Microbicidal effect and storage stability of neutral HOCl-containing aqueous gels with different thickening/gelling agents

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Electrolyzed waters, containing mainly hypochlorous acid, are used in dental practice because of their high microbicidal effect. For wider use, three neutral electrolyzed water-based gels, namely, HOCl-containing aqueous gels were prepared with a thickening/gelling agent in this study. We evaluated their microbicidal effects against four strains and storage stabilities indicated by available chlorine concentration. Immediately after preparation, all gels (70 ppm) could completely remove microbes by a 3-min treatment. The gel prepared with xanthan gum remarkably reduced its available chlorine concentration even under shaded and refrigerated storage conditions, failing to maintain its microbicidal effect following 1-day storage, whereas other gels, prepared with carboxyvinyl polymer or agar, maintained effective concentration (>20 ppm), with high microbicidal effects following 9-day and 21-day storage, respectively. Neutral electrolyzed water-based gels might be useful to remove oral microbes. Based on our results, agar is the most suitable thickening/gelling agent from the viewpoint of storage stability.

Keywords: Neutral HOCl-containing aqueous gels, Neutral electrolyzed water, Storage stability, Thickening/gelling agent, Microbicidal effect

INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) has heightened the importance of infection control in homes and schools, as well as in medical institutions and facilities for the elderly. As a countermeasure against COVID-19, electrolyzed waters containing mainly available chlorine can be used to clean and disinfect surfaces, such as door knobs, as they inactivate the novel coronavirus (SARS-CoV-2)⁹-²⁰. In clinical dentistry, these electrolyzed waters have been widely used for the prevention of nosocomial infections via dental instruments, impressions, and prostheses because of their wide-range antimicrobial spectrum²¹-²⁸ and high microbicidal effect as a result of hypochlorous acid (HOCl) and hypochlorite ion (OCl⁻) in free available chlorine. Most electrolyzed waters are prepared by the electrolysis of aqueous sodium chloride (NaCl) solutions, a raw material, which returns to a dilute aqueous NaCl solution on losing its microbicidal activity⁹,¹⁰. Therefore, they are less likely to affect the human body and environment than are common disinfectants¹¹,¹². In addition, due to their high biological safety¹³-¹⁴, they are highly effective as mouthwashes¹⁵,¹⁶ and for root canal cleaning¹⁷,¹⁹, sterilization of periodontal disease-causing bacteria⁶, and cleaning and disinfection of wound sites²⁰.

Regarding several electrolyzed waters containing mainly HOCl, we reported their storage stability²¹, corrosion behaviors²², and their ability to remove bacteria from root canals¹⁹, dental instruments²³, and prostheses²⁰. Based on our results, neutral electrolyzed water (NW) might be the electrolyzed water that is most acceptable for clinical use because of its high storage stability²¹ and lower corrosive effect on metals²², showing equivalent bactericidal activity²¹,²²,²⁴,²⁵ while disinfecting denture bases²⁶, impressions²⁶-²⁸, and impression trays²⁷. In our previous study, we reported that strongly acidic electrolyzed water showed marked decalcification, and the weakly acidic type showed slight decalcification following a 1-week immersion, whereas the neutral type showed no harmful effects on human enamel²⁹. From our research results so far, we recommend the use of NW to prevent infection and improve the oral environment.

Similar to other electrolyzed waters, NW, which is in the liquid state, is used for washing and wiping to remove microbes. In the form of a gel or steam, it is easier to work with and finds applications in various treatments. An electrolyzed acidic gel (pH<2.7) prepared by the electrolysis of aqueous glycerin solution had been reported by Ito et al. in 2002, and its bactericidal activity against gram-negative bacteria was reported by Bucko et al. in 2004. In 2015, Bucko et al.³¹ reported that a HOCl gel for postprocedural treatment and scar prevention (pH: 5.2–7.8, 120 ppm), which was prepared by combining a modified silicon oil and stabilized HOCl, shows higher microbicidal and antibiofilm properties, in addition to potency as a topical wound healing agent superior to current regimes based on results of their clinical study. In the present study, we focused on gels for dental use and aimed to easily prepare neutral HOCl-containing aqueous gels by using HOCl water for oral care, which
have high antimicrobial activity and are safe without causing adverse effects on accidental ingestion. Aqueous solutions, such as electrolyzed waters, which contain available chlorine (HOCl and OCl\(^{-}\)), greatly differ in pH and the concentration of available chlorine, which are the microbicidal components, depending on the method of preparation, derived based on the principle of the apparatus. In aqueous solutions containing available chlorine, species and their fractions differ depending on the pH values, and their properties differ depending on the storage condition\(^{21}\). For each electrolyzed water and other available chlorine-containing aqueous solutions, it is important to carry out a detailed examination of two chemical properties, pH and available chlorine concentration, which indicate the efficacy of the microbiological properties of the prepared gel.

For wider use in dental practice, we prepared a prototype of a semisolid NW-based gel (pH, ~7; available chlorine concentration, ~70 ppm), namely, HOCl-containing aqueous gels and reported that the gel enhanced root canal cleaning and bactericidal effects, following 5-min contact on irrigating the root canals with NW\(^{19}\). The gel increases the contact time with the treated surface, thereby enhancing sterilization with prolonged bactericidal action. Thus, the previous report concluded that further use in the root canal after irrigation with NW enhanced the bactericidal effect\(^{19}\). We aimed to prepare NW-based gels for not only root canal cleaning but also other applications such as oral care for the elderly requiring nursing care to improve their oral hygiene (e.g., gels with microbicidal effect for cleaning natural tooth and oral mucosa). In this study, the NW-based gels were prepared by our original methods that were improved the previous reported gel for root canal cleaning\(^{19}\).

For the preparation of the gel with our recommended NW, NW containing a larger amount of available chlorine such as HOCl and OCl\(^{-}\) (NW\(_{\text{high}}\)) than does conventionally prepared NW (NW\(_{35}\); standard concentration, 30–40 ppm) was used. Thus, the preparation methods were modified to compensate for the decrease and/or deactivation of available chlorine expected due to thickening/gelation by increasing their concentration in the electrolyzed water, which was the main raw material. Considering the biosafety of gels, we chose three components used in foods, cosmetics, and medical materials as thickening/gelling agents. In this study, the microbicidal effects and chemical properties of NW-based gels, which were prepared with one of three thickening/gelling agents, namely xanthan gum (XT), carboxyvinyl polymer (CB), and agar (AG), were compared to determine the most suitable thickening/gelling agent and to consider the applicability for removing oral microbes in dental practice.

### MATERIALS AND METHODS

#### Preparation of NW-based gels

Three types of NW-based gels were prepared with each thickening/gelling agent and other materials by

| Material (Molecular weight) | Code | Product/Apparatus | Manufacturer |
|---------------------------|------|-------------------|--------------|
| Xanthan gum               | XT   | Xanthan gum powder| MP Biomedicals (Solon, OH, USA) |
| Carboxyvinyl polymer      | CB   | Hiviswako 104     | Wako Pure Chemical (Osaka, Japan) |
| Agar                      | AG   | Agar powder       | Asahi (Kawasaki, Kanagawa, Japan) |
| High-concentration electrolyzed water\(^{a2}\) | NW\(_{\text{high}}\) | AP aqua21\(^a\) | Asahi Pretec (Kobe, Hyogo, Japan) |
| Neutral electrolyzed water\(^{a3}\) | NW\(_{35}\) | AP aquaNeoEX\(^a\) | Asahi Pretec |
| Sterile distilled water   | DW   | —                 | —            |
| pH adjuster\(^{a4}\)      | KOH  | Potassium hydroxide| Wako Pure Chemical |

\(^{a1}\): Typical value of molecular weight after thickening/gelation based on references\(^{32-34}\).

\(^{a2}\): Electrolyzed water showing a high available chlorine concentration. It was prepared by the apparatus with partial modification by the manufacturer at the desired pH (7–8) and concentration (800–1,200 ppm) in this study.

\(^{a3}\): Electrolyzed water prepared by normal operation of the apparatus (pH: 7, available chlorine concentration: 30–40 ppm). It is theoretically equal to NW\(_{\text{CL}}\) (CL=30–40) which was prepared to 30–40 ppm with NW\(_{\text{high}}\) and DW.

\(^{a4}\): 10% aqueous KOH solution was used.

\(^{a}\): NW apparatuses on the same principle with both diaphragmless electrolysis and diaphragmatic electrolysis.
For each type of thickening/gelling agent, seven kinds of gel with varying available chlorine concentration (0, 10, 15, 20, 30, 50, and 70 ppm) were prepared for microbiocidal effect tests described below (TEST 1) to compare between the types of gels and with NWs of the same concentration prepared as test waters (NWCL, CL: 0, 10, 15, 20, 30, 50, and 70 (ppm)). Subsequently, each type of test gel/water prepared with an available chlorine concentration of 70 ppm (XTJ70, CBJ70, and AGJ70; Fig. 3) was examined for changes in chemical properties, pH, and available chlorine concentration (TEST 2) and microbiocidal effects during storage as shown below (TEST 3).

To prevent contamination, each test gel/water was prepared using sterile distilled water (DW) and the devices (spatula, magnetic stirrer and containers for mixing and storage) which were sterilized by autoclaving with a high-pressure steam sterilizer (BS-245, TOMY SEIKO, Tokyo, Japan) or disinfected with a 70–80 vol% aqueous solution of ethanol (FUJIFILM Wako Pure Chemical, Osaka, Japan).
Preparation for microbicidal effect tests (TEST 1 and TEST 2)

Four standard microbial strains were tested to examine the microbicidal effects of the test gels/water: Staphylococcus aureus NBRC12732 (S. aureus), Streptococcus mutans NBRC13955 (S. mutans), Enterococcus faecalis NBRC100481 (E. faecalis), and Candida albicans NBRC100481 (C. albicans). Each microbial suspension was obtained from single colony isolation on an agar plate and inoculated in overnight microbial suspension was obtained from single colony isolation on an agar plate and inoculated in overnight broth cultures at 37°C. Three bacterial strains were grown in a brain-heart infusion (BHI; Becton, Dickinson broth cultures at 37ºC. Three bacterial strains were grown in a brain-heart infusion (BHI; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) broth, and C. albicans were grown in a yeast and mold (YM; Becton, Dickinson and Company) broth. Only S. mutans were anaerobically cultured; the other three microbes were aerobically cultured. For each strain, bacteria or fungi of the resultant cultures were collected, washed twice with sterile phosphate-buffered saline (PBS; AS ONE, Osaka, Japan), centrifuged at 3,000 r.p.m for 15 min, and subsequently resuspended in fresh PBS to prepare approximately 2×10⁷ colony forming units (CFU)/mL.

TEST 1. Microbicidal effect test to examine the relationship between available chlorine concentration and microbicidal activities

Immediately after the preparation of the seven types of gels with varying concentrations for each thickening/gelling agent (XTJ, CBJ, and AGJ), i.e., a total of 21 test gels, 9.0 mL of the gel was added to 1.0 mL of bacterial or fungal suspension while repetitive pipetting using a sterile glass pipette and vortexed for 3 min using a vortex mixer (Thermolyne Maxi Mix II Vortexer, Barnstead Thermolyne, Dubuque IA, USA) to examine its microbicidal effect. Test gels were colorless and transparent (CBJ) or translucent white (XTJ, AGJ), and were different in color from the microbial suspensions (brown or pale yellow). Therefore, it could be roughly confirmed with the naked eye that each gel and the microbial suspension were mixed almost uniformly in the process of repetitive pipetting and vortex mixing. After treatment, a total of 0.1 or 1.0 mL of gel, which was collected 0.02 or 0.2 mL from any 5 areas per gel, was appropriately diluted with fresh sterile PBS while repetitive pipetting using a sterile glass pipette, the diluted solution was added to each agar culture medium and incubated at 37ºC for 24 h. A nutrient agar medium (Nissui, Nissui Pharmaceutical, Tokyo, Japan) was used for S. aureus and E. faecalis, a BHI agar medium (Becton, Dickinson and Company) was used for S. mutans, and a YM agar medium (Becton, Dickinson and Company) was used for C. albicans. The total number of surviving microbes in each gel was calculated from the CFU in the media after incubation. The microbial removal rate (%)=100(1−N_{surviving}/N_{initial})

Based on the microbial removal rates calculated from these results, the available chlorine concentration of the gel effective for microbicidal treatment was determined. The seven NWCL (CL: 0, 10, 15, 20, 30, 50, and 70 (ppm)) were also tested in the same way.

TEST 2. Test to examine the storage stability

For each test gel with an available chlorine concentration of 70 ppm (XTJ70, CBJ70, and AGJ70), changes in the pH and available chlorine concentration were examined during storage, as shown below.

Immediately after preparation, 20 mL of each gel was poured into a polypropylene syringe (Terumo syringe, SS-01T10, Terumo, Tokyo, Japan) and stored under either one of the following storage conditions: shaded and refrigerated condition (4ºC) for up to 28 days, shaded condition (25±2ºC), or non-shaded condition (25±2ºC). Under the latter two conditions, storage was terminated when no available chlorine concentration was detected. The pH of the gel was examined with a pH meter (PHL-20, DKK-TOA, Tokyo, Japan). The concentration of available chlorine, which is an indicator of microbicidal activity, was measured by a chlorine comparator (Chlorine Comparator for Free Chlorine in Water, Sibata Scientific Technology, Saitama, Japan) by the N, N-diethyl-p-phenylenediamine method (Sibata Scientific Technology). This method is often used for analysis of water quality such as tap water. Each test gel/water was appropriately diluted (1 to 50 times) with distilled water so that the concentration range was 0.1–1.5 ppm (colorimetric measurement range: 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.3, 1.5, 2.0 ppm), and then the value was measured. It was multiplied by the degree of dilution to obtain the available chlorine concentration of each test gel/water.

NW70, which had the same available chlorine concentration as the gels in TEST 2 and TEST 3, and NW0, which was prepared at a standard concentration of the apparatus under normal operating conditions, were also tested in the same way.

For each test gel/water after the 28-day storage, rates of changes in the pH and available chlorine concentration were calculated.

TEST 3. Microbicidal effect test for gels stored for 28 days

The gels that had an available chlorine concentration of 15 ppm or higher after the 28-day storage were examined for microbicidal effects in the same way as TEST 1.

All tests (TEST 1, TEST 2, and TEST 3) were repeated five times per test gel/water and per storage condition.

Statistical analysis

After analysis of the normality of data distribution using the Shapiro-Wilk normality test, the results of TEST 1 were analyzed by a one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test at a level of α=0.05 for comparison among gels and comparison with NWCL. Analysis of the number of
surviving bacteria or fungi obtained in TEST 1 and TEST 3 was performed following a logarithmic conversion.

The rates of change in the pH and available chlorine concentration due to the 28-day storage under refrigerated conditions were also analyzed in the same way as mentioned above, at a level of $\alpha=0.05$, for comparison among gels and with NWCL.

All statistical analyses were performed using EZR statistical software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

RESULTS

Microbicidal effects of test gels/water in TEST 1

For each test gel/water, the effects of its available chlorine concentration on the number of surviving microbes, i.e., the three bacterial strains and one fungal strain, after the 3-min treatment are shown in Fig. 4 with a logarithmic scale. Additionally, microbial removal rates of each test gel/water calculated based on the numbers of the surviving bacteria or fungi are also shown in Table 2. For all types of test gels and NWCL, the numbers of surviving bacteria or fungi significantly decreased with an increase in the available chlorine concentration ($p<0.05$). Only NWCL showed a high removal rate (>99%) against every microbe, even with an available chlorine concentration of 10 ppm. On the other hand, no gels of the same concentration (10 ppm) could significantly remove microbes, thus showing no significant difference from each gel type with an available chlorine concentration of 0 ppm in a logarithm of the number of surviving bacteria or fungi ($p>0.05$). Each type of gel with an available chlorine concentration of 15 ppm or higher could significantly remove $S. aureus$, $S. mutans$, and $E. faecalis$ compared with gels with an available chlorine concentration less than 15 ppm.
Table 2  Microbial removal rates of each test gel/water

| Test gel/water | Microbe       | Available chlorine concentration in each test gel/water |
|----------------|---------------|----------------------------------------------------------|
|                |               | 10 ppm | 15 ppm | 20 ppm | 30 ppm | 50 ppm | 70 ppm |
| XTJ            | S. aureus     | 15.5(13.5)| 96.4 (0.9)| 99.7 (0.1)| >99.99 | >99.999| ≈100*  |
|                | S. mutans     | 10.8(9.2) | 97.5 (0.7)| >99.9 | >99.999| >99.999| ≈100*  |
|                | E. faecalis   | 21.0(11.1)| 98.3 (1.0)| >99.9 | >99.999| >99.999| ≈100*  |
|                | C. albicans   | ≈0 (0.0) | 52.8 (14.0)| >99.9 | >99.999| >99.999| ≈100*  |
| CBJ            | S. aureus     | 13.0 (9.7)| 97.1 (0.7)| >99.9 | ≈100*  | ≈100*  | ≈100*  |
|                | S. mutans     | 16.4 (10.2)| 98.4 (0.7)| >99.9 | ≈100*  | ≈100*  | ≈100*  |
|                | E. faecalis   | 21.0 (11.8)| 98.7 (0.5)| >99.9 | ≈100*  | ≈100*  | ≈100*  |
|                | C. albicans   | ≈0 (0.0) | 67.9 (10.1)| >99.9 | >99.999| >99.999| ≈100*  |
| AGJ            | S. aureus     | 15.6 (11.3)| 97.5 (0.9)| >99.99 | ≈100*  | ≈100*  | ≈100*  |
|                | S. mutans     | 8.8 (7.5) | 98.2 (0.7)| >99.99| ≈100*  | ≈100*  | ≈100*  |
|                | E. faecalis   | 15.9 (11.1)| 99.3 (0.7)| >99.99| ≈100*  | ≈100*  | ≈100*  |
|                | C. albicans   | ≈0 (0.0) | 81.0 (6.6)| >99.99| ≈100*  | ≈100*  | ≈100*  |
| NW CL          | S. aureus     | 99.9±0.1 | >99.999 | ≈100*  | ≈100*  | ≈100*  | ≈100*  |
|                | S. mutans     | 99.8±0.9 | >99.999 | >99.999| ≈100*  | ≈100*  | ≈100*  |
|                | E. faecalis   | 99.8±0.1 | >99.999 | ≈100*  | ≈100*  | ≈100*  | ≈100*  |
|                | C. albicans   | 99.8±0.1 | >99.999 | >99.999| ≈100*  | ≈100*  | ≈100*  |

Data are presented as mean (standard deviation) (n=5).
*: The surviving bacteria or fungi were not detected, that is, <50 cells of surviving bacteria or fungi.

(p<0.05). For C. albicans, AGJ showed a higher fungicidal effect (removal rate: >80% at 15 ppm, >99.999% at 20 ppm) than the two other types of gels. XTJ and CBJ showed the same effect (>99.999%) at 50 ppm and 30 ppm or higher, respectively. No microbes were detected in XTJ with an available chlorine concentration of 70 ppm and CBJ with an available chlorine concentration of 50 ppm or higher; however, in AGJ, it was not detected even at a concentration of 30 ppm, similar to NW CL.

Storage stability of test gels/water in TEST 2
The changes in pH and available chlorine concentration depending on the storage condition for each type of test gel/water are shown in Fig. 5. In addition, their rate of change calculated based on each value of the test gels/water depending on the condition during the 28-day storage are shown in Fig. 6. For all the test gels/water, the decrease in available chlorine concentration was the least in the case of the shaded and refrigerated storage condition. With each passing day of the storage time, all test gels showed significantly greater reductions in pH and available chlorine concentration than NW CL prepared at the same concentration (p<0.05). Regarding pH, AGJ showed the greatest decline among the test gels (p<0.05); however, it maintained a pH more than 6 (6.09±0.04) after the 28-day storage under the shaded and refrigerated condition (4°C). XTJ showed a remarkable decline in the available chlorine concentration even under the shaded and refrigerated condition (2.80±0.75 ppm) and could not maintain the concentration that exhibited microbicidal effects during the 1-day storage. In contrast, CBJ 70 and AGJ 70 maintained an available chlorine concentration higher than 20 ppm, which exhibited high microbicidal effects, during the 9-day and 21-day shaded and refrigerated storages, respectively. After the 28-day storage, available chlorine was not detected in XTJ 70, and only 1.60±0.49 ppm was detected in CBJ 70 stored under the shaded and refrigerated condition. On the other hand, AGJ 70 could maintain a concentration of 15.40±0.49 ppm under the same storage conditions.

Microbicidal effects of gel stored for 28 days in TEST 3
Only AGJ 70 stored under the shaded and refrigerated condition maintained an available chlorine concentration higher than 15 ppm after the 28-day storage, and its microbicidal effects are shown in Fig. 7. AGJ 70 stored for 28 days under the shaded and refrigerated condition was as effective for removing microbes as the gel of the same concentration tested in TEST 1, decreasing the number of surviving bacteria from the level of 10⁶ to 10⁴ for each bacterial strain (removal rate: >95%) and to the level of 10⁸ for C. albicans (>80%).
Fig. 5 Changes in the properties of each test gel/water during the 28-day storage, results of TEST 2. Vertical bars indicate the standard deviation ($n=5$).

Fig. 6 Rates of change in properties of each test gel/water containing 70 ppm of available chlorine during the 28-day storage. Vertical bars indicate the standard deviation. In each of the four graphs, different letters represent statistically different groups ($p<0.05$) according to one-way ANOVA ($n=5$). *1 Under shaded and refrigerated condition at 4ºC, *2 Under shaded condition at 25±2ºC, *3 Under non-shaded condition at 25±2ºC.
Fig. 7 Microbicidal effects of agar gel (AGJ) after the 28-day storage under shaded and refrigerated condition at 4°C, results of TEST 3. Each vertical axis shows Log_{10} (the number of surviving bacteria or fungi in AGJ after treatment). Vertical bars indicate the standard deviation (n=5).

DISCUSSION

Available chlorine species, especially HOCl (pKa: 7.5 at 25°C), show high microbicidal effects by oxidizing microbial cells with adequate concentration and handling. In aqueous solutions concluding available chlorine, the fraction of each component in free available chlorine (Cl_2, HOCl, and OCl^-) varies greatly with pH\textsuperscript{35-37}, as shown in Fig. 8. HOCl, which is the main component of electrolyzed water commonly used for disinfection, has microbicidal effects several tens of times those of OCl^-, which is the main component of aqueous sodium hypochlorite (NaOCl) solution. Therefore, it can exhibit equivalent microbicidal effects at several tens of ppm, which is the concentration of a few tenths of OCl^-\textsuperscript{36-38}. This advantage of HOCl is due to its ability to penetrate not only the microbial cell but also the plasma membrane. In other words, it can be oxidized by both from the outside and inside of the cell\textsuperscript{37,38}. Another advantage is that it does not exert harmful effects on eyes and skin, which can occur with NaOCl as it is strongly alkaline\textsuperscript{10,14,39-41}. Since OCl^- cannot penetrate the plasma membrane of cells, it has a lower microbicidal effect than HOCl. However, there is an advantage that OCl^- has better cleaning effects against organic substances than HOCl. Due to the difference in the method of electrolysis, depending on the apparatus, the changes in the pH of electrolyzed water ranges from strongly acidic to alkaline, and the available chlorine concentration ranges from 10 to several hundred ppm. In this study, we used conventionally prepared NW with an approximately available chlorine concentration of 30–40 ppm using NW_{35} and NW_{high} (800–1,200 ppm) prepared as per manufacturer’s modification in terms of its flow rate for preparations of test gels/water. The available chlorine in NW_{35} (pH: 7.00±0.02) comprises approximately 70% (25 ppm) of HOCl and approximately 30% (10 ppm) of OCl^-.

Similar to other reports concerning electrolyzed water containing HOCl\textsuperscript{7,17,19,21,27}, NW_{CL} showed effective microbial removal rates (>99%) against three bacterial strains and one fungal strain even at the minimum available chlorine concentration of 10 ppm in TEST 1. The surviving microbes were practically not detected at 15 ppm (removal rates>99.999%) and were not detected at 20 or 30 ppm or higher (~100%). Regarding test gels, no surviving microbes were detected in XTJ, CBJ, and AGJ at concentrations of 70, 50, and 30 ppm or higher, respectively. In general, the addition of thickening/gelling agents tends to lower rather than reinforce the effects of the main component, such as a disinfectant. Other researchers reported that alcohol gels have a significantly lower bactericidal activity than the liquid form with the same alcohol content\textsuperscript{42-44}. Similarly, Sogawa et al.\textsuperscript{45} reported that inhibition of bactericidal activity is caused in a dose-dependent manner by gelation of chlorhexidine gluconate-containing disinfectant using a carboxyvinyl polymer. In the present study, it is considered that the thickening/gelling component in the test gels lowered the action of available chlorine on bacterial and fungal cells, and the degree of reduction differed depending on the thickening/gelling agent used.

XT is identical to the naturally occurring polysaccharide produced by a fermentation process using the bacteria Xanthomonas camppestris. It is highly stable because the side chains have a very long molecular structure relative to the main chain. We selected XT as a thickening agent for hydrophilic colloids because of these properties of stabilizing foams, emulsions, and suspensions generally used. However, mixing NW_{100} with XT deactivated approximately 90% of its available chlorine. Therefore, it was necessary to use NW_{CL} at a concentration higher than 100 ppm for preparation, and each gel was prepared with appropriate dilution of NW_{CL} (CL: 200–700 (ppm)) with distilled water (DW).
For example, we prepared gels with a concentration of 10 and 15 ppm by mixing NW_{200}, DW, and XT while controlling its available chlorine concentration and pH. XTJ was deactivated following the storage time, and it lost its microbicidal effect. The decrease in concentration was most remarkable among the test gels with a concentration of 70 ppm, and even after the 1-day shaded and refrigerated storage, all the available chlorine was deactivated. XT is a thickening agent that is frequently used in foods, cosmetics, and pharmaceuticals; however, it is extremely challenging to thicken while maintaining the available chlorine, HOCl, and OCl\(^{-}\), for a certain time. It is thought that the microbicidal actions deteriorated immediately due to the consumption and decomposition of HOCl and OCl\(^{-}\) through contact with XT, which is an organic substance. Even if XTJ has low storage stability, it can find applications in dental practice if it can be prepared quickly and easily each time for immediate use. However, the preparation of XTJ is complicated because vigorous stirring is necessary to avoid spotting when XT is dispersed in NW. It is presumed that XT is not useful as a thickening agent without further significant improvement considering its disadvantages of low storage stability and the need for pH adjustment.

CB has acrylic acid as the main chain and is a watersoluble polymer with a carboxyl group, which is used as a thickening agent in cosmetics and disinfectants. Among the three test gels, CBJ has maximum transparency and glossiness and closely resembles the appearance of commercial oral care gels. It is often used as a binder in commercial oral care gels. In the preparation with CB, we first prepared a water-based gel (0 ppm) without available chlorine while controlling its pH, and then mixed the gel with one-sixth to one-fifth the amount of NW 100 at approximately ten times the final concentration. For example, for CBJ of 10 ppm, when 20 mL of NW_{100} was added to 100 mL of the water-based gel, the theoretical final concentration should be approximately 17 ppm; however, it was approximately 10 ppm due to the deactivation of approximately 40% of the available chlorine. The rate of deactivation is less than half of that in the preparation with XT. However, the preparation of CBJ requires not only the final precise adjustment of pH as in XTJ but also the addition of alkali for neutralization prior to gelation. Therefore, as with XTJ, the preparation is complicated, and it is challenging to prepare from scratch as the present method of preparation for immediate use. On the other hand, the change in pH was less than that of AGJ, and the available chlorine concentration of 15 ppm, which is effective for removing microbes, could be maintained for 9 days under the shaded and refrigerated condition. Based on this point, it is expected that to improve the preparatory method, the addition of a small amount of high concentration NW_{100} immediately before each use to compensate for the reduction in the available chlorine concentration will make it possible to find applications in dental practice.

AG is a hydrophilic colloid and a complex sulfated polymer of galactose units. It serves as the main component of a reversible hydrocolloid impression material for many years in dental practice. We focus on AG as a gelling agent because it is already used in several fields, thus ensuring biological safety and easy gelation. Regarding the preparation of AGJ, we could prepare the gels with the desired and theoretical levels of available chlorine concentration with less deactivation of available chlorine by mixing NW_{100} at a concentration twice that of the final concentration with an equal amount of the first prepared water-based gel (0 ppm). For example, 100 mL of NW_{100} was used to prepare 200 mL of AGJ with a concentration of 50 ppm. We considered that AGJ had the highest storage stability because the available chlorine was scarcely deactivated by the addition of AG. Compared with the other two types of gels, it was not necessary to consider the significant decrease in the available chlorine due to thickening/gelation, and the concentration could be easily adjusted.

Generally, gels prepared with AG have lower water retentivity than those prepared with other thickening/gelling agents. It is considered that in water-based aga...
study is required. We should aim to extend to several times that, for example, 3 months or more, preferably about 12 months, so of course, within the concentration range where safety is ensured, by increase the available chlorine concentration at the time of preparation, addition of other effective components, degas etc. We plan to improve its preparatory method while also considering the addition of components that enhance water retention, if necessary.

Some oral care products contain medicinal components to remove oral microbes and to strengthen and/or whiten teeth. Since NW has not only excellent microbicidal effects\(^{19,21,24,27}\), but also a hemostatic effect\(^{17}\), we aim to establish prolonged contact with each treated body by thickening/gelling it for cleaning teeth and the mucous membrane. Based on the results of our study, AGJ\(_{70}\) could be used as an oral care gel to remove microbes, as it maintained a sufficient available chlorine concentration after storage for a month, thereby maintaining its microbicidal effects. However, it is necessary to add gels with an available chlorine concentration ≥70 ppm to evaluate their detailed storage stability and other properties such as biological safety in future studies. Additionally, we will conduct further microbicidal effect tests using obligate anaerobic bacteria not used in the present study.

CONCLUSION

An NW-based gel might be useful as a gel to remove oral microbes due to its bactericidal and fungicidal effects. Agar was the most suitable thickening/gelling agent for preparing NW-based gel from the viewpoint of storage stability.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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