Original Article

A Novel Approach to Gutta-Percha Disinfection: Evaluating Hypochlorous Acid Against Standard Endodontic Disinfectants

Didar S. Hama Gharib[*1], *Safeen O. Mahmood*[1]

Abstract

Objective: It is advisable to decontaminate gutta-percha cones before their insertion into the root canal system. The prevalence of contamination remains a contentious issue. This study aimed to assess and compare the effectiveness of several chemical agents for disinfecting gutta-percha cones (GP).

Methods: One hundred and ninety size F3 GP cones were used. The cones were contaminated with Enterococcus faecalis and Candida albicans following immersion. Three chemical agents were used: 2% chlorhexidine gluconate (CHX), 5.25% sodium hypochlorite (NaOCl), and 200ppm hypochlorous acid (HOCL). GP cones were immersed in the chemical agents for periods of 1 and 5 minutes. Following disinfection, the cones were incubated in thioglycolate broth, and the turbidity of the medium was used to indicate bacterial growth.

Results: CHX and NaOCl showed time-dependent regrowth of both microbes after short (1-minute) exposures; only a 5-minute NaOCl exposure achieved sustained bacterial elimination. In contrast, 200 ppm HOCl demonstrated immediate and complete eradication of both pathogens with all exposure times, showing no regrowth over 14 days.

Conclusions: HOCl proved superior to CHX and NaOCl, exhibiting rapid, stable, and prolonged antimicrobial efficacy without significant reduction over time.

Keywords: *Candida albicans, Enterococcus faecalis, Gutta-percha cone, Sodium hypochlorite, Chlorhexidine, Hypochlorous acid.*

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1. *Operative Dentistry and Endodontics Department, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.*
2. *Basic Medical Sciences Department, College of Medicine, University of Sulaimani, Sulaimani, Iraq.*

** Corresponding author*: *didar.hamagharib@univsul.edu.iq.*

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Introduction

In dentistry, root canal therapy has long been utilized to protect and save teeth[1]. The essential goals of endodontic treatment are to eliminate microbial infection from the root canal system and prevent reinfection through effective cleaning, shaping, and obturation[2]. Despite advances in disinfection protocols, persistent infections remain a significant challenge, often attributed to resilient microorganisms such as Enterococcus faecalis and Candida albicans[3]. E. faecalis, a Gram-positive facultative anaerobe, is frequently isolated from failed root canal procedures due to its ability to form biofilms, survive in nutrient-deprived environments, and resist common antimicrobial agents[4]. In the same vein, C. albicans, an opportunistic fungal pathogen, has been more frequently associated with refractory endodontic infections, particularly in cases of compromised immune responses or inadequate disinfection[5]. The prevalence of these microorganisms in root canals can lead to periapical inflammation, delayed healing, and, ultimately, endodontic failure[6].

The most common core obturation material is gutta-percha (GP) cones because they are biocompatible and thermoplastic[7]. Despite being produced under aseptic conditions and sold in sealed packages, their sterilization is questionable and can be easily contaminated when they are handled, stored, or used in a clinical setting[8]. Effective disinfection before obturation is important for long-term treatment effectiveness because contaminated gutta-percha cones can bring bacteria back into the root canal system[9]. Multiple compounds, such as sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), hydrogen peroxide, and alcohol solutions, have been utilized for the disinfection of gutta-percha[10]. 5% NaOCl is recognized for its strong antibacterial and tissue-dissolving capabilities [11], while 2% CHX is appreciated for its ability to adhere to surfaces and address a variety of bacteria and fungi[12]. However, each of these agents has its own set of challenges: NaOCl has the potential to cause cytotoxicity and compromise the structural integrity of gutta-percha[13]. Conversely, CHX is ineffective against certain bacterial strains and may produce precipitates when it interacts with NaOCl residues[14].

Considering these limitations, there is increasing interest in alternative disinfectants that provide superior antibacterial effectiveness while minimizing negative effects. Hypochlorous acid (HOCl), a naturally occurring compound produced by neutrophils during immunological reactions, has surfaced as a viable candidate[15]. HOCl is biocompatible and non-toxic to host tissues, and it exhibits rapid bactericidal and fungicidal efficacy at minimal concentrations (e.g., 200 ppm)[16]. It is effective against a wide range of infections, including antibiotic-resistant forms, by inflicting oxidative damage on microbial cell walls, proteins, and DNA[17]. Recent studies have demonstrated the potential of HOCl in the fields of wound care[18] and surface disinfection[19]. However, its efficacy in disinfecting gutta-percha cones, particularly compared with conventional agents, has been scarcely examined.

This research aims to assess and compare the antimicrobial effectiveness of 2% CHX, 5.25% NaOCl, and 200 ppm HOCl in disinfecting gutta-percha cones contaminated with E. faecalis and C. albicans. This study seeks to determine an appropriate disinfection technique by evaluating the relative death rates and residual antimicrobial effects, ensuring microbiological eradication without affecting material characteristics or biocompatibility. The findings may provide insight into how clinical endodontic techniques could be improved, leading to better treatment outcomes and reduced failure rates due to microbial persistence.

Materials and Methods

Sample preparation

A total of 190 ISO size (F3) GP cones (Dentsply Sirona, Ballaigues, Switzerland) were used in this study. All cones were handled aseptically throughout the experimental procedures using sterile gloves and instruments. Prior to contamination, the cones were exposed to ultraviolet (UV) light for 30 minutes to minimize pre-existing microbial contamination. The Ethical Committee at the College of Dentistry, University of Sulaimani, approved the research project with Code No. (COD-EC-24-0062) on December 16, 2024.

Microbial strains and inoculation

Two standard microbial strains, E. faecalis (ATCC 29212) and C. albicans (ATCC 10231), were used to simulate typical endodontic pathogens. The strains were individually cultured in Brain Heart Infusion (BHI) broth at 37°C for 24 hours to reach an approximate concentration of 10^8 CFU/mL. Each GP cone was submerged in a microbial suspension containing either E. faecalis or C. albicans for 30 minutes under sterile conditions to ensure uniform contamination.

Disinfection protocol

Next to contamination, 180 GP cones were randomly divided into three experimental groups (n=60/group) based on the disinfectant used:

Group A: 5.25% NaOCl, (CHLORAXID 5.25%, CERKAMED, Poland) / n=60 (30 for E. faecalis + 30 for C. albicans).

Group B: 2% CHX, (GLUCO-CHEX 2%, CERKAMED, Poland) / n=60 (30 for E. faecalis + 30 for C. albicans).

Group C: 200 HOCl, (SulOX, Sulox for antimicrobial disinfectants production, Iraq) / n=60 (30 for E. faecalis + 30 for C. albicans).

Then each group was further subdivided into two subgroups according to the duration of disinfection:

Subgroup 1: 1-minute immersion

Subgroup 2: 5-minute immersion

Following disinfection, each cone was rinsed in sterile distilled water for 10 seconds to remove residual disinfectant and prevent carryover into the culture medium.

Post-disinfection incubation and evaluation

The GP cones were aseptically transferred into sterile glass vials containing 5 mL of sterile thioglycolate broth (Condalab, Madrid, Spain) and incubated at 37°C under aerobic conditions. For each subgroup, samples were evaluated for microbial growth at three different time intervals: 24 hours, 7 days, and 14 days. At each interval, the turbidity of the broth was visually assessed as an indicator of microbial growth. In addition, 100 µL aliquots were taken from each vial and streaked onto blood agar plates to confirm microbial presence and identify persistent contamination.

Control groups

Positive control: A total of five Contaminated GP cones without disinfection were directly placed into thioglycolate broth.

Negative control: A total of five Sterile, uncontaminated GP cones were directly placed into thioglycolate broth to confirm asepsis during handling.

Outcome assessment

In each incubation period, the presence or absence of turbidity in the broth was the major outcome measure, indicating viable microbial growth. Agar culture confirmation was used as a secondary outcome to validate turbidity results. All procedures were performed in triplicate for each group to ensure reproducibility.

Statistical analysis

The data were expressed as absolute values and percentages [n (%)]. Statistical comparisons were conducted using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA). To evaluate differences in paired binary outcomes across incubation intervals, the non-corrected McNemar test was applied. This test was used to detect statistically significant changes in microbial growth status following exposure to chemical disinfectants at different time points (1, 7, and 14 days). Separate p-values were calculated to compare antimicrobial performance across time intervals: Pa (1-day vs. 7-day), Pb (1-day vs. 14-day), and Pc (7-day vs. 14-day). Statistical significance was indicated by a P-value < 0.05.

Results

The bactericidal efficacy of 2% CHX, 5.25% NaOCl, and 200 ppm HOCl against E. faecalis was evaluated across three incubation intervals as shown in Table 1 and Figure 1. One-minute exposure to CHX resulted in persistent bacterial survival across all time points (1 day: 40.0%; 7 days: 46.7%; 14 days: 60.0%), with no statistically significant differences among time points (p = 0.804, 0.439, and 0.814, respectively). By contrast, exposure to CHX for 5 min resulted in a marked growth inhibition at 1 day (6.7%), whereas incomplete regrowth was detected at 7 and 14 days (20.0% and 26.7%, respectively), with significant differences between the results of the assays after incubation for 24 h versus those obtained after longer incubation (p = 0.013 or p = 0.031).

The initial efficacy at one minute exposure was higher with NaOCl (5.25%) in comparison to the other tested solutions (0.0% growth at 1 day), however, bacterial regrowth occurred after 7 days (20.0%) and 14 days of storage time (40.0%), being statistically different from 1 day to 7-day contact time intervals, p = 0.008). Five-minute exposure to NaOCl resulted in sustained absence throughout all incubation periods (0.0% growth) and was not significantly different among any [p = 1.000]. HOCl (200 ppm) completely suppressed bacterial growth at all exposure times and time points tested (0.0% growth), and temporal trends in any comparison were not significant (p = 1.000). A total of 90 infected GP cones with C. albicans were treated by disinfection techniques (2% CHX, 5.25% NaOCl, and 200 ppm HOCl). After 1-min exposure, CHX and NaOCl resulted in a complete inhibition at day 1 (0%) but gradually regrew by day 7 (CHX: 33.3%, NaOCl: 33.3%) and by day 14 (CHX: 46.7%; NaOCl: 40%), with significant differences being found between the incubation periods of day 1 and both day 7 (CHX, p =.041; NaOCl, p =.041) and day 14 (CHX, p =.023; NaOCl, P =.049). After 5-minute exposure to 2% CHX and 5% NaOCl, sustained inhibition was observed at all time points, with mild

regrowth of NaOCl at day 14 (13.3%), which was statistically significant compared with that at the 1- and 7-day incubation periods (P = .002). In contrast, HOCl (200 ppm) maintained 100% antifungal efficacy at all exposure times, regardless of incubation duration; no regrowth was observed, and no significant differences among comparisons were observed (p = 1.000). These results highlight HOCl's stability as a fast and sustained antifungal agent, surpassing CHX and NaOCl in immediate and residual disinfection efficiency, as shown in Table 2 and Figure 2.

Table 1: Comparative effectiveness of the three chemical disinfectants on E. faecalis growth across incubation periods.

Groups	Exposure time	Microbial growth	Incubation time			p-value		
			Day 1	Day 7	Day 14	P a	P b	P c
2%CHX, n=30	1 Minute, n=15	Growth	6 (40.0%)	7 (46.7%)	9 (60.0%)	0.804	0.439	0. 814
		No growth	9 (60.0%)	8 (53.3%)	6 (40.0%)			
	5 Minutes, n=15	Growth	1 (6.7%)	3 (20.0%)	4 (26.7%)	0.013*	0.031*	0.077
		No growth	14 (93.3%)	12 (80.0%)	11 (73.3%)			
5%NaOCl, n=30	1 Minute, n=15	Growth	0 (0.0%)	3 (20.0%)	6 (40.0%)	0.008	0.078	0.238
		No growth	15 (100.0%)	12 (80.0%)	9 (60.0%)			
	5 Minutes, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			
200 ppm HOCl, n=30	1 Minute, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			
	5 Minutes, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			

n; number of the samples, %; percentage, *; statistically significance, p-value; Significant level, Pa; P-value for (1-day vs. 7-day

Table 2: Comparative effectiveness of chemical disinfectants on C. albicans growth across incubation periods.

Groups	Exposure time	Microbial growth	Incubation time			p-value		
			Day 1	Day 7	Day 14	P a	P b	P c
2%CHX, n=30	1 minute, n=15	Growth	0 (0.0%)	5 (33.3%)	7 (46.7%)	0.041 *	0.023 *	0.629
		No growth	15 (100.0%)	10 (66.7%)	8 (53.3%)			
	5 Minutes, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			
5%NaOCl, n=30	1 minute, n=15	Growth	0 (0.0%)	5 (33.3%)	6 (40.0%)	0.041 *	0.049 *	0.454
		No growth	15 (100.0%)	10 (66.7%)	9 (60.0%)			
	5 Minutes, n=15	Growth	0 (0.0%)	0 (0.0%)	2 (13.3%)	1.000	0.002 *	0.002 *
		No growth	15 (100.0%)	15 (100.0%)	13 (86.7%)			
200 ppm HOCl, n=30	1 minute, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			
	5 Minutes, n=15	Growth	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1.000	1.000
		No growth	15 (100.0%)	15 (100.0%)	15 (100.0%)			

n; number of the samples, %; percentage, *; statistically significance, P-value; Significant level, Pa; P-value for (1-day vs. 7-day incubation), Pb; P-value for (1-day vs. 14-day), and Pc; P-value for (7-day vs. 14-day).

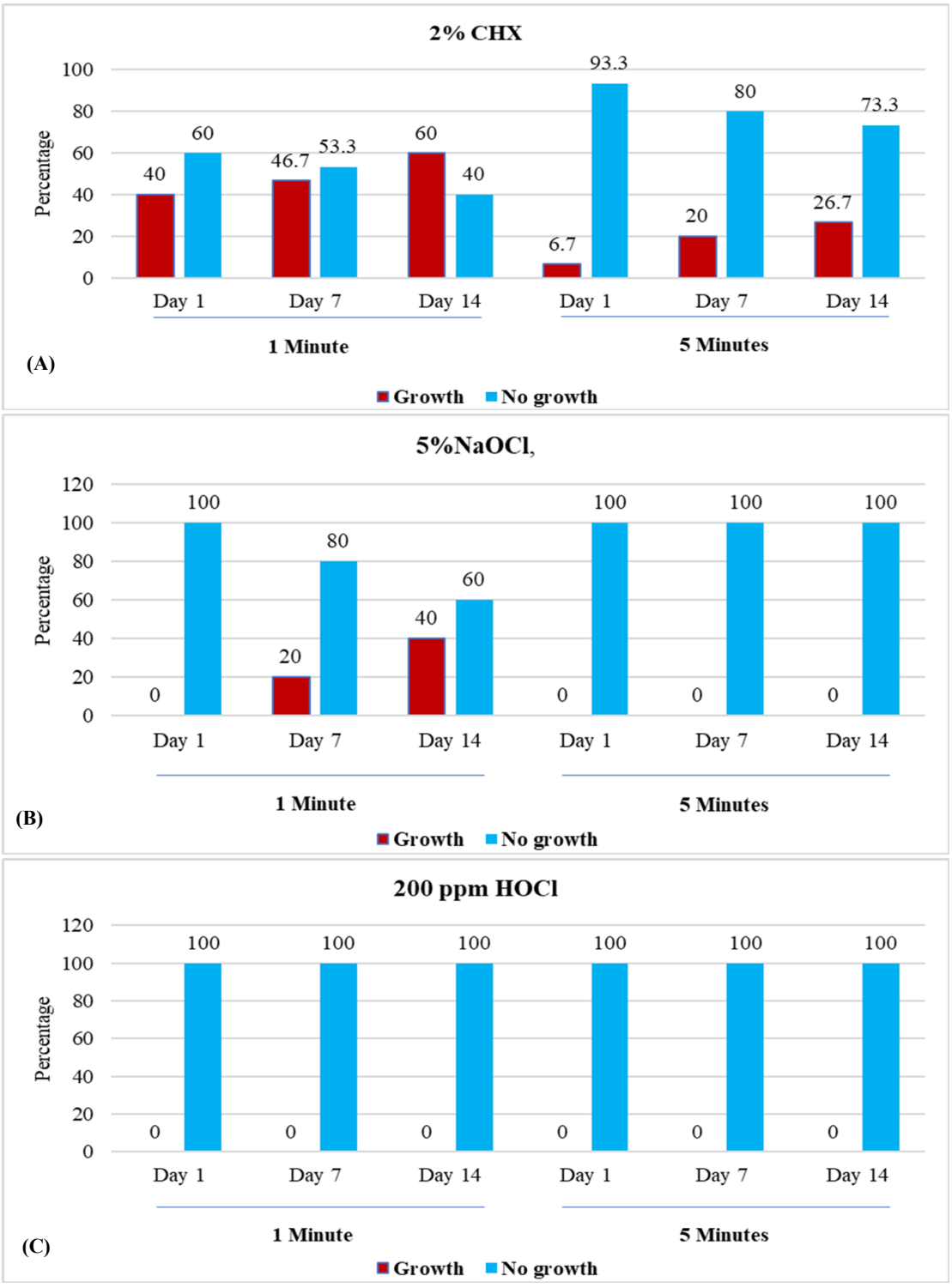

Figure 1: Comparative efficacy of 2% CHX (A), 5.25% NaOCl (B), and 200ppm HOCL (C) on Enterococcus faecalis growth across different incubation periods.

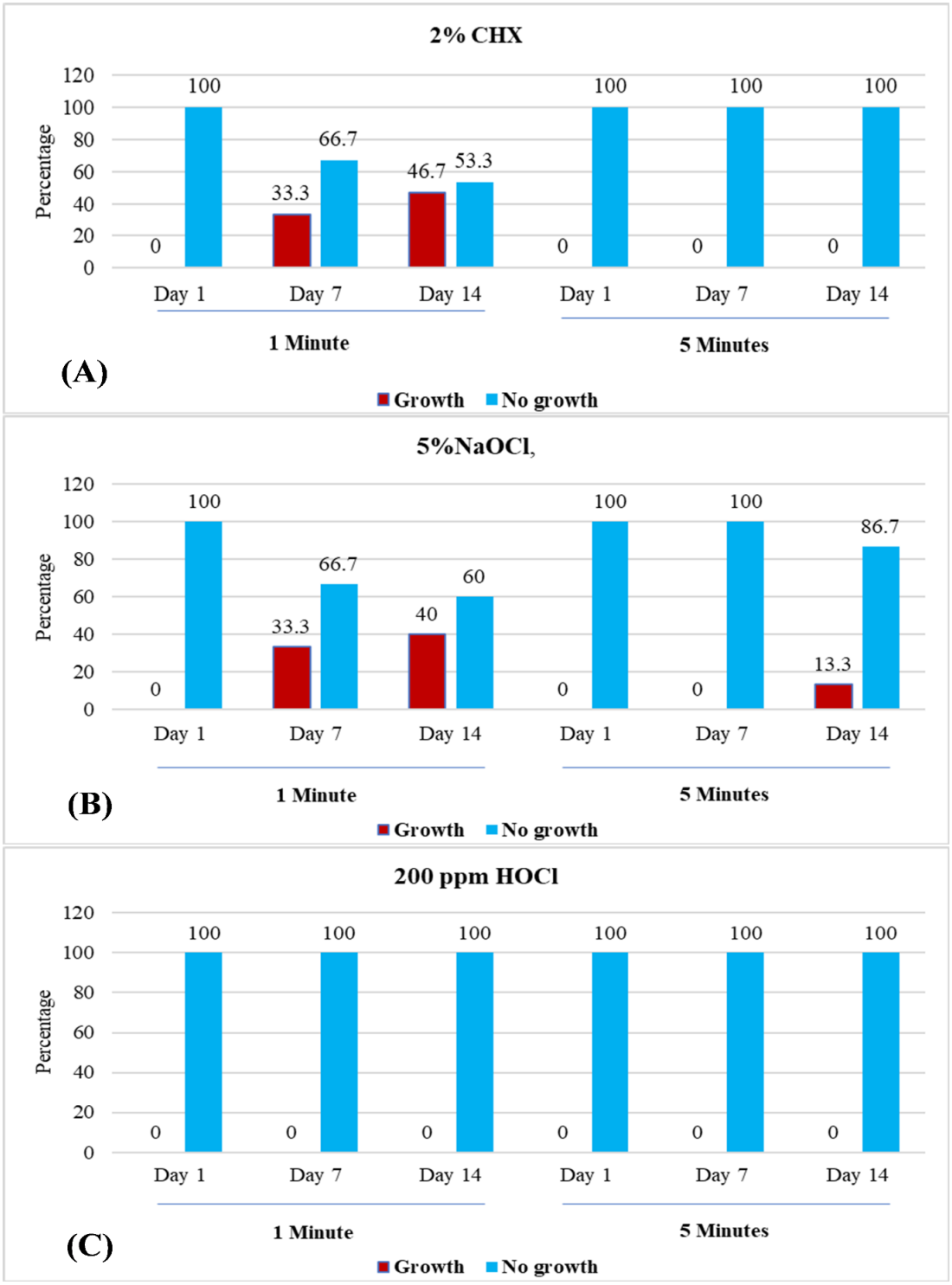

Figure 2: Comparative efficacy of 2% CHX (A), 5.25% NaOCl (B), and 200ppm HOCL (C) on *Candida albicans* growth across different incubation periods.

Discussion

This study assessed the antibacterial effectiveness of 2% CHX, 5.25% NaOCl, and 200 ppm HOCl in disinfecting GP cones contaminated with E. faecalis and C. albicans, over two exposure durations (1 and 5 minutes) and three incubation periods (1, 7, and 14 days). The results reveal substantial disparities in the short- and long-term antimicrobial actions of these agents, with 200 ppm HOCl exhibiting higher sustainability and enhanced antimicrobial activity compared to 2% CHX and 5.25% NaOCl.

Antibacterial Efficacy Against Enterococcus faecalis
A 1-minute exposure to 2% CHX resulted in persistent bacterial viability (40–60%) throughout all incubation periods, exhibiting no significant decline over time. Despite a 5-minute treatment, 2% CHX exhibited partial regrowth (20–26.7%) by days 7 and 14, demonstrating its low residual efficacy against E. faecalis. These results are consistent with a study showing that CHX is less effective against biofilm-embedded E. faecalis[4].

Strong initial bactericidal activity was shown by NaOCl (5.25%) (0% growth at 1 day after 1-minute exposure), but regrowth occurred at 7 and 14 days (20–40%), indicating that short-term exposure might not ensure long-term disinfection. Nonetheless, a 5-minute exposure to NaOCl resulted in complete and long-lasting elimination, underscoring the importance of prolonged exposure for dependable disinfection. These results are supported by studies demonstrating that NaOCl's efficacy decreases in the presence of organic debris[20,21].

HOCl (200 ppm) was outstanding, sustaining 100% inhibition at all exposure times and incubation intervals, with no regrowth. This consistency shows that HOCl is more stable and works as an antimicrobial for longer than CHX and NaOCl. This finding aligns with a study that reveals that neutral charges of HOCl molecules lead to deeper penetration into biofilms, which enhances its bactericidal effects[15].

Antifungal Efficacy Against Candida albicans
Both CHX and NaOCl exhibited complete inhibition at a one-day incubation period after a 1-minute exposure, but time-dependent regrowth was observed at 7 and 14 days, reaching 33.3–46.7% for CHX and 33.3–40% for NaOCl. This regrowth suggests that short exposures to these agents may not provide prolonged antifungal protection. These findings align with a previous study indicating that CHX loses its efficacy over time due to protein binding and neutralization by organic tissue[22]. Similarly, NaOCl's antimicrobial activity reduces as chlorine is consumed[11].

In contrast, 5-minute exposures resulted in prolonged inhibition for 2% CHX, however, 5.25% NaOCl still showed modest regrowth at 14 days (13.3%), indicating that its antimicrobial effectiveness reduced over time. This supports the theory that prolonged exposure times improve disinfection but may not totally prevent bacterial regrowth[23].

HOCl (200 ppm) demonstrated complete and persistent antifungal activity at all exposure times and incubation intervals, with no regrowth observed. These results coincide with a study that exhibits HOCl's superior performance, which may be attributed to its strong oxidative properties and rapid microbial membrane disruption[24]. This confirms HOCl's stability and long-lasting efficacy, making it a more reliable alternative for preventing C. albicans from contributing to endodontic reinfection.

The present findings suggest that, when used for long periods of time, HOCl will be more effective than either CHX or NaOCl in their broad-spectrum antimicrobial activity with non-toxicity and stability, indicating it as the most appropriate alternative to conventional endodontic irrigation or adjunct agent[25].

The present findings indicate the promising impact of hypochlorous acid in root canal disinfection; however, its long-term effectiveness necessitates validation through rigorously designed in vivo and clinical studies. Investigating the effects of higher concentrations or the synergistic application with other disinfectants may further enhance the antimicrobial efficacy of endodontic protocols.

Conclusion

In summary, 200 ppm HOCl outperformed 2% CHX and 5.25% NaOCl in both immediate and residual antibacterial and antifungal efficacy against E. faecalis and C. albicans. Its consistent, long-lasting inhibition without regrowth establishes it as a viable disinfectant in endodontic therapy, necessitating additional clinical investigation.

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