes)	Standard deviation	Observations
Group A	6.252	0.277	High dissolution time, body temperature NaOCl. (a)
Group B	0.369	0.034	Fastest dissolution time, NaOCl at 80°C. (b)
Group C	6.389	0.410	Similar to Group A, Dual Rinse HEDP at body temperature. (a)
Group D	0.377	0.037	Similar to Group B, Dual Rinse HEDP at 80°C. (b)
Group E	0.0	0.0	No dissolution observed, saline control. (c)

Statistical analysis indicated that heating the irrigants at 80°C demonstrated a statistically faster dissolution time compared to the groups tested at body temperature ($p<0.05$), without any significant difference between the groups tested at the same temperature ($p>0.05$). No pulp dissolution occurred in the control group, which significantly differed from all other groups ($p<0.05$). Statistical significance is indicated with letters: Groups tested at body temperature (A, C) share the same significance (a), Groups tested at 80°C (B, D) share the same significance (b), and the control group (E) has a distinct significance (c). NaOCl: Sodium hypochlorite, HEDP: 1-hydroxyethylidene-1,1-diphosphonic acid

pairwise differences among groups. SPSS software version 23.0 (Armonk, New York: IBM Corp.) was utilized for the statistical analysis and the significant difference was set at $p<0.05$.

RESULTS

The mean pulp tissue dissolution time and standard deviation for the different groups are reported in Table 1 and Figure 3. Statistical analysis indicated that heating the irrigants to 80°C resulted in a statistically faster dissolution time compared to the groups tested at body temperature ($p<0.05$), with no significant differences among the groups tested at the same temperature ($p>0.05$). No pulp dissolution occurred in the control group, which significantly differed from all other groups ($p<0.05$).

DISCUSSION

An ideal irrigating solution for endodontic treatment should meet several critical criteria: a strong antibacterial effect, the ability to dissolve vital and necrotic organic tissues and the capability to remove inorganic debris and the smear layer from endodontic walls without damaging root dentine (16, 17). These properties are essential to ensure effective cleaning and disinfection of the complex root canal system, contributing to successful clinical outcomes. Several studies have investigated the properties of novel irrigating solutions and the efficacy of modern activation protocols (18–22). Among these, the concept of continuous chelation has emerged, which involves the application of a mild chelating agent mixed to sodium hypochlorite throughout the entire instrumentation phase (8, 23). The continuous chelation protocols have been shown to enhance the adhesion of epoxy resin-based sealers to root dentine (24) and improve the penetration of bioceramic root canal sealers into dentinal tubules (25).

Dual Rinse HEDP has garnered attention for its favourable biological profile, including low cytotoxicity and the absence of genotoxic effects (26). When combined with sodium hypochlorite (NaOCl), its toxicity levels are comparable to NaOCl alone, making it a biocompatible adjunct in clinical endodontics (26). Furthermore, a randomised clinical trial demonstrated that Dual Rinse HEDP does not compromise the clinical efficacy of NaOCl in root canal disinfection (27).

In the present *in vitro* study, the performance of Dual Rinse HEDP at different temperatures were evaluated and compared to conventional NaOCl. The null hypothesis tested was rejected, as Dual Rinse HEDP mixed with 5.25% NaOCl demonstrated comparable efficacy to 5.25% NaOCl alone in dissolving pulp tissue at different temperatures, also highlighting the effectiveness of increased temperatures for pulp tissue dissolution,

The key findings obtained in the present study were that NaOCl at 80°C (Group B) achieved the fastest dissolution time among all groups, Dual Rinse HEDP at 80°C (Group D) performed similarly to NaOCl at 80°C, groups tested at body temperature (A and C) required significantly longer time to obtain a complete pulp dissolution and the control group (E) using saline solution demonstrated no pulp dissolution, thus confirming the need to use active irrigants.

Since 2015, a protocol has been developed for activating irrigants involving the controlled internal heating of sodium hypochlorite (28). The benefits of heated NaOCl are well-documented, including enhanced tissue dissolution capabilities and a stronger antibacterial effect (29, 30). However, pre-heating NaOCl outside the oral cavity has proven ineffective, as the solution rapidly stabilises to body temperature upon introduction into the root canal (31). The internal heating technique overcomes this limitation by utilising a heat carrier device positioned 3 mm short of the working length and operated at 180°C for 6 seconds (30).

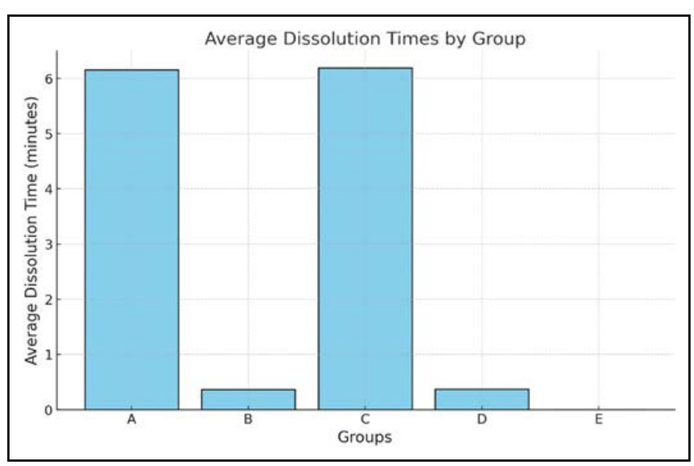

Figure 3. The bar chart for average dissolution time in minutes highlights the effectiveness of NaOCl and Dual Rinse HEDP at higher temperatures (80°C) compared to body temperature and the control group

NaOCl: Sodium hypochlorite, HEDP: 1-hydroxyethylidene-1,1-diphosphonic acid

This method effectively raises the temperature of sodium hypochlorite to 80°C during its active application (30).

Several studies have investigated the clinical and biological advantages of this technique (31, 32). Iandolo et al. (33) have demonstrated that this protocol effectively removes the smear layer and debris from dentinal walls, even without EDTA. Moreover, adherence to the controlled internal heating protocol ensures that external root surface temperatures remain below 40°C (30), safeguarding the periodontal ligament and surrounding tissues from thermal damage. Importantly, this method does not compromise the compressive strength of root dentine, preserving its structural integrity for long-term function (32).

Given these advantages, the temperature reached with the internal heating protocol was applied to the new irrigant Dual Rinse HEDP. The results confirmed that its tissue dissolution was comparable to NaOCl alone under similar conditions. Giardino et al. (34) observed that Dual Rinse HEDP increases the surface tension of NaOCl, which could theoretically limit its penetration into challenging anatomical regions, such as the apical third, isthmuses, fins and dentinal tubules. Conversely, internal heating decreases the viscosity of NaOCl at 80°C, improving its flow characteristics and enabling better penetration into the intricate microanatomy of the root canal system (35).

According to the Arrhenius equation (36, 37), reaction rates approximately doubled with every 10°C increase in temperature. In the present study, the temperature increase from 36.8°C to 80°C represents a rise of approximately 43°C, which corresponds to a theoretical reaction rate increase of approximately 2(43.2/10)≈16.982^{(43.2/10)} \approx 16.982(43.2/10)≈16.98-fold. The results of the present study closely align with this prediction, demonstrating a reaction rate increase of 16.94-fold. This agreement validates the theoretical model and highlights the high impact of temperature on chemical reactions in endodontic irrigation.

These findings underscore the critical role of elevated temperatures in improving the efficiency of irrigants such as NaOCl and Dual Rinse HEDP in pulp tissue dissolution. By combining the principles of continuous chelation (38, 39) and controlled internal heating, clinicians can achieve better tissue dissolution in the complex root endodontic space.

A relevant study by De-Deus et al. (40) could serve as an interesting model for future studies.

CONCLUSION

Despite the inherent limitations of this *in vitro* study including the use of bovine pulp tissue, the absence of debris and complex root canal anatomy, as well as the lack of blood interaction—it can be concluded that Dual Rinse HEDP mixed with 5.25% NaOCl demonstrated comparable effectiveness to 5.25% NaOCl alone in dissolving pulp tissue at different temperatures. Heating the irrigants to 80°C resulted in a statistically faster dissolution time compared to the groups tested at body temperature. Continuous chelation may serve as a viable alternative to traditional irrigation protocols.

Disclosures

Authorship Contributions: Concept – A.I., D.A., E.E.; Design – A.I., E.E., D.M.; Supervision – A.I., G.P.; Materials – A.I., D.A., D.M.; Data collection and/or processing – A.I., D.A., D.M., G.P.; Data analysis and/or interpretation – A.I., D.A., D.M., G.P.; Literature search – A.I., G.P.; Writing – A.I., D.A.; Critical review – A.I., D.A., D.M., G.P.

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