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Review Article

Systematic Review of the Microbiological Impact of Sodium Hypochlorite Concentrations in Endodontic Treatment

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ABSTRACT

Sodium hypochlorite (NaOCl) is considered the most effective solution for irrigating root canals due to its powerful disinfecting properties, including its ability to dissolve tissue, disrupt microorganisms, and eliminate bacterial biofilms in endodontic treatments. Typically, NaOCl is used at concentrations ranging from 0.5% to 5.25% during procedures. The objective of this research was to evaluate the bactericidal effects of different concentrations of NaOCl on biofilms and their influence on the outcomes of root canal treatment. This review compiles studies published between 2000 and 2023, utilizing databases like Medline, PubMed, and ScienceDirect. A total of 10 studies were included to assess how varying NaOCl concentrations impact root canal disinfection and patient outcomes. While higher concentrations were more successful in reducing bacterial counts, they did not significantly affect healing rates or patient discomfort. NaOCl exhibited strong antibacterial effects against several bacterial species, including *Enterococcus faecalis*, *Candida albicans*, *Actinomyces naeslundii*, and *Streptococcus aureus*. The findings suggest that NaOCl's efficacy is dependent on using appropriate irrigation protocols and adequate solution exchange. Further research is needed to better understand how different concentrations of NaOCl and its combination with other irrigants influence root canal treatment outcomes.

Keywords: Sodium hypochlorite, Microbiological effect, Endodontics, Systematic review

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Introduction

Endodontic treatment aims to eradicate bacteria from the root canal system and prevent the spread of infection to the surrounding pulp and periapical tissues. The primary technique for achieving this involves chemomechanical cleaning, which combines mechanical instrumentation with chemical agents [1-3]. Despite thorough cleaning, certain bacterial strains, particularly those that are resistant, may survive in the root canal, leading to persistent infections. While planktonic bacteria are generally sensitive to antibacterial agents, bacteria embedded in biofilms attached to the canal walls or within more difficult-to-reach areas, like the tips of dentinal tubules and lateral canals, are much harder to treat. This creates a significant challenge, requiring specialized treatment strategies to address these issues effectively [2-5].

One of the most commonly used root canal irrigants is sodium hypochlorite (NaOCl), a powerful disinfectant known for its ability to dissolve tissue, disrupt microbial cells, and eradicate bacterial biofilms. NaOCl is typically used in concentrations ranging from 0.5% to 5.25% during endodontic procedures. When biofilm growth, particularly that of Enterococcus faecalis, increases, it becomes mineralized, making it more resistant to traditional cleaning methods and increasing the likelihood of persistent infection [2-4]. Biofilms composed of *E. faecalis* begin to show signs of mineralization and full maturity after approximately six weeks. As biofilms mature, they become harder to remove, posing a challenge for effective treatment. Most research conducted on biofilms focuses on younger formations, but in clinical settings, biofilms in root canals are often weeks or even months old while being treated. Understanding the stages of biofilm development is crucial in determining how biofilms respond to antimicrobial agents and improving treatment strategies [3-6].

The objective of this research was to evaluate the bactericidal effects of different concentrations of NaOCl on biofilms and their influence on the outcomes of root canal treatment.

Materials and Methods

A thorough literature search was performed using databases such as Medline, PubMed, and ScienceDirect, covering the period from 2000 to 2023. The search utilized terms such as “sodium hypochlorite,” “systematic review,” and “root canal treatment.” The selection process for included studies was outlined using the PRISMA flowchart **(Figure 1)**.

Inclusion criteria

Studies included in this review were case-control and randomized controlled trials published in English from 2000 to 2023.

Exclusion criteria

Exclusion criteria consisted of expert opinion articles, systematic reviews, narrative reviews, studies outside the specified time frame, research conducted in languages other than English, and in vitro studies.

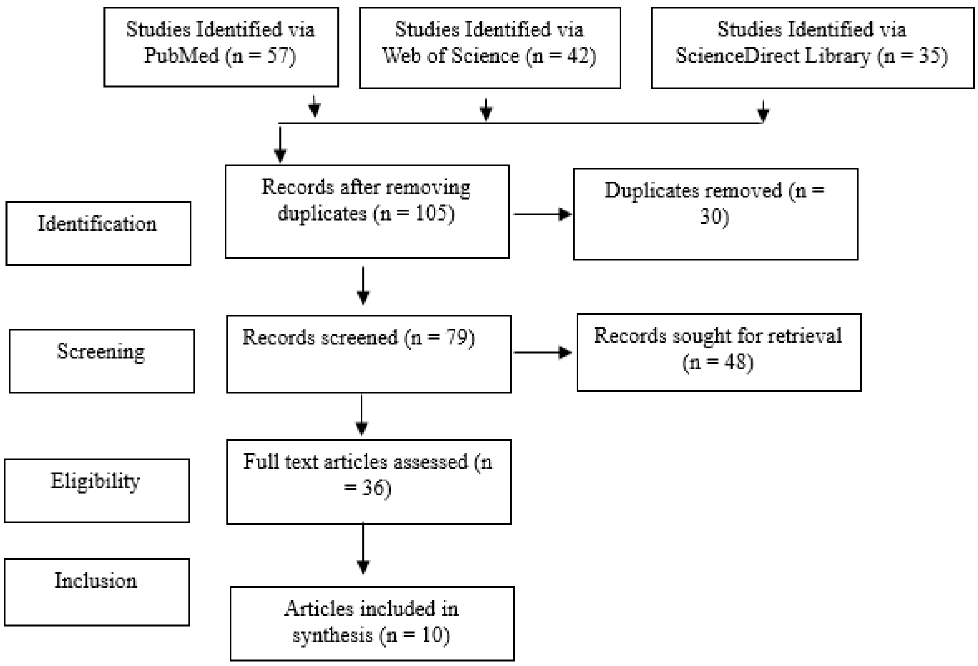

Figure 1. PRISMA flow diagram

The quality of the included studies was evaluated using the Cochrane risk of bias assessment method **(Table 1)**.

Table 1. Summary of Cochrane risk of bias assessment

Reference	Reporting bias/selective reporting of outcomes	Detection bias: blinding of outcome assessors	Performance-related bias in blinding	Selection bias in allocation concealment	Selection bias in randomization	Selection bias/appropriate control selection/baseline characteristics similarity	Accounting for confounding bias
Ulin *et al.* [6]	+	+	-	+	+	+	+
Radcliffe *et al.* [7]	-	+	+	-	+	+	+
Siqueira *et al.* [8]	+	+	+	-	+	+	+

Verma *et al.* [9]	+	+	+	+	+	+	+
Reyhani *et al.* [10]	+	+	+	+	+	+	-
Shakouie *et al.* [11]	+	+	+	-	+	+	+
Câmara *et al.* [12]	+	+	+	+	-	+	+
Rôças *et al.* [13]	+	+	-	+	+	+	+
Zand *et al.* [14]	+	-	+	+	+	+	-
Torabinejad *et al.* [15]	+	+	+	+	-	+	+

Results and Discussion

The study by Ulin *et al.* [6] involved 298 participants who were randomly divided into two groups: one treated with 0.5% NaOCl and the other with 3% NaOCl. The trial included individuals with a variety of endodontic diagnoses. The results indicated that different concentrations of NaOCl did not significantly impact the numbers of positive bacterial cultures, nor did they affect the frequency or severity of postoperative pain. However, patients treated with the higher concentration (3% NaOCl) showed a notably increased incidence of postoperative edema.

In Radcliffe *et al.* [7], the effects of different NaOCl concentrations on bacterial growth were examined. The study found that all NaOCl concentrations effectively reduced the colony-forming units (cfu) of *A. naeslundii* and *Candida albicans* below detection limits within 10 seconds. When compared to *E. faecalis*, NaOCl showed stronger antibacterial activity. Specifically, a 0.5% NaOCl solution completely eradicated *E. faecalis* after 30 minutes, outperforming higher concentrations in terms of time required to achieve a similar effect ($P = 0.001$).

Siqueira *et al.* [8] evaluated the efficacy of various NaOCl concentrations (1%, 2.5%, and 5.25%) and saline for reducing bacterial levels in the root canal. Bacterial samples were taken before and after irrigation. The results demonstrated that all NaOCl concentrations significantly reduced bacterial presence compared to saline ($P = 0.05$), with no significant difference observed between the NaOCl solutions themselves ($P > 0.05$).

Verma *et al.* [9] compared the post-treatment recovery and pain levels associated with two NaOCl concentrations following primary endodontic treatment. Patients rated their pain on a visual analog scale daily for a week, and radiographic examinations and clinicals were conducted every three months for a year. After 12 months, 86 teeth were available for evaluation, showing a healing rate of 76.7%. The healing rate for the high-concentration (HC) group was 72.1%, compared to 81.4% for the low-concentration (LC) group, although the difference was not significant ($P > 0.05$).

Reyhani *et al.* [10] investigated the impact of NaOCl on *E. faecalis* biofilms in root canals. Chemomechanical debridement was performed on 104 patients' maxillary central incisors, followed by irrigation with 5.25% NaOCl for three minutes to remove the smear layer. This treatment eliminated *E. faecalis* biofilms in groups treated with higher concentrations of NaOCl, while group 1 showed lower bacterial counts at 4, 6, and 10 weeks compared to the control. The study found that biofilms in more mature stages were more resistant to the 1% NaOCl solution, but both 2.5 and 5.25% NaOCl effectively inhibited *E. faecalis* growth at all stages of biofilm development.

The study by Shakouie *et al.* [11] aimed to compare the antibacterial activity of various concentrations of sodium hypochlorite (NaOCl) (0.5%, 1%, 2.5%, and 5%) with Triphala, a plant-derived solution, against *Enterococcus faecalis* (*E. faecalis*). The results showed that the inhibition zones produced by Triphala were notably larger than those produced by the 0.5% and 1% NaOCl concentrations. Statistically, Triphala demonstrated stronger antibacterial effectiveness than the lower concentrations of NaOCl ($P = 0.05$).

In another study by Rôças *et al.* [13], fifty single-rooted teeth with apical periodontitis were treated using BioRaCe rotary instruments and irrigated with 2.5% NaOCl. Despite thorough cleaning, bacterial remnants were found in each S1 sample. After using the 2.5% NaOCl, bacterial traces were still detected in 44% and 40% of the root canals. However, a significant reduction in bacterial counts was observed in the S2 samples ($P < 0.001$), showing a marked decrease in bacterial cell equivalents from 3.7×10^5 to 5.49×10^2.

Zand *et al.* [14] carried out an in vitro study using sixty extracted single-rooted teeth. These teeth were contaminated with E. faecalis and cultured for six weeks. It was found that the NaOCl solutions at concentrations of 2.5% and 5.25% were significantly more effective in reducing bacterial levels than the 2.5% NaOCl gel ($P < 0.05$).

In the research by Torabinejad *et al.* [15], the combination of low NaOCl concentrations as an intracanal irrigant followed by MTAD as a final rinse proved to be more effective in eliminating the smear layer. Importantly, this method did not cause noticeable damage to the dentinal tubules.

Câmara *et al*. [12] conducted a study to investigate the antibacterial properties of NaOCl at concentrations of 0.5%, 1%, and 2.5% in root canals, using the ProTaper Universal system for instrumentation. The study included five groups: three groups received NaOCl solutions (0.5%, 1%, 2.5%), and two control groups were treated with sterile saline solutions. The results indicated that all the NaOCl concentrations effectively eliminated bacteria in the root canals, with one exception at 0.5% NaOCl in the S1 group, which showed bacterial growth. There were no significant differences between the efficacy of the NaOCl concentrations (P = 1.000).

An overview of the key findings from the studies is presented in **Table 2**.

Table 2. Overview of the key findings from the studies

Reference	Sample size	NaOCl concentration tested	Study aim	Key findings
Ulin *et al*. [6]	298	0.5% NaOCl and 3% NaOCl	To evaluate if 3.0% NaOCl results in fewer post-treatment bacterial cultures than 0.5% buffered NaOCl	A significant mean difference of 12.7 (P = 0.0084) was observed between the two concentrations.
Siqueira *et al*. [8]	-	1%, 2.5%, and 5.25%	To assess the reduction of intracanal bacteria with saline or NaOCl	No significant difference was found between the three NaOCl concentrations (P > 0.05).
Radcliffe *et al*. [7]	-	0.5%, 1.0%, 2.5%, and 5.25%	To investigate the resistance of bacteria in refractory endodontic infections to NaOCl	0.5% NaOCl reduced bacterial counts to zero within 30 minutes for both strains.
Reyhani *et al*. [10]	104	5.25% NaOCl	To examine how various NaOCl concentrations affect the development of *Enterococcus faecalis* biofilms in root canals	Complete elimination of *E. faecalis* biofilms was achieved in groups 2 and 3 (P = 0.0001).
Torabinejad *et al*. [15]	80	5.25% NaOCl	To explore the effects of NaOCl as an intracanal irrigant followed by MTAD	No significant damage to the dentinal tubule structure was observed.
Zand *et al*. [14]	60	2.5% and 5.25% NaOCl	To evaluate the antibacterial efficacy of NaOCl solutions on *E. faecalis* biofilms	No significant difference was found between 2.5% and 5.25% NaOCl (P > 0.05).
Shakouie *et al*. [11]	38	0.5%, 1%, 2.5%, and 5%	To compare the antibacterial activity of NaOCl solutions with Triphala against *E. faecalis*	Triphala showed significantly larger inhibition zones than 0.5% and 1% NaOCl.
Câmara *et al*. [12]	50	0.5%, 1%, and 2.5%	To assess the antibacterial effects of NaOCl at various concentrations in root canals	No significant difference between NaOCl concentrations was found (P = 1.000).
Verma *et al*. [9]	86	1% and 5%	To compare the recovery and discomfort following endodontic treatment with different NaOCl concentrations	No statistically significant difference was found between the groups (P > 0.05).
Rôças *et al*. [13]	50	2.5% NaOCl	To examine the antibacterial effects of NaOCl versus chlorhexidine (CHX) during infected root canal preparation	No major findings were provided for the NaOCl group specifically.

The study demonstrated that the difference in NaOCl concentration (0.5% vs. 3%) did not have a significant effect on the number of positive cultures or the level of postoperative discomfort experienced [16]. However, the higher concentration of NaOCl (3%) led to a noticeable increase in postoperative edema [17]. Participants in the study were consecutively recruited, but many initially considered candidates were ineligible, mostly due to experiencing "clinical discomfort" [6]. A potential weakness in the study's validity, both internally and externally, arises from the huge number of patients who declined participation. This study design therefore exists as a cross between efficacy and real-world effectiveness studies.

In total, ten dentists with varying clinical experience—from 2 years to 35 years—were involved in the study, with a mix of experts and post-graduate students. This diversity in expertise can be viewed as a strength. However, when comparing the results of specialists to post-graduate students, no significant differences were observed [18]. For randomization, a simple odd/even day approach was employed to facilitate smooth survey completion without disrupting daily routines, although this method introduces a potential risk of selection and bias of performance [19].

The study results revealed that bacterial counts in biofilms aged over ten weeks were higher than those in younger biofilms treated with a 1% NaOCl solution. This indicates that as biofilms age and develop more complex, calcified structures, removing bacteria from them becomes increasingly difficult [20]. Previous research has shown that NaOCl effectively kills *E. faecalis* biofilms. While NaOCl demonstrates antibacterial properties against *E. faecalis* at all concentrations, the effectiveness varies depending on how long the biofilm is exposed to the solution. Research by Gomez *et al*. showed that 2.5% NaOCl could eliminate all bacteria within 10 minutes. Our study confirmed that both 2.5% and 5.25% NaOCl successfully eliminated mature E. faecalis biofilms at all tested time intervals, while 1% NaOCl only partially reduced the bacterial count compared to PBS [21].

The ideal irrigating solution for root canals would possess the strongest antibacterial and tissue-removal abilities with minimal negative side effects [22]. NaOCl has proven potent in fighting *E. faecalis* but its effectiveness depends on both concentration and exposure time. This study also explored the gel form of NaOCl versus its liquid counterpart, noting that the gel is less likely to be extruded through the apex. Using #4 Gates-Glidden drills and #35 K-files for canal preparation and coronal flaring improved the penetration of both gel and solution into the canals. Therefore, if the solutions did not fully reach the apical regions of the canals, it should not be assumed that antibacterial activity was absent [23].

This study highlights the advantages of using natural alternatives, such as Triphala, in comparison to the limitations of NaOCl. The results demonstrated that Triphala exhibited superior antibacterial properties compared to 0.5% and 1% NaOCl, making it a promising option as an irrigation solution in endodontic treatments. Further research is necessary to confirm these findings. A key distinction of this study was the use of *E. faecalis* biofilms, which are more resilient to antibacterial treatments than free-floating (planktonic) bacteria, setting this investigation apart from previous studies [24]. The results also showed no significant difference in the antibacterial effects between NaOCl solutions at concentrations of 2.5% and 5.25% when applied to *E. faecalis* [25]. Previous studies had indicated that 5.25% NaOCl was the most effective, followed by 2.5%. In contrast, other research revealed that using 2.5% NaOCl left about 40% of the canal surfaces contaminated, suggesting its inefficacy against E. faecalis [26]. The discrepancies in previous research regarding NaOCl's antibacterial effectiveness at different concentrations could be attributed to factors such as variations in study methodology, the microbial characteristics of the biofilm, exposure time, and chemical concentration [25, 26].

When assessing the therapeutic efficacy of NaOCl, it's crucial to account for the complex anatomy of root canals, the polymicrobial nature of infections, and the presence of biofilms [27]. Biofilms are known to impede the diffusion of solutes, which further complicates treatment [28]. Additionally, the smear layer present in the root canal may prevent effective penetration of the irrigant into the dentinal tubules. Moreover, the interaction of NaOCl with substances like blood, dentin, tissue fluids, and other organic materials can reduce its effectiveness. The short contact time of NaOCl within the root canal during chemomechanical preparation limits its ability to penetrate the tubules. As observed in this study, the concentration and exposure time of NaOCl within the canal significantly influence its antibacterial action. Consequently, a solution that is effective in vitro against a specific bacterium in the dental root canal may not demonstrate the same efficacy in clinical settings [29].

It is important to note that the value of NaOCl in endodontic procedures is not limited to its antibacterial properties. An effective root canal irrigant must also have strong detergent properties, low surface tension, ease of handling, and the ability to break down proteins and tissue. Since NaOCl is unstable and decomposes in sodium chloride and oxygen—both naturally occurring in the body—it leaves no harmful residues behind when used as an irrigant.

Conclusion

In summary, the reviewed studies offer essential information about the use of sodium hypochlorite (NaOCl) as an irrigation solution in root canal therapy. The research indicates the concentration of NaOCl, ranging from 0.5% to 5.25%, did not have a significant impact on the number of bacterial cultures or severity of postoperative discomfort. However, higher concentrations of NaOCl (3% and 5.25%) were associated with an increased incidence of postoperative swelling. NaOCl proved effective in eliminating various bacteria, including *Candida albicans*, *Enterococcus faecalis*, *Actinomyces naeslundii*, and *Streptococcus aureus*. The findings highlight that proper irrigation techniques, coupled with adequate solution exchange, are critical for ensuring maximum antibacterial effectiveness when using NaOCl as an irrigant. Future research is needed to explore the effects of varying NaOCl concentrations and how they interact with other irrigants to improve root canal treatment outcomes.

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