The Sterilization Effect of Hard Oxidized Water in Toothbrush Washing with an Ultrasonic Cleaning Device

AKIRA SATO, MASAHIRO KANDA, TOMOHISA OGAWA, HIROKI KAWAMURA and KYUICHI KAMOI

Abstract: Hard oxidized water is obtained through electrolysis of water with a small amount of sodium chloride. We examined the bactericidal effect of hard oxidized water, to investigate the possibility of using hard oxidized water and an ultrasonic device to wash a toothbrush. The results revealed that hard oxidized water had a strong bactericidal effect for anaerobic bacteria on a toothbrush. Moreover, it was revealed that use of the ultrasonic device increased the bactericidal effect. The findings suggest that hard oxidized water is useful for washing a toothbrush.

Key words: hard oxidized water, ultrasonic cleaning device, bactericidal effect

Introduction

One cause of periodontal disease is bacteria in plaque, and therefore it is important to control plaque not only in the clinic setting but also in home care. The toothbrush is usually the main form of plaque control. The toothbrush is usually washed in ordinary tap water and kept in the lavatory. Thus, bacteria are not removed and can even proliferate. On the other hand, gas sterilizers are expensive, and the brush must be left in the sterilizer for at least 6 hours to properly use the ethylene oxide gas and formaldehyde, which have toxicity for the organism. After brushing, the toothbrush is contaminated by microorganisms in plaque, even if the brush is thoroughly washed in tap water. The friction of brushing applies physical force not only on the tooth surface but also on the gingiva. The pressure and friction of brushing can injure healthy gingiva, as well as inflamed gingiva, and microorganisms on the toothbrush can bring about infection and bacteremia. Therefore, it is necessary for the toothbrush to be kept as aseptic as possible. Hard oxidized water can be obtained from electrolysis of water with a small amount of sodium chloride. This has a strong bactericidal effect at a pH of less than 2.7, or at an oxidation-reduction potential greater than 1100 mV. This has various applications, because hard oxidized water has adverse effects on tissues, and its usage is easy and convenient. Much research has been carried out in the field of dentistry, and various applications are expected.

In the present study, we applied hard oxidized water in ultrasonic washing, to investigate the bactericidal effect of hard oxidized water on the toothbrush after brushing, and the influence of ultrasonic washing in hard oxidized water.

Materials and methods

The hard oxidized water used in this study was generated by the OXILYZER OXMOI (Miura Electron Co., Ltd., Tokyo). Output was adjusted to an oxidation-reduction potential of greater than 1100 mV and a pH less than 2.5, with an available chlorine concentration of 30 ppm.

Ten members (8 men, 2 women; average age 28.5) of the department of periodontology at the Nippon Dental University brushed their teeth for 3 minutes, using the Bass method, and the brushes were washed under the conditions listed in Table 1.

1. Measurement of the number of viable microorganisms
Sheep blood agar medium, with 1% Hemin (SIGMA Co., USA) and 0.05% Menadion (SIGMA Co., USA), was cultured for 72 hours at 37°C under aerobic and anaerobic conditions (85% N₂, 10% H₂, 5% CO₂ mixed gas). After brushing, the toothbrushes were washed under each condition by stirring in 10 mL of physiological saline for 1 minute using a Voltex mixer. A fungus suspension was diluted at 10²- and 10³-fold, respectively, and 0.1 mL of the solution was pipetted on blood agar.

After culturing using the above method, cell growth was quantified based on the number of colonies in colony forming units/mL (number of viable microorganisms, CFU/mL).

2. Observation by scanning electron microscopy (SEM)

Part of the toothbrush surface was dehydrated in alcohol and then fixed with 2.5% glutaraldehyde, 0.1 M phosphate buffer (pH 7.4) for 1 hour and post-fixed with 1% osmium tetroxide, 0.1 M phosphate buffer (pH 7.4). The brush samples were then immersed in t-butyl alcohol after dehydration, followed by freeze-drying. The sample surface was ion-sputtered with gold and observed by scanning electron microscopy (SEM).

3. Statistical analysis

A multiple comparison test was carried out using Fisher's PLSD, and the number of viable microorganisms (CFU/mL) in each group was analyzed by one-way analysis of variances.

Results

1. Bactericidal effect on aerobic bacteria

In the group washed in plain tap water, the number of viable microorganisms was $4.0 \times 10^5 \pm 1.7 \times 10^5$ CFU/mL; in the group washed ultrasonically in tap water, the count was $2.1 \times 10^5 \pm 1.2 \times 10^5$ CFU/mL; in the group washed in hard oxidized water, the count was $2.3 \times 10^4 \pm 4.8 \times 10^4$ CFU/mL; and in the group washed ultrasonically in hard oxidized water, the count was $3.2 \times 10^5 \pm 7.1 \times 10^5$ CFU/mL. Statistically significant differences existed between the group washed in tap water and the groups washed ultrasonically in tap water, in hard oxidized water, and ultrasonically in hard oxidized water. There was a statistically significant difference between the group washed ultrasonically in tap water and the groups washed ultrasonically in hard oxidized water (Fig. 1).

2. Bactericidal effect on anaerobic bacteria

In the group washed in plain tap water, the number of viable microorganisms was $4.9 \times 10^5 \pm 2.0 \times 10^5$ CFU/mL; in the group washed ultrasonically in tap water, the number was $2.6 \times 10^4 \pm 1.8 \times 10^5$ CFU/mL; in the group washed in hard oxidized water, the count was $2.3 \times 10^4 \pm 4.8 \times 10^4$ CFU/mL; and in the group washed ultrasonically in hard oxidized water, the count was $5.0 \times 10^3 \pm 7.0 \times 10^3$ CFU/mL. There was a statistically significant difference between the group washed in tap water and the groups washed ultrasonically in tap water, in hard oxidized water, and ultrasonically in hard oxidized water. There was a statistically significant difference between the group washed ultrasonically in tap water and the groups washed in hard oxidized water and ultrasonically in hard oxidized water (Fig. 2).

Table 1 Washing conditions for toothbrush

| (1) Washed under tap water, 2000 mL/min for 1 minute |
| (2) Immersed in tap water and washed ultrasonically for 5 minutes |
| (3) Washed under hard oxidized water, 2000 mL/min for 1 minute |
| (4) Immersed in hard oxidized water and washed ultrasonically for 5 minutes |

Fig. 1 Bactericidal effect on aerobic bacteria

(1) Groups washed ultrasonically in hard oxidized water, (2) groups washed in hard oxidized water, (3) groups washed ultrasonically in tap water, (4) groups washed in tap water.
3. Observation by scanning electron microscopy (SEM)

Relatively few bacteria were observed in the group washed ultrasonically in hard oxidized water (Fig. 3). Several colonies were observed, but clumping was not observed, in the group washed in hard oxidized water (Fig. 4). Some bacteria were observed on the toothbrush surface in the group washed ultrasonically in tap water. They seemed to have been lysed (Fig. 5).

Bacteria and their exenzymes were observed both on and around the toothbrush surface in the group washed in tap water (Fig. 6).

Discussion

The broad bactericidal effect\(^{(4-9)}\) of hard oxidized water on pathogenic microorganisms is as strong as that of conventional disinfectants. Additionally, hard oxidized water has been used in various fields, in such applications as the...
washing and sterilization of food, and to maintain hygienic conditions in food processing facilities, since it has a high degree of safety and does not injure skin, mucosa, or the surface of damaged tissue in humans or animals\(^6\). Hard oxidized water has also been used for the disinfection of medical devices and sanitary cleaning of hands, as well as for washing the oral cavity in dentistry. However, this bactericidal effect is reduced by organic substances, such as serum\(^7\), and hard water does corrode metals\(^8\).

It is said that the level of bacteria detected on the toothbrush after use\(^9\) is usually only \(1.11 \times 10^6\). It has been reported that the presence of bacteria on the surface of the toothbrush is not only bad for health but also causes infection, as bacteria can be introduced into a wound by brushing excessively\(^3\). Therefore, we examined the bactericidal effect of hard oxidized water and ultrasonic cleaning of the toothbrush after the teeth were brushed for 3 minutes. The results suggest that hard oxidized water is effective for sterilization of the toothbrush in the groups washed in running water and by an ultrasonic device, compared with the group washed in tap water. It has been reported that hard oxidized water acts on bacterial reproduction through an oxidation mechanism of hypochlorous acid, as well as the pH and oxidation-reduction potential, and that this raises the membrane potential of cells above stabilization, killing cells by disturbing energy metabolism and respiration\(^{10}\). Therefore, the bactericidal effect of hard oxidized water also seems to be related to pH, the oxidation-reduction potential, and oxidation by hypochlorous acid. A stronger bactericidal effect was obtained by ultrasonic washing in tap water compared with regular washing in tap water.

The bactericidal effect was also obtained by ultrasonic washing in hard oxidized water, but the difference was not significant. It is well known that various foams are continuously generated when ultrasonics is applied in liquids\(^{14}\). Therefore, it seems that the stronger bactericidal effect of ultrasonic washing was due to the foam and various generated by the ultrasonics.

These results were also evident even in the observation of the toothbrush by scanning electron microscopy. In the group washed ultrasonically in hard oxidized water, hardly any bacteria were observed. The bactericidal effect was strong. In the group washed in hard oxidized water, some bacteria were observed, but there was no agglomeration. The bactericidal effect obtained by washing in hard oxidized water without ultrasonic washing was not as strong as the bactericidal effect obtained by washing in hard oxidized water with ultrasonic washing, but a sufficient bactericidal effect was obtained. Few bacteria were observed in the group washed ultrasonically in tap water. The bacteria observed by the scanning electron microscope seemed to have been destroyed. This seems to be due to the foaming and vibration arising from the ultrasonics. In the group washed in tap water, bacteria and their intracellular materials were observed. There was virtually no bactericidal effect.

In this case, the number of viable bacteria was not significantly different but was greater under anaerobic conditions compared with aerobic conditions. Shiba et al\(^5\) reported that the bactericidal effect of hard oxidized water was lower for anaerobes compared with aerobes. They pointed out that protein could not be avoided in the fungus suspension in their experiment, so the bactericidal action of hard oxidized water was weakened. However, further investigations are needed to clarify why differences were seen, because the same blood agar was used for both conditions in this study.

It is clear that hard oxidized water exhibits an almost 100% bactericidal effect in only 10
In this study, the number of viable colonies decreased considerably in the group washed in hard oxidized water, and a strong bactericidal effect was apparent. A bactericidal effect was also seen after washing for 1 minute in tap water and after washing ultrasonically for 5 minutes.

It has also been reported that the bactericidal effect of hard oxidized water is reduced by blood and saliva. In the present study, because the subjects did not suffer from periodontitis, they did not bleed from their gingiva, and so very little blood adhered to the toothbrush. It can be assumed that the bactericidal effect of hard oxidized water is not weakened by washing in tap water, and ultrasonic washing seems to have washed out any saliva that could weaken the effect. Therefore, in ordinary households and dental clinics, it seems to be more effective to wash out any adherent blood and saliva in tap water, when hard oxidized water is later used to sterilize the toothbrush.

It is virtually impossible to prevent bacteria from adhering to the toothbrush, because the brush is used to remove plaque. However, this study suggests that washing the toothbrush in hard oxidized water after use was effective in removing bacteria, and this effect could be increased by using an ultrasonic device.

References

超音波洗浄法を用いた強酸性水による歯ブラシの殺菌効果

佐藤 昭、神田 昌宏、小川 智久
川村 浩樹、鴨井 久一

強酸性水は、少量の食塩水を電気分解して得られる。われわれは、歯ブラシの洗浄に強酸性水と超音波洗浄器を用いて強酸性水の殺菌効果について検討した。その結果、強酸性水は歯ブラシに付着した嫌気性菌に強い殺菌効果を有することがわかった。さらに、超音波洗浄器によって殺菌効果は増強した。強酸性水は、歯ブラシの洗浄に有益であることが示唆された。

キーワード：強酸性水、超音波洗浄器、殺菌効果