

Long-Term Efficacy of Chlorine-Based Disinfectants Vs Multi-Enzymatic Detergent with Hypochlorous Acid for Waterline Disinfection in Inactive Dental Chair Units

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Objective: This study aimed to evaluate the long-term efficacy of two disinfection methods for waterline systems in inactive dental chair units (DCUs).

Methods: On-site sampling and quantitative bacterial analysis were conducted to compare the effectiveness of chlorine-based disinfectants and a combination of multi-enzymatic detergent with hypochlorous acid in controlling microbial contamination in waterline systems of inactive DCUs.

Results: No statistically significant differences in disinfection efficacy were observed between the treatment and control groups immediately after disinfection or on the third day post-disinfection ($p > 0.05$). However, significant differences were observed on the fifth day and seventh day following disinfection ($p < 0.001$).

Conclusion: Both chlorine-based disinfectants and the combination of multi-enzymatic detergent with hypochlorous acid effectively controlled contamination in waterline systems for DCUs inactive for up to three days. For periods of inactivity exceeding five days, the combination of multi-enzymatic detergent with hypochlorous acid demonstrated superior efficacy by reducing biofilm adhesion over time.

Keywords: chlorine-containing disinfectant, dental chair unit, hypochlorous acid, inactive, multienzyme, waterline disinfection

Introduction

The dental chair unit (DCU) is a complex system integrating water, electrical, and pneumatic pipelines, all essential for supporting various dental procedures. A vital component of the DCU, the dental unit waterline (DUWL), consists of interconnected plastic hoses and metal control valves.¹ An inactive DCU refers to a unit temporarily underutilized due to specific circumstances in dental clinics or hospitals, such as limited patient appointments, rapid expansion of practices, altered clinician schedules, or seasonal fluctuations in patient volume. Unlike permanently decommissioned units, inactive DCUs remain operational but unused for a specified duration.

Data from the American Dental Association (ADA) indicate that disparities in patient volume across clinics, as of the third quarter of 2024, have affected DCU utilization. Although some facilities experience overwork, approximately 24% to 28% of dentists have reported insufficient patient volumes, resulting in unused capacity and higher DCU inactivity rates.² Similar trends are evident in China, where DCU inactivity rates surpass 50% in dental clinics within cities such as Beijing. Despite this underutilization, certification reports from Global Market Intelligence (GMI) highlight continued growth in the DCU market.³ However, the global economic downturn and ongoing supply-demand imbalances are expected to further increase DCU inactivity rates.

The increasing rates of inactivity in DCUs present significant challenges to maintaining disinfection of the waterline systems. Unlike frequently used DCUs, inactive units are more susceptible to management and hygiene issues, particularly within their waterline systems. Reduced or halted water flow promotes microbial growth and biofilm formation in pipelines, thereby elevating the risk of clinical infections.^{4,5} Existing physical interventions, such as flushing, filtration, and anti-backflow devices, provide moderate improvements in water quality but are ineffective against established biofilms. Both domestic and international guidelines recommend intermittent, regular, or continuous application of chemical disinfectants as an effective strategy to prevent biofilm formation in inactive DUWLs.⁶ Two methods including conventional chlorine-based disinfectants and a combination of multi-enzymatic detergent and hypochlorous acid disinfectant were used for disinfection. Hypochlorous acid is a weakly acidic and strongly oxidizing disinfectant. In aqueous solution, it dissociates into H^+ and OCl^- , followed by denaturing and aggregating protein. Hypochlorous acid also destroys the virus by chlorination to form chloramines and nitrogen-centered free radicals, resulting in single- and double-stranded DNA breaks and inactivation of virus. It has strong penetration of biofilm and may effectively clean the surface of implants contaminated by biofilm.^{7,8} It is widely used in water system disinfection, but its high concentration may cause corrosion of instruments and produce by-products. Multi-enzymatic detergent includes proteases, lipases, and amylases. These enzymes degrade proteins, lipids, polysaccharides and other organic components in biofilms through specific catalytic reactions, disintegrate biofilm structure and enhance the permeability of chemical disinfectants. Hypochlorous acid, a subsequent microbicide, can rapidly inactivate free microorganisms and inhibit biofilm regeneration. The combination of the two can quickly remove biofilms in the pipeline and reduce microbial contamination.⁹ However, the priority of the two disinfection methods was not determined. The selection of appropriate chemical disinfectant is therefore of critical importance. The comparative efficacy of a combination of multi-enzymatic detergent and hypochlorous acid disinfectant versus conventional chlorine-based disinfectants was evaluated for the disinfection of inactive DUWL. Additionally, the effectiveness of continuous disinfection strategies in controlling waterline contamination was assessed to provide a reference framework for selecting optimal disinfection methods for inactive DUWLs.

Materials and Methods

Materials

Experimental Units

Forty-five inactive DCUs of the same model were selected from a tertiary Class A stomatological hospital in Hubei Province. The units were randomly allocated into either the treatment or control group, with 15 DCUs in each group. Furthermore, the experimental design additionally incorporated a blank control group (15 DCUs) to ensure methodological rigor. Test specimens included independent water storage tanks, three-way spray lances, and water samples collected from the dental handpiece connection tubing.

Main Reagents

- (1) Treatment group: Multi-enzymatic detergent combined with hypochlorous acid disinfectant.
- (2) Control group: Chlorine-containing effervescent tablets.
- (3) Blank control group: No disinfectant or treatment applied.

Methods

Study Protocol

Disinfection procedures were conducted weekly.¹⁰ Full-time nurses, trained in hospital infection control protocols, performed waterline disinfection of inactive DCUs every Monday morning prior to the commencement of clinic operations. Samples

were collected before disinfection and on the third, fifth, and seventh day post-disinfection, with additional collections performed four times per week. Three sampling sites were established for each dental chair, and continuous sampling was conducted over a one-week period. The total sample size was determined by multiplying the number of dental chairs, sampling sites, and collection time points, resulting in 360 samples. These samples were equally distributed between the treatment and control groups, with 180 samples allocated to each group.

Disinfection methods

- (1) Treatment group: A disinfectant solution was prepared by combining sterile distilled water and multi-enzymatic detergent (the MEDICOM ANIOS Endoscope-Specific Multi-Enzyme Cleaner; A.R.Medicom Inc. Healthcare (Shanghai) Limited; Shanghai; China) in a ratio of 1500 mL to 22.5 mL. The enzyme activity unit was ≥ 800 U/mL with protease being 300 U/mL, lipase being 250 U/mL, and amylase being 250 U/mL. Samples were subsequently immersed in isolated water storage tanks for initial disinfection. The water volume was adjusted to maximum capacity, and the foot pedal was activated to ensure thorough filling of all waterlines. The system was left to soak for 30 minutes.¹¹ The tank was drained, and a secondary solution was prepared by mixing sterile distilled water with sodium hypochlorite disinfectant (Bolanconi No-Rinse Hypochlorous Acid Disinfectant; Shanghai SEC JIE Technology Co., LTD; Shanghai; China) in a 1000 mL to 500 mL ratio. The available chlorine concentration was 50 ppm. The same procedural steps were repeated to complete the disinfection process.
- (2) Control group: Chlorine-containing effervescent tablets (Jianzhisu; Beijing Jianzhisu Health Element Disinfection Technology Service Co. LTD; Beijing; China) were dissolved in sterile distilled water to prepare a disinfectant solution with a chlorine concentration of 500 mg/L.^{12,13} This solution was used to disinfect the isolated water storage tank through immersion. The water volume was subsequently adjusted to maximum capacity, and the foot pedal was activated to ensure all waterlines were completely filled and soaked for 30 minutes. Following the soaking period, the water storage tank and waterlines were thoroughly rinsed with sterile distilled water to eliminate any residual disinfectant.

Water Sample Collection

- (1) Collection from the isolated water storage tank: The DCU power supply was disconnected, and the isolated water bottle was removed. A sterile pipette was used to collect 10 mL of water from the tank.¹⁴
- (2) Collection from the three-way spray lance: The water outlet of the three-way spray lance, without the nozzle, was disinfected using 75% ethanol. The water was drained for 30 seconds before 10 mL was collected in a sterile container.¹⁴
- (3) Collection from the dental handpiece connection tube: The metal cap was removed from the connection port, ensuring no handpiece was attached. The post was disinfected with 75% ethanol or a sterile water sampler. The water was then drained for 30 seconds, and 10 mL was collected in a sterile container.¹⁴

Outcome Measures

Bacterial cultures were quantified using the plate counting method. Water samples or their dilutions were inoculated directly onto solid media, and bacterial colonies were enumerated after incubation. Compliance was determined based on the total bacterial colony count, with a threshold of 500 colony-forming units per milliliter (CFU/mL), as specified by the oral hygiene water standards set by the Centers for Disease Control and Prevention (CDC).¹⁵ The logarithmic transformation of the colony count was subsequently calculated to facilitate statistical analysis.

Statistical Methods

The data accuracy was independently verified by two individuals prior to analysis. Statistical analyses were conducted using SPSS version 27.0. The Shapiro–Wilk test was applied to assess the normality of measurement data, with *p*-values exceeding 0.05, indicating that the data followed a normal distribution. Subsequently, an independent samples *t*-test and one-way ANOVA analysis were employed for further data evaluation.

Results

Disinfection Efficacy of Inactive DCUs on Day 3 Post-Disinfection

No statistically significant difference in bacterial colony counts was observed between the treatment group and the control group on the third day post-disinfection ($p > 0.05$), as presented in Table 1. However, the two disinfection procedures demonstrated beneficial effects (both $p < 0.001$).

Disinfection Efficacy of Inactive DCUs on Day 5 Post-Disinfection

On the fifth day post-disinfection, the two disinfection procedures also demonstrated beneficial effects (both $p < 0.001$). In addition, colony counts in the treatment group were significantly lower than those in the control group, with a statistically significant difference observed ($p < 0.001$), as detailed in Table 1.

Disinfection Efficacy of Inactive DCUs on Day 7 Post-Disinfection

On the seventh day post-disinfection, the bacterial colony counts in the treatment group and the control group were lower than that in the blank group. In addition, the treatment group demonstrated a significantly lower bacterial colony count compared to the control group, with a statistically significant difference ($p < 0.001$), as presented in Table 1.

Differences in Bacterial Colony Counts Among the Sampling Areas

There were no significant differences among the three sampling areas (the dental handpiece connection tubing, three-way spray lances and independent water storage tanks) within each group at all time points before and after disinfection ($P > 0.05$), as presented in Tables 2–4.

Table 1 Bacterial Colony Counts (CFU/mL) Across Groups

Group	N	Day 0 (Pre-Disinfection)	Day 3	Day 5	Day 7
Treatment group	15	227.73 ± 58.294	29.07 ± 6.273	43.33 ± 7.669	76.47 ± 9.448
Control group	15	240.87 ± 70.358	37.60 ± 14.875	80.47 ± 12.112	173.80 ± 20.936
Blank control	15	228.47 ± 7.676	268.40 ± 82.455	340.20 ± 97.160	430.93 ± 93.452
P value		>0.05 ^{a,b,c}	<0.05 ^{b,c}	<0.001 ^{a,b,c}	<0.001 ^{a,b,c}
		a: TvsC $t = -0.557$ $p = 0.582$	a: TvsC $t = -2.047$ $p = 0.055$	a: TvsC $t = -10.032$ $p < 0.001$	a: TvsC $t = -16.412$ $p < 0.001$
		b: TvsB $t = -0.035$ $p = 0.973$	b: TvsB $t = -11.216$ $p < 0.001$	b: TvsB $t = -11.797$ $p < 0.001$	b: TvsB $t = -14.616$ $p < 0.001$
		c: CvsB $t = 0.528$ $p = 0.602$	c: CvsB $t = -10.649$ $p < 0.001$	c: CvsB $t = -10.274$ $p < 0.001$	c: CvsB $t = -10.399$ $p < 0.001$

Notes: Data were presented as mean ± standard deviation. a: Comparison between the Treatment group and Control group using an independent samples t-test. b: Comparison between the Treatment group and Blank control using an independent samples t-test. c: Comparison between the Control group and Blank control using an independent samples t-test. The t-value and corresponding p-value are reported, and $P < 0.05$ was considered of significant difference. All superscript symbols (a, b, c) in the table correspond to the statistical comparisons defined above.

Table 2 Bacterial Colony Counts (CFU/mL) in Treatment Group

Sampling Areas of the Dental Units	N	Day 0 (Pre-Disinfection)	Day 3	Day 5	Day 7
The dental handpiece connection tubing	15	227.27 ± 59.31	29.40 ± 6.20	41.47 ± 7.75	74.60 ± 10.15
Three-way spray lances	15	227.93 ± 57.22	29.93 ± 6.76	42.33 ± 7.13	78.53 ± 9.40
Independent water storage tanks	15	227.47 ± 59.06	27.93 ± 6.54	46.20 ± 9.14	76.27 ± 9.78
F statistic		0.001	0.381	1.469	0.610
P value		0.999	0.686	0.241	0.548

Notes: Data were presented as mean ± standard deviation. $P < 0.05$ was considered of significant difference.

Table 3 Bacterial Colony Counts (CFU/mL) in Control Group

Sampling Areas of the Dental Units	N	Day 0 (Pre-Disinfection)	Day 3	Day 5	Day 7
The dental handpiece connection tubing	15	241.13±71.15	37.93±15.55	80.20±14.26	172.53±20.96
Three-way spray lances	15	236.80±67.65	36.47±15.15	79.13±12.93	176.27±20.92
Independent water storage tanks	15	243.00±73.85	38.40±14.38	82.07±9.83	172.60±21.71
F statistic		0.030	0.068	0.212	0.152
P value		0.970	0.935	0.810	0.859

Notes: Data were presented as mean ± standard deviation. $P < 0.05$ was considered of significant difference.

Table 4 Bacterial Colony Counts (CFU/mL) in Blank Group

Sampling Areas of the Dental Units	N	Day 0 (Pre-Disinfection)	Day 3	Day 5	Day 7
The dental handpiece connection tubing	15	228.47±58.07	271.00±84.72	340.40±97.80	429.47±92.78
Three-way spray lances	15	226.47±59.06	267.80±81.50	338.53±96.15	434.47±94.65
Independent water storage tanks	15	226.40±57.35	266.40±81.75	341.67±98.04	428.87±93.33
F statistic		0.295	0.012	0.004	0.016
P value		0.746	0.988	0.996	0.984

Notes: Data were presented as mean ± standard deviation. $P < 0.05$ was considered of significant difference.

Discussion

Opportunistic pathogens, such as *Legionella* and *Pseudomonas aeruginosa*, are the primary contaminants in inactive DCUs.^{16–18} As these DCUs are not used by patients, retrograde contamination is an unlikely source; instead, contamination predominantly originates from the DUWLs.¹⁹ Without prompt elimination of microorganisms and biofilms, subsequent use of the DCUs can pose a significant infection risk, particularly for individuals with compromised immune systems.^{20,21} As indicated in Tables 1–3, bacterial counts post-disinfection in both groups consistently remained below 500 CFU/mL, confirming the substantial bactericidal efficacy of both disinfection methods. Notably, the data (as shown in Table 1) indicated no statistically significant differences between disinfection methods for DCUs inactive for up to three days, suggesting that either method is equally effective under such conditions.

As presented in Tables 2 and 3, the treatment group exhibited a statistically significant difference from the control group ($p < 0.01$). The combined use of multi-enzymatic detergent and hypochlorous acid disinfectant demonstrated superior efficacy and prolonged effectiveness compared to conventional chlorine-based disinfectants, particularly in addressing contamination in inactive DUWLs. This dual approach proved highly effective in mitigating biofilm adhesion, especially in DCUs that had remained inactive for extended periods (5 days or more).

Biofilm accumulation within inactive DUWLs represents a significant infection risk for both dental staff and patients.²² While traditional disinfection protocols can remove biofilms, their frequent application may result in waterline corrosion and a reduction in service life. Pre-treatment with multi-enzymatic detergents effectively degrades the organic components of biofilms, thereby enhancing the subsequent action of chemical disinfectants. Unlike harsher chemical agents, multi-enzyme formulations help preserve the integrity of dental equipment, reduce material corrosion, and are suitable for long-term use.

Hypochlorous acid, a potent broad-spectrum antibacterial agent, has demonstrated significant efficacy against prevalent DUWL pathogens, including *Pseudomonas*, *Legionella*, and non-*Mycobacterium tuberculosis*.²³ Its antimicrobial mechanism involves the rapid destruction of microbial cells by disrupting cell membranes and intracellular components. In DUWL systems, hypochlorous acid effectively controls microbial contamination, eliminates biofilms, and maintains water quality within the prescribed safety limits. Its relatively low corrosivity and minimal impact on equipment materials make it well-suited for long-term use, minimizing damage to dental instruments. Clinically, hypochlorous acid is frequently used in conjunction with adjunctive treatments, such as multi-enzymatic detergents, to optimize disinfection outcomes. A sequential approach—where multi-enzymatic detergents first remove organic matter and biofilm, followed by hypochlorous acid for sterilization—has proven to achieve superior microbial control within

DUWL systems. This combination also extends the contact time of the disinfectant with the waterlines, thereby improving its efficacy against opportunistic pathogens.^{9,24,25}

A variety of disinfection methods have been developed for DCU waterlines, yet few specifically target inactive DCUs. Effective infection control strategies are essential for mitigating risks associated with the disinfection for inactive equipment.^{17,26} Effective and long-lasting disinfection strategies are critical to minimize potential hazards during diagnosis and therapeutic procedures. The selection of an optimal method disinfection enables the implementation of systematic protocols specifically tailored to inactive DCUs. These protocols reduce microbial growth within waterline systems, prevent cross-contamination during oral procedures, and maintain high standards of medical service quality. Additionally, efficient disinfection practices ensure that frequency of interventions aligns with varying periods of inactivity, mitigating both the risks of excessive disinfection and bacterial proliferation due to delayed intervention. Adopting appropriate disinfection methods also improves operational efficiency by ensuring that inactive equipment remains hygienically safe for use while extending its lifespan through the prevention of waterline-related contamination and associated malfunctions. Collectively, these measures help reduce operational costs for dental clinics and hospitals while optimizing the use of inactive resources.

The combination of multi-enzyme cleaners and sodium hypochlorite disinfectants offers several advantages over traditional chlorine-based disinfectants. It provides stronger cleaning power by breaking down organic pollutants such as blood, mucus, and grease, while traditional chlorine-based disinfectants mainly rely on oxidation and have weaker cleaning abilities. Additionally, this combination is less corrosive, making it safer for precision instruments and medical devices. It also has less irritating odor and is safer to use, with reduced risk to the respiratory system, skin, and eyes. Furthermore, multi-enzyme cleaners are more environmentally friendly, as they are biodegradable and cause less pollution compared to traditional chlorine-based disinfectants.

The study has some limitations. The study only included 45 DCUs of the same type, and did not cover different brands or old equipment, which might limit the generalizations of the conclusions. The influence of external factors such as water hardness, environmental temperature and humidity on the disinfection effect has not been systematically analyzed, and such variables need to be included in subsequent studies to improve the evaluation system. The experiment used fixed concentration parameters, and the influence of different concentration gradients on disinfection efficacy was not evaluated, which might affect the applicability of the conclusion. The current data are based on the observation of a single disinfection cycle, and the stability of the results of multiple repeated experiments needs to be performed in the further study. This study did not detect and observe the biofilm in the pipeline straightly, which might lead to bias.

Conclusion

For DCUs with shorter periods of inactivity, either chlorine-based disinfectants or the combination of multi-enzymatic detergents and hypochlorous acid can effectively maintain waterline hygiene. However, for DCUs that remain inactive for more than five days, the combination of multi-enzymatic detergent and hypochlorous acid exhibits superior efficacy in preventing biofilm adhesion and ensuring long-lasting disinfection.

Abbreviations

DCU, Dental Chair Unit; DUWL, Dental unit waterline; ADA, American Dental Association; GMI, Global Market Intelligence; CDC, Centers for Disease Control and Prevention.

Data Sharing Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author Huan Sun.

Ethics Approval and Consent to Participate

This study was conducted with approval from the Ethics Committee of The School & Hospital of Stomatology, Wuhan University (Approval number is WDKQ2024B115). This study was conducted in accordance with the declaration of Helsinki. Written informed consent was not appropriate for this research and was waived by the Ethics Committee.

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Disclosure

The authors declare that they have no competing interests.

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