Bactericidal efficacy of glycine-type amphoteric surfactant as a denture cleaner and its influence on properties of denture base resins

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The bactericidal efficacy of 1.00–4.50% glycine-type amphoteric surfactant (Gly) was evaluated by measuring its microorganism removal rate in denture plaque. Physical and mechanical properties such as surface roughness, color difference, and bending strength of two different denture base resins were determined before and after cleaning in Gly solutions, a commercial denture cleaner, and tap water. The microorganism removal rates of all the Gly solutions were higher than those of a commercial enzymatic denture cleaner (Polident) (p>0.05). The removal rate of Candida spp. by Polident was not significantly different from the removal rate using water. Changes in the surface roughness and color difference among the specimens were slight. There were no significant differences in the bending strengths of the two resins for all concentrations of Gly solution (p>0.05). These results suggested that glycine-type amphoteric surfactant solution may be effective as a denture cleaner in conjunction with an ultrasonic cleaning device.

Keywords: Resin, Surfactant, Microorganism

INTRODUCTION

In the growing ranks of the elderly population, oral care is important for prevention not only of caries and periodontal disease, but also of systemic diseases such as aspiration pneumonia1,2. In particular, poor denture hygiene results in the accumulation of debris and bacterial plaque on the surface of prostheses, causing malodor and inflammatory changes to the adjacent mucosa3. For elderly patients who are susceptible to infection, a poorly cleaned oral cavity or prosthesis may induce fatal infections such as aspiration pneumonia and endocarditis4. Bacteria causing aspiration pneumonia, opportunistic infections, and endocarditis are known to occur on the dentures and the pharynx of the dependent elderly; and it is thought that the denture may act as a reservoir for these bacteria4. Yoneyama et al. reported that oral care lowered the risk of pneumonia in the institutionalized elderly5. Therefore, maintaining denture cleanliness is very important for elderly patients whose resistance to infection is lowered. Currently, many kinds of denture cleaners are available; however, their efficacy as sterilization or disinfection agents is unclear6. The current expectation is for cleaning agents to clean simply and effectively, with no risk to human health, and with no adverse effects on the properties of the denture material7. As most denture materials are porous acrylic resins, water is easily absorbed, so that when denture hygiene is neglected, the denture becomes stained and unpleasant-smelling, with resulting bad breath8,9. While the denture is being worn, bacteria easily proliferate between the mucous membrane and the denture10, and the denture plaque containing bacteria adheres more easily to a denture surface that is scratched due to an incorrect cleaning method11. We use Raverrack D (Sundental, Osaka, Japan) as the denture cleaner in our hospital. Raverrack D is composed of 2% sodium hypochlorite with a glycine type amphoteric surfactant (polyoctylpolyaminoethylglycine), and is known to be highly efficacious in removing stains, organic substances and tartar. Its bactericidal efficacy is also excellent because of its high removal rate of denture plaque. Furthermore, Raverrack D has a relatively insignificant influence on physical and mechanical properties such as surface roughness, color difference, and bending strength12. However, Raverrack D cannot be used as a home denture cleaner because it contains 2% sodium hypochlorite. It is unclear whether commercial denture cleaners containing only glycine-type amphoteric surfactant are currently available.

In this study, we investigated the bactericidal efficacy of glycine-type amphoteric surfactant
(polyoctylpolyaminoethylglycine) against oral microorganisms, and its effect on physical and mechanical properties such as bending strength, surface roughness, and color difference of self-cured and heat-cured denture base resins, and discussed the viability of simple glycine-type amphoteric surfactant as a denture cleaner.

MATERIALS AND METHODS

Microbiological evaluation

The subjects of this study were 90 patients of the Denture Prosthodontics Restoration, Advanced Dentistry Center, Kagoshima University Medical and Dental Hospital. The 90 subjects all wore complete resin dentures containing overdenture with no metal attachments, and all subjects were satisfied with their dentures and cleaned the dentures themselves. The subjects were divided into six groups according to cleaning agent: glycine-type amphoteric surfactant (Toho Chemical Industry, Tokyo, Japan) diluted to 4.50%, 2.25%, 1.50%, 1.00%, tap water and a commercial enzymatic denture cleaner (Polident, Glaxo Smith Kline, England) (Table 1). Dentures being cleaned in Gly solution and tap water, and Po were subjected to ultrasonic cavitation (Branson yamato 3200, 47 kHz) and immersed, respectively, for 15 min each. After cleaning, the dentures were rinsed in tap water.

The denture was removed and the surface carefully dried by air syringe to avoid contamination by saliva. Specimens of microorganisms were collected before cleaning (right side) and after cleaning (left side) by rubbing a sterile swab (Fukifuki check 2, Eiken Kagaku, Tokyo Japan) over the denture plaque on the mucosal surface of the upper or lower denture. The samples were immediately transported to the laboratory for identification of microorganisms by standard culture methods. Specimens were inoculated onto sheep’s blood agar plates, chocolate agar plates, BTB lactose agar plates, and Chrom agar Candida plates for aerobic organisms (Table 2). The plates were incubated at 37°C for 48 hours under aerobic conditions, and the microorganisms were identified by standard methods. To determine the cleaning effect, the microorganism removal rate was calculated using the following equation:

\[
\text{Removal rate (\%) } = \frac{(C_{\text{before}} - C_{\text{after}})}{C_{\text{before}}} \times 100 \quad (1)
\]

<table>
<thead>
<tr>
<th>Code</th>
<th>Cleaning solution</th>
<th>Composition</th>
<th>Cleaning condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>4.50 % Gly solution</td>
<td>Amphoteric type surfactant</td>
<td>Ultrasonic cleaning, 15 min</td>
</tr>
<tr>
<td>2.25</td>
<td>2.25 % Gly solution</td>
<td>(Polyoctylpolyaminoethylglycine)</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>1.50 % Gly solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.00 % Gly solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>Tap water (pH 6.9)</td>
<td></td>
<td>Ultrasonic cleaning, 15 min</td>
</tr>
<tr>
<td>Po</td>
<td>Polident</td>
<td>Enzyme, Surfactant</td>
<td>50 °C, warm water</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150 ml, immersion, 15 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Incubate condition</th>
<th>Time</th>
<th>Isolated microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep’s blood agar plate</td>
<td>Aerobic condition, Carbon dioxide 37 °C</td>
<td>48 hr</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Chocolate agar plate</td>
<td>Aerobic condition, Carbon dioxide 37 °C</td>
<td>48 hr</td>
<td>Bacteria</td>
</tr>
<tr>
<td>BTB lactose agar plate</td>
<td>Aerobic condition, 37 °C</td>
<td>48 hr</td>
<td>Enterobacteria</td>
</tr>
<tr>
<td>Chrom agar Candida plate</td>
<td>Aerobic condition, 37 °C</td>
<td>48 hr</td>
<td>Candida spp.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Code</th>
<th>Heat</th>
<th>Self</th>
</tr>
</thead>
<tbody>
<tr>
<td>Products</td>
<td>GC Acron</td>
<td>Tokuyama Rebase 2 normal</td>
</tr>
<tr>
<td>Composition</td>
<td>Monomer</td>
<td>Methylmethacrylate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polymer</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>Curing condiiton</td>
<td>100 °C, 40 min</td>
<td>Room temperature, 15 min</td>
</tr>
</tbody>
</table>
where $C_{\text{before}}$ represents the colony forming units per ml (CFU/ml) before cleaning, and $C_{\text{after}}$ represents CFU/ml after cleaning.

**Physical and mechanical properties evaluation**

Two types of denture base resins (heat-cured and self-cured acrylic resins) were used to evaluate the effects of cleaners on the physical properties of the resins (Table 3). Each material was manipulated according to the manufacturers’ instructions. The resin was cured in Teflon molds to maintain dimensions of $35 \times 75 \times 2$ mm, and the plates were then cut to $35 \times 10 \times 2$ mm. Thirty specimens of each resin type were prepared and polished with 800, 1000, 1200 and 1500 grit abrasive papers, and big silicone points (Exa Technik R10a, Yoshida), and then buffed with rouge (Resin Poli, Misu Chemical Laboratory). The width and thickness of each specimen were measured with a digital caliper. The specimens were stored in water for 24 hours at 37ºC to release residual monomer.

Glycin-type amphoteric surfactant (Gly) diluted to 4.50, 2.25, 1.50, 1.00 %, tap water, and commercial product of enzymatic denture cleaner (Polident, Glaxo Smith Kline, Po) were used as cleaners (Table 1). Specimens being cleaned in Gly solution and tap water, and Po were subjected to ultrasonic cavitation and immersed, respectively, for 15 min each. The specimens being measured for surface roughness and color difference were cleaned at 10, 30 and 60 times, and those being measured for bending strength were cleaned 60 times.

The average surface roughness ($R_a$) of the specimens was measured using a surface profilometer (Surfcom 130A, Accretech, Tokyo, Japan), which scanned a sample length of 2.4 mm at 0.6 mm/s, and cut-off value of 0.8 mm before and after cleaning.

The color of the specimens was measured using a color meter (Shade Eye NCC, Shofu, Kyoto, Japan). Color difference ($\Delta E$) was calculated using the following equation:

$$\Delta E = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

where $L^*$, $a^*$, and $b^*$ represents lightness, redness-greenness, and yellowness-blueness, respectively, and $\Delta L^*$, $\Delta a^*$, and $\Delta b^*$ represent the differences between $L^*$, $a^*$, and $b^*$ values of the specimens before and after cleaning.

After cleaning 60 times, specimens were tested for bending strength using a universal testing machine (Model 1310 NW, Aikoh Engineering, Osaka, Japan) at a crosshead speed of 5 mm/min and span length of 20 mm. Five specimens for each solution were measured for physical and mechanical properties.

Microscopic observation was conducted on specimens of both resin types before and after 60 times cleaning using a scanning electron microscope (SEM, JSM-5510LV, JEOL, Tokyo, Japan) operating at an accelerating voltage of 20 kV.

**Statistical analysis**

The results were analyzed by a two-way or three-way analysis of variance (ANOVA) and multiple comparisons were performed using the Tukey post-hoc test ($\alpha=0.05$). All analyses were computed with the SPSS for Windows operating system (SPSS 14.0 j, SPSS Inc, Chicago, IL, USA.).

**RESULTS**

*Microorganism removal rate*

Fig. 1 shows the detection rates for aerobic microorganisms in denture plaque. The predominant aerobic organisms on dentures were *Streptococcus* spp. (detection rate: 95.5%), *Candida* spp. (54.5%),...
The microorganism removal rates of each cleaning solution for total microorganism, Streptococcus spp., Neisseria spp., and Candida spp. are shown in Fig. 2. The removal rates for the 2.25%, 1.50% and 1.00% Gly solutions were all higher than 90%. The removal rate in tap water was significantly lower than that of all the Gly solutions against any microorganism ($p<0.01$). There were significant differences between Po and the 4.50% and 2.25% Gly solutions ($p<0.05$), but there was no significant difference between tap water and Po for Candida spp. ($p>0.05$).

### Physical and mechanical properties

Fig. 3 shows the surface roughness of the denture base resin after cleaning in various solutions. There were significant differences between denture base resin, concentrations of solution, and cleaning time as shown in Table 4 ($p<0.01$). Although the change was small, the surface roughness of the heat-cured resin cleaned in 4.5% Gly solution was significantly higher than those cleaned in other solutions and Po ($p<0.01$). The surface roughness of the self-cured denture base resin increased with cleaning times ($p<0.01$).

Fig. 4 shows the color difference of denture base resins after cleaning in various solutions. There were significant differences between denture base resins, concentrations of solution, and cleaning times as shown in Table 5 ($p<0.01$). The color difference of the heat-cured denture base resin was significantly different between the solution and the cleaning times ($p<0.01$). In the self-cured resin, there was a significant difference between the solutions ($p<0.01$), but no significant difference between cleaning times ($p>0.05$). However, all the values were less than 1.0, indicating that the color changes were slight according to the ISO ranking.

Fig. 5 shows the three-point bending strength of the denture base resins after cleaning in various solutions. There was no significant difference among the different Gly solutions ($p>0.05$), although there was significant difference between the heat-cured
Table 5  Result of 3-way ANOVA for color difference of denture base resins after cleaning in various solutions

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denture base resin (A)</td>
<td>1</td>
<td>1.717</td>
<td>75.340</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Concentration of solution (B)</td>
<td>5</td>
<td>0.167</td>
<td>7.325</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cleaning time (C)</td>
<td>2</td>
<td>0.266</td>
<td>11.687</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>A × B</td>
<td>5</td>
<td>0.017</td>
<td>0.756</td>
<td>0.583</td>
</tr>
<tr>
<td>A × C</td>
<td>2</td>
<td>0.143</td>
<td>6.262</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B × C</td>
<td>10</td>
<td>0.010</td>
<td>0.445</td>
<td>0.922</td>
</tr>
<tr>
<td>A × B × C</td>
<td>10</td>
<td>0.019</td>
<td>0.847</td>
<td>0.584</td>
</tr>
<tr>
<td>Error</td>
<td>141</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4  Color differences of heat- and self-cured denture base resin after 10-, 30-, and 60-time cleaning in 4.50, 2.25, 1.50, and 1.00 % Gly solution, tap water, and Polident.

Fig. 5  Three-point bending strength of heat- and self-cured denture base resin after 60 times cleaning in 4.50, 2.25, 1.50, and 1.00 % Gly solution, tap water, and Polident.
and self-cured denture base resins (p<0.01) (Table 6).

Fig. 6 shows the SEM images of the surface of the specimen before and after cleaning 60 times in 4.50% Gly solution. The surface of both resin types before cleaning shows many scratch lines due to the polishing. After cleaning 60 times, both resin types are significantly roughened. Many voids can be seen around the scratches; the voids of the self-cured resin are larger than those of the heat-cured resin.

**DISCUSSION**

Amphoteric surfactant is used for the disinfection of instruments, and is known to have strong bactericidal efficacy\(^{13}\). Amphoteric surfactant reduces the boundary tension of the bacterial cell membrane surface, and causes damage to the cell membrane and denaturation of the protein. The pH of amphoteric surfactant ranges from neutral to alkaline, but its toxicity is low. It possesses the washing action of anionic surfactant and the bactericidal action of cationic surfactant. The structure of the glycine-type amphoteric surfactant (polyoctylpolyaminoethylglycine) used in this study is:

\[(\text{NH}_2\text{CH}_2\text{CH}_2\text{)}_2 \text{– NH – CH}_2 \text{– COO}^-]\]

This surfactant is a neutral (pH 5.8-6.5), clear liquid, soluble in water, and lemon yellow in color. It is effective against bacteria, fungi, and *Mycobacterium tuberculosis*, but not against viruses and spores. The bactericidal activity of this surfactant is higher than general disinfectants such as chlorhexidine and benzalkonium chloride.

The present study demonstrated that Gly...
solution has excellent bactericidal efficacy. Microbiological evaluation indicated that Gly was more effective for removing microorganisms in denture plaque than Po. An ultrasonic device was used for cleaning with the Gly solution and tap water, but not for cleaning with Po. The study of Palenik et al. using an ultrasonic device and tap water alone for cleaning demonstrated that the cleaning action was related to sonic cavitation alone, and not to special chemicals. Use of ultrasonic devices has been reported to reduce cleaning time by 1/420 to 2/3. However, in the present study, the removal rate using tap water was lower than for every Gly solution. These results suggest that Gly solution may be highly effective in removing microorganisms in denture plaque. The removal rate for Candida spp. was lower for Po (without an ultrasonic device) than for tap water (with an ultrasonic device). Nikawa et al. reported that Po was less effective as fungicide on C. albicans for relatively short cleaning periods (10 to 15 minutes), but its efficacy improved with a longer incubation period of 2 hours. These findings suggest that mechanical cleaning such as brushing and ultrasonic cleaning, was more effective for removal of Candida spp. than simple chemical cleaning. This may be because denture plaque forms a drug resistant biofilm on the denture surface. If the biofilm is not broken, the chemical agent does not come into contact with the microorganisms in denture plaque. It has been reported that meticulous brushing in tap water is effective in removing artificial discolorations and plaque from acrylic resin dentures. He et al. reported that heat-cured resins in general tended to attract significantly lower numbers of yeast organisms than self-cured resins. For this reason, dentures should be made using heat-cured resins.

Denture cleaners need to be tested for their effects on the properties of denture base resins as well as for their bactericidal efficacy. Our assessment of the effects of Gly solution on the physical and mechanical properties of denture base resins revealed that the surface roughness increased by 0.02–0.05 μm after cleaning. This slight increase in roughness seems to be due to chipping of the polymer particles by ultrasonic cavitation as shown in Fig. 6. The surface roughness of self-cured resin was greater than heat-cured resin after cleaning, possibly because of the larger voids that formed due to the size, distribution and hardness of the polymer particles. Otake et al. reported that denture base resin was eluted by denture cleaners and decreased in weight. Therefore, our findings suggest that a component of the denture base resin is eluted, and polymer particles at the site of the elution are chipped by ultrasonic cavitation; self-cured denture base resin is eluted to a greater degree than heat-cured resin because of its lower degree of polymerization.

Radford et al. reported that C. albicans adheres less to smooth surfaces than to rough surfaces. In the present study, the 4.50% Gly solution produced the greatest roughness (0.05 μm) and it was reported that the surface roughness of real denture used for a long time was 0.15–0.20 μm, but the microorganism removal rate was 100%. These results indicate that surface roughness of 0.05 μm does not decrease the cleaning effect. Moreover, saliva in the real oral cavity would reduce adhesion of C. albicans. The data related to the effects of cleaners on color difference indicated that color difference at all concentrations was less than 1.0 and was therefore negligible. Aysun et al. reported that the greatest whitening effect was observed in self-cured resins, but the difference between heat-cured and self cured resins was statistically insignificant. In the present study, there was a significant difference in color difference between Po and the Gly solutions. However, the reason for this was that the surface of the self-cured resin became rougher than that of the heat-cured resin. The data relating to bending strength indicated that there was no significant difference between resins before cleaning and after cleaning 60 times. However, Yamaki et al. reported that the bending strength was influenced by the temperature of the solution, time, and the thickness of the specimens. In our study, the bending strength was unaffected by cleaning time.

These results revealed that the Gly solution has an excellent bactericidal efficacy against microorganisms and is almost ineffective on properties of denture base resin. Then, it is concluded that glycine type amphoteric surfactant is available as denture cleaner in conjunction with ultrasonic device.

CONCLUSION

The present study demonstrated that the 4.50% Gly solution exhibited the greatest bactericidal efficacy, although the difference was not statistically significant. Gly solutions did not affect the bending strength of denture base resins, and had little effect on their surface roughness and color difference. Therefore, we suggest that a simple Gly solution should be considered as a denture cleaning agent in conjunction with an ultrasonic cleaning device.

REFERENCES

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