

Short Communication

Bactericidal Effects of Acidic Electrolyzed Water on the Dental Unit Waterline

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SUMMARY: Many studies have been conducted in the United States regarding the microbial contamination of dental unit waterline, but not in Japan. Recently, acidic electrolyzed water has been used in the medical and dental fields. In this study, we investigated the bactericidal effects of the temporary inflow of acidic electrolyzed water on microbial contamination of the dental unit waterline. First, in order to observe the daily bacterial contamination of the dental unit waterline, water samples were collected at the end of handpieces and three-way syringes before the inflow of acidic electrolyzed water. They were cultured to detect viable bacteria. Later, the inflow of acidic electrolyzed water was conducted through the piping box of the dental unit. Before starting operation on next day, water samples were collected and cultured, as described above. The mean viable bacteria count was 910 ± 190 CFU/ml at the end of handpieces, and 521 ± 116 CFU/ml at the end of three-way syringes before the inflow of acidic electrolyzed water. However, bacteria were detected in only small numbers at the end of handpieces and three-way syringes on the next day. These results indicated that acidic electrolyzed water could be applied as an appropriate measure against bacterial contamination of the dental unit waterline.

In the United States, several studies have clarified the microbial contamination of dental unit waterline (1,2). In Japan as well, a recent study reported the detection of many viable bacteria in the dental unit waterline (3).

The bactericidal activities of acidic electrolyzed water prepared by the electrolysis of water containing a small amount of sodium chloride have been investigated (4). Acidic electrolyzed water showed effective bactericidal activity for even a brief immersion time (5). Moreover, it has been clarified that acidic electrolyzed water does no harm to the human body (6). Therefore, recently, acidic electrolyzed water has come to be applied in the medical and dental fields. In this study, we speculated that acidic electrolyzed water could be used as a disinfectant for the dental unit waterline and investigated the bactericidal effects of the inflow of acidic electrolyzed water on the dental unit waterline. A previous study showed (7) that dental unit waterline could be sterilized by piping acidic electrolyzed water into it. We assumed that the constant introduction of acidic electrolyzed water into the dental unit waterline would cause environmental pollution and corrosion of the dental unit water line. Therefore, in the present study we introduced acidic electrolyzed water only temporarily into the dental unit waterline and examined its bactericidal effect on microbial contamination.

The acidic electrolyzed water used in this study was produced by QueenH-AP (AQUA medical, Hiroshima). This water was produced by the electrolysis of water from a municipal water system. The acidic electrolyzed water used in this study has the following physical properties: pH 2.7, an oxidative-reduction potential of 1100 mV, a concentration

of dissolved chlorine of 32 ppm, and a concentration of dissolved oxygen of 16.

We selected six dental units in the general dentistry clinics as experimental units. First, in order to determine the daily bacterial contamination of dental unit waterline, water samples were collected at the end of handpieces and three-way syringes before the inflow of acidic electrolyzed water. Sample collection was conducted 3 times in each dental unit. Each sample of 500 µl was spread on R2A agar plates, using a micropipette with a sterile disposable plastic tip and a sterile bent glass rod. These media were incubated aerobically at 24°C for 2 days. We counted the colonies manually on three sample plates and converted the results to CFU/ml. Later, we selected three of these six dental units and flowed acidic electrolyzed water through the piping box (Figs. 1-A and B). We poured about 2L of acidic electrolyzed water for 30 min. The remaining three units did not receive an inflow of acidic electrolyzed water, serving as the controls. On 1, 2, and 5 days after the acidic electrolyzed water was introduced into the waterline, water samples were collected at the ends of the handpieces and three-way syringes of all six dental units and cultured as described above. Furthermore, water samples were collected from three spots from a municipal water system in our clinic before the acidic electrolyzed water was introduced into the dental unit waterline and also cultured as described above.

Statistical analysis of the data was performed using Student's t test.

Immediately after the inflow of acidic electrolyzed water, the pH of the water discharged from the handpiece was 6.57, indicating that this water was neutral. On the other hand, on 8 min after the inflow of acidic electrolyzed water, the pH of the water was 3.0, indicating that this water was acidic (Fig. 2). Based on these findings, it was thought that the water in the dental unit waterline exerted a strong bactericidal activity. Figures 4, 5, and 6 show the numbers of colonies in the

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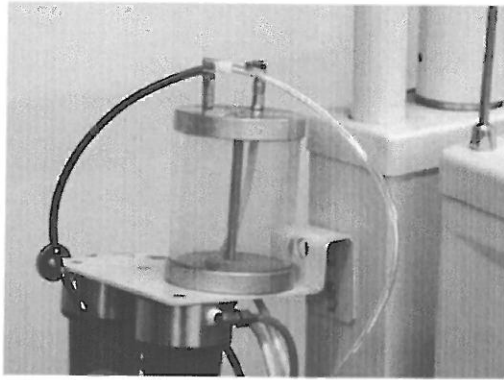


Fig. 1-A. Piping box of dental unit.



Fig. 1-B. Inflow of acidic electrolyzed water into the piping box.

Piping box

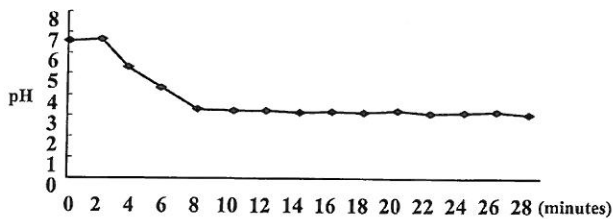
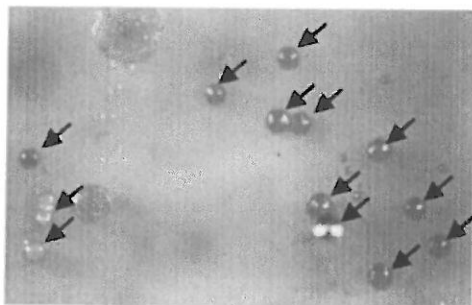


Fig. 2. pH of water discharged from the handpiece after inflow of acidic electrolyzed water.

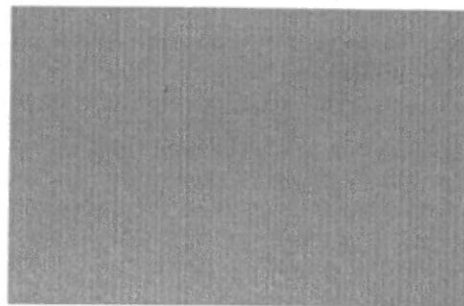
dental unit waterline and in the city water before and after the inflow of acidic electrolyzed water. The mean viable bacteria count was  $910 \pm 190$  CFU/ml at the handpiece, and  $521 \pm 116$  CFU/ml at the three-way syringe before the inflow of acidic electrolyzed water (Figs. 3-6). These results showed that high levels of bacterial contamination still existed. This condition appears to be nearly universal, regardless of whether the dental units are connected to municipal water suppliers or are equipped with a separate water system (8-9). In a previous study conducted in the general dental clinics of the University Hospital, Faculty of Dentistry, Tokyo Medical and Dental University, although no deleterious bacteria (*Legionella*, *Streptococcus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were detected in the dental unit waterline, the following were identified as dominant bacteria: *Sphingomonas paucimobilis*, *Methylobacterium mesophilicum*, and *Pseudomonas stutzeri* (3). Because our examination was also conducted at general dental clinics, we assumed that the same types of bacteria existed in the samples collected in this study. However, recently it has become a public health problem that *Legionella* was detected in the dental unit waterline. Therefore,

we must conduct the effective bactericidal measures for the dental unit waterline, immediately.

On the next day after the inflow of acidic electrolyzed water, bacteria were detected in only negligible amounts on the handpiece and three-way syringe, showing significant differences from the control chair (Figs. 5 and 6). A previous study demonstrated that acidic electrolyzed water exerted a strong bactericidal activity on various bacteria in a short time (5). The present study also showed that the pH of water in the dental unit line indicated acidity at 8 min after the inflow of acidic electrolyzed water, as mentioned above. Based on these findings, it was thought that the dental unit line was sterilized by the inflow of acidic electrolyzed water even for a short time. Moreover, it was clarified that acidic electrolyzed water has strong bactericidal effects on all bacteria including methicillin-resistant *S. aureus*, viruses including human immunodeficiency virus, hepatitis B virus (HBV), hepatitis C virus, and cytomegalovirus, and fungi (10). In recent years, it has been reported that HBV invaded handpieces from patients with HBV. The bactericidal activity of acidic electrolyzed water would be effective in attacking HBV in such locations. Although several experimental approaches have been used, the above findings indicate that the temporary inflow of acidic electrolyzed water is also an extremely effective bactericidal measure for sterilizing the dental unit waterline. Furthermore, this sterilization method is thought to be environmentally friendly and inexpensive, since it requires only a small amount of acidic electrolyzed water. However, on 2 days after the inflow of acidic electrolyzed water, a small number of bacteria were detected. Furthermore, on 6 days after the inflow of acidic electrolyzed water, many



Before inflow



Next day after inflow

Fig. 3. Colony on the R2A agar plate



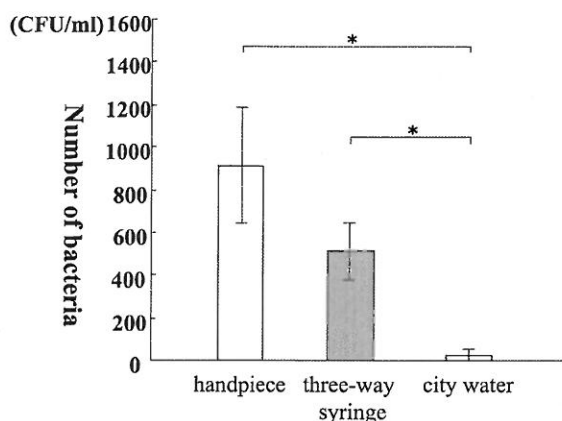


Fig. 4. The number of bacteria in the dental unit water line and in city water.

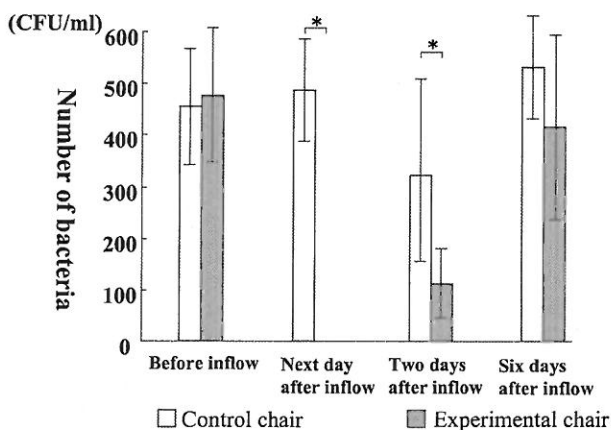


Fig. 5. The number of bacteria in the three-way syringe.

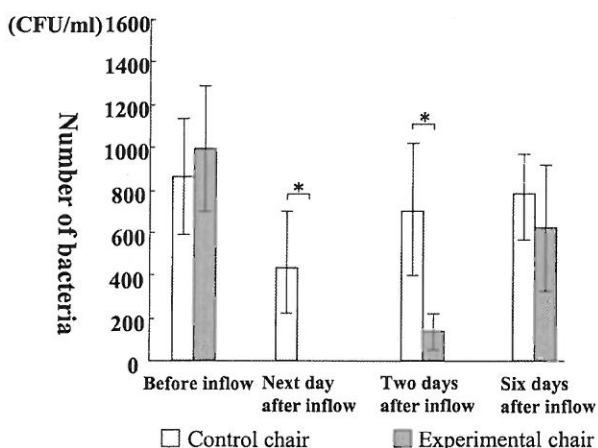


Fig. 6. The number of bacteria in the handpiece.

bacteria were found (Figs. 5 and 6). As shown in Figure 4, there were few bacteria in the city water. Therefore, the dental unit waterline was not polluted by the city water after the inflow of acidic electrolyzed water. We guessed that some bacteria formed biofilms and remained in the dental unit line. Moreover, it was also suggested that the saliva of patients

became mixed into the dental unit waterline from handpieces or three-way syringes. These findings indicated that acidic electrolyzed water must be introduced into the waterline at least every other day. However, in order to maintain the complete sterility of the dental unit line, this process must be performed on the line following the treatment of each patient. Thus, we considered that piping acidic electrolyzed water directly into the dental waterline itself would be the most efficient way to achieve this. However, as mentioned, this process might cause environmental pollution and corrodes the line. This would necessitate the neutralization of the acidic electrolyzed water before is discharged into a municipal waste water system. In regard to the latter problem, corrosion could be prevented by constructing the line of stainless steel. On the other hand, in a previous study, it was demonstrated that oyster shell neutralized acid rain (11). The dominant component of oyster shell is calcite ( $\text{CaCO}_3$ , wt%98) can be dissolved easily in neutral or weak acidic aqueous solutions. Therefore, we expect that oyster shell could be applied to neutralize the acidic electrolyzed water after using it for disinfection.

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