Review

ANNALS OF DENTISTRY
UNIVERSITY OF MALAYA

www.adum.edu.my

Ann Dent UM 2024, 31: 13-25
DoI: 10.22452/adum.vol31no3

Comparative Analysis of Antimicrobial Efficacy of Electrolyzed Water with Sodium Hypochlorite Solution against Root Canal Pathogen: A Scoping Review

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KEYWORDS

Antibacterial, electrolyte solution, Enterococcus faecalis, root canal irrigants, sodium hypochlorite

ABSTRACT

Electrolyzed water (EW) has been introduced as a surface disinfectant due to its antimicrobial properties without cytotoxic effects to oral tissues contrary to sodium hypochlorite (NaOCl), which is cytotoxic and can be detrimental if extruded beyond the root canal. This scoping review aimed to compare antimicrobial efficacy of EW with NaOCl on root canal pathogens and to assess effect of concentration and exposure time on antimicrobial efficacy. The review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews protocol guidelines. Electronic databases were searched for eligible articles published between 2011-2021 in PubMed, PLOS, Science Direct, and Google Scholar. Joanna Briggs Institute Critical Appraisal Tool was used for quality assessment. Of the 784 articles recovered, only 8 were eligible based on inclusion criteria. The included studies assessed several types of EW, which showed antimicrobial potential against *E. faecalis* in vitro in suspension and biofilm forms. EW demonstrated antimicrobial efficacy comparable with NaOCl in 5 of 8 studies. Only one study found that higher concentration and exposure time increased antimicrobial efficacy of EW; other studies showed otherwise. It can be concluded that EW can be a potential alternative solution for NaOCl as an endodontic irrigant.

INTRODUCTION

The anatomy of the root canal system is highly complex and variable. During non-surgical root canal treatment (NSRCT), the use of endodontic irrigant is crucial to facilitate the elimination of bacterial biofilms [1]; studies have shown 35%–50% of the root canal surfaces were left untouched by endodontic files [2].

The most widely used endodontic irrigant for NSRCT is sodium hypochlorite (NaOCl) due to its outstanding antimicrobial properties and tissue-dissolving capabilities. However, the use of NaOCl in the root canal comes with several disadvantages: cytotoxicity [3], reduced root dentin fracture strength [4], reduced restorative bond strength [5], unpleasant smell, and bleaching of items [6]. The severity of these disadvantages depends on the concentration of NaOCl [3–5], with a higher concentration causing more severe condition. Currently, there is no conclusive evidence on the difference in antimicrobial efficacy of low or high NaOCl concentration; however, there is a trend in using a higher concentration to achieve better

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disinfection and tissue-dissolving effect [4]. There are other methods to improve the disinfection and tissue-dissolving efficacy of NaOCl, such as agitation and increasing exposure time; however, these methods come with increased risk of extrusion [7] and reduced root dentin microhardness [8,9] respectively. Therefore, an alternative irrigant with better properties than NaOCl needs to be further explored.

Recently, electrolyzed water (EW) has been verified as an effective disinfectant that can be used as alternative to NaOCl [10]. EW has high free-activated chlorine concentration and oxidation–reduction potential (ORP), which contribute to its high sterilization ability. Furthermore, EW has been shown to have little in vivo toxicity [11] and has been used in dentistry for disinfection of dental instruments, endodontic and periodontic irrigation, and mouthwash [12].

Far too little attention has been paid within the last 10 years to study the effectiveness of EW against root canal pathogens. Hence, a review that summarizes and synthesizes all related evidence on efficacy of EW toward endodontic microbiota and its potential as an alternative endodontic irrigant is needed. Thus, this scoping review aims to identify the antimicrobial efficacy of EW in comparison with NaOCl solution against root canal pathogens, to explore the types of EW available, and to investigate the potential effect of EW concentration and exposure time on its antimicrobial efficacy.

MATERIALS AND METHODS

Protocol guideline

The scoping review was conducted from July to November 2021. This research design was chosen because of the broad nature of the research questions. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) protocol guideline [13] was followed (Figure 1). It aims to improve the quality of scoping review protocols, similar to the impact achieved by other reporting guidelines [14].

Formulation of the review questions

The review questions were formulated according to the PICO formulation. These frameworks have been suggested as a model for developing review questions and search terms [15]. In this scoping review, the formulated questions were "Does EW have comparable antimicrobial efficacy as NaOCl on root canal pathogen?", "Do different concentrations show different antimicrobial activity of EW against root canal pathogen?", and "Do different durations show different antimicrobial activity of EW against root canal pathogen?".

Search strategy

Articles published from January 2011 to September 2021 were systematically searched through PubMed, PLOS, Science Direct, and Google Scholar. The articles were restricted to journal articles published in English. Boolean phrases were applied to improve article search. The following terms were used in the search: (root canal pathogen OR root canal bacteria) AND (electrolyzed water OR electrochemically activated water OR electrolyte solution) AND sodium hypochlorite AND (antimicrobial OR antibacterial). The search aimed to identify all quasi-experimental study designs, which would later be summarized into the antimicrobial efficacy of EW in comparison with NaOCl against root canal pathogens.

Eligibility criteria

Original studies that discuss the antimicrobial efficacy of EW and NaOCl against root canal pathogens were included in the review. The inclusion criteria of this scoping review were based on PICO strategy, where P: root canal pathogen, I: electrolyzed water, C: sodium hypochlorite, and O: antimicrobial efficacy of electrolyzed water and sodium hypochlorite. Studies that did not focus on PICO strategy were excluded from this review. Gray studies, case reports, letters, conference abstracts, and review papers were also excluded.

Data extraction

The title, abstract, and full text were screened independently by two authors (SNA, SNAR). Any disagreements were resolved by discussion among authors. The characteristics of the included studies in this scoping review were analyzed by SNA, SNAR, and SMK and summarized in Tables 1, 2, and 3.

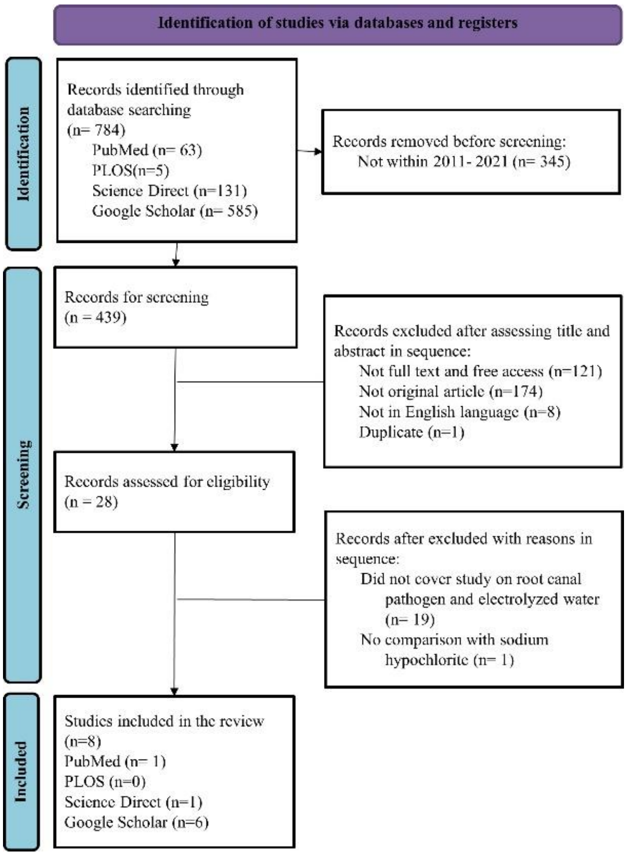
Assessment of risk of bias in included studies

The methodological quality of the included studies was assessed by four authors (SNA, SNAR, SMK, MHA) using the Joanna Briggs Institute (JBI) Critical Appraisal Tools: Checklist for Quasi-Experimental Studies (Supplementary Table). This assessment determined the extent to which a study has addressed the possibility of bias in its design, conduct and analysis [16]. Kappa score among the authors showed a high level of agreement on the included studies ($\kappa > 0.90$).

RESULTS

Study selection and characteristics

Initially, 784 articles with the search terms were identified from the literature search. The studies were then checked for their relevancy and duplicates, resulting in 28 potentially eligible studies for inclusion. The titles and the abstracts of the articles were assessed to select the relevant studies to be included in the review. Based on the inclusion criteria, only 8 of the 28 studies were qualified to be included in the review. The reasons for exclusion of other studies are listed in Figure 1.



The included articles discussed the antimicrobial properties of EW and NaOCl solution against root canal pathogens. The included articles were quasi-experimental studies. The characteristics of the included studies are reported in Table 1.

Assessment of the included studies

JBI Critical Appraisal Tool for Quasi-Experimental Studies was used to evaluate the quality of the included studies. The overall quality score for the assessment of the included studies was more than 60%.

Antimicrobial efficacy of EW in comparison with NaOCl against root canal pathogens

Majority of the studies found that EW were able to eradicate a significant percentage of bacteria from the samples [11,17–22] but bacterial reduction was less compared to NaOCl [17–22]. Statistical analysis showed that the bacterial reduction difference between EW and NaOCl was not significant in 7 of the 8 studies (p>0.05) (Table 2) depending on the exposure time [17,23] and type of EW used [20].

Type of EW used

Five different types of EW were used in the included studies: electrolyzed oxidizing water (EOW) [11], electrochemically activated water (ECAW) [24,25], strong acid electrolyzed water (SAEW) [26], super-oxidized water (SPOW) [27–29], and electrolyzed-functional water (FW) [30]. Some studies used ECAW produced by Medilox and Envirolyte devices [25], some studies used SPOW with different brands such as OxOral [27,29] and Microdacyn [27]. Different EW types have different pH values, which are listed in Table 1.

Effect of EW concentration on its antimicrobial efficacy

Only 2 studies [22,31] included findings on the effect of EW concentration toward antimicrobial efficacy (Table 3). However, both studies have contradictory results; Okamura et al. concluded that higher EW concentration yields better antimicrobial efficacy (p<0.01) [22], while the other study showed no significant difference in antimicrobial efficacy of EW when higher concentration was used (p>0.05) [31].

Effect of EW exposure time on its antimicrobial efficacy

Three studies [17,23,31] included the findings on antimicrobial efficacy of EW for different exposure times (Table 3). Only one study showed significant effect of different exposure time on the antimicrobial efficacy of EW (p<0.05) [17]. The other 2 studies showed no statistically significant difference in antimicrobial efficacy of EW with increased exposure time (p>0.05) [23,31].

Table 1 Overview of the included articles

Author	Root canal pathogen	Methodology	Biofilm formation	Type and amount of EW used	Comparator	Exposure time	Sampling method
Mena-Mendivil et al. (2013)	*S. sobrinus*, *P. gingivalis*, *S. intermedius*, *T. forsythia*, *E. faecalis*	33 extracted single rooted human teeth inoculated with 10 µL (0.5 × 10^8 CFU/mL) mixture of anaerobic bacteria. Root canals were irrigated with experimented solutions.	Yes	10 mL SPOW: 1. Microdacyn 60, neutral pH 2. OxOral, neutral pH	5.25% NaOCl	5 min	Sterile paper points
Cheng et al. (2016)	*E. faecalis*	48-well BioFlux plate inoculated with 10^8 CFU/mL of flowing biofilm and 6-well plate with presterilized coverslips seeded with 10^8 CFU/mL static biofilms. Biofilms were then treated with the experimented solutions.	Yes	SAEW, pH 2.3 ± 0.15 Amount NM	5.25% NaOCl, 0.9% saline	10 min	(Not available)
Lata et al. (2016)	*E. faecalis*	48 freshly extracted single rooted human teeth were inoculated with bacterial suspension. The experimented solutions were irrigated into the root canals.	NM	2 mL ECAW, pH 6.81	1% NaOCl, 3% NaOCl Distilled water	5 min	BHI solution injected into root canal and aspirated
Zan et al. (2016)	*E. faecalis*	120 extracted single rooted teeth were inoculated with 10 µL bacterial suspension. The experimented solutions were irrigated into the root canals.	Yes	10 mL SPOW, pH 5.5	0.9% saline, 5.25% NaOCl	1,2,3,5 min	Paper points
Saucedo et al. (2017)	*E. faecalis*	36 bacterial cultures were mixed with experimented solutions.	No	1 mL OxOral	5.25% NaOCl	15 s, 60 s	1 mL of solution was extracted from mixture
Akbulut & Eldeniz (2019)	*E. faecalis*	100 extracted single rooted teeth were inoculated with bacterial suspension. The experimented solutions were irrigated into the root canals.	Yes	5 mL ECAW produced by: 1. Medilox device, pH 5.0–6.5 2. Envirolyte device, pH 7.0–7.5	2% CHX, 2.5% NaOCl	NM	Dentin chips taken from canal walls under aseptic condition
Okamura et al. (2019)	*S. mutans*, *P. gingivalis*, *E. faecalis*, *C. albicans*	10 µL (1×10^8 CFU/mL) of each species was mixed with experimented solutions.	No	1 mL EW: 1. Acidic FW, pH 2.7 2. Alkaline FW, pH 11.5	5% NaOCl	30 s	(Not available)
Hsieh et al. (2020)	*E. faecalis*, *S. mutans*	100 µL (1×10^8 CFU/mL) of each species was mixed with experimented solution.	No	10 mL EOW diluted to 0.0125% and 0.0250% HOCl	1.5% NaOCl, 5.25% NaOCl	0.5,1,5 min	(Not available)

CFU, colony forming unit; CHX, chlorhexidine; EW, electrolyzed water; ECAW, electrochemically activated water; EOW, electrolyzed oxidizing water; FW, functional water; GG; Gates Glidden, NaOCl, sodium hypochlorite; SAEW, strong acid electrolyzed water; SPOW, super-oxidized water; NM, not mentioned

Table 2 Antimicrobial efficacy of electrolyzed water compared with sodium hypochlorite against root canal pathogens

Author	Bacterial count before treatment	Bacterial count after treatment		Comparability with NaOCl	
		EW	NaOCl	Type of EW	*p* value
Mena-Mendivil et al. (2013)	0.5×10^8 CFU/mL	OxOral: mean CFU count = 0.26 ± 0.46 CFU/mL Microdacyn 60: mean CFU count = 2.57 ± 1.58 CFU/mL	Mean CFU count = 0 ± 0 CFU/mL	OxOral Microdacyn 60	$p = 0.924$ $p = 0.408$
Cheng et al. (2016)	1×10^8 CFU/mL	Flow biofilm bacteria reduction = 88.2% Static biofilm bacteria reduction = 93.9%	Flow biofilm bacteria reduction = 90% Static biofilm bacteria reduction = 96.2%	SAEW	$p > 0.05$
Lata et al. (2016)	997.12 µL	Mean CFU count = 19.808 µL	1% NaOCl: mean CFU count = 17.885 µL 3% NaOCl: mean CFU count = 10.808 µL	ECAW	$p > 0.05$
Zan et al. (2016)	1.5×10^8 CFU/mL	1 min: mean CFU count = 4400 ± 960 CFU/mL 2 min: mean CFU count = 805 ± 123 CFU/mL 3 min: mean CFU count = 39.5 ±19.6 CFU/mL 5 min: mean CFU count = 7.50 ± 5.96 CFU/mL	2 min: mean CFU count = 0 ± 0 CFU/mL	SPOW at 1 & 2 min SPOW at 3 & 5 min	$\boldsymbol{p < 0.05}$* $p > 0.05$
Saucedo et al. (2017)	NM	15 s: CFU > 100 in all 9 cultures 60 s: CFU > 100 in all 9 cultures	15 s: CFU 2–100 on 3 cultures and CFU > 100 on 6 cultures 60 : No CFU on 4 cultures, CFU 2–100 on 3 cultures, and CFU > 100 on 1 culture	OxOral at 15 s OxOral at 60 s	$p = 0.065$ $\boldsymbol{p < 0.01}$*
Akbulut & Eldeniz (2019)	OD600 = 0.6	ECA-EN: mean CFU count = 0 ± 0 CFU/mL ECA-MX: mean CFU count = 3.75 ± 0.65 CFU/mL	Mean CFU count = 0 ± 0 CFU/mL	ECA-EN ECA-MX	$p > 0.05$ $\boldsymbol{p < 0.001}$*
Okamura et al. (2019)	1×10^8 CFU/mL	Acidic FW: mean CFU count = 20% (*S. mutans*), 0% (*P. gingivalis*), and 3.8% (*E. faecalis*) Alkaline FW: mean CFU count = 72.7% (*S. mutans*), 0% (*P. gingivalis*), and 84.6% (*E. faecalis*)	Almost no bacteria were observed	Acidic FW Alkaline FW	$p > 0.01$ $p > 0.01^{\dagger}$
Hsieh et al. (2020)	1×10^8 CFU/mL	More than 99.9% bacterial reduction	More than 99.9% bacterial reduction	EOW	$p > 0.05$

CFU, colony-forming unit; ECA-EN, electrochemically activated water produced by Envirolyte device; ECA-MX, electrochemically activated water produced by Medilox device; ECAW, electrochemically activated water; EOW, electrolyzed oxidizing water; EW, electrolyzed water; FW, functional water; NaOCl, sodium hypochlorite; SAEW, strong acid electrolyzed water; SPOW, super-oxidized water; NM, not mentioned

*Statistically significant (NaOCl has a better antimicrobial efficacy than EW)

†Only for *P. gingivalis*

Table 3 Effect of concentration and exposure time of electrolyzed water on its antimicrobial efficacy

Author	**Effect of different EW concentrations on antimicrobial efficacy**			**Effect of different EW exposure times on antimicrobial efficacy**		
	Concentration	**Bacterial count after treatment with EW**	***p*-value**	**Exposure time**	**Bacterial count after treatment with EW**	***p*-value**
Mena-Mendivil et al. (2013)		Not included			Not included	
Cheng et al. (2016)		Not included			Not included	
Lata et al. (2016)		Not included			Not included	
Zan et al. (2016)		Not included		1 min [a] 2 min [a] 3 min [b] 5 min [b]	4400 ± 960 CFU/mL 805 ± 123 CFU/mL 39.5 ±19.6 CFU/mL 7.50 ± 5.96 CFU/mL	$\mathbf{p < 0.05}$*
Saucedo et al. (2017)		Not included		15 s 60 s	CFU > 100 on all cultures CFU > 100 on all cultures	$p > 0.05$
Akbulut & Eldeniz (2019)		Not included			Not included	
Okamura et al. (2019)	10% acidic FW 30% acidic FW	51.5% of *E. faecalis* remained Less than 5% remained	$\mathbf{p < 0.01}$*		Not included	
Hsieh et al. (2020)	0.0125% HOCl 0.025% HOCl	More than 99.9% bacterial reduction More than 99.9% bacterial reduction	$p > 0.05$	30 s 60 s	More than 99.9% bacterial reduction More than 99.9% bacterial reduction	$p > 0.05$

EW, electrolyzed water; FW, functional water; HOCl, hypochlorous acid
*Statistically significant, [ab]values with the same superscript letter are statistically no different

Supplementary Table JBI Critical Appraisal Tools: Checklist for Quasi-Experimental Studies

Criteria	Mena-Mendivil et al. (2013)	Cheng et al. (2016)	Lata et al. (2016)	Zan et al. (2016)	Saucedo et al. (2017)	Akbulut & Eldeniz (2019)	Okamura et al. (2019)	Hsieh et al. (2020)
1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e., there is no confusion about which variable comes first)?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Were the participants included in any comparisons similar?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4. Was there a control group?	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?	Yes	Yes	Yes	Yes	No	Yes	Yes	No
6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	NA	NA	NA	NA	NA	NA	NA	NA
7. Were the outcomes of participants included in any comparisons measured in the same way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Were outcomes measured in a reliable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9. Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

NA, Not Applicable

DISCUSSION

Antimicrobial efficacy of EW in comparison with NaOCl against root canal pathogens

Electrolyzed water is a solution that is produced by the transformation of low mineral salt solutions into an activated metastable state via electrolysis in an electrochemical cell [32]. Many types of EW were used in the included studies with different pH values, ORP values, and available chlorine concentration (ACC) or hypochlorous acid (HOCl) concentration, all of which can affect the antimicrobial efficacy of EW [33].

Seven out of eight studies in this review reported that EW has comparable antimicrobial efficacy with NaOCl to eliminate root canal pathogens (Table 2). Studies that used EOW [31], OxOral EW [19], SPOW [17], and ECAW [20] reported that both NaOCl and EW eliminated more than 99.9% bacterial population from the samples. Meanwhile, studies that used ECAW [18], acid-electrolyzed FW [22], and SAEW [21] reported that EW eliminated a significant percentage of bacterial population up to at least 80% with comparable antimicrobial efficacy with NaOCl. However, one of the 7 studies showed that EW has comparable antimicrobial efficacy to NaOCl only when EW was used for a longer period compared to NaOCl [17]. Zan et al. reported that when the bacterial samples were exposed to EW for 3 and 5 min, the antimicrobial efficacy was comparable to 2 min treatment time of NaOCl [17].

Only Saucedo et al. [23] found NaOCl to have significantly better antimicrobial efficacy compared to EW. They used OxOral EW and demonstrated extended bacterial growth on all culture samples. Mena-Mendivil et al. [19] used the same solution, but in their study, OxOral EW demonstrated comparable antimicrobial efficacy with NaOCl and eliminated a significant percentage of bacterial population from the samples. These conflicting results may be due to the differences in the methodology. Mena-Mendivil et al. [19] used 10 mL OxOral for 5 min on a mixture of anaerobic bacteria samples, while Saucedo et al. [23] used only 1 mL and limited to 60 s treatment time on *E. faecalis* culture. The longer exposure time, higher disinfectant volume, and bacterial interaction might have contributed to the better antimicrobial effect of EW in the study by Mena-Mendivil et al. Additionally, only the study by Saucedo et al. [23] showed the inability of 5.25% NaOCl to eradicate more than 90% of bacterial population.

The mechanism of bacterial destruction by EW is through ORP, which differs from NaOCl. The level of ORP in EW ranges from +600 to +1200 mV [18] depending on the pH value [33]. This ORP range can interrupt the important metabolic processes of bacteria as it is not in the normal working range. Solutions with powerful oxidants may rupture biochemical bonds in bacteria, which can lead to cell function loss. Furthermore, bacterial membrane structures can also be destroyed due to unstable osmotic concentration between ions available in the solution and the bacteria in a high ORP environment [18,33].

Types of EW used

Different EW types possess different antimicrobial activities, although generally, their differences are not statistically significant when compared with NaOCl. Mena-Mendivil et al. [19] in their study showed that OxOral possessed more prominent effect than Microdacyn 60 and was comparable to NaOCl. Both OxOral and Microdacyn 60 are SPOW with neutral pH. The authors claimed that this difference in antimicrobial effectiveness could be because OxOral came in a secured container and the Microdacyn 60 was in an exposed container. Further investigation is needed to confirm this possibility. In a preceding study done by Cui et al. [34], reduction of ORP, ACC, and dissolved oxygen were detected in EW stored in an open container compared with EW stored in a closed container. EW undergoes massive reduction in ACC when exposed to the environment; thus, the bactericidal activity is affected.

Furthermore, Lata et al. [18] mentioned the difference of antimicrobial efficacy between ECAW produced by the anolyte and catholyte. During the production of EW in an electrolysis cell, the anode produces the anolyte which possesses oxidation potential, while the cathode produces the catholyte which possesses reduction potential. The anolyte, which is brownish and acidic, has lower toxic effects towards human tissue cells with proven bactericidal, antiviral, antifungal, non-allergenic, and non-inflammatory characteristics. Meanwhile, the catholyte, which is alkaline, has lesser bactericidal properties in comparison with the anolyte.

Akbulut and Eldeniz [20] also demonstrated that different types of ECAW have different antimicrobial efficacies. They reported that ECAW produced by Envirolyte device (ECA-EN) had more significant antibacterial activity (100% bacterial reduction) against *E. faecalis* when used alone or combined with EndoActivator sonication compared with ECAW produced by Medilox device (ECA-MX). Although these solutions were produced by an identical electrolysis process, they had distinct antimicrobial properties since the ORP and pH values of the solutions were dissimilar [35]. Comparable ORP values were observed in both ECA-EN and ECA-MX solutions, but ECA-EN had a neutral pH and ECA-MX had a lower pH. This can also be compared with the study by Lata et al. [18]. They used ECAW with slightly lower pH than ECA-EN, and the result showed slightly lower bacterial reduction (19.808 µL) compared to ECA-EN (0 ± 0 CFU/mL). These differences in results by different ECAW could be explained by the difference in pH, where EW with neutral pH showed more potent bacterial reduction. The neutral pH could be accountable for the longer shelf-life and the preservation of the antimicrobial effectiveness [36].

Okamura et al. [22] also observed difference in antimicrobial efficacy between FW with different pH values. They showed that acidic FW have identical bactericidal outcomes to NaOCl on *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Enterococcus faecalis*. However, alkaline FW portrayed profound bactericidal effects only for *P. gingivalis*. A previous study supported this finding in which alkaline FW had relatively less bactericidal and biofilm-removing effects [37]. The bactericidal effects of FW are thought to be influenced by the chlorine-related substances such as chlorine, HOCl, and hypochlorous acid ions (ClO^-) [36,38], which contribute to the amount of ACC. Okamura et al. [22] showed that ACC must be higher than 10 ppm for the acidic FW to have efficient bactericidal effect. Furthermore, the level of ORP can also decrease with increasing in pH of the EW solution [33].

Besides the different properties of the EW solution, the types of bacterial samples used in each study may also affect antimicrobial efficacy; bacteria in the form of biofilms are protected and much more impervious to biocides compared to the planktonic form of the same microbes [39]. Among the studies included in this review, only the study by Cheng et al. [21] investigated the differences in the antimicrobial effect of NaOCl and EW on flowing and static biofilms. The flowing biofilms were produced under a continuous shear flow in a microfluidic system while the static biofilms were produced under a fixed environment on coverslip surfaces. Both the flowing and static biofilms were mixed with SAEW and NaOCl for 10 min. The bacterial reduction after treatment with SAEW and NaOCl was significant on both static and flowing biofilms with comparable antimicrobial efficacy, although static biofilms showed higher bacterial reduction compared to flowing biofilms. The bactericidal mechanism of SAEW is based on its low pH, high ORP, and the interactions of HOCl, chlorine, hydrogen peroxide, and hydroxide. The low pH influences cell membrane porosity, which then inhibits bacterial reproduction. Hydrodynamic condition in the microfluidic system contributes to continuous flow of fluid which facilitates the development of robust biofilm. This type of biofilm cannot be destroyed easily compared to static biofilm.

In the included articles, ex vivo and in vitro methods were used. Ex vivo method involved experimenting in extracted tooth model was used in 4 of the included studies [17–20], and only 3 studies mentioned biofilm formation [17,19,20]. This methodology can closely resemble the clinical situation with respect to root canal ecology. However, variables such as the dentinal property and the degree of bacterial invasion into dentinal tubules are difficult to be standardized [21]. Furthermore, the sampling method after treatment with experimented solutions on ex vivo method can be misleading and give false negative result; some bacteria may have entered the dentinal tubules and ramifications and were left undetected [40]. On the other hand, in vitro method was used by the other 4 studies [21–23,31], and only one study confirmed biofilm formation [21]. This method is less accurate in mimicking the natural environment in the root canal system and the bacteria are easier to be eliminated, but the bacterial count would be more accurate.

Effect of EW concentration on antimicrobial efficacy

The effect of different EW concentrations was investigated in 2 studies [22,31]. Okamura et al. [22] found that antimicrobial efficacy of acidic FW was significant at concentrations of 30% and higher, but no antimicrobial effect was observed when 10% acidic FW was used. In contrast, Hsieh et al. [31] found no significant difference between EOW at 0.0125% and 0.025% HOCl. At both concentrations, EOW eliminated more than 99.9% of the bacterial population in the samples. This different finding from the two studies can be due to the difference in ACC used. Hsieh et al. [31] used high ACC at 330–350 ppm, while Okamura et al. [22] only used ACC between 20 and 100 ppm.

Other studies in the literature also have opposing results. Some studies showed high EW concentration have better antimicrobial efficacy than low concentration [41–45], while other studies showed similar antimicrobial efficacy between high and low EW concentrations [46,47]. Yanik et al.

observed that *E. faecalis* were susceptible to higher EW concentrations (dilutions of 1/1, 1/2, and 1/10), compared to lower EW concentrations (dilutions of 1/20, 1/50, and 1/100) when exposed for 1 min [41]. In another study, Gunaydin et al. observed that *E. faecalis* was susceptible to EW diluted 1/1 and 1/2, while dilutions of 1/5, 1/10, 1/50, and 1/100 were ineffective with similar exposure time of 1 min [42]. The variation in antimicrobial efficacy between the concentrations can be due to the type of EW used; Yanik et al. used ECAW produced by Envirolyte (ECA-EN) with chlorine content of 500–700 ppm, and Gunaydin et al. used ECAW produced by Medilox (ECA-MX) with chlorine content of only 80 ppm. Akbulut and Eldeniz mentioned that the pH values play a role in antimicrobial efficacy of ECAW [25]. Even though the ECAW used in studies by Gunaydin et al. and Yanik et al. have similar pH, the chlorine content was vastly different, hence contributing to better antimicrobial efficacy of ECA-EN in the study by Yanik et al. Gunaydin et al. also experimented on other microbes such as *Aspergillus niger* and *Aspergillus flavus*, but both fungi were not susceptible to high nor low EW concentrations when exposed for 1 min. However, when exposed at longer periods, high EW concentration was more effective than low EW concentration on both fungi [42].

Effect of EW exposure time on its antimicrobial efficacy

Only 3 articles discussed the effect of exposure time on the antimicrobial efficacy of EW [17,23,31]. Only Zan et al. [17] found increased antimicrobial efficacy with increasing exposure time of EW. Another 2 studies by Saucedo et al. [23] and Hsieh et al. [31] found that increasing the treatment time did not affect the antimicrobial efficacy of EW. This contradictory finding is due to the difference in exposure time used between the studies. Hsieh et al. [31] and Saucedo et al. [23] used shorter exposure time of maximum 60 s (Table 3) and Zan et al. [17] used a longer treatment time which compared between 1–2 and 3–5 min, resulting in a better antimicrobial efficacy. Furthermore, Zan et al. [17] experimented on extracted human teeth, whereas Saucedo et al. [23] and Hsieh et al. [31] used bacterial culture samples in planktonic form; thus, longer treatment time was required to achieve better antimicrobial efficacy in the study by Zan et al. [17]. Furthermore, Mena-Mendivil et al. [19] also showed that at 5 min exposure time using OxOral, EW showed significant bacterial reduction compared to 60 s OxOral exposure in the study by Saucedo et al. [23], where no bactericidal effect was observed.

Other studies in the literature showed similar patterns in their results. Yanik et al. found that EW exposure time between 1, 2, 5, and 30 min had no significant difference in antimicrobial effect on *E. faecalis* when using high concentration of EW. But at low concentrations (dilution of more than 1/20), a significant difference was observed in *E. faecalis* reduction for exposure times between 1–2 and 5–30 min [41]. Similarly, Yamada et al. found no significant difference in exposure times of 0.25, 1, 5, 10, 15, and 30 min to eliminate *P. gingivalis* when using high concentration of EW, but when using low concentration, a significant difference in *P. gingivalis* reduction can only be observed after 15 min of exposure [45]. Yamada et al. also experimented on other microbes such as *Streptococcus sobrinus* and *Candida albicans*; *S. sobrinus* required longer exposure time for significant reduction compared to *P. gingivalis* using the same EW concentration. For *C. albicans*, there was no significant difference in the reduction between 1 min and 1 h for high and low concentrations [45].

Hence, it can be inferred that EW concentration and exposure time have different antimicrobial efficacies depending on the type of microbial species, EW type, pH level, chlorine level, and ORP value to influence the antimicrobial efficacy.

There are a few limitations in this review. First, few articles were included due to the strict inclusion criteria. Furthermore, the studies included had substantial methodological heterogeneity: 1) microbial samples, 2) *in vitro* and *ex vivo* studies, 3) different types of EW used with different preparations, 4) methods of calculating microbial reduction, and 5) different exposure times. Furthermore, the quality assessment of included studies suggests medium quality, hence the results should be interpreted cautiously and robust conclusions could not be drawn.

CONCLUSION

Based on *in vitro* studies, EW has a potential to be an antimicrobial endodontic irrigant alternative to NaOCl. It is shown to be effective against different types of root canal pathogen including *E. faecalis*, which is common in persistent apical periodontitis. Furthermore, increasing the concentration and exposure time of EW may have a potential to heighten the antimicrobial efficacy. To further validate these findings and determine the clinical applicability of EW, it would be beneficial to conduct clinical studies.

ACKNOWLEDGEMENT

This paper was supported by UMP-IIUM-UiTM Sustainable Research Collaboration 2020 Research Grant (SRCG20-014-0014). The authors would also like to thank International Islamic University Malaysia, Sr. Nurul Alia Risma Rismayuddin, and Sr. Aini Rasidda Norazmi for assisting with the database search.

DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

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Editorial History

Date of Submission: 27 Nov 2023
Review & Revision: 14 Dec 2024 – 20 Feb 2024
Accepted: 20 Feb 2024
Published: 18 Mar 2024