Bactericidal activities of electrolyzed strong and weak acid waters for acrylic denture base resin were evaluated in order to discuss the applicability of these waters for sterilization of denture base. Only 1-minute immersion in the electrolyzed strong or weak acid water could completely eliminate the attached bacteria, Staphylococcus aureus 209P, on the resin plate. When the resin was relined with tissue conditioner, 5-minute immersion or 1- to 2-minute ultrasonic cleaning reduced the number of the bacteria from $10^5$/cm$^2$ level to $10^1$/cm$^2$ and no surviving bacteria could be detected after 10-minute treatment. These findings suggest that both the electrolyzed strong and weak acid waters are well applicable to the disinfectant for acrylic denture base showing excellent bactericidal activities in a significantly shorter treatment as compared with the conventional denture cleaning.

Key words: Electrolyzed acid water/Denture base acrylic resin/Sterilization

INTRODUCTION

The concepts of disinfection and sterilization of dental materials and devices have more and more been of interest with prevalence of nosocomial infection. On the used acrylic denture, heavy accumulations of debris, stains and bacteria are often observed. Soft reliner for denture base may be much more likely to be subjected to such contaminations than the denture base. Those dentures frequently come in contact with the dentist and/or co-dental staff for adjustment, repair or relining. It is, therefore, preferable to sterilize them prior to working with them in order to avoid unexpected infection. Many denture cleaners and chemical disinfectants are now commercially available. It was reported, however, that some of them caused problems such as color change, porosity and surface roughness of the denture base resin surface$^{1-3}$. It was also demonstrated that some denture cleaners and chemical disinfectants showed the possibilities to induce surface roughness and porosity on the tissue conditioners$^{4}$.

Recently, the marked bactericidal activity of the electrolyzed acid water has been successfully utilized in dental practice$^{5-9}$. The authors and co-workers previously reported its effectiveness for sterilization of alginate and silicone impressions$^{10-12}$ and several dental instruments$^{13}$. Electrolyzed acid water is classified broadly into electrolyzed strong acid water and weak acid water.
This study was undertaken to apply these acid waters to sterilization of denture base. For the basic study, in this first report, sterilization efficiencies of these acid waters on the denture base acrylic resin plates with or without lining a tissue conditioner were examined by counting the surviving bacteria before and after treatment in these acid waters.

MATERIALS AND METHODS

Preparation of bacteria solution
As a fundamental study, the bacteria used were Staphylococcus aureus 209P, which have been commonly used for evaluating sterilization effects. The bacteria incubated in a brain heart infusion (Lot 103476JC, DIFCO, Detroit, MI, USA) at 37°C for 24 hr. Bacteria suspensions were prepared to be $5.0 \times 10^6$/ml in saline solution.

Preparation of acrylic resin plate
Resin materials used were a heat-curing acrylic resin (UR) and a self-curing acrylic resin (QR) as listed in Table 1. Resin plate (30\times30\times3.0 mm) was cured\textsuperscript{14}, polished with #400 emery paper and finished by buffing (left in Fig. 1). A tissue conditioning material (TC in Table 1) was mixed at the standard P/L ratio and placed onto one side of the UR resin plate by applying a load of 15 kgf for 5 min to be 2 mm in thickness using a spacer (right in Fig. 1). The plate was entirely immersed in 50 ml of bacteria-suspended solution ($5.0 \times 10^6$/ml) for 3 hr in order to shift the bacteria to the surface of the plate.

<table>
<thead>
<tr>
<th>Materials used</th>
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<tbody>
<tr>
<td>Materials (Shade)</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Heat curing acrylic resin for denture base URBAN(#3)</td>
</tr>
<tr>
<td>Fast setting self-curing resin for denture base Quick resin B(2)</td>
</tr>
<tr>
<td>Tissue conditioning material TISSUE CONDITIONER</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Kyoto, Japan

Fig. 1 Acrylic resin plates tested.
left: resin plate not relined,
right: resin plate relined with TC
Two types of electrolyzed acid waters were used for sterilizing the resin plate. They were the strong acid water (SW) and the weak acid water (WW) as shown in Table 2. The strong acid water was prepared by electrolyzing 0.05% sodium chloride aqueous solution with an electrolyzing apparatus (SUPER WATER mini, Hirata Corp., Osaka, Japan). The weak acid water was prepared by electrolyzing tap water containing specified electrolysis with an electrolyzing apparatus (ACIDENT, J. Morita Tokyo MFG. Corp., Tokyo, Japan). The pH value and oxidation-reduction potential (ORP) were examined with a pH meter (D-22S, HORIBA Ltd., Tokyo, Japan) and the concentration of residual chlorine was determined with a chlorine comparator (Portable Type II, Toyo ENGINEERING WORKS LTD., Chiba, Japan) based on the O-triginn method. Those values are shown in Table 2. As the properties change with time by light- or air-exposure, the acid water was supplied for experiment within 1-hour storage in a sealed and shaded tank after preparation. The preparation and storage of the water were carried out at 23±2℃.

The plate specimen with the bacteria being attached to its surface was entirely immersed in the acid water with or without adding ultrasound by an ultrasonic cleaner (VS-150, IUCHI SEIEIDO CO., LTD.) under the conditions shown in Table 3 at 23±2℃. The same treatments were performed with distilled water (DW) for comparison. After treatment, the plate was ultrasonically cleaned in 50 ml of fresh saline solution for 30 sec, and 1 ml from the solution was added to the agar culturing media (Nutrient Agar, lot. No.057907, Nissui, Tokyo, Japan). The number of the surviving bacteria in the media was counted after incubation at 37℃ for 24 hr. As the control, the bacteria attached on the specimen were counted without any sterilization treatment. In order to estimate the effect of the ultrasonic cleaning on bactericidal activity

<table>
<thead>
<tr>
<th>Water</th>
<th>Code</th>
<th>pH</th>
<th>ORP(mV)</th>
<th>Residual chlorine(ppm)</th>
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<tr>
<td>Electrolyzed strong acid water</td>
<td>SW</td>
<td>2.3±0.3</td>
<td>+1,170±5</td>
<td>50±25</td>
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<tr>
<td>Electrolyzed weak acid water</td>
<td>WW</td>
<td>5.7±0.2</td>
<td>+873±5</td>
<td>75±25</td>
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<tr>
<td>Distilled water</td>
<td>DW</td>
<td>6.6±0.4</td>
<td>+456±20</td>
<td>0±0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Amount for treatment (ml)</th>
<th>Time for treatment (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin plate (Code: UR and QR)</td>
<td>50</td>
<td>1, 2, 5, 10</td>
</tr>
<tr>
<td>Resin plate relined with TC (Code: UR with TC)</td>
<td>50</td>
<td>1, 2, 5, 10</td>
</tr>
</tbody>
</table>
against the inside of the tissue conditioner, the penetration of the water into TC relined on the resin plate was examined by means of staining with crystal violet (lot. No. LKJ1095, Wako Pure Chemical Ind, Osaka, Japan) incorporated in the water. All the experiments were repeated 5 times and the results were statistically compared by ANOVA and t-test.

RESULT

The numbers of the bacteria attached to the resin plates are shown in Figs. 2 and 3, referring to as the control without treatment. The bacteria shifted from the bacteria solution \(5.0 \times 10^6 / \text{ml}, 50 \text{ ml}\) to the resin plate was about \(5 \times 10^6 (2 \times 10^4 / \text{cm}^2)\) out of \(2.5 \times 10^8\) in the solution. The numbers of surviving bacteria on the resin plate after several treatments are also shown in Figs. 2 and 3. As the number of the bacteria was expressed by logarithm in these graphs and the value of zero could not be plotted, the notation of \(0 \pm 0\) was put in the figures if no residual bacteria were detected on the plate.

When the resin plate was immersed in the distilled water (DW), very few bacteria were removed from the plate. Addition of ultrasonic cleaning in DW showed a larger effect for removing bacteria than the simple immersion treatment \((p<0.01)\). However, the level of \(10^2 / \text{cm}^2\) of the bacteria still remained after 10-minute treatment. On the other hands, the immersion treatment in the electrolyzed acid water, SW and WW, showed a marked bactericidal effect. No surviving bacteria were found on all the resin plate specimens after 1-minute treatment. There were no significant differences among the resin materials used \((p>0.05)\).

The numbers of the bacteria attached to the UR resin plate which was relined with TC are shown in Fig. 4, referring to as the control without treatment. The bac-

![Fig. 2](image-url)  
**Fig. 2** Number of surviving bacteria on UR after treatment.  
- : immersion for 1 min  
- : ultrasonic cleaning for 1 min  
- : control (without sterilization treatment)  
0±0: no surviving bacteria were detected on the plate

![Fig. 3](image-url)  
**Fig. 3** Number of surviving bacteria on QR after treatment.  
- : immersion for 1 min  
- : ultrasonic cleaning for 1 min  
- : control (without sterilization treatment)  
0±0: no surviving bacteria were detected on the plate
Sterilization effect was significantly smaller for UR with TC with every acid water than for UR and QR. The level of $10^2$ and $10^1$/cm$^2$ of surviving bacteria still remained on UR with TC after 1-minute immersion in SW and WW, respectively. The number dropped to the level of $10^1$/cm$^2$ and less than 10 by ultrasonic cleaning in the respective acid waters. At the treatment conditions of 1-minute immersion with or without ultrasonic cleaning and 2-minute ultrasonic cleaning, WW showed a greater
sterilization effect on UR with TC than SW (p<0.01). When the treatment time was prolonged to 5 min, the number of surviving bacteria was reduced to almost zero by any treatment and in any acid water. No more bacteria could be detected on UR with TC after 10-minute treatment regardless of the treating method and the type of acid water.

Fig. 5 shows the depth of penetration of the water into TC under each treating condition. The longer the treatment time was, the deeper the penetration of the treatment water was. Ultrasonic cleaning showed a tendency to accelerate the penetration of every water. The penetration by ultrasonic cleaning was 4 to 10 times deeper than that by simple immersion.

DISCUSSION

It has been pointed out that the conventional denture cleaning and sterilization often induced some problems such as color change, porosity and surface roughness on the denture base resin as well as porosity and surface roughness on the tissue conditioner. The use of the electrolyzed acid water for denture cleaner, on the contrary, seems to solve those problems. Kishii et al. reported that the properties (Knoop hardness, surface roughness and color change) of denture base resin and resilient denture liner were unchanged by 2-week immersion in electrolyzed strong acid water and that this acid water would be apply to the denture cleaner. It was also reported that no changes were observed in the properties of artificial resin teeth by 4-week immersion in this water. These findings suggest that the electrolyzed acid water will be safely adopted as a denture cleaner without affecting the denture base, artificial resin teeth and soft reliner from the chemical, physical and mechanical point of views.

In addition to such advantages with the electrolyzed acid water, the present study could clarify its bactericidal effects on the denture base resin and the soft reliner in order to avoid nosocomial infection. The acrylic resin plate specimens easily made the bacteria attach to their polished surfaces when they were immersed in the bacteria-suspended solution. The number of the bacteria attached to the resin surface was in the order of 10^4/cm^2 out of 10^8 in the solution. Once the bacteria were attached to the resin surface, it was difficult to remove them by simple cleaning with tapping water. Even after ultrasonic cleaning in distilled water for 10 min, the 10^2/cm^2 level of surviving bacteria still remained. The use of the electrolyzed strong or weak acid water could drastically reduce the number of the bacteria and all of them on the resin plate completely disappeared by only 1-minute immersion in the acid water.

When the resin plate was relined with the tissue conditioner, the sterilization treatment by 1-minute immersion in the acid water was not always sufficient, leaving the order of 10^3/cm^2 surviving bacteria although the percent bactericidal effect reached 99.0%. It was probably because the bacteria might still survive in the porous surface layers of the tissue conditioner. The sterilization effect significantly
increased with increase in the immersion time. The number of the surviving bacteria were reduced to almost zero by 5-minute immersion or ultrasonic cleaning in both the strong and weak acid waters and completely eliminated by 10-minute immersion.

The authors and co-workers reported that the addition of the ultrasonic cleaning action in electrolyzed acid water was effective for sterilization of dental instruments having complicated configuration such as dental burs and diamond stones\(^{13}\). Similar effects of the ultrasonic cleaning in the acid water were observed especially in shorter treating term in this study. The number of the bacteria on the resin plate relined with the tissue conditioner rapidly dropped to less than 10/cm\(^2\) by 2- and 1-minute ultrasonic cleaning in electrolyzed strong and weak acid water, respectively. Two processes might account for the distinct effect of the ultrasonic cleaning in acid water when compared to the simple immersion. One is that the bacteria existing in the surface layer of the tissue conditioner involving porosities might be ejected out by the ultrasonic action, and another, the ultrasonic action might accelerate the penetration of the acid water into the surface layer of the tissue conditioner and attack the bacteria there. The penetration depth significantly increased with treating time while no significant difference in the depth was found among distilled, strong acid and weak acid waters. However, the number of the surviving bacteria became almost zero in 5 min by both the simple immersion and ultrasonic cleaning in every acid water, and no more differences were found between the two treatments. Finally, the resin plate relined with the tissue conditioner was thoroughly sterilized in 10 min in both types of the acid waters.

In the practical application of the electrolyzed acid water for sterilization of the denture, it is recommended that a plenty of fresh acid water should be supplied on the surface of the denture since the bactericidal effects will markedly fall if the water contacts protein. It is, therefore, better to preliminarily take off large debris and other protein or to make the treating time somewhat longer than the estimation.

The use of the electrolyzed acid water for sterilization of the denture has several advantages when compared to the conventional denture cleaning and sterilization. It costs very little and takes a reasonably short time for sterilization treatment causing no measurable adverse effects. Furthermore, the acid water has a unique feature that it is neutralized to be plain water with time and hence there is little fear of drainage contamination, while discharge of the waste denture cleaner and disinfectant consisting of chemicals may have a risk of environmental contamination through the drainage water. Owing to these advantages, it can be declared that the acid water is much superior to the denture cleaner and chemical disinfectants in rapid, safe and certain sterilization of the denture base.

It is one of the problems, however, that the bactericidal activities of the electrolyzed acid waters may be rapidly reduced by the presence of protein or organic substances. Thus in the next report, their bactericidal efficiencies will be discussed for the denture base used in service as compared with those of the conventional denture cleaner and chemical disinfectant.
ACKNOWLEDGMENT

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REFERENCES


