Journal of Microbiology, Immunology and Infection (2017) 50, 270–276

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ORIGINAL ARTICLE

Monitoring the decontamination efficacy of the novel Poseidon-S disinfectant system in dental unit water lines

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Received 9 February 2015; received in revised form 10 April 2015; accepted 5 May 2015
Available online 14 May 2015

KEYWORDS
cytotoxicity;
dental unit water lines (DUWLs);
disinfection system;
electrolyzed water;
microbial contamination

Abstract *Background:* Contaminated dental unit waterlines (DUWLs) are a known source of specific health care-acquired infections because of the difficulty in keeping them clean during routine dental practice. Recently, an electrolysis apparatus that uses only the chlorine normally present in municipal water, the Poseidon-S system, was developed as a novel additive-free disinfectant system to control microbial contamination in DUWLs.
Methods: The microbiological quality of water samples collected from DUWLs was assessed before and after installation of the Poseidon-S system in terms of the total viable counts (TVCs) of microorganisms. The microbicidal effects of the electrolyzed water against oral organisms and its cytotoxicity against human oral-derived cell lines were also examined.
Results: Water samples from the DUWLs initially had average microbial TVCs of 10^3–10^6 colony-forming units (CFU)/mL. After installation of the Poseidon-S system, the number of microorganisms in the water samples decreased to less than 1×10^2 CFU/mL. The electrolyzed water also exhibited remarkable microbicidal effects on the microorganisms present in the DUWLs as well as microorganisms commonly isolated from human oral cavities, but showed low cytotoxicity towards human oral-derived cells.
Conclusion: This study demonstrated that routine use of the Poseidon-S system can effectively maintain low microbial levels in DUWLs.
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http://dx.doi.org/10.1016/j.jmii.2015.05.006
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Introduction

Studies conducted over the last 40 years have demonstrated that the water output from dental unit waterlines (DUWLs) is often contaminated with high densities of environmental microorganisms such as aerobic heterotrophic bacteria.[1–3] The American Dental Association (ADA) established a recommendation that, by the year 2000, water used for nonsurgical procedures should contain no more than 200 colony-forming units per milliliter (CFU/mL) of aerobic, mesophilic, or heterotrophic bacteria in unfiltered output from DUWLs (American Dental Association 1996).[4] Nevertheless, DUWLs typically used in dental practices are rarely disinfected, and bacterial contamination levels $>10^2$—10^6 CFU/mL have regularly been reported.[1,5,6] In particular, the formation of biofilms presents difficulties in maintaining clean DUWLs during routine dental practice.[5] Many contaminating microbial genera have been isolated and identified in water samples collected from dental units (DUs).[1–3]

Previous studies have focused on the effectiveness of numerous disinfectants used for the cleaning and maintenance of DUWLs.[7–9] In recent years, the use of herbal disinfectants such as *Aloe vera* solutions, as an alternative to chemical disinfectants has also been examined.[10,11] However, their efficacy is not well recognized. Due to its low toxicity, electrolyzed water has also attracted attention as an effective disinfectant in various fields, including agriculture, food industries,[12,13] medicine,[14] and dentistry.[15–17] Recently, an electrolysis apparatus known as the Poseidon-S (Self Medical Co., Kyoto, Japan) was developed as a novel disinfectant system. The Poseidon-S system controls microbial contamination in DUWLs without the need for sodium chloride solutions and provides high quality water to patients. In this system, a sensor detects the flow of water and supplies an electric current to the electrolyzer to oxidize the chloride ions (Cl^-) in water to chlorine (Cl_2). The chlorine immediately reacts with water to form hypochlorous acid (HClO) and hypochlorite ions (ClO^-), which, like free chlorine, have strong microbicidal effects.[18,19] Typically, electrolyzed neutral water is produced by mixing municipal water and a solution obtained by electrolyzing sodium chloride.[20] Such water is characterized by a pH of 5.5—7.5, an oxidation-reduction potential (ORP) of 600 mV—800 mV, and a chlorine concentration of ~20 ppm.[15] However, the advantage of the Poseidon-S system is that sodium chloride is not required to produce electrolyzed water, allowing for a direct connection to DUWLs and eliminating the cost of additives.

The purpose of the present study was to investigate the microbicidal effects of the electrolyzed water produced by the Poseidon-S system (hereafter, P-water) on microorganisms from DUWLs and assess any cytotoxic effects on cell lines derived from the human oral cavity. This study was performed to verify the safety and efficacy of the Poseidon-S system applied to DUs for reducing the microbial contamination of the water output from DUWLs.

Materials and methods

Measurement of residual chlorine

Residual chlorine levels in municipal water and P-water were measured using a Chlorine Meter (Hach Chlorine Pocket Colorimeter II 58700-00; Hach Company, Loveland, CO, USA). pH and ORP were determined using a pH meter (F-51; Horiba Co. Ltd., Kyoto, Japan), with a pH electrode (9680-10D; Horiba Co. Ltd.) or an ORP electrode (9300-10D; Horiba Co. Ltd.), respectively.

Collection of water samples from DUWLs before installation of the Poseidon-S system

Two DUs (DU-A and DU-B; Yoshida Dental Mfg. Co., Ltd., Tokyo, Japan) were selected from the available units at the Dental Hospital of the Health Sciences University of Hokkaido in Hokkaido, Japan. The DUs were linked to the municipal water system and had been used for daily dental work for 20 years. Although the DUs were regularly flushed, they were only cleaned for the first time during the present study. However, this is not surprising since typically DUWLs are rarely disinfected, and bacterial contamination levels greater than 10^2—10^6 CFU/mL have been regularly reported.[1,5,6] The total viable counts (TVCs) in the DUWLs in the current study were evaluated before installation of the Poseidon-S system, and it was found that the measured TVCs were within this range. These DUs were not used for dental work during the water sample collection period. Water samples (5 mL) were collected from the DUs at three sampling locations within the system: the air/water syringe (dentist's side), the high-speed dental handpiece, and the cup filler.

During the first 3-week period, both DUWLs were flushed four times a day (at 09:00, 12:00, 15:00, and 18:00) for 3 minutes each, every day from Monday to Friday. The water samples were collected after flushing at 09:00 on Tuesdays and Thursdays for the weekday water samples (WD-water; six samples per DU, 12 total). After weekends, during which no flushing occurred, weekend water samples were collected after flushing at 09:00 on Monday (WE-water; three samples per DU, six total). During the second 3-week period, the DUWLs of both DUs were flushed only on Monday at 09:00, 12:00, 15:00, and 18:00. Then, they were left undisturbed for 7 days, after which they were flushed on the following Monday at 09:00, and "after-vacation" water samples were collected (AV-water; three samples per DU, six in total).

Evaluation of the TVCs of microorganisms in water samples collected before installation of the Poseidon-S system

The microorganisms in 1 mL water samples collected from the DUs before installation of the Poseidon-S system were harvested, and serial 10-fold dilutions of the samples were prepared. Then, 0.1 mL aliquots of each sample were inoculated onto R2A agar plates.[21] The microorganisms were then cultured aerobically at 25°C for 5—7 days to

determine the TVCs, according to the guidelines established for culturing heterotrophic bacteria in the Standard Methods for the Examination of Water and Wastewater.

Cleaning of the DUWLs and installation of the Poseidon-S system

Before installation of the Poseidon-S system, the DUWLs of DU-A and DU-B were filled with a 1.25% (w/v) sodium hydroxide (alkaline) solution for 1 hour to clean the lines. The DUWLs were then thoroughly rinsed by running municipal water through them until neutralized. After cleaning, DU-A was reconnected to the municipal water system via a Poseidon-S system, whereas DU-B was reconnected to the municipal water system without a Poseidon-S.

Collection of water samples from DUWLs after installation of the Poseidon-S system

Water samples were collected from the two DUs using the same protocol as described above, except they were collected over a period of 12 weeks (with WE and WD samples taken in the first 6 weeks, and AV samples in the second 6 weeks). The TVCs of the microorganisms in each water sample (12 WD-water samples per DU, 24 samples total; six WE-water samples per DU, 12 samples total; and six AV-water samples per DU, 12 samples total) were determined using the same method used to assess the water samples collected before installation of the Poseidon-S system.

Growth conditions for typical oral microbial species

Seven Gram-positive bacteria, four Gram-negative bacteria, and one fungus (Table 1) were used as typical oral microorganisms to investigate the microbicidal activity of P-water. The oral bacterial species were maintained at 37°C in an anaerobic chamber with an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 on brain—heart infusion broth (BHI broth; BD Diagnostic Systems, Sparks, MD) plates containing 1.5% (w/v) agar and supplemented with 5 μg/mL hemin, 4 μg/mL menadione, and 5% (v/v) defibrinated sheep blood (BHI-HM blood agar plate). *Candida albicans* was maintained on Sabouraud agar plates at 37°C under aerobic conditions.

Assay for microbicidal activity against oral microorganisms

Oral microbial cells were collected from agar plates using sterilized cotton swabs, dispersed into sterilized phosphate-buffered saline (PBS), and washed three times by centrifugation. The cells were then suspended in PBS at a concentration of 10^{7-8} CFU/mL ($OD_{660} = 1$). One milliliter of the microbial cell suspension was centrifuged and incubated with 1 mL of freshly prepared P-water at room temperature. After 5 minutes, the treated microorganisms were washed with PBS three times by centrifugation and then resuspended in PBS. After preparing serial 10-fold dilutions, 0.1 mL of the suspension was inoculated onto agar plates and incubated for 5—7 days to estimate the TVCs of each microorganism. PBS was used as a negative control.

Measurement of P-water cytotoxicity

To confirm the safety of using P-water in oral care, cytotoxicity studies were performed using established human gingival fibroblasts (HGF) and a human oral epidermoid carcinoma cell line (KB). Cytotoxicity was determined by using a water-soluble tetrazolium salt (WST-1) (Roche

Table 1 List of microorganisms used for the microbicidal assay.

Type of microorganism	Species	Source/strain
Gram-positive bacteria	*Streptococcus mutans* (Sm)	Ingbritt
	Streptococcus sanguinis (Ss)	ATCC 10556
	Enterococcus faecalis (Ef)	ATCC 19433
	Actinomyces naeslundii (An)	ATCC 12104
	Mogibacterium timidum (Mt)	ATCC 33093
	Lactobacillus casei (Lc)	ATCC 4646
	Propionibacterium acnes (Pa)	ATCC 6919
Gram-negative Bacteria	*Veillonella parvula* (Vp)	ATCC 10790
	Porphyromonas gingivalis (Pg)	ATCC 33277
	Prevotella intermedia (Pi)	ATCC 25611
	Fusobacterium nucleatum (Fn)	JCM 6328
Fungus	*Candida albicans* (Ca)	2S2 (Clinical isolate)

ATCC = American Type Culture Collection (Manassas, VA, USA); JCM = Japan Collection of Microorganisms (Saitama, Japan).

Diagnostics, Mannheim, Germany) cell proliferation assay, which is based on the conversion of WST-1 to formazan by mitochondrial dehydrogenases. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured in 96-well plates and incubated with P-water, municipal water (pH 7.2 ± 0.1, residual chlorine concentration of 0.1 ppm), strong acid electrolyzed water[22] (SAEW; pH 2.8, residual chlorine concentration of 10 ppm; Aoi Engineering Inc., Shizuoka, Japan), or 0.05% chlorhexidine gluconate (CHX) for 5 minutes. After the cells were washed three times with DMEM and incubated for an additional 0.5—2 hours, the resulting formazan product was quantified by measuring the absorbance at 450 nm using a scanning multiwell spectrophotometer (TECAN Infinite 200; Tecan Deutschland GmbH, Crailsheim, Germany). Values are expressed as mean ± SD of three independent experiments.

Statistical analysis

Data were analyzed by using Student *t* test, one-way ANOVA followed by Tukey's *post hoc* test using IBM SPSS Statistics 22 (IBM Japan Inc., Tokyo, Japan). In all analyses, differences with $p < 0.05$ were considered statistically significant.

Results

Characteristics of P-water

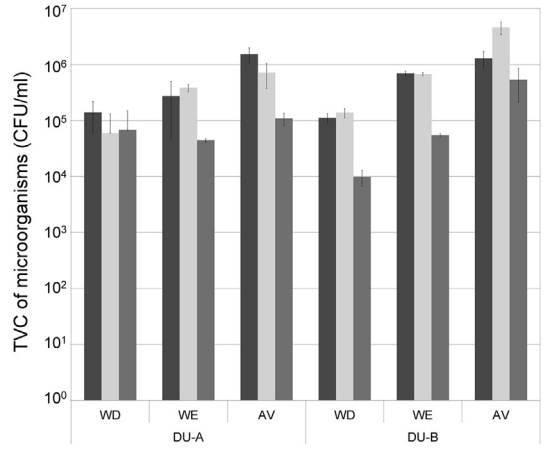
The residual chlorine concentration of municipal water was 0.1—0.2 ppm, and the mean residual chlorine concentration in freshly obtained P-water was 21 ± 1 ppm. The mean pH and ORP (mV) values of the P-water were 7.2 ± 0.1 and 793.7 ± 9.3 mV, respectively. No changes were observed in the residual chlorine concentration, pH, or ORP values of the P-water following storage in stoppered polypropylene tubes at room temperature for 18 hours (data not shown).

Microbial contamination of DUWLs

The TVCs of microorganisms detected in the water samples collected from DU-A and DU-B prior to installation of the Poseidon-S system ranged from 9.8×10^3 to 4.6×10^6 CFU/mL, and no significant differences in TVCs were found between the two DUs, although they varied slightly according to sampling location (Figure 1). After 18 hours of exposure to P-water, the microorganisms from these samples failed to form colonies on R2A agar plates (data not shown).

Effect of the Poseidon-S disinfectant system on the TVCs of microorganisms

The TVCs of the microorganisms in water samples from DU-A and DU-B after installation of the Poseidon-S in DU-A are shown in Figure 2. The TVCs of WD-water from the handpiece and cup filler line of DU-A were reduced to <10 CFU/mL. The TVCs in the WD-water from the syringe, all WE-waters, and all AV-waters from DU-A, were 10—100 CFU/mL. In contrast, the microbicidal effect of cleaning DU-B with an alkaline solution was short lived (Figure 2). This shows that the Poseidon-S system achieved a significantly better long-term (i.e., at least 6 weeks) antimicrobial effect than the control (i.e., no system) after cleaning with an alkaline solution (syringe, $n = 24$, $p < 0.01$; handpiece, $n = 24$, $p < 0.01$; cup filler, $n = 24$, $p < 0.01$; total, $n = 72$, $p < 0.01$). The high TVCs in the DU-B samples suggest that cleaning with an alkaline solution is not sufficient to control microbial growth in DUWLs.



	DU-A WD	DU-A WE	DU-A AV	DU-B WD	DU-B WE	DU-B AV
syringe	1.4×10^5	2.7×10^5	1.5×10^6	1.1×10^5	7.0×10^5	1.3×10^6
dental handpiece	5.9×10^4	3.8×10^5	7.1×10^5	1.4×10^5	6.7×10^5	4.6×10^6
cup filler	6.8×10^4	4.4×10^4	1.1×10^5	9.8×10^3	5.4×10^4	5.4×10^5

Figure 1. Microbial quality of output water from test dental units (DU-A and DU-B). Mean total viable counts (TVCs) of microorganisms (CFU/mL) in water samples collected from each sampling location at various sampling intervals.

The microbicidal activity of P-water against oral microorganisms

P-water was found to reduce the viability of typical oral microbial cells from 10^8 to 10^6 CFU/mL, and it exhibited similar microbicidal activity against Gram-positive and Gram-negative species (Figure 3). This reduction represents a microbicidal rate of >98.1%. No growth of *C. albicans* was observed after treatment with P-water.

Cytotoxicity of P-water

The effect of P-water on the survival of human oral-derived cells was assessed. P-water was most toxic for HGF cells and least toxic for KB cells. However, a 5-minute treatment with P-water exhibited lower cytotoxicity than a 5-minute treatment with 0.05% CHX (Figure 4). There was no significant difference between the cytotoxicity of P-water and municipal water.

Discussion

The pH and ORP values of the water produced by the Poseidon-S indicate that, despite the lack of additives, this

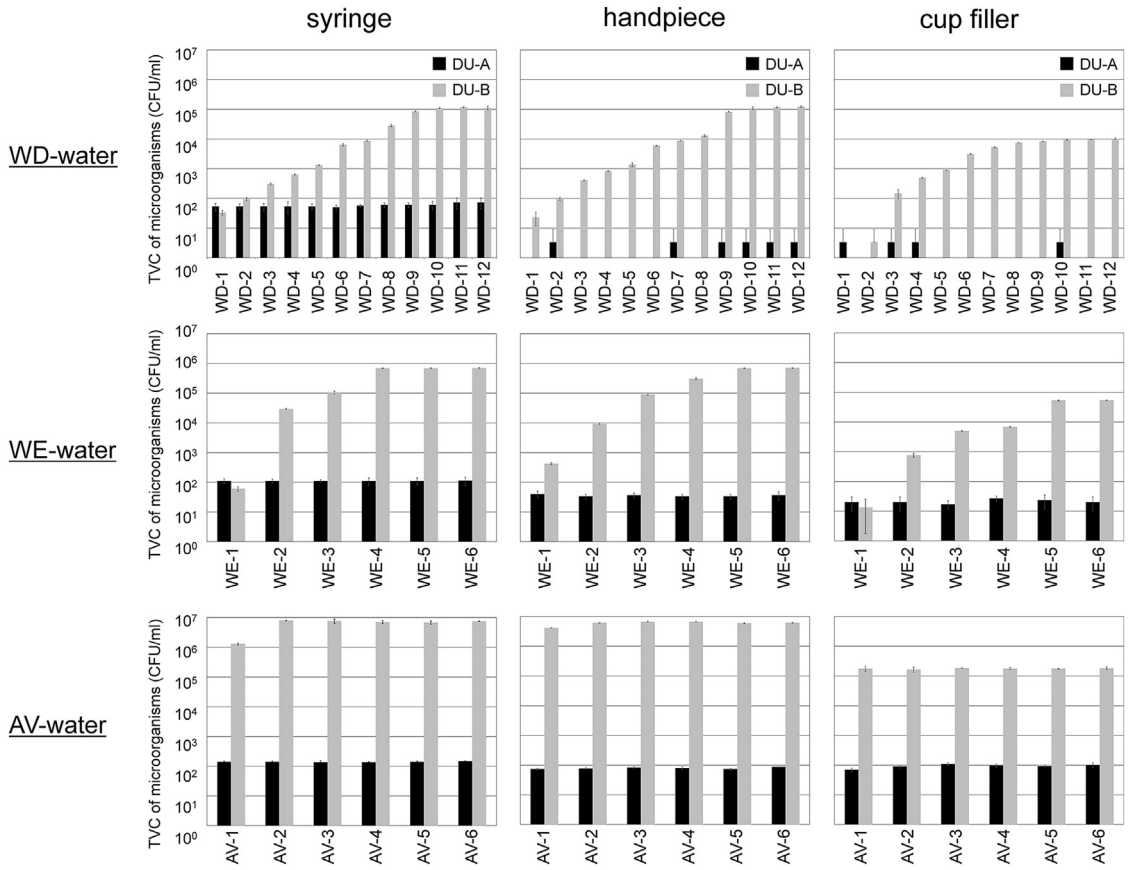


Figure 2. Total viable counts (TVCs) of microorganisms (mean ± SD, CFU/mL) in weekday (WD), weekend (WE), and "after-vacation" (AV) water samples from dental unit waterlines (DUWLs) after installation of the Poseidon-S system.

water can be classified as electrolyzed neutral water, which is known to exhibit excellent stability and bactericidal effects.[18,23] The mean residual chlorine concentration in the P-water was 21 ± 1 ppm, which was higher than that in the municipal water samples (0.1—0.2 ppm). Before installation of the Poseidon-S system, the TVCs of the microorganisms in the DUs ranged from 10^3 to 10^6 CFU/mL (Figure 1). Even after cleaning, the TVCs returned to precleaning levels within 3 months, suggesting that regularly sanitized DUs may be just as contaminated as those that are never sanitized. In many studies, the levels of microbial contamination in DUWLs have been shown to exceed the maximum recommended levels in the ADA guidelines (200 CFU/mL). Although the microorganisms isolated from the water

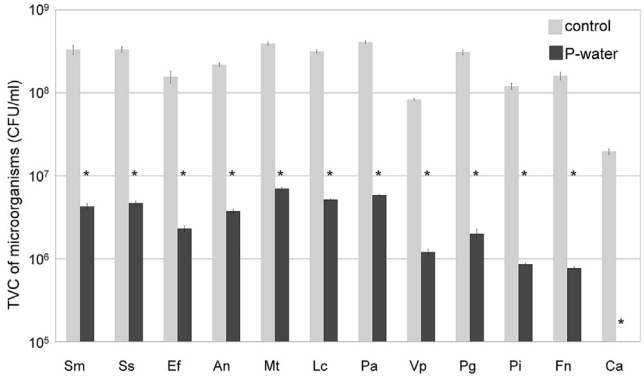


Figure 3. Microbicidal effect of P-water on oral microorganisms *in vitro*. TVCs of oral microorganisms (mean ± SD, CFU/mL) after treatment with P-water for 5 minutes, compared with control values. *$p < 0.05$.

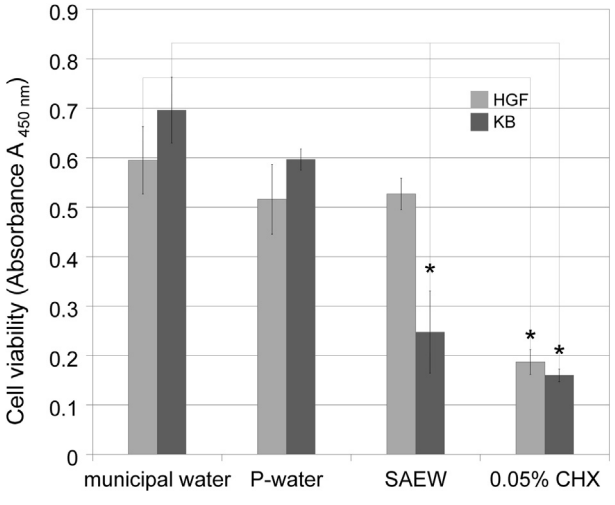


Figure 4. Influence of P-water on the survival of human oral-derived human gingival fibroblasts and human oral epidermoid carcinoma cells (KB cells). Cells were incubated with municipal water, P-water, strong acid electrolyzed water (SAEW), or 0.05% chlorhexidine gluconate (CHX) for 5 minutes.

samples in the present study were not identified, their growth on R2A agar under aerobic conditions suggests that they may be aerobic, heterotrophic microorganisms.[1–3]

Lower levels of contamination were detected in the cup filler water samples than in those from the syringes and dental handpieces, and no significant differences were observed between the latter two, which was in agreement with previous reports.[24] Although the exact reason for this difference is not known, it may be attributed to the fact that the cup filler is made of materials that are less hospitable to microbes and that water runs through its lines more frequently. In addition, the current study results indicate that prolonged periods without using or cleaning the DUs result in higher levels of contamination.

After the DUs were cleaned and the Poseidon-S system was installed, the TVCs of the water samples from DU-A decreased to <100 CFU/mL. These contamination levels easily met the 200 CFU/mL criterion specified in the ADA guidelines. Furthermore, although the TVCs of the WD-water and WE-water collected from DU-B were very low soon after cleaning with an alkaline solution, they quickly increased over time, reaching precleaning levels within 1 week. The current study findings indicate that the Poseidon-S system effectively reduced the number of microorganisms in DUWLs by at least 99.8–99.9%; this is as effective as the acidic electrolyzed water used by Kohno et al.[17]

P-water was shown to significantly reduce the viability of 11 species of oral bacteria, including both Gram-positive and Gram-negative bacteria (Figure 3). These findings corroborate previous reports of electrolyzed municipal water reducing the viability of periodontopathogens such as *Actinobacillus actinomycetemcomitans*[25] and bactericidal action against organisms isolated from infected root canals.[15] In the present study, *C. albicans* was completely undetectable after treatment with P-water for 5 minutes, suggesting that P-water is efficacious against not only oral bacteria, but also against pathogenic fungi. These findings echo a previous study in which treatment with electrolyzed neutral water for 1 minute substantially reduced the TVCs of *C. albicans*.

The cytotoxicity of SAEW against pulp cells has been reported as milder than a sodium hypochlorite solution.[22] Although the current study did not include the latter as a comparison, P-water was found to be substantially less cytotoxic to oral-derived cells than 0.05% CHX (Figure 4). CHX, a cation-active compound that remains on the skin, may have more lasting effects than P-water, which functions through negatively charged hydroxyl radicals that oxidize key metabolic systems. Further study is needed to test this hypothesis.

In addition to its substantial microbicidal properties, the advantages of P-water include the fact that it does not create environmental pollution because it regains the characteristics of "normal," unelectrolyzed water upon contact with organic materials. Therefore, P-water is useful and safe not only for patients but also for the environment. The current study showed that the DUs used had a high level of microbial contamination prior to cleaning. The TVCs of water samples from these DUWLs ranged from 10^3 to 10^6 CFU/mL; however, this contaminated water was completely disinfected by treatment with the water produced by the Poseidon-S system. Moreover, after installation of the Poseidon-S system, the TVCs in water samples from the DUWLs were significantly reduced. P-water exhibited microbicidal effects on oral microorganisms, and its microbicidal activity against *C. albicans* was especially potent. Furthermore, P-water exhibited very low cytotoxic activity against human oral-derived cells, even compared to 0.05% CHX.

Conclusion

All of the results obtained in this study indicate that the Poseidon-S system is an effective, additive-free disinfection system that reduces the microbial contamination of DUWLs and provides high quality water that is clean and safe for both patients and the environment.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Professor Takanori Shibata (Department of Reconstructive Surgery for Oral and Maxillofacial Region, School of Dentistry, Health Sciences University of Hokkaido), Professor Takeo Maida (Institute of Medical Science, Health Sciences University of Hokkaido), and Dr. Satsuki Kato (Department of Periodontology and Endodontology, School of Dentistry, Health Sciences University of Hokkaido) for their cooperation. Financial support from the Self Medical Corporation is also gratefully acknowledged.

References

1. Williams JF, Johnston AM, Johnson B, Huntington MK, Mackenzie CD. Microbial contamination of dental unit waterlines: prevalence, intensity and microbiological characteristics. *J Am Dent Assoc* 1993;**124**:59–65.
2. Barbeau J, Gauthier C, Payment P. Biofilms, infectious agents, and dental unit waterlines: a review. *Can J Microbiol* 1998;**44**:1019–28.
3. Singh R, Stine OC, Smith DL, Spitznagel Jr JK, Labib ME, Williams HN. Microbial diversity of biofilms in dental unit water systems. *Appl Environ Microbiol* 2003;**69**:3412–20.
4. American Dental Association. ADA Statement on Dental unit waterlines. *J Am Dent Assoc* 1996;**127**:185–6.
5. Walker JT, Bradshaw DJ, Bennett AM, Fulford MR, Martin MV, Marsh PD. Microbial biofilm formation and contamination of dental-unit water systems in general dental practice. *Appl Environ Microbiol* 2000;**66**:3363–7.
6. Walker JT, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, ØStergaard E, et al. Microbiological evaluation of dental unit water systems in general dental practice in Europe. *Eur J Oral Sci* 2004;**112**:412–8.
7. Walker JT, Bradshaw DJ, Fulford MR, Marsh PD. Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. *Appl Environ Microbiol* 2003;**69**:3327–32.

8. Schel AJ, Marsh PD, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, et al. Comparison of the efficacies of disinfectants to control microbial contamination in dental unit water systems in general dental practices across the European Union. *Appl Environ Microbiol* 2006;**72**:1380—7.
9. Walker JT, Marsh PD. Microbial biofilm formation in DUWS and their control using disinfectants. *J Dent* 2007;**35**:721—30.
10. Saleem M, Nazir M, Ali MS, Hussain H, Lee YS, Riaz N, et al. Antimicrobial natural products: an update on future antibiotic drug candidates. *Nat Prod Rep* 2010;**27**:238—54.
11. Pareek S, Nagaraj A, Sharma P, Atri M, Walia S, Naidu S, et al. Disinfection of dental unit water line using aloe vera: In vitro study. *Int J Dent* 2013. ID618962.
12. Deza MA, Araujo M, Garrido MJ. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Lett Appl Microbiol* 2003;**37**:482—7.
13. Abadias M, Usall J, Oliveira M, Alegre I, Viñas I. Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. *Int J Food Microbiol* 2008;**123**:151—8.
14. Tagawa M, Yamaguchi T, Yokosuka O, Matsutani S, Maeda T, Saisho H. Inactivation of a hepadnavirus by electrolyzed acid water. *J Antimicrob Chemother* 2000;**46**:363—8.
15. Horiba N, Hiratsuka K, Onoe T, Yoshida T, Suzuki K, Matsumoto T, et al. Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999;**87**:83—7.
16. Nagamatsu Y, Tajima K, Kakigawa H, Kozono Y. Application of electrolyzed acid water to sterilization of denture base part 1. Examination of sterilization effects on resin plate. *Dent Mater J* 2001;**20**:148—55.
17. Kohno S, Kawata T, Kaku M, Fuita T, Tsutsui K, Ohtani J, et al. Bactericidal effects of acidic electrolyzed water on the dental unit waterline. *Jpn J Infect Dis* 2004;**57**:52—4.
18. Deza MA, Araujo M, Garrido MJ. Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* on stainless steel and glass surfaces by neutral electrolysed water. *Lett Appl Microbiol* 2005;**40**:3416.
19. Hricova D, Stephan R, Zweifel C. Electrolyzed water and its application in the food industry. *J Food Prot* 2008;**71**:1934—47.
20. Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M, et al. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *J Microbiol Methods* 2002;**49**:285—93.
21. Reasoner DJ. Heterotrophic plate count methodology in the United States. *Int J Food Microbiol* 2004;**92**:307—15.
22. Gomi K, Makino T, Suzuki S, Hasegawa M, Maeda N, Arai T. Microbicidal and cytotoxic effects of functional water in vitro. *Quintessence Int* 2010;**41**:166—72.
23. Nagamatsu Y, Chen KK, Tajima K, Kakigawa H, Kozono Y. Durability of bactericidal activity in electrolyzed neutral water by storage. *Dent Mater J* 2002;**21**:93—104.
24. Nikaeen M, Hatamzadeh M, Sabzevari Z, Zareh O. Microbial quality of water in dental unit waterlines. *J Res Med Sci* 2009;**14**:297—300.
25. Lee SH, Choi BK. Antibacterial effect of electrolyzed water on oral bacteria. *J Microbiol* 2006;**44**:417—22.