Effect of Inhibition of Bacterial Proliferation by Neutral Electrolytic Water in Dental Unit Waterlines

Sayaka Mishima[1], Junya Sonobe[1], Katsu Takahashi[1], Miki Nagao[2], Satoshi Ichiyama[2], Kazuhisa Bessho[1]

[1]Department of Oral and Maxillofacial Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [2]Department of Clinical Laboratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Abstract

Background: Microbial contamination in dental unit waterlines (DUWLs) has recently become an important issue in the field of dental infection control. Using neutral electrolytic water as a new disinfecting method has attracted considerable attention. However, long term data about the effect of using neutral electrolytic water in clinical settings are scarce. This is the first study to evaluate the long term effectiveness of inhibition of bacterial proliferation using the purification system to supply neutral electrolytic water to refine the waterworks in DUWLs. Methods: Before the study, we investigated the actual levels of bacterial contamination in DUWLs. Then we did thorough cleaning of DUWLs and water samples were collected 6 dental units. Three dental units assigned as Group A had purification systems that used neutral electrolytic water, and the other 3 units were the Control group. Water samples were collected from the gargle water, high-speed handpieces and the three-way syringe. We utilized the equipment in Group A, and both groups were maintained for daily clinic work for 14 months. We counted the bacterial colony forming units (cfu) for each sample and identified the pathogenic bacterial species. Results: 3 and 14 months later, no microbes were detected during the study period in Group A whereas numbers of cfu which grew from the Control group increased and glucose non-fermenting gram-negative rod of possible pathogenic organisms to human were identified in the control groups. Conclusions: The water purification system using neutral electrolytic water was effective to control the proliferation of bacteria and could maintain a hygienic environment in DUWLs.

Key Words: Dental Infection Control, Dental Unit Waterlines (DUWLs), Microbial Contamination, Heterotrophic bacterium, Neutral Electrolytic Water

Introduction

With the advances in medical care and an ageing society, the prevention of nosocomial infections is a very important issue. Nosocomial infections are classified as either endogenous infection, which originate from the bacteria carried within a patient, or exogenous infections, which originate from other patients, medical staff and/or medical equipment. In hospital-based dentistry, we have to manage the oral health care of pre-operative patients, aged patients immunocompromised patients, and patients with multiple devices to reduce the risk of endogenous infections. Contamination in dental unit waterlines (DUWLs) in dentistry is regarded as one of the major sources of exogenous infections. Because the water supply is stagnant for a long time after daily clinic work, bacteria in the retention water of the DUWLs can slowly grow. Thereby, the bacteria form biofilms, and adhere to the surface. Subsequently, bacteria that desquamate from the surface of the waterlines of dental units may be released and colonized in the mouth of the patient [1,2]. Electrolyzed water has excellent bactericidal and virucidal activities [3]. Therefore, it has been used for sterilization and sanitation [4,5]. Now, neutral electrolytic water may be utilized as a new sterilization method [6]. Tap water was electlized by purification systems hypochlorous acid (HClO) and hypochlorous acid ions (ClO^-) were generated. These show antibacterial activity as free residual chlorine. It is a water solution that includes hypochlorous acid (HClO) and hypochlorous acid ion (ClO^-) and has excellent bactericidal effects at low chlorine concentrations. However, long term data about the effect of using neutral electrolytic water in the clinical practice are scarce. In this study, we evaluated the effectiveness of inhibition of bacterial proliferation using the purification system to supply neutral electrolytic water for refining the waterworks in DUWLs.

Methods

Study design

This study was conducted at Kyoto University Hospital, a 1182-bed tertiary-care university hospital with 21 departments including bone marrow transplantation and solid organ transplantation units, and is located in Kyoto, Japan. Dental units (J.MORITA MFG.Co.,Kyoto, Japan) were used for 10 years, and were directly connected to main water. Before the study, we investigated the actual levels of bacterial contamination in DUWLs. We collected 10 ml of water from high-speed handpiece, low-speed handpiece, and three-way syringe as baseline sampling and counted each sample for bacterial colony forming unit (cfu). After that, we did thorough cleaning of DUWLs and all water supply tubes of the DUWLs, which were made of soft fluorine, were replaced with new ones. We confirmed that the number of heterotrophic bacteria was <30 cfu/mL, which was the detection limit, and water samples were collected 6 dental units. Poseidon S is purified electrolytic water from the tap water. It is characterized by the PH of 6.5-7.5 and adjusting the residual chlorine concentration of 5ppm. Furthermore, this equipment does not require a membrane in the electrolysis cell, additives such as salt and chlorine. In the equipment, tap water is electrolyzed. It is to change chloride ions (Cl^-) to chlorine (Cl_2). Immediately Cl_2 reacts with water, it is change to a water solution that includes hypochlorous acid (HClO)

Corresponding author: Sayaka Mishima, Department of Oral and Maxillofacial Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan, Tel.: +81 (75) 751-3729; Fax: +81 (75) 751-4550; E-mail address: sayakams@kuhp.kyoto-u.ac.jp

and hypochlorous acid ion (ClO^-) and has excellent bactericidal effects at low chlorine concentrations. Three dental units were equipped with water purification systems using the neutral electrolytic water (Poseidon S, Self Medical Co., Ltd, Osaka, Japan) as Group A. The other three dental units were the Control group. Water samples were collected from the high-speed handpiece, the three-way syringe and the gargle water in 3 months and 14 months period each 10 ml of water. Before starting of the clinic work, all samples were collected after flushing the DUWLs for 1 minute each for draining the residual water.

Microbiological method

We counted the bacterial cfu of each sample and identified the pathogenic bacterial species in the DUWLs. The bacterial cfu was measured after incubation for 7 days at 20°C onto peptone-glucose-yeast extract (PGY) agar plates at Japan Food Research Laboratories, Tokyo, Japan. At preliminary study water samples of DUWLs did not grow *Mycobateria spp.* and *Legionella spp.*. We used Middlebook 7H11 agar (Kyokuto Pharmaceutical industrial Co. Ltd., Japan) for *Mycobateria spp.* and Buffered Charcoal Yeast Extract (BCYE) agar for *Legionella spp.* (Kyokuto Pharmaceutical industrial Co. Ltd, Japan). Then the aim of this intervention was to prevent contamination by pathogenic microbes so that we decided to detect bacteria which might cause nosocomial infections to our patients.

The identification process for the pathogenic bacterial species was as follows: each sample was centrifuged for 15 minutes at 3000 rpm, the sediment was removed and the sample was cultured for 3 days in thioglycollate broth (TGC broth; Nikken Biomedical Laboratory, Japan). The samples that were culture-positive were then cultured in Columbia 5% Sheep Blood agar (SB agar; Eiken chemical Co., Ltd, Japan) and Drigalski agar (Eiken chemical Co., Ltd, Japan) at 37°C and identified with the Vitek2 (bioMérieux, Marcy l'Etoile, France).

Results

Before cleaning of DUWLs, the number of heterotrophic bacterium discharged from high-speed handpiece was 2.3×10^5 cfu/mL, low-speed handpiece was 3.2×10^4 cfu/mL, and three-way syringe was 1.3×10^5 cfu/mL. After 3 months and 14 months, the number of heterotrophic bacteria discharged from the high-speed handpieces, three-way syringes and gargle water was <30 cfu/mL in Group A (*Figures 1,2*).

After 3 months, the number of bacteria from the three-way syringes was <30 cfu/mL in the Control group. The number of the bacteria from the high-speed handpieces was 74 cfu/mL, and the number of bacteria from two other units was <30 cfu/mL. The numbers from the samples of gargle water were 540 cfu/mL, 170 cfu/mL and 220 cfu/mL (*Figure 1*).

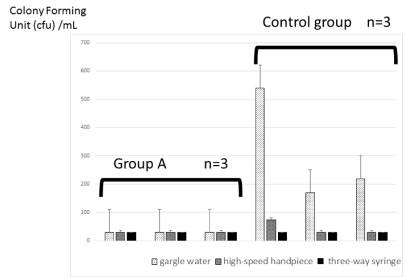

Figure 1. *Group A (n=3) had purification systems that used neutral electrolytic water, and the other 3 units were Control (n=3). The graph shows the number of hetero-trophic bacteria discharged from the gargle water, high-speed handpieces and three-way syringes after the establishment of purification with neutral electrolytic water after three months. Error bars: Stand error.*

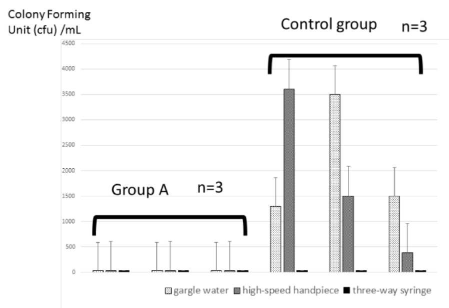

Figure 2. *The graph shows the number of heterotrophic bacteria discharged from the gargle water, high-speed handpieces and three-way syringes after the establishment of purification with neutral electrolytic water after 14 months. Error bars: Stand error.*

After 14 months, the number of bacteria from the three-way syringes was <30 cfu/mL in the Control group. The numbers of the bacteria from the high-speed handpieces was 3.6×103cfu /mL, 1.5×10^3cfu/mL, 3.8×10^2cfu/mL. The numbers from the samples of gargle water were 1.3×10^3cfu /mL, 3.5×10^3cfu /mL, 1.5×10^3cfu /mL (*Figure 2*). *Chryseobacterium indologenes* and *Sphingomonas paucimobillis* (identification rate 96～98%), which are glucose non-fermenting gram-negative rods and pathogenic to immune compromised individual were identified in the gargle water in all control groups and from the high-speed handpiece in Control group.

Discussion

The literature has reported a very large variability in the total bacterial counts obtained from DUWLs [7,8]. These counts have ranged from 1.5×10^2 to 1×10^6 cfu/mL. Sampling time, culture medium and the time and temperature of incubation, among other variables, have been responsible for some of these variations [9]. In this study, the bacterial counts were within the limits of this range before the cleaning of the DUWLs.

The Japan Ministry of Health, Labour and Welfare guidelines have recommended to maintain a reduced number of bacteria in dental unit water supply systems as from the point of view of prevention nosocomial infection. But it is difficult to remove bacteria biofilms by disinfectant once they grow [10].

The 1996 American Dental Association (ADA) statement on DUWLs challenged the dental manufacturing industry to develop methods to control the development of biofilms in dental unit water systems. In this statement, a goal was established for dental water to contain no more than 200 cfu/mL of heterotrophic bacteria in the unfiltered output [11]. In addition, the Centers for Disease Control and Prevention (CDC) suggested that the number of heterotrophic bacterial discharged from DUWLs was less than 500 cfu/mL in 2003 [12]. However, our result showed that the output was drastically higher than this index before the cleaning of the DUWLs.

In this study, the number of bacteria for all samples was <30 cfu/mL after the cleaning of DUWLs in the Control groups. After 3 months, the numbers of heterotrophic bacteria discharged from each unit significantly increased whereas the number of bacteria discharged from all three-way syringes was under the detection limit. All numbers were less than 2000 cfu/mL, which was within the range of the criteria for the Ministry of Health, Labour and Welfare in Japan, but some were exceeded the limit proposed by CDC and ADA. After 14 months, the numbers of cfus were further increased in the Control groups.

On the contrary, in Group A, both 3 and 14 months later, the numbers of heterotrophic bacteria discharged from the gargle water, high-speed handpiece and the three-way syringe were <30 cfu/mL under the detection limit. Thus, we were able to control the proliferation of bacteria in the DUWLs and maintain a hygienic environment using neutral electrolytic water.

The microbial contamination of DUWLs principally originates from two sources. First, it can result from the suck-back of saliva from the oral cavity of the patients [13]. Therefore, the most frequently isolated oral microorganisms from DUWLs belong to the genera *Lactobacillus, Streptococcus, Actinomyces, Staphylococcus, Bacteroides, Veillonella* and *Candida* [14,15]. Second, microbial contamination of the DUWLs may also be associated with water from a municipal system or a water reservoir [16]. After 3 months, the pathogenic bacterial species, which were found in the gargle water in the Control groups and high-speed handpiece in the Control group, were identified as glucose non-fermenting gram-negative rods, such as *Chryseobacterium indologenes* and *Sphingomonas paucimobillis*. Boyle et al reported a study showing a five-week period of microbiological testing of water tank supply that were heavily contaminated with bacteria, such as *Chryseobacterium indologenes* and *Sphingomonas paucimobillis* [17] which was in consistent with our report. Non-fermented gram-negative bacilli inhabit the soil and the fresh water, and these can multiply and survive for a long term in wet and poor-nutrition environments. It has been found that most of these bacteria form biofilms, which causes decreased effect of antimicrobials. These bacteria are considered to be those which we should be careful about for opportunistic infections in the hospital.

Previous studies have demonstrated that DUWLs can provide a favorable environment for microbial proliferation and biofilm formation, and that water is consequently often contaminated with high densities of various microorganisms (bacteria, fungi, protozoa, and viruses). The presence of high levels of microbial contamination may be a health problem for dentists and patients, especially immunocompromised ones. Thus, the quality of water is of considerable importance [18].

The electrolyzed water available in the market is roughly classified into 3 categories: strong acid water, weak acid water and neutral water according to their pH values. Among the various types of electrolyzed water, the strong acid water is the most acidic and it has been feared that metals might be corroded if they were treated with it. Dong et al reported the precious metal alloys showed the largest amount of dissolution of their constituent elements in acidic electrolyzed water [19]. There is a risk that strong acid water and weak acid water pollute the environment with its drainage and corrode metallic materials. On the other side, neutral electrolytic water appeared the least corrosive to metals among the 3 types electrolyzed waters showing equivalent bactericidal activity, [19] and have used for the sterilization of dentistry equipment parts and materials for late years. It is a water solution that includes hypochlorous acid (HClO) and hypochlorous acid ion (ClO^-) and has excellent bactericidal effects at low chlorine concentrations. In addition, it can maintain its bactericidal activity for more than 3 months, and it has less harmful effects on humans or the environment [20]. In this study, it is suggested usefulness of neutral electrolytic water as with previous reports.

The limitations of this study were that this study was performed at a single center. Distrubution of the microbes in water may vary among the institutions and by the frequencies of DUWLs use. Further study is needed to evaluate the efficacy of neutral Electrolysed water by multicentre study including hospital dentistry as well as dental clinics.

Conclusions

For dentistry in the tertiary care hospital, we have to manage oral health care of pre-operative patients, aged patient or immunocompromised patients. Bacterial contamination of DUWLs can cause infections including pneumonia in these compromised patients. The findings demonstrated that the water purification system using neutral electrolytic water was effective to control the proliferation of bacteria and could maintain a hygienic environment in DUWLs.

Conflict of Interest

None declared

Funding Source

In this study, equipment's for the purification systems using neutral electrolytic water were provided by Poseidon S, Self Medical Co., Ltd, Osaka, Japan.

Ethical Approval

We confirm that ethical approval was given by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee

References

1. Mills SE. The dental unit waterline controversy: defusing the myths, defining the solutions. *The Journal of the American Dental Association.* 2000; **131:** 1427–1441.

2. Mayo JA, Oertling KM, Andrieu SC. Bacterial biofilm: a source of contamination in dental air-water syringes. *Clinical Preventive Dentistry.* 1990; **12:** 13–20.

3. Shimizu Y, Sugawara H. Virucidal and bactericidal effects of electrolyzed oxidizing water: Comparison of disinfectant effect with electrolyzed oxidizing water and hypochlorous acid. *Japanese Journal of Oral Biology.* 1996; **38:** 564-571.

4. Ito K, Nishida T, Murai S. Inhibitory effects of acid water prepared by an electrolysis apparatus on early plaque formation on specimens of dentine. *Journal of Clinical Periodontology.*1996; **23:** 471-476.

5. Meakin NS, Bowman C, Lewis M, Dancer SJ. Cleaning efficacy between in-use disinfectant and electrolysed water in an English residential care home. *Journal of Hospital Infection.* 2012; **80:** 122–127.

6. Stewart M, Bogusz A, Hunter J, Devanny I, Yip B, et al. Evaluating use of neutral Electrolysed water for cleaning near-patient surfaces. *Infection Control and Hospital Epidemiology.* 014; **35:** 1505-1510.

7. O'Donnell MJ, Boyle M, Swan J, Russell RJ, Coleman DC. A centralized, automated dental hospital water quality and biofilm management system using neutral Ecasol maintains dental unit waterline output at better than potable quality: a 2-year longitudinal study. *Journal of Dentistry.* 2009; **37:** 748-762.

8. Walker JT, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, et al. Microbiological evaluation of dental unit water systems in general dental practice in Europe. *European Journal of Oral Sciences.* 2004; **112:** 412e8.

9. Barbeau J, Tanguay R, Faucher E et al. Multiparametric Analysis of Waterline Contamination in Dental Units. *Applied and Environmental Microbiology.* 1996; **62:** 3954–3959.

10. http://www.mhlw.go.jp/seisakunitsuite/bunya/kenkou_iryou/iryou/shika_hoken_jouhou/dl/02-01.pdf

11. ADA Council on Scientific Affairs. Dental unit waterlines: Approaching the year 2000. *The Journal of the American Dental Association.*1999; **130:** 1653–1664.

12. Kohn WG, Collins AS, Cleveland JL, Harte JA, Eklund KJ, et al, Centers for Disease Control and Prevention (CDC). Guidelines for Infection Control in Dental Health-Care Settings 2003. *MMWR Recommendations and Reports.* 2003; **52:** 1–61.

13. Coleman DC, O'Donnell MJ, Shore AC, Russell RJ. Biofilm problems in dental unit water systems and its practical control. *Journal of Applied Microbiology.* 2009; **106:** 1424–1437.

14. Martin MV. The significance of the bacterial contamination of dental unit water systems. *British Dental Journal.* 1987; **163:** 152–154.

15. Pankhurst CL, Johnson NW, Woods RG. Microbial contamination of dental unit waterlines: the scientific argument. *International Dental Journal.* 1998; **48:** 359–368.

16. Szymańska J, Sitkowska J, Dutkiewicz J. Microbial contamination of dental unit waterlines. *Annals of Agricultural and Environmental Medicine.* 2008; **15:** 173–179.

17. Boyle MA, O'Donnell MJ, Miller A, Russell RJ, Coleman DC. Control of bacterial contamination of washbasin taps and output water using Ecasol: a one-year study. *Journal of Hospital Infection.* 2012; **80:** 288–292.

18. Barbot V, Robert A, Rodier MH, Imbert C. Update on infectious risks associated with dental unit waterlines. *FEMS Medical Microbiology and Immunology.*2012; **65:** 196–204.

19. Dong H, Nagamatsu Y, Chen KK, Tajima K, Kakigawa H, et al. Corrosion Behavior of Dental Alloys in Various Types of Electrolyzed Water. *Dental Materials Journal.* 2003; **22:** 482–493.

20. Nagamatsu Y, Chen KK, Tajima K, Kakigawa H, Kozono Y. Durability of Bacterial Activity in Electrolzyed Neutral Water by Strage. *Dental Materials Journal.* 2002; **21:** 93–104.