The aim of this study was to examine the bactericidal effects and bactericidal time of an acidified sodium chlorite compound gel (ASC-Gel) on bacteria isolated from the peri-implant sulci of 10 patients who received implants 3–27 years previously, and the depth of each peri-implant sulcus was 5 mm or less. Porphyromonas gingivalis (ATCC33277) was used as the control bacterium. Five ASC-Gel preparations were created by adding 3.3%, 5.0%, 7.0%, 9.0%, and 11.0% citric acid (CA) (condition a, b, c, d, and e, respectively) into an oral moisturizing gel containing sodium chlorite. The concentrations of chlorine dioxide (ClO₂) generated in ASC-Gel under conditions (a) to (e) were 12.1, 14.1, 17.2, 21.2, and 39.3 ppm, respectively. We examined the bactericidal effects of the 5 ASC-Gel preparations at volumes of 0.5, 1.0, and 2.0 mL, and measured the bactericidal time when 2.0 mL of ASC-Gel was used under condition (e). The bactericidal effects of ASC-Gel became significantly greater with increased concentrations of CA and ClO₂ and with increased usage (0.5–2.0 mL) of the gel. All bacteria were killed by using 2.0 mL of ASC-Gel under condition (e). ASC-Gel also needed between 45 and 90 minutes to kill all microbes under condition (e). Within the limits of the present investigation, these results suggest that ASC-Gel is useful as a chemical disinfectant against bacteria in the peri-implant sulcus. Further studies are also required to protect teeth, the surface of hydroxyapatite-coated implants, and the surrounding soft tissues from effects of chemical dissolution such as acid erosion due to the low pH of ASC-Gel.

**Key Words:** bactericidal effect, acidified sodium chlorite (ASC), chlorine dioxide (ClO₂), oral moisturizing gel, peri-implant sulcus

**INTRODUCTION**

Maintenance of good occlusal loading and infection control measures are crucial for the long-term success of implant prostheses. For the latter, management of the soft tissue around implants and of the remaining teeth is important. Treatment protocols that are based on the system of cumulative interceptive supportive therapy have 5 stages: (1) mechanical cleaning, (2) antiseptic therapy, (3) antibiotic therapy, (4) surgical approach, and (5) explantation. These protocols have been proposed to improve the condition of patients with peri-implantitis. Chemical cleanings have been reported to be as effective as mechanical cleaning. Citric acid (CA), chlorhexidine digluconate (CHX), and chlorine dioxide (ClO₂) have demonstrated efficacy as antiseptics in postoperative management of peri-implant pathogens. CHX-containing and ClO₂-containing mouthrinses have been used in the treatment of oral malodor and chronic atrophic candidiasis, and as an antibacterial agent against methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, a combination of in vitro chemical and mechanical cleaning, involving the use of an acidified ClO₂ mouthrinse supplemented with CA and ultrasonic cleaning of the implant fixtures, achieved bacterial elimination in 3 minutes. The acidified ClO₂ mouthrinse has demonstrated effec-
tiveness as a disinfectant, but the risk of accidental aspiration remains for the elderly.

In Japan, a rapidly aging society is developing, and some patients who received implant therapy require nursing care. For these patients, gel-type agents rather than water-soluble mouthrinse, would be easier and safer to apply for peri-implant tissue management. CHX-containing and ClO2-containing gels are commercially available as gel-type agents. In this study, we focused our attention on a ClO2-containing gel because ClO2, unlike CHX, has demonstrated antibacterial activity against bacterial biofilms. Few studies have demonstrated the bactericidal effects of ClO2-containing gels using evidence-based medicine.

The purpose of this study was to examine bactericidal effects and bactericidal times of acidified ClO2 gel on bacteria isolated from the peri-implant sulcus.

**Materials and Methods**

**Preparation of an acidified sodium chlorite gel**

In this pilot study, acidified sodium chlorite gel (ASC-Gel) was used as a disinfectant. Five ASC-Gel preparations were created by adding 1 mL of CA at 5 concentrations of 3.3%, 5.0%, 7.0%, 9.0%, and 11.0% (Yoshida Pharmaceutical Co Ltd, Tokyo, Japan) to 10 g of an oral moisturizing gel, the ClO2 Fresh gel (Pinemedical Co, Tokyo, Japan), which is composed of purified water, glycerin, carboxymethyl cellulose (CMC), polyethylene glycol, sodium chlorite, peppermint, and methylparaben; sodium chlorite was formulated at a concentration of 0.16% (available ClO2, 1000 ppm). After mixing sufficiently by stirring, the mixture was left standing for 30 minutes to allow the reaction to be constant. The mixtures were used as disinfectants under 5 conditions (a, b, c, d, and e, respectively).

**Measurements of the ClO2 concentrations, pH, and oxidation-reduction potential of ASC-Gel preparations under conditions (a) to (e)**

The ClO2 concentration generated by dissolving 5 g of ASC-Gel in 500 mL of purified water was measured using a hydrogen ion concentration indicator with glass electrodes for high viscosity (D-52), a pH electrode meter (9621-10D and 9677-10D), and a platinum electrode (9300-10D) (HORIBA, Kyoto, Japan). The tests were repeated twice, and the average values were determined as the ClO2 concentration and pH and ORP values of ASC-Gel disinfectants under each condition.

**Collection of bacteria and preparation of the bacterial solution for experimentation**

The experimental bacteria were collected from the peri-implant sulci of 10 systemically healthy men and women aged 61–79 years (mean 72.8 ± 5.8 years). Informed consent was obtained from each patient before sampling the experimental bacteria. Two sterile paper points were inserted into the deepest point of the peri-implant sulcus of each participant for 60 seconds, after which they were removed and suspended in 0.1 mL of sterilized saline solution. Immediately afterward, the suspensions were inoculated on a trypticase soy (TS) blood agar plate medium containing hemin and vitamin K by using an bacteria spreader. Subsequently, each bacterium was cultured in an anaerobic culture medium (Anaeromate-P, Nissui, Tokyo, Japan) for 48 hours at 37°C (anaerobic culture test).

From colonies that had the same color and shape on the culture medium for each participant, the best-growing colony was selected and used as the bacterium for experimental use. The bacteria isolated from the participants were as follows (Table 1): Streptococcus (No. 1, 4, 5, and 10), Peptostreptococcus (No. 9), Prevotella (non–black-pigmented anaerobic microbes) (No. 2 and 3), Eikenella (No. 7 and 8), and black-pigmented anaerobic rods (No. 6). Each of the isolated bacteria was placed in brain heart infusion (BHI) broth for anaerobic culture and used as the bacterial solution for experimentation. Porphyromonas gingivalis (ATCC33277), which is considered the most typical periodontal pathogenic anaerobic bacterium, was used as the control bacterium. Table 1 shows the characteristics of subjects and isolated bacteria.

**Bactericidal Effect Test**

In total, 0.03 mL of each experimental bacterial suspension isolated from each patient was added to tubes containing 5 mL of sterilized BHI broth. After 0.5, 1.0, and 2.0 mL of ASC-Gel under 5 conditions
(a) to (e) were immediately mixed, the tubes were inoculated anaerobically for 48 hours at 37°C. The presence or absence of bacterial growth was assessed visually from the turbidity of the broth. When the result was negative (ie, the broth was clear), 0.03 mL of bacterial suppression was taken from the incubated BHI broth and inoculated on sterilized TS blood agar plate medium for another anaerobic test (as described previously) to reexamine the bacterial growth. A series of this test was performed twice. The bactericidal effect was determined to be effective when no bacterial growth was found after both attempts.

**Bactericidal Time**

A 0.03-mL aliquot of each experimental bacterial suspension was added into tubes containing 5 mL of sterilized BHI broth. Subsequently, 2 mL of ASC-Gel under condition (e) was mixed sufficiently. Afterward, the tubes were inoculated anaerobically for 10, 20, 30, 45, 60, and 90 minutes at 37°C. When the result was negative (ie, the broth was clear), another anaerobic test by using sterilized TS blood agar plate medium was performed (as described above) to reexamine the bacterial growth. The bactericidal time of ASC-Gel was determined when the absence of bacterial growth was confirmed in an agar plate medium after a second anaerobic culture test.

**Statistical analysis**

The mean and standard deviation values of the generated ClO₂ concentrations, pH values, and ORP values for each added concentration of CA were assessed using Student t test. Statview software 4.02 (Abacus Concepts Inc, Berkley, Calif) was used for statistical analysis.

**RESULTS**

Table 2 shows the mean and standard deviations of the generated ClO₂ concentrations, pH values, and ORP values in ASC-Gel under conditions (a) to (e). When CA concentration increased, the ClO₂ concentration and ORP value increased, and the pH decreased. Table 3 shows the bactericidal effects of ASC-Gel on the isolated bacteria and *P. gingivalis* under 5 conditions (a–e). The bactericidal effect increased as the concentrations of CA and ClO₂ increased. The bactericidal effect of ASC-Gel was enhanced as its volume was increased from 0.5 to 2.0 mL. By using 0.5 mL of ASC-Gel, almost no bactericidal effects were observed under conditions (a) to (d); however, bactericidal effects were observed under condition (e). By using 1.0 mL of ASC-Gel, bactericidal effects were observed in more than half of the patients under condition (d), whereas under condition (e), bacteria excluding No. 6 were killed. Regarding the bactericidal effect of 2.0 mL of ASC-Gel, bacteria excluding No. 6, No. 10, and *P. gingivalis* were killed under condition (a). Under condition (e), all bacteria were killed.

Table 4 shows the bactericidal time for 2.0 mL of ASC-Gel. A bactericidal effect was observed after 20 minutes; however, at least 45 minutes were required for the effect in more than half of the
bacteria, including 60 minutes for *P. gingivalis* and 90 minutes for No. 6.

**DISCUSSION**

The results of this study revealed that a bactericidal effect occurred when the concentration of ClO2 in the ASC-Gel was 12.1 ppm (condition a) or higher, and the bactericidal effect became remarkable when the concentration of ClO2 was increased (Table 3). These results were similar to those obtained from the use of water-containing ASC solution on food-related bacteria (*Escherichia coli*).28 A ClO2 concentration of 39.3 ppm (condition e) or higher was necessary for bacterial elimination in all patients. Furthermore, the bactericidal effect of the ASC-Gel was influenced by the amount used and was greater in 2.0 mL than in 0.5 mL of ASC-Gel. The bactericidal time was 45–90 minutes when 2.0 mL of ASC-Gel was used.

For peri-implant tissue management, chemical cleaning (using an CA, pH 1.0, and a 35% phosphate gel, pH 1, for 1 minute) is effective because it does not cause damage to the interface between the gingival tissue around the implant and the abutment.3,4,29 In addition, CHX-containing mouthwash and gel and hyaluronic acid gel exhibit bactericidal effects.12,13,30 Mouthrinses containing ClO214,15,31 or

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**TABLE 2**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CA (%)</th>
<th>ClO2 (ppm)</th>
<th>pH</th>
<th>ORP (mV)</th>
</tr>
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<tr>
<td>a</td>
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<td>4.26±0.01</td>
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<td>5.0</td>
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<td>9.0</td>
<td>21.2±0.4</td>
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<tr>
<td>e</td>
<td>11.0</td>
<td>39.3±1.5</td>
<td>3.65±0.02</td>
<td>648±3.1</td>
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</tbody>
</table>

†ClO2 indicates chlorine dioxide; ORP, oxidation-reduction potential; ASC-Gel, acidified sodium chlorite Gel; CA, citric acid. *P < .05; **P < .01.

**TABLE 3**

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<th>Condition</th>
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<th>3</th>
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<th>5</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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*+* indicates positive; –, negative
†See Table 2 for description of conditions (a) to (e).
ASC\textsuperscript{19,22,24,32} are known to inhibit the growth of plaques and exhibit bactericidal effects against bacterial biofilms.\textsuperscript{31,32} Therefore, ASC-Gel can be considered a chemical cleaning tool for soft tissue management around implants, especially for patients requiring nursery care. However, the results of this study cannot be compared with those of previous studies because the other studies did not use bacteria isolated from peri-implant sulci.

It has been reported that the bactericidal effects of acidified ClO\textsubscript{2} solution after the addition of CA or malic acid were greater than those of ClO\textsubscript{2} containing solution alone.\textsuperscript{15,26,32} The results obtained in these studies are similar to those obtained in the present study.\textsuperscript{15,26,32} The ORP value was 57 mV higher under condition (e) than under condition (a). It has been reported that low ORP values of \(-100\) mV were one of the conditions for the growth of anaerobic bacteria in old dental plaques (which show a tendency of being deoxidized), whereas the ORP value was \(+200\) mV in saliva immediately after cleaning the mouth.\textsuperscript{33} Our findings are in agreement with those previous findings. It was demonstrated that there is a positive relation between ClO\textsubscript{2} concentration and ORP value.\textsuperscript{34} When an acid with low pH is added, the available concentration of ClO\textsubscript{2} increases, reduces odor by acting on odiferous bacteria and malodor-causing substances, and increases the bactericidal effects.\textsuperscript{35,36} The pH values of the ASC-Gel used in our pilot study were as low as 3.65–4.26 (Table 2) and appeared to have contributed to the bactericidal effect, whereas the pH in the investigations by Zablotsy et al\textsuperscript{3} and Strooker et al\textsuperscript{29} was 1.0. Meanwhile, much attention has to be paid to the risk of erosion or decalcification of teeth, the surface of hydroxyapatite-coated implants, and the surrounding soft tissues.

Forty-five to 60 minutes were required for 2.0 mL of highly concentrated ASC-Gel (39.3 ppm) under condition (e) to kill the bacteria isolated from the peri-implant sulcus without inflammation (pocket depth \(\leq 4\) mm). These results were similar to those of a report according to which the duration of the bactericidal effect of a 600-ppm ClO\textsubscript{2} solution examined in 7 bacterial species was a maximum of 60 minutes for MRSA.\textsuperscript{36} However, 90 minutes were required to eliminate bacterium No. 6 that was isolated from an inflamed peri-implant sulcus (pocket depth \(\geq 5\) mm). Sixty minutes were required to eliminate \textit{P. gingivalis} (control bacterium) (Table 4). In other words, very active black-pigmented anaerobic rods might exhibit resistance to highly concentrated ASC-Gel. The other factors responsible for these findings are presumed to be as follows: (1) the available ClO\textsubscript{2} in ASC-Gel was surrounded by CMC and glycerin, and therefore, ClO\textsubscript{2} was no longer in direct contact with the bacteria; and (2) the CMC in the gel acted as a reducing agent against ClO\textsubscript{2} and thereby inhibited or decreased its oxidizing power.

ASC-Gel is more effective in prolonging the duration of the bactericidal effect than mouthrinses containing ClO\textsubscript{2},\textsuperscript{22,23} but to accelerate the time of onset of the bactericidal action,\textsuperscript{13} the concentration of CA must be increased.\textsuperscript{26} Therefore, to increase the fast-acting property of ASC-Gel, the buffer action of CMC on ClO\textsubscript{2} has to be improved. In addition, attention is required to properly handle highly concentrated ASC-Gel under condition (e). In the future, improvements in materials are required to reduce the damage caused by CA on the surface.

<table>
<thead>
<tr>
<th>Time (min)</th>
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<th>P. gingivalis</th>
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<td>60</td>
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</tr>
<tr>
<td>90</td>
<td>-</td>
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</table>

\*+ indicates positive; −, negative
of teeth, hydroxyapatite-coated implants, and surrounding soft tissues.

**Conclusions**

In this study, the bactericidal effects of ASC-Gel increased as its volume increased (from 0.5 to 2.0 mL). By using 2.0 mL of ASC-Gel under condition (e), it was able to kill all the bacteria isolated from the peri-implant gingival sulcus and *P. gingivalis* because of the interactions between the concentrations of CA (11.0%) and ClO₂ (39.3 ppm). Therefore, ASC-Gel appears to be a prospective chemical cleaning agent for dental use. However, 45–90 minutes were required for its bactericidal effects. Further studies are required to improve the fast-acting property of ASC-Gel and to protect teeth, implants, and soft tissues from chemical damage such as acid erosion.

**Abbreviations**

ASC-Gel: acidified sodium chlorite gel  
BHI: brain heart infusion  
CA: citric acid  
CHX: chlorhexidine digluconate  
ClO₂: chlorine dioxide  
CMC: carboxymethyl cellulose  
Gel: ClO₂ Fresh gel  
MRSA: methicillin-resistant *Staphylococcus aureus*  
TS: trypticase soy

**Acknowledgments**

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**References**


