

Antimicrobial Effect and Environmental Impact of Controlled Release Iodine Water Disinfectant

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Abstract—The dental chair unit is used by many patients during dental treatment every day, so dental unit waterlines (DCWs) should be disinfected. DCWs often use disinfecting cartridges containing iodine. The aim of this study was to measure iodine concentration and to evaluate its antimicrobial effect. Distilled water flowed through the iodine release cartridge, and iodine concentration of the water pumped through the cartridge was collected and measured every day for 5 days. The antibacterial effect was evaluated by Quantitative PCR and PMA dye PCR method. The iodine concentration was 1.1 ppm at the collection time and started to decrease after two days. After 5 days the concentration was reduced to 0.4 ppm. The iodine water showed an antibacterial effect 12 h after application. The iodine-release DCW disinfectant product killed 99.99 % of the bacteria within 48 h, and the concentration of iodine was lower than that mentioned the drinking water guideline recommended by the WHO.

Index Terms—antibacterial effect, dental chair water line, iodine, quantitative PCR

I. INTRODUCTION

Dental Chair Units (DCUs) are used during the treatment of many patients throughout each day, so they are a significant potential source of cross-infection [1]. DCU equipment that is in direct contact with the patient's oral cavity, such as dental unit handpieces, three-in-one air/water syringes, and suction hoses, are of particular concern. This equipment and the patient rinse cup filler of DCUs are supplied with water via narrow tubes called Dental Unit Waterlines (DUWs) [2], [3]. The droplets and aerosols generated by DCUs may be inhaled by patients and dental healthcare personnel [4]–[7]. A case report in 2012 [8], described an instance where an older woman in Italy acquired *Legionella* infection. Therefore, disinfection of water in the DCU is vital to prevent negative impacts on human health.

Chlorine and iodine are classified as halogen chemicals. All the halogens are oxidants as they have seven electrons in their outer shell. The suitability of halogens as disinfectants is based on both their oxidizing power and substitution reactions. Elemental iodine is less soluble in water than chlorine or bromine. Iodine was widely used in

disinfectants such as 10 % povidone-iodine solution, tincture of iodine, Lugol's iodine, and iodophor. It was also used as a water disinfectant and iodine-based water disinfection has an extensive history. More recently, there are several DCW treatment systems that use iodine [9].

Iodine supplementation must be carefully monitored to ensure adequate iodine intake while avoiding iodine excess [10], [11]. Instances of excessive iodine uptake are caused by numerous factors, including high levels of salt iodization and high iodine levels in water. Occurrence of the negative effects associated with excessive iodine, such as hypothyroidism, hyperthyroidism, and Autoimmune Thyroid Disease (ATD) have become more frequent [10]–[12]. Therefore, we have to consider the effect of iodine on humans.

There are two common procedures used to evaluate the antibacterial effect of a substance, namely the culture method and Quantitative-Polymerase Chain Reaction (qPCR). The culture method is the most common procedure, but the use of culture methods to assay bacteria from an environmental sample is problematic because many of these organisms are difficult to culture. On the other hand, qPCR can rapidly detect bacteria with high sensitivity, but it also detects the DNA of dead bacteria. Propidium Monoazide (PMA) dye permeates through the disrupted cell wall of dead bacteria, binds to DNA, and inhibits PCR, thus identifying only living bacteria [13]. Therefore, if PMA is applied to bacteria before amplification using qPCR, only living bacteria can be detected, and the ratio of living/dead bacteria can be determined.

The aim of study was to evaluate the iodine concentration and antibacterial effect of the iodine cartridge water in DCUs.

II. MATERIALS AND METHODS

A. Iodine Concentration in Water

A DentaPure™ DP365M Municipal Water Cartridge was used in this study. Distilled water was pumped through the cartridge (DPW) and after every 2 L, 200 mL of the treated water was collected in a plastic bottle (N = 3) and iodine concentration was determined using an Iodine Colorimeter – Checker® HC (HANNA, CA, U.S.A.)

B. Change in Iodine Concentration

Changes in iodine concentration were determined by placing the plastic bottles containing 200 mL of treated water at 15-20 °C and measuring the iodine concentration every 24 h (N = 3).

C. Heterotrophic Bacteria Sampling

Water samples (100 mL) were taken from the three-in-one air/water syringes (SW) of the DCU in the dental clinic at the Health Science University of Hokkaido. The DCU was more than 10 years old and the three-in-one air/water syringes had not been used for 6 months.

D. Antibacterial Test

Five hundred microliters of SW was collected in 1.5 mL centrifuge tubes and it was centrifuged (22000 RCF). After that, supernatant was discarded and 1 mL DPW was added into the tube (N = 3). After 12, 24, and 48 hours, 500 μ L of sample water was taken and centrifuged in tubes (22000 RCF). Another 500 μ L of sample water were treated with propidium monoazide (PMA) (Biotium, Hayward, CA, USA). PMA was dissolved in 20 % dimethyl sulfoxide (DMSO) to produce a 24 mM stock solution. Following incubation with the dye for 5 min under the dark conditions, the cells were exposed for 15 min to Glo-Plate™ Blue LED Illuminator (Biotium, Hayward, CA, USA). After this treatment, total genomic DNA was extracted from each aliquot using GenCheck DNA Extraction Reagent (Fosmac, Kanagawa, Japan) as described by the manufacturer. PCR was performed with a LightCycler Nano (Roche Biochemicals, Mannheim, Germany) using universal primers for 16S rDNA 5'TCCTACGGGAGGAGCAGCAG-3' and 5' - GGACTACCAGGGTATCTA-3'. The total reaction volume was 20 μ L containing 10 μ L of DNA master SYBR Green (DNA Master SYBR Green I Kit; Roche Diagnostics), 0.2 μ L (50 μ M) of forward and reverse primer, 4.6 μ L of distilled water, and 5 μ L of extracted DNA. Each PCR included sterile distilled water as a negative control. The amplification conditions were: an initial denaturation step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 20 s, annealing at 47 °C for 20 s, and extension at 72 °C for 50 s, with fluorescence acquisition in single mode. The number of DNA copies obtained after incubation of bacteria with iodine water was determined using standard curves for *Streptococcus mutans* OD1 concentration. Antibacterial activity was defined as the absence of growth with antibiotic as compared with growth in the control. Conversely, resistance to an antibiotic was defined as an increase in the number of DNA copies during the time of incubation.

E. Bacterial Cultures and Growth Conditions

To confirm the relationship between real-time PCR results and bacterial growth, the control group and 48 treated samples were grown anaerobically at 25 °C in R2A medium.

III. RESULTS

The iodine concentration in water at the first pick up was 2.3 ± 0.1 ppm and that at every 2 L until 10 L was

1.0 ± 0.1 ppm. The iodine concentration measured daily remained stable for the 48 h, then decreased to 0.5 ppm (Fig. 1). According to the 1.0 ppm iodine concentration of fresh DPW, 1.0 ppm iodine DPW was used for the antibacterial effect test.

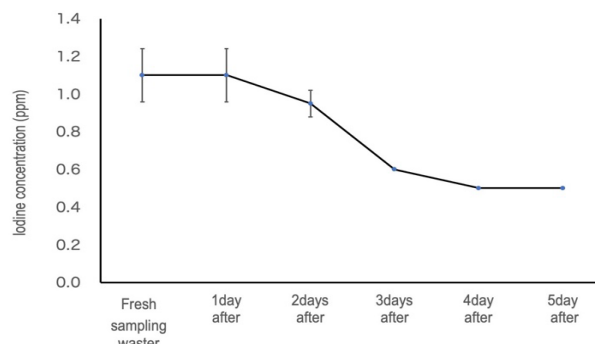


Figure 1. Change in iodine concentration, determined every 24 h. After 48 h iodine concentration began decreasing until 120 h (5 days) after sampling.

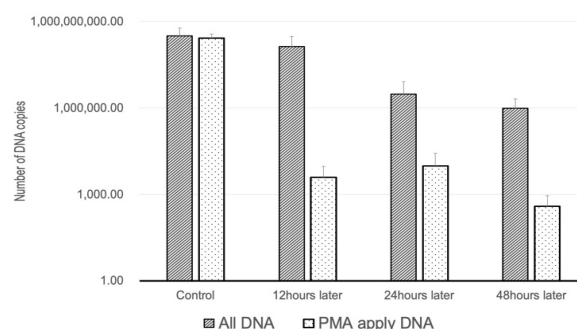


Figure 2. Results of the Quantitative-Polymerase Chain Reaction (qPCR) method, identifying DNA from all bacteria present, and the Propidium monoazide dye with Quantitative-Polymerase Chain Reaction method (PMA-qPCR) (identifying only DNA from living bacteria).

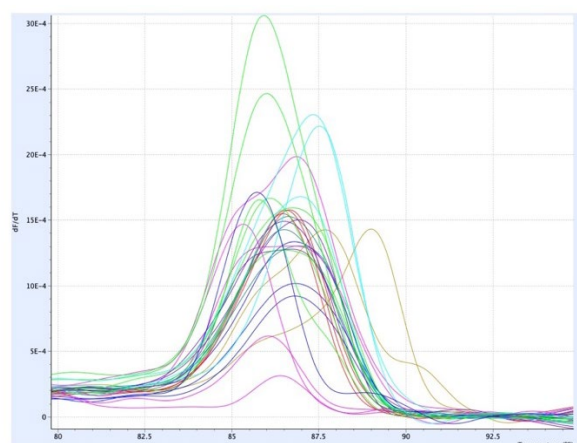


Figure 3. Melt peak analysis of real-time polymerase chain reaction (PCR). The melt peak temperature was different for each well.

There were 3.2×10^8 and 2.7×10^8 copies of bacterial DNA, in the control group and PMA treatment, respectively (Fig. 2).

After 12-h iodine treatment, the DNA copies detected were 1.3×10^8 ; however, after PMA application, only 3900 were detected. After 24-h iodine treatment, the

DNA copies detected were 3.0×10^6 ; however, after PMA application, only 9700 were detected. After 48-h iodine treatment, the DNA copies detected were 1.0×10^6 ; however, after PMA application, only 390 were detected.

Fig. 3 shows the melt-peak of each well, and each peak point is different.

Cultured samples from the control group showed bacterial growth, but 12-h treatment group did not show bacterial growth on R2A culture plates (Fig. 4).

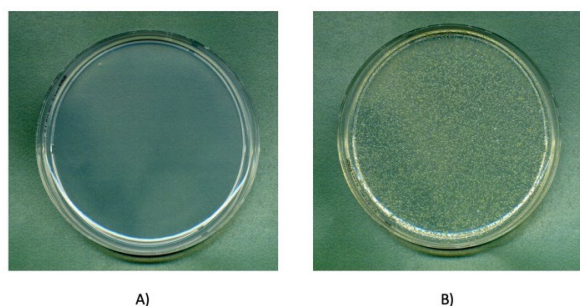


Figure 4. Bacterial growth on R2A culture plates. A) 48 h after application of iodine-water, showing no bacterial growth. B) control group showing bacterial growth.

IV. RESULTS

These results suggest that the DPW contains lower iodine levels than those mentioned in the WHO guidelines for drinking water, and it also exhibits antibacterial effects. The serious infection caused by the DUW bacteria [8] will indicate that the water in the DUC should be disinfected.

There are several sterilization systems that use UV [14], low concentration hydrogen peroxide [15], povidone-iodine [16], and iodine. UV sterilizes the water, but the water does not have any antibacterial properties, so there will be no environmental effects. Hydrogen peroxide has an antibacterial effect, but is susceptible to decomposition. However, iodine, a halogen element, has antibacterial properties and remains in the water. The iodine concentration of DPW was lower than that stated in the manufacturer's information, but this was also observed in previous studies [10]. In this study, the iodine concentration in the water started to decrease after 48 h and continued to decrease for five days. These results suggest that the iodine was used by bacteria or volatilized into the air.

In the natural environment, iodine exists everywhere. The oceans are the most significant source of natural iodine, and from the sea, iodine spreads to the air, water, and soil. Iodine can remain in the soil for a long time because it combines with organic material in the soil. Therefore, the decrease of iodine in the DPW is a natural occurrence. The concentration of iodine in seawater, rainwater, and in groundwater is 0.064 mg/L, 0.0015mg/L, and 0.001 mg/L respectively. Therefore, the iodine concentration in DPW (1.0 ppm) is 15 times higher than the natural iodine concentration. However, iodine is an essential element, and the National Institute of Health recommends an intake of at least 90 $\mu\text{g/day}$ for

children aged 1–8 years, and with age, the need for iodine increases [17].

In the guideline for the concentration of iodine in drinking water by the WHO, oral doses of 2000–3000 mg of iodine (about 30–40 mg/kg of body weight) is estimated to be lethal to humans, but survival has been reported after ingestion of 10,000 mg in short-term exposure. However, after long-term exposure of approximately five years to 1 mg/liter per day of iodine, no cases of hyper- or hypothyroidism, urticaria, or iodism were seen [18]. In 1988, Joint Expert Committee on Food Additives set a Provisional Maximum Tolerable Daily Intake of iodine of 1 mg/day (17 $\mu\text{g/kg}$ of body weight per day) from all sources [19]. A 0.5–1.0 ppm iodine concentration in DPW is lower than these guidelines, so iodine in DPW has no critical effect on human and environment health.

The antibacterial effect of iodine was confirmed by the PMA procedure. The DNA number in PMA-treated bacteria, was 3900. Samples treated for 12 h showed a similar number of DNA to the control groups. These results show that DPW killed 99.99 % of bacteria. After 12 to 48 h, the sample shows a similar number of DNA by PMA treatment, and the concentration of iodine was decreased after 24 h. Adequate concentrations of iodine killed most of the bacteria within 12 h, and the iodine concentration relates to the antibacterial effect. Regular PCR could detect bacterial DNA with high sensitivity, but this procedure detects dead bacteria as well. PMA dye could screen dead bacteria, so using both procedures could find the living bacteria and disrupted bacterial cells. The control group shows that bacterial DNA copies are 3.2×10^8 , with 84.3 % living bacteria. Within 12 h after iodine application, there were 1.3×10^8 bacterial DNA copies and 0.03 % living bacteria. After 24 and 48 h, later, there was less than 1 % living bacteria. The total number of DNA copies also decreased, and after 12, 24, and 48 h, it was 33, 1, and 0.3 %, respectively, of that of control groups. These differences suggest that iodine first killed bacteria, and after that, the bacteria caused autolysis.

Usually, the melt-peak shows one clear peak because the primer pair is sequence-specific, but in this study, melt-peak shows a broad peak, since the universal primer pair was used. These results suggest that there are many different bacteria in DCW water, and some of these bacteria may be difficult to culture. At this point, the PMA-qPCR procedure has the advantage to the culture procedure. The R2A culture plate data supported the PMA-qPCR results. DNA was also broken down after 24 h, because the number of DNA copies decreased.

Around the DCU, the water-drop and aerosol can spread with bacteria in DCW and patient. If the Dentapure is used, the water contains low, but enough iodine, so this system will work to avoid cross-infection. In addition, the iodine concentration in these aerosols is safe as it is lower than several guidelines such as Agency for Toxic Substances and Disease Registry and Food and Nutrition Board. The Dentapure cartridge should be used for disinfecting the DUW and chairside environment and avoiding severe infection and cross-infection.

V. CONCLUSION

The concentration of iodine was safe enough, according to the Guidelines for drinking-water quality, Agency for Toxic Substances and Disease Registry and Food and Nutrition Board. The iodine-included water by Dentapure showed the antibacterial effect and could killed 99.999999% bacteria after 12 h application.

CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the content of this article.

AUTHOR CONTRIBUTIONS

Yasuhiro Matsuda and Kenichi Koshiro conducted the research; Yasuhiro Matsuda and Mari Fujita analyzed the data; Yasuhiro Matsuda wrote the paper; Yasuhiro Matsuda and Takashi Saito supervised this research; all authors had approved the final version.

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REFERENCES

- [1] R. Ireland, *Clinical Textbook of Dental Hygiene and Therapy*, Ames, Iowa: Blackwell Munksgaard, 2006, p. 181-207.
- [2] M. J. O'Donnell, A. C. Shore, and D. C. Coleman, "A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: A one-year study," *J. Dent.*, vol. 34, no. 9, pp. 648-661, Oct. 2006.
- [3] M. J. O'Donnell, A. C. Shore, R. J. Russell, and D. C. Coleman, "Optimisation of the long-term efficacy of dental chair waterline disinfection by the identification and rectification of factors associated with waterline disinfection failure," *J. Dent.*, vol. 35, pp. 438-451, May 2007.
- [4] P. A. Leggat and U. Kedjarune, "Bacterial aerosols in the dental clinic: A review," *Int. Dent. J.*, vol. 51, pp. 39-44, Feb. 2001.
- [5] S. C. Siegel and J. A. V. Fraunhofer, "The effect of handpiece spray patterns on cutting efficiency," *J. Am. Dent. Assoc.*, vol. 133, pp. 184-188, Feb. 2002.
- [6] S. K. Harrel and J. Molinari, "Aerosols and splatter in dentistry: A brief review of the literature and infection control implications," *J. Am. Dent. Assoc.*, vol. 135, pp. 429-437, Apr. 2004.
- [7] M. J. O'Donnell, C. M. Tuttlebee, F. R. Falkiner, and D. C. Coleman, "Bacterial contamination of dental chair units in a modern dental hospital caused by leakage from suction system

- hoses containing extensive biofilm," *J. Hosp. Infect.*, vol. 59, no. 4, pp. 348-360, Apr. 2005.
- [8] M. L. Ricci, S. Fontana, F. Pinci, E. Fiumana, M. F. Pedna, P. Farolfi, M. A. Sabattini, and M. Scaturro, "Pneumonia associated with a dental unit waterline," *Lancet*, vol. 379, pp. 84, Feb. 2012.
- [9] American Dental Association Council on Scientific, "A laboratory evaluation of dental unit water treatment systems," *J. Am. Dent. Assoc.*, vol. 145, pp. 379-380, Apr. 2014.
- [10] M. B. Zimmermann, "Iodine deficiency and excess in children: Worldwide status in 2013," *Endocr. Pract.*, vol. 19, pp. 839-846, Jun. 2013.
- [11] M. B. Zimmermann and M. Andersson, "Update on iodine status worldwide," *Curr. Opin. Endocrinol. Diabetes Obes.*, vol. 19, pp. 382-387, Oct. 2012.
- [12] E. N. Pearce, M. Andersson, and M. B. Zimmermann, "Global iodine nutrition: Where do we stand in 2013?" *Thyroid*, vol. 23, no. 5, pp. 523-528, May 2013.
- [13] A. Yasunaga, A. Yoshida, K. Morikawa, K. Maki, S. Nakamura, I. Soh, S. Awano, and T. Ansai, "Monitoring the prevalence of viable and dead cariogenic bacteria in oral specimens and in vitro biofilms by qPCR combined with propidium monoazide," *BMC Microbiol.*, vol. 13, no. 1, pp. 157, Dec. 2013.
- [14] R. Leger and P. Sorensen, "UV-light apparatus integrated dental unit. An ergonomic proposal," *Inf. Orthod. Kieferorthop.*, vol. 8, pp. 424-427, Apr. 1976.
- [15] S. M. Lin, K. K. Svoboda, A. Giletto, J. Seibert, and R. Puttaiah, "Effects of hydrogen peroxide on dental unit biofilms and treatment water contamination," *Eur. J. Dent.*, vol. 5, no. 1, pp. 47-59, Jan. 2011.
- [16] J. Mungara, N. C. Dilna, E. Joseph, and N. Reddy, "Evaluation of microbial profile in dental unit waterlines and assessment of antimicrobial efficacy of two treating agents," *J. Clin. Pediatr. Dent.*, vol. 37, no. 4, pp. 367-371, Jul. 2013.
- [17] Institute of Medicine (US) Panel on Micronutrients, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, Washington: Academy Press, 2001, pp. 258-289.
- [18] W. C. Thomas, A. P. Black, G. Freund, and R. N. Kinman, "Iodine disinfection of water," *Int. Arch. Occup. Environ. Health*, vol. 19, no. 1, pp. 124-128, Jul. 1969.
- [19] Safety Evaluation of Certain Food Additives and Contaminants, World Health Organization Technical Report Series, vol. 896, pp. 1-128, Dec. 2002.

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