Original Study

IN VITRO EVALUATION OF IONIZED WATER AS A POTENTIAL ENDODONTIC IRRIGANT

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ABSTRACT

Objective: This study aimed to evaluate the antimicrobial and tissue-dissolving properties of ionized water in comparison with other endodontic irrigants, which were sodium hypochlorite solutions (medical, conventional, and flavored) and two typ of oxyg n ba d solution

Methodology: *E. faecalis* bacteria (ATCC No. 29212) were cultured in a hundred Petri dishes in conjunction with ten types of irrigants placed in disks. The inhibition zone for each sample was measured twice at 24 and 48 hours by a ruler to assess the antimicrobial effect of the irrigants. The dissolving effect of irrigants was assessed by comparing the weights of hundred standardized pieces of bovine muscular tissue before and after the dissolution experiment. After recording the preoperative weight, the irrigants were applied and experimental weighing was carried out at 30, 60, 90 minutes, 8, 24 hours, and 12 days if the complete dissolving is not achieved earlier. Sterile saline solution was served as a negative control in both experiments.

Results: The oxygen-based solutions significantly showed the highest antimicrobial effect ($p < 0.05$), unlike all other experimental groups, which were comparable to each other. With regard to the dissolution effect, only sodium hypochlorite solutions showed significant effect over time ($p < 0.05$) while other groups were not effective in tissue dissolution. Saline showed no antimicrobial or tissue-dissolving abilities.

Conclusion: Ionized water solutions show antimicrobial potential but not as a tissue-dissolving solution. Further studies and improvements are needed to investigate and possibly optimize the use of ionized water as an endodontic irrigant.

Key words: Antibacterial, Dissolution, Ionized Water, Sodium Hypochlorite, Root Canal Irrigation.

Introduction

Irrigation plays a critical part in the course of endodontic treatment, and different irrigant solutions have different methods of action; hence, they have various indications of usage [1, 2]. An ideal irrigation solution would be one that would aid in flushing debris, lubricating canals, dissolving organic and inorganic tissue, removes smear layer, has a strong antimicrobial effect and the ability to deactivate endotoxins, and is non-toxic [3]. Unfortunately, to date, this ideal irrigant does not exist. For many years, sodium hypochlorite has been the most-used irrigation solution in endodontic therapy due to its antimicrobial effect and its ability to dissolve organic tissue [4, 5]. Although sodium hypochlorite exhibits beneficial features in endodontic therapy, it is still a toxic solution [6]. Hydrogen peroxide, sometimes used in household bleach, has also been used as an endodontic irrigant; its main advantage is the bubbling effect that causes disruption of the debris and the release of nascent oxygen producing a germicidal activity mainly against anaerobic organisms [7]. However, the major disadvantage is that this agent does not dissolve tissue [8]. Anosteralyth and Neutrosteralyth (AQUIS GmbH, Marburg, Germany) are both ionized water solutions used for wound irrigation. According to the manufacturer, they are electrochemically activated water. In an in vitro study conducted by the manufacturer, they found a reduction of species including resistant *Staphylococcus aureus* and *Candida albicans* by six log levels. This study aimed to investigate the effectiveness of electrochemically-activated water as an endodontic irrigant solution in terms of its antibacterial effectiveness and tissue dissolution ability and compare it to different hydrogen peroxide and sodium hypochlorite solutions. Table-1 shows the ten irrigation solutions used in this study.

Materials and Methods:

Ten irrigation solutions were investigated under in vitro settings for their antibacterial and tissue dissolution abilities. A summary of the solutions used is presented in Table-1.

Antibacterial experiment:

Enterococcus faecalis isolates (*E. faecalis* ATCC- 29212) were used in this study. Agar diffusion test was used to measure the inhibition zone of the bacterial growth and 100 sterile Petri dishes (Oxoid England) were divided over ten groups each containing ten samples. The *E. faecalis* bacteria were spread on brain heart infusion (BHI) using a sterile swab and incubated at 37°C for 24h. Each sterile Petri dish was immersed in the corresponding irrigation solution then mounted over the center of the agar plate. Measurement of the inhibition zone in each sample was recorded at 24 h and 48 h using a ruler. The experiment was carried under rigorous aseptic conditions [9].

Tissue dissolution experiment:

Fresh bovine muscular tissue was prepared by Slider machine (Minerva Omega, Italy) and modified for standardization purposes using a surgical scalpel blade No.11 (Swann-Morton, England). A hundred tissue samples were prepared and kept in a 100% humidity refrigerator. Handling the specimen was done with a tweezer (Hawe-Neos No 820) and the weight was measured using an electronic weighing device (RADWAG, Wegi Electroniczne, PS 1000/C/2). The mean preoperative weight of each sample was 0.4 grams, (SD= 0.1 g). Each tissue sample was placed in an individual medicine cup then 20 ml of the corresponding irrigation solution was applied using a plastic syringe (BU KWANG, Korea). Samples' weights were recorded after 30, 60, and 90 minutes, and then after 8 and 24 hours. Before weighing, all pieces were blotted on a filter paper. The trial continued for the undissolved tissue until it was deemed to be dissolved or for 12 days if 24 h tissue dissolution was not achieved [10]. All experiments were conducted at room temperature.

Statistical analysis:

One-way ANOVA followed by a multiple comparison Tukey's test was utilized for the comparison of the mean values of different irrigation solutions. A p-values < 0.05 was deemed significant.

Results:

Antibacterial results:

The saline group showed no antibacterial effect at 24 or 48 hours. Household bleach for colored clothes and white clothes groups recorded the significantly highest antibacterial effect at both time intervals ($p < 0.05$) and there was not any significant difference between them. Seven groups including (Medical-Use Sodium Hypochlorite, Regular Household Bleach, Orange-Flavored Household Bleach, Low-Concentration Anosteralyth, High-Concentration Anosteralyth, Low-Concentration Neutrosteralyth, High-Concentration Neutrosteralyth) showed significantly higher antibacterial effect than the saline group but significantly lower antibacterial effect than the hydrogen-based bleaches at both 24 and 48 hours ($p < 0.05$). The mean and standard deviation of the inhibition zones of all groups at both 24 and 48 hours are presented in Figure-1.

Dissolution results:

The dissolution effect was measured by comparing the preoperative weight of tissue sample to the weight after 30, 60, and 90 minutes, and 8 and 24 hours from the application of the corresponding irrigation solution. The chlorine-based groups were the only groups that showed a significant dissolution effect over time ($p<0.05$) with no significant differences between them. However, there was no dissolution effect in the saline, household bleaches for colored and white clothes, Neutrosteralyth, and Anosteralyth groups at any time interval. Moreover, the latter solution type showed a significant reverse reaction and an increase in the sample weight ($P < 0.05$), which indicates an absorption behavior. Figure-2 shows the dissolution effect of all tested solutions at each interval.

Discussion:

To the best of our knowledge, no previous study has evaluated the potential use of Anosteralyth and/or Neutrosteralyth (AQUIS GmbH, Marburg, Germany) as endodontic irrigants. They are both electrochemically activated "ionized" water solutions and their use was suggested for wound irrigation because they are bactericidal and biocompatible with the tissues. This study aimed to evaluate their dissolution and antimicrobial efficacy against endodontic microbes in comparison to different brands of household bleach.

Generally, using electro-chemically activated water as an endodontic irrigation solution has been proposed many years ago. Lata et al. in 2016 investigated the antimicrobial effect of Electro-Chemically Activated water (ECA) against *E. faecalis* and found comparable results to sodium hypochlorite solution. They concluded that ECA water could be used as root canal irrigant, which is in agreement with our results [11]. Another recent in vitro study evaluated the antibacterial efficacy of two different electrochemically activated solutions produced by two different devices; Envirolyte® (ECA-EN) and Medilox® (ECA-MX) in root canals inoculated with *E. faecalis* for 4 weeks. The results showed that Envirolyte® (ECA-EN) was more effective than Medilox® (ECA-MX) and was also comparable to 2.5% NaOCl in the elimination of *E. faecalis*. Therefore, it was proposed that ionized water could serve as an alternative

endodontic irrigant [12]. Interestingly, electrolyzed saline demonstrated a significant capability in smear layer removal [13] or even greater efficacy in the cleaning of root canal surfaces over sodium hypochlorite [14]. Another study agreed with the previously mentioned investigations and found that the use of ECA resulted in a slight reduction in the number of obligate anaerobic and facultative bacteria within the root canal system, but this was not statistically significant in comparison to sodium hypochlorite [15]. Overall, the assessment of the ionized water efficacy against oral bacteria has been evaluated in numerous studies. One study reported that electrolyzed tap water inhibited the growth of several aerobic and anaerobic salivary and periodontal bacteria and suggested its use as a mouthwash for daily oral hygiene and toothbrush disinfection [16].

A household bleach solution containing sodium hypochlorite was previously proposed as an endodontic irrigant and claimed to be as effective as dental sodium hypochlorite [4]. In a study, it was also concluded that commercial household "regular" bleach and "fresh scent" sodium hypochlorite (Clorox) are equally effective against *enterococcus faecalis* and *Candida albicans* when exposed to either 5.25% or 2.62% concentrations of bleach solution for periods ranging from 15 to 120 seconds [17]. This is in agreement with the present study as both household bleach solutions containing sodium hypochlorite and the dental sodium hypochlorite have a comparable antimicrobial impact against *E. faecalis.* However, it is generally recommended not to use them due to their added products and to avoid any possible allergic reactions [17]. Ionized water solutions and sodium hypochlorite-containing solutions could not overcome the antimicrobial efficacy of hydrogen peroxide-based bleaches. This would be explained by the greater bactericidal effect of hydrogen peroxide as a potent oxidant by producing the hydroxyl free radicals (OH), which react directly with DNA and proteins of bacterial cells [18]. Despite the fact that hydrogen peroxide had a history of being used as root canal irrigant, the literature does not recommend its use over other endodontic irrigants [19].

The tissue dissolution effect is a favorable property in any irrigation solution. It fulfills the requirement of an ideal endodontic irrigant [1, 3]. It was investigated in the present study using pieces of bovine muscular tissue, although the rate of dissolution might not be similar to the human pulpal tissue, such an effect can be explored among the solutions. This method has been used previously in a classic study by Hasselgren et al. using porcine muscular tissue [10]. It gives an overall idea of the dissolution capacity of tested solutions. In this study, there was no dissolution effect observed in the Neutrosteralyth and Anosteralyth groups at all time intervals compared to the sodium hypochlorite groups. Similarly, in a study the dissolution of bovine pulp tissue by one type of electro-chemically activated solution, branded as "HealOzone, Aquatine Alpha Electrolyte®" was investigated [20]. It was found that only sodium hypochlorite was able to dissolve the bovine pulp tissue while HealOzone did not. Furthermore, the only solutions in the present study that showed a significant dissolution effect over time were sodium hypochlorite-containing solutions despite its type. This finding is in line with the study by Jungbluth et al., which found a similar dissolution ability among different brands of household bleach compared to dental sodium hypochlorite solution [4].

It is well known that some microorganisms are markedly resistant to being eradicated by commonly used antibacterial solutions. In failed root canal treatment cases, *E. faecalis* is considered one of the most commonly isolated microorganisms that display resistance to elimination [21]. Several virulence factors grant *E. faecalis* the ability to persist as a pathogen in the root-filled canals [22]. Therefore, *E. faecalis* was selected to be the tested microorganism using the agar diffusion method for antimicrobial assessment. Despite the limitation of this technique, it enables the simple and direct assessment of the antimicrobial effect and allows direct comparison between different experimental solutions against selected organisms. However, evaluating the antimicrobial effect of irrigation solution on planktonic cells might not represent its effectiveness as in vivo conditions. Therefore, further laboratory and clinical investigations are required to evaluate these possible irrigants before recommending their usage as root canal irrigants.

Conclusion:

Within the limitations of the present in vitro study, it can be concluded that ionized water has potential use as endodontic irrigant since it possesses a great antimicrobial effect, however, it lacks the dissolution ability. Further improvements are needed on such solutions to optimize their usability in the endodontic irrigation field.

Declaration of Interest:

Nothing to declares

References

1. Zehnder M. Root canal irrigants. Journal of endodontics. 2006 May 1;32(5):389-98.
2. Manojkanna K, Chandana CS. Nanoparticles in endodontics–A review. JAPER. 2017;7(2):58-60.

3. Basrani B, Haapasalo M. Update on endodontic irrigating solutions. Endodontic topics. 2012 Sep;27(1):74-102.
4. Jungbluth H, Peters C, Peters O, Sener B, Zehnder M. Physicochemical and pulp tissue dissolution properties of some household bleach brands compared with a dental sodium hypochlorite solution. Journal of endodontics. 2012 Mar 1;38(3):372-5.
5. Fard EM, Ghaffari T, Zarei K. Evaluation of the Effect of Two Denture Disinfectants on the Surface Roughness of Conventional Heat-Cured Acrylic and TiO2-Reinfirced Acrylic Resins. J. Biochem. Technol. 2019, (2):106-12.
6. Hülsmann M, Hahn W. Complications during root canal irrigation–literature review and case reports. International endodontic journal. 2000 May;33(3):186-93.
7. Martin DM. Irrigation and medication of the root canal. International Endodontic Journal. 1979 Apr;12:55-62.
8. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dental Clinics. 2010 Apr 1;54(2):291-312.
9. Siddique R, Anjaneyulu K, Muralidharan NP. Antimicrobial Efficacy of Garlic-Lemon in Comparison with Sodium Hypochlorite against E. faecalis. Journal of Clinical & Diagnostic Research. 2019 Jan 1;13(1).
10. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. Journal of Endodontics. 1988 Mar 1;14(3):125-7.
11. Lata S, Mohanty SK, PrADHAN PK, PATri G, SiNHA SP, AGrAwAL P. Anti bacterial effectiveness of electro-chemically activated (ECA) water as a root canal irrigant-An in-vitro comparative study. Journal of clinical and diagnostic research: JCDR. 2016 Oct;10(10):ZC138.
12. Akbulut MB, Eldeniz AU. In vitro antimicrobial activity of different electrochemically-activated solutions on enterococcus faecalis. European Oral Research. 2019 Jan;53(1):44.
13. Dube K, Jain P. Electrolyzed saline… An alternative to sodium hypochlorite for root canal irrigation. Clujul Medical. 2018 Jul;91(3):322.
14. Marais JT. Cleaning efficacy of a new root canal irrigation solution: a preliminary evaluation. International Endodontic Journal. 2000 Jul;33(4):320-5.
15. Marais JT, Williams WP. Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution. International Endodontic Journal. 2001 Apr;34(3):237-43.
16. Lee SH, Choi BK. Antibacterial effect of electrolyzed water on oral bacteria. The Journal of Microbiology. 2006 Aug;44(4):417-22.
17. Harrison JW, Wagner GW, Henry CA. Comparison of the antimicrobial effectiveness of regular and fresh scent Clorox. Journal of endodontics. 1990 Jul 1;16(7):328-30.
18. Block SS. Peroxygen compounds. Disinfection, sterilization, and preservation. 2001 Dec 31;5.
19. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic topics. 2005 Mar;10(1):77-102.
20. Rossi-Fedele G, Steier L, Dogramaci EJ, Canullo L, Steier G, de Figueiredo JA. Bovine pulp tissue dissolution ability of HealOzone®, Aquatine Alpha Electrolyte® and sodium hypochlorite. Australian Endodontic Journal. 2013 Aug;39(2):57-61.
21. Poordast T, Alborzi S, Askari E, Samsami A, Najib FS, Hamedi M, Naghmeh Sanj Z. A Rare Case of Tubo-Ovarian Abscess by Enterococcus SPP, Following Intrauterine Insemination. Int. J. Pharm. Phytopharm. Res. 2019; 9(1):32-5.
22. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1998 Jan 1;85(1):86-93.

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Name	Company
Normal Saline (negative control)	production countries (Baxter, Hospira, Braun)
Medical Use Sodium Hypochlorite (Positive control) (Chlorine-based)	National Medical Solutions EST for Medical Devices & Cosmetics, KSA
Regular Household Bleach (Chlorine based)	Clorox registered trademark for the Clorox company, Manufactured in KSA by M.A Abudawood and Partners for industry, Jeddah
Orange Flavored Household Bleach (Chlorine-based)	
Household Bleach for Colored clothes (Hydrogen-based)	
Household Bleach for White clothes (Hydrogen-based)	
Low Concentration Anosteralyth (30%)	(Eckhardt + Hedderich GmbH) Am Wall 17, D35041 Marburg
High Concentration Anosteralyth (100%)	
Low Concentration Neutrosteralyth (30%)	
High Concentration Neutrosteralyth (100%)	

Table 1: List of all irrigation solutions used in the study and their producing companies

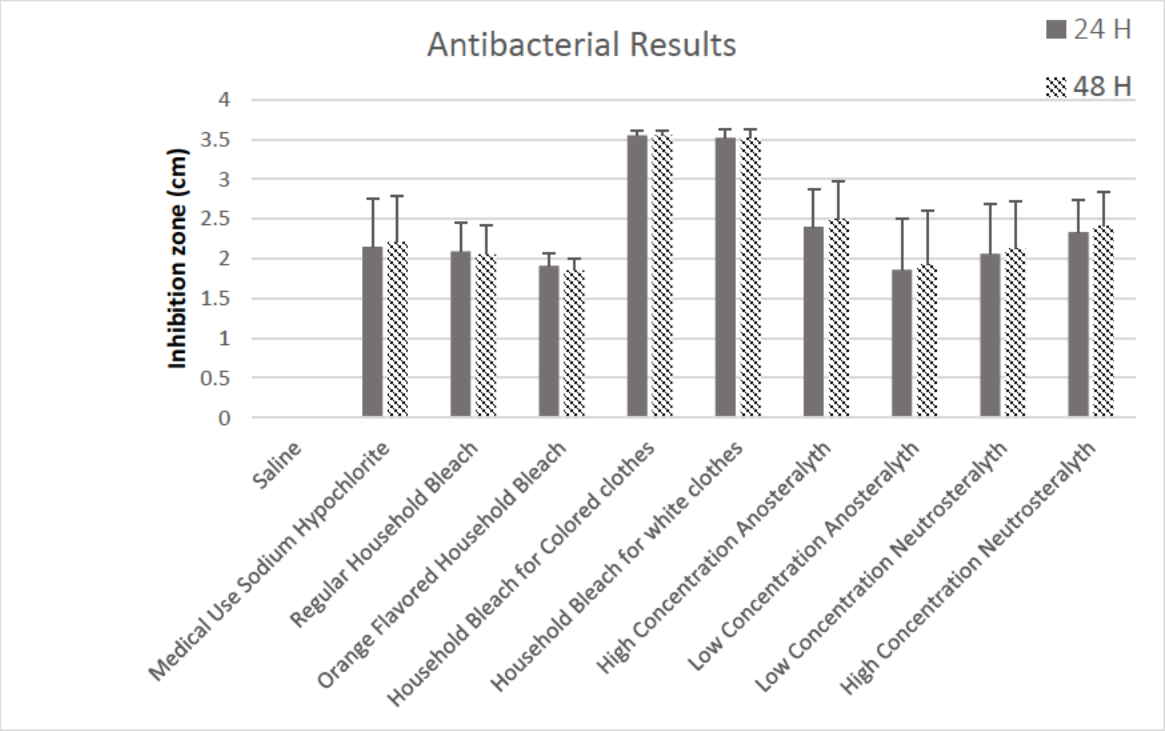

Figure 1: The mean and standard deviation of the inhibition zones in cm of each irrigation solution at 24 and 48 hours.

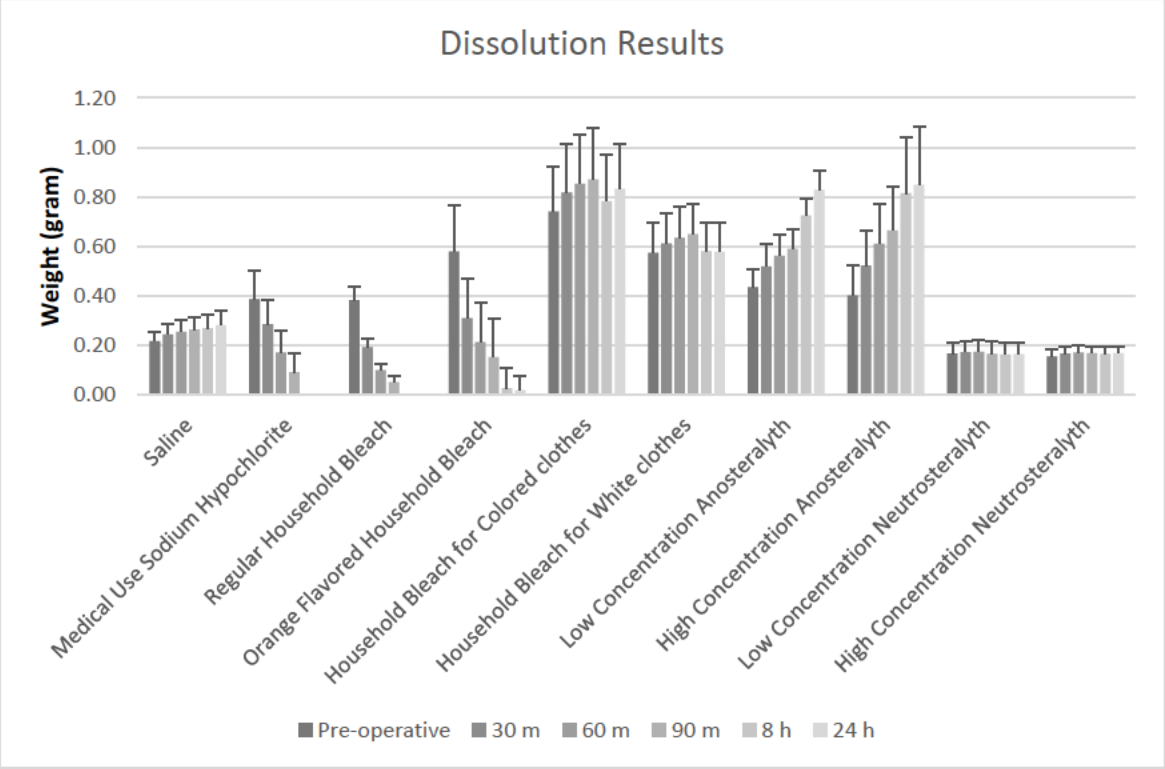

Figure 1: The mean and standard deviation of the weight over time in grams