Pharmaceutical properties of a tinted formulation of a biguanide antiseptic agent, olanexidine gluconate

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Summary

Olanexidine gluconate-containing preoperative antiseptic (OLG-C) is colorless, which makes it difficult to determine its area of application. To overcome this drawback, we realized a stable orange-tinted antiseptic (OLG-T) by adding new additives to OLG-C and investigated its pharmaceutical properties compared with OLG-C and povidone iodine (PVP-I). We evaluated the influence of the additives on the antimicrobial activity and adhesiveness of medical adhesives to OLG-T-applied skin by in vitro time-kill/ex vivo micropig skin assays and a peel test using excised micropig skin, respectively. In the in vitro time-kill assay, the bactericidal/fungicidal activity of OLG-T and OLG-C were equivalent. In the ex vivo micropig skin assay, their fast-acting and persistent bactericidal activities against vancomycin-resistant Enterococcus faecalis were higher than that of PVP-I. In the peel test, the adhesion force of the incise drape and the amount of stripped corneocytes on the peeled drape were comparable between OLG-T- and OLG-C-applied skin, but both were less than those of PVP-I-applied skin. The drapes for OLG-T- and OLG-C-applied skin had moderate adhesion force, and the drape-related injuries were expected to be weak. These results suggest that OLG-T performs no worse than OLG-C in terms of its antimicrobial activity and medical adhesive compatibility. Therefore, we expect OLG-T to lead to more convenient preoperative skin preparation and further contribute to lowering SSI rates.

Keywords: olanexidine, antiseptic, antimicrobial activity, ex vivo skin model, peel test, persistence
INTRODUCTION

Surgical site infection (SSI) increases the hospital stay length, mortality, and cost after surgical procedures. (1-3) Despite the implementation of a myriad of preventive measures including enhanced nutritional support, improved surgical techniques, antimicrobial drug use, wound dressing, use of surgical incise drapes, and protecting the wound from contamination by preoperative bathing, SSI remains a problem. (3,4) Surgical site antisepsis, which reduces the number of microorganisms present on the skin of the surgical site, is a classic procedure that is easier to perform than other interventions. Skin antisepsis is essential for preventing SSI in all types of surgery. Chlorhexidine gluconate (CHG)-alcohol, aqueous and alcoholic povidone-iodine (PVP-I) are the most commonly used preoperative antiseptics. (3)

Alcohol-based antiseptics are frequently used, but they may be ignited by electric scalpels, and they are not suitable patients with alcohol sensitivity. Therefore, we believe that offering a choice of non-alcoholic antiseptic formulations is necessary.

We discovered a biguanide antiseptic agent, olanexidine gluconate [1-(3,4-dichloro-benzyl)-5-octylbiguanide mono-D-gluconate, OLG], which has antimicrobial activity against a wide range of microorganisms (5-7), and especially higher efficacy against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) compared to CHG and PVP-I. An aqueous solution containing OLG (OLG-C) was introduced in Japan in 2015 for use as a preoperative skin preparation for surgical sites (Fig. 1A). (8) As of writing, some surgeons have reported that OLG-C reduces the risk of SSI after surgery. (9-12) However, since the formulation is colorless, it is difficult to see the actual area on which OLG has been applied preoperatively; thus, complete coverage cannot be assured. (13) A tinted OLG formulation is expected to lead to more convenient preoperative skin preparation and further contribute to lower SSI rates, but the addition of colored dye to colorless OLG-C produces insoluble salts from cationic olanexidine and anionic colored dye.
One solution is to add surfactants that dissolve the insoluble salts to prepare a stable tinted OLG formulation, but many surfactants reduce the antimicrobial activity and adhesion force of surgical incise drapes to skin. Ensuring that incise drapes properly adhere at the wound edge, where the skin and incise drape are adjacent to the surgical incision site, is important.

Separation of incise drapes from the skin has been reported to be associated with a six-fold increase in infection rates compared with surgical procedures in which the drape did not lift off. (14) However, it has also been reported that if the adhesion force between the skin and incision drape is too strong, skin stripping, that is, medical adhesive-related skin injury (MARSI), will occur during peeling. (15) It is important to determine whether proper adhesion force is maintained between the antiseptic-applied skin and medical adhesives such as incision drapes. In this study, we determined a surfactant composition that will allow the tinted solution, OLG-T (Fig. 1B) to have the same antimicrobial activity and efficiency as colorless OLG-C.

We also demonstrated a new method of assessing the compatibility of antiseptics and medical adhesives using excised micropig skin.

MATERIALS AND METHODS

Antiseptic solutions and reagents

OLG-T, an orange-tinted antiseptic containing 1.5% OLG, polyoxyethylene(20) polyoxypropylene(20) glycol, lauromacrogol, N,N-dimethyldodecylamine N-oxide, D(-)-mannitol, partially hydrolysed polyvinyl alcohol and Sunset Yellow FCF (Yellow No. 5), was prepared at the Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan); it is available under the brand name, Olanexidine® antiseptic solution 1.5% OR. Olanedine® antiseptic solution 1.5% (Otsuka Pharmaceutical Factory, Inc.) containing 1.5% OLG and polyoxyethylene(20) polyoxypropylene(20) glycol (as a solubilizing agent), and Isodine® solution 10% (Mundipharma K.K., Tokyo, Japan) containing 10% povidone-iodine, were used.
as OLG-C and PVP-I, respectively.

Other reagents were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan).

**Microorganisms**

The following bacterial strains were purchased from American Type Culture Collection (Manassas, VA, USA), Japan Collection of Microorganisms (RIKEN BioResource Research Center, Ibaragi, Japan), or NITE Biological Resource Center (National Institute of Technology and Evaluation, Tokyo, Japan): *Staphylococcus aureus* subsp. aureus (ATCC® 29213™), *Staphylococcus aureus* subsp. aureus (ATCC® 33591™) (MRSA), *Staphylococcus epidermidis* (ATCC® 12228™), *Brevibacterium epidermidis* (ATCC® 35514™), *Micrococcus luteus* (ATCC® 4698™), *Enterococcus faecalis* (ATCC® 29212™), *Enterococcus faecalis* (ATCC® 51575™) (VRE), *Corynebacterium minutissimum* (ATCC® 23348™), *Acinetobacter baumannii* (ATCC® 19606™), *Escherichia coli* (ATCC® 25922™), *Klebsiella pneumoniae* subsp. pneumoniae (ATCC® 13883™), *Pseudomonas aeruginosa* (ATCC® 27853™), *Serratia marcescens* (ATCC® 14756™), *Candida albicans* (ATCC® 90028™), *Cutibacterium acnes* (ATCC® 11827™), *Candida auris* (JCM 15448), and *Malassezia furfur* (NBRC 0656). *C. acnes* was grown on reinforced clostridial agar (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) at 35°C under anaerobic conditions. The two yeasts, *C. albicans* and *C. auris*, were grown on Sabouraud dextrose agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 35°C and *M. furfur* was grown on Sabouraud dextrose agar overlaid with olive oil at 30°C. The remaining strains were grown on soybean-casein digest agar (Nihon Pharmaceutical Co., Ltd.) under aerobic conditions at 35°C, and the grown colonies were suspended in distilled water to prepare the test bacterial suspensions at appropriate concentrations.

**In vitro evaluation of fast-acting antimicrobial activity**

Fast-acting antimicrobial testing was performed using an *in vitro* time-kill method.
An aliquot of test antiseptic solution was added to 0.05 parts of each test microorganism suspension at 23°C, and a part of which was removed 30 s or 60 s after inoculation. The recovered reaction mixture was diluted with a diluting fluid containing a neutralizer (10% polysorbate 80, 1.167% soybean lecithin, 0.5% sodium thiosulfate hydrate, and 1% Tamol® NN8906) to neutralize the biocidal activity of the antiseptic. Viable microorganisms in the serially diluted fluids were counted by the pour-plate method or surface-spread method using a suitable agar medium containing 0.1% lecithin and 0.7% polysorbate 80. The lower detection limit of the viable microbial count was 10 and 100 colony-forming units (cfu)/mL for the pour-plate and surface-spread methods, respectively. Microbial log$_{10}$ reductions were calculated by subtracting the mean log$_{10}$ recovery of each surviving microbial population from that of the initial microbial population. When the determined viable microbial count was less than the lower detection limit, the log$_{10}$ reduction was calculated with a lower detection limit and expressed with an inequality sign. We also confirmed that the neutralizer used in the microbial count effectively quenched the antimicrobial activity of the test antiseptic solutions and was not toxic to the test microorganisms according to ASTM E1054-08.

Pig skin preparation

Commercially available Yucatan micropig (YMP) skin sets were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Each YMP skin set, consisting of 16 sheets (approximately 10 cm × 10 cm per sheet), was excised from one female YMP (5 months old). The YMP skin sets were stored at −80°C until use. Immediately before use, the sheets were thawed in an incubator maintained at 35°C for 10 min, and the subcutaneous fat was removed from all the sheets. Then, one sheet was cut into pieces (2 cm × 2 cm or approx. 5 cm × 5 cm) with a surgical knife, and the resulting skin pieces were disinfected by soaking them in 70% ethanol for 5 min.
**Ex vivo evaluation of fast-acting bactericidal activity**

The *ex vivo* skin infection model was established by modifying previously reported methods. (19-22) Briefly, a YMP skin piece (2 cm × 2 cm) was inoculated with 10 μL of the test bacterial suspension (VRE ATCC 51575) using an inoculation loop and air-drying. Subsequently, 20 μL of each antiseptic solution was dropped onto the infected YMP skin piece and spread using an inoculation loop. After 30 s or 3 min of antiseptic application, the skin piece was recovered in 10 mL of neutralizer-containing diluting fluid to neutralize the bactericidal activity of the antiseptics. To establish a control, untreated infected YMP skin pieces were recovered using the same procedure. Viable bacteria in the diluting fluids were quantified using the above-mentioned methods. Log₁₀ reduction was calculated from the determined number of viable cells (cfu per skin piece).

**Evaluation of remaining bactericidal activity on the skin after rinsing and drying**

A 20-μL aliquot of each test antiseptic solution was dropped onto a YMP skin piece (2 cm × 2 cm) and spread using an inoculation loop. The antiseptic-treated skin pieces were incubated at 35°C for 4 h, rinsed with 1 mL of sterilized normal saline which could sufficiently wash the skin surface, and then incubated at 35°C for a further 4 h. After incubation, the pieces were uniformly inoculated with 10 μL of the test bacterial suspension (VRE ATCC 51575) with an inoculation loop to estimate the persistent bactericidal activity. After 1 min of inoculation, each skin piece was recovered in 10 mL of a neutralizer-containing diluting fluid. To establish a control, an untreated YMP skin piece was rinsed, incubated, and recovered using the same procedure. Viable bacteria in the diluting fluids were quantified using the above-mentioned methods. Log₁₀ reduction was calculated from the determined number of viable cells (cfu per skin piece).

**The 180° peel adhesion test and measurement of the amount of stripped corneocytes**

We evaluated the adhesion force of surgical incise drapes to YMP skin by modifying the...
A 100-μL aliquot of each test antiseptic solution was dropped onto a YMP skin piece (approx. 5 cm × 5 cm) and spread using an inoculation loop before air-drying for 30 min. Then, adhesive strips (size including gripping margin: 10 cm × 4 cm, adhesive part: 4 cm × 4 cm) prepared from an incise drape (3M Ioban2 Antimicrobial Incise Drape, 3M Japan Ltd., Tokyo, Japan), were uniformly applied to the antiseptic-treated skin piece. After 30 min of dry application, the skin piece was held at the upper chuck of the peeling tester (ZTS-5N, MX2-500N; Imada Co., Ltd, Toyohashi, Japan), and the strip on one side was folded back at a 180° angle so that it overlapped with the backside of the strip. The strips were mechanically removed from the skin piece at a rate of 100 mm/min, and the peel force required for strip removal was measured using a peeling tester. The mean adhesion force per 1 cm of an adhesive strip width (N/cm) was the average of the peel force from 25% to 62.5% of the drape-removing time after starting the measurement.

After removing the adhesive strips, the staining solution, an aqueous solution containing Gentian Violet B and Brilliant Green, was dropped onto the adhesive part of the strip due to the staining of corneocytes. The amount of stripped corneocytes was measured by binarizing the stained corneocytes and the background (incise drape) using a digital microscope (VHX-5000; Keyence Co., Osaka, Japan) and calculating the area ratio of stained corneocytes. The measurements were repeated three times for each drape sample.

**Statistical analysis**

In each test of excised micropig skin, the data were analyzed by Tukey’s multiple comparison test when the homogeneity of variance in Bartlett’s test was homogeneous. Otherwise, the data were analyzed using the Steel-Dwass multiple comparison test. The significance level was set to P < 0.05. EXSUS (version 10.0.3; CAC Exicare Corporation, Tokyo, Japan) was used for the statistical analysis.
RESULTS

Colored preoperative skin antiseptics are important for determining the disinfection area, but adding only an orange-colored dye, Sunset Yellow FCF to colorless OLG-C resulted in the production of insoluble salts. To prevent the production of insoluble salts, we added four additives, lauromacrogol, \(N, N\)-dimethyldodecylamine \(N\)-oxide, mannitol and polyvinyl alcohol, which allowed us to realize a stable formulation, OLG-T.

To confirm the \textit{in vitro} antimicrobial activity of OLG-T, a time-kill assay was carried out for 30 and 60-s exposure times against 17 tested bacterial and fungal strains, which are known resident microorganisms of human skin or pathogens isolated from SSI.\(^{(25,26)}\) After OLG-T and OLG-C treatments, no viable microorganisms remained from all test strains (Table 1).

Although PVP-I had weak bactericidal activity against enterococci, it showed strong antimicrobial activity against other microbial species. Therefore, the \textit{in vitro} fast-acting bactericidal/fungicidal activity of OLG-T did not differ from that of OLG-C. Furthermore, OLG-T and OLG-C had a broader bactericidal spectrum than PVP-I at short exposure times.

We also evaluated the bactericidal activity of OLG-T against VRE in \textit{ex vivo} skin. YMP is hairless and its morphological and functional characteristics are comparable to those of human skin; many reports have shown that YMP skin is used for drug permeability and pharmacological studies.\(^{(22,27,28)}\) The fast-acting bactericidal activity was evaluated on YMP skin contaminated with vancomycin-resistant \textit{E. faecalis} ATCC 51575. The mean inoculum dose was \(1.47 \times 10^6\) cfu per skin piece. The bactericidal activities of OLG-T, OLG-C and PVP-I expressed in mean log\(_{10}\) reductions [95\% confidence interval (CI)] were 3.74 [2.74, 4.74], 2.92 [2.07, 3.77] and 0.23 [0.00, 0.45] after 30 s of exposure, respectively. At 3-min exposure, the activities of OLG-T, OLG-C and PVP-I were 3.85 [3.01, 4.70], 3.92 [2.59, 5.24] and 1.02 [0.34, 1.71], respectively. The values for OLG-T were significantly higher than those for PVP-I, but did not differ from those of OLG-C (Fig. 2A).
In clinical practice, OLG-T applied to the skin is wiped off after surgery, so it was rinsed with saline after 4 h, which was the average surgery time, and its substantivity (i.e. remaining bactericidal activity after rinsing) a further 4 h after antiseptic removal was evaluated. Viable bacteria remaining after 1 min of VRE ATCC 51575-inoculation (mean inoculum dose: $1.12 \times 10^6$ cfu per skin piece) on the YMP skin, were counted to measure the degree of antiseptic molecules retaining bactericidal activity. When the substantivity evaluated using VRE as test bacteria was expressed in mean log_{10} reductions [95% CI], those for OLG-T, OLG-C and PVP-I were 2.97 [1.84, 4.10], 4.35 [3.62, 5.07] and 0.31 [0.22, 0.41], respectively. The substantivity for OLG-T was significantly higher than that for PVP-I, but did not differ from that of OLG-C (Fig. 2B). From the above results, we can see that OLG-T has a fast bactericidal activity and substantivity similar to those of OLG-C and stronger than those of PVP-I on ex vivo skin.

Surgical incise drapes are routinely used in surgery, although there is still no convincing evidence that they reduce SSI rates. The following tests were performed to verify the compatibility of the incision drape with OLG-T-applied skin.

To measure the adhesion force for drape removal from YMP skin treated with an antiseptic, we performed the 180° peel test on the incise drapes. The mean adhesion forces [95% CI] for the skin treated with OLG-T, OLG-C and PVP-I were 0.14 [0.11, 0.17], 0.14 [0.06, 0.23] and 0.36 [0.25, 0.47] N/cm, respectively. The adhesion force for OLG-T-applied skin was significantly lower than that for PVP-I, but did not differ from that of OLG-C (Fig. 3A).

Furthermore, after removal of the drape from YMP skin treated with antiseptics, the amount of stripped corneocytes was evaluated by measuring the area ratio of stained corneocytes on the adhesive surface of the drape. The mean amount [95% CI] of stripped corneocytes from the skin treated with OLG-T, OLG-C and PVP-I were 19.5% [16.9, 22.1], 15.9% [14.6, 17.3] and 32.0% [28.0, 36.0], respectively. The amount of stripped corneocytes for skin treated with OLG-T was significantly lower than skin treated with PVP-I, but did not differ from that of
DISCUSSION

In the tinted OLG solution, OLG-T became stable by the addition of an orange dye and four additives to a colorless OLG solution. Some additives inhibit the antimicrobial activity of biguanide compounds, such as CHG and OLG. (19, 29) We investigated the influence of multiple additives contained in OLG-T on antimicrobial effects. From the results of the in vitro time-kill assay, the antimicrobial spectrum and bactericidal/fungicidal activity of OLG-T and OLG-C were considered equivalent. The bactericidal activity of OLG-C against VRE in a fast-acting ex vivo skin assay was significantly higher than that of PVP-I, as previously reported. (22) Although only a limited number of bacterial species were examined, OLG-T and OLG-C exerted an equivalent strong and fast-acting bactericidal effect on the skin, reflecting the results of the in vitro test. The remaining bactericidal activity after removing the antiseptic from the skin, that is, substantivity, was also examined. The test method was slightly modified from our previous report, considering clinical conditions such as surgical operating time and wiping fluid. (22) As a result, it seems that the substantivities of OLG-T and OLG-C are also equivalent. We consider that the combination of lauromacrogol and N,N-dimethyldecylamine N-oxide is important for maintaining the antimicrobial activity of OLG, but the mechanism of this combination effect is unknown.

Next, considering that adding multiple additives also influences the adhesion force of the medical adhesive, the effect was examined by a peel test using YMP skin. We investigated the effect of OLG-T- and OLG-C-application on incise drape adhesion force, finding comparable results, albeit weaker than that for PVP-I-applied skin. The amount of stripped corneocytes on the peeled drape was correlated with the adhesion force. It has been reported that there is a correlation between the amount of stripped corneocytes in the peel test and skin irritation,
which has been termed MARSI.\(^{(23,30)}\) OLG-C has been used clinically for more than five years at present, but there have been few complaints related to incise drape-lift and there are no reports related to MARSI. These findings suggest that for OLG-T-applied skin, the adhesion force of the drapes was moderate and drape-related injury was weak, and there will be no problem with the compatibility of OLG-T and adhesives, as with OLG-C. Polyvinyl alcohol and mannitol provided moderate adhesion of drapes to OLG-T-applied skin, because the drape was significantly less adhesive when the formulation did not contain polyvinyl alcohol and mannitol (data not shown). Since polyvinyl alcohol is used as an adhesive for various products, it also functions as an adhesive in OLG-T.\(^{(31,32)}\) The function of mannitol is unknown, but we estimate it as follows: after OLG-T dries, the components except mannitol in OLG-T will be present in semisolid or waxy forms, but mannitol may improve medical adhesives by precipitating as a solid. In addition, because mannitol precipitated as a solid without absorbing the moisture of the surrounding environment, it can remain dry.\(^{(33,34)}\) We suggest that precipitated mannitol contributes to adhesiveness by keeping the skin dry and filling the skin grooves to increase the adhesive area. This may be because the critical relative humidity of mannitol is high.\(^{(33,34)}\)

At the moment, the issue of MARSIs has not been widely addressed, and there are only a few study reports. The peel test and quantification of stripped corneocytes using YMP skin in this study need further studies on extrapolation to humans, but offer the following advantages: YMP skin is commercially available and can be kept frozen for a long period of time, thus allowing flexibility in experiment initiation and fewer ethical issues than human clinical trials. Therefore, we consider it particularly useful for preclinical pharmaceutical research on topical agents.

CONCLUSIONS
We think it is important to increase the range of choices for easy-to-use, water-based antiseptics that avoid risk of ignition by electric scalpels, are suitable alcohol-intolerant patients, and prevent the development of antiseptic-resistant microorganisms. OLG-T is a tinted water-based antiseptic containing a relatively novel active ingredient, which showed fast-acting bactericidal/fungicidal activity and substantivity, similar to those of colorless formulation, OLG-C. In addition, we demonstrated a new method of assessing the compatibility of antiseptics and medical adhesives using YMP skin, showing that OLG-T and OLG-C had similar results. It seems that OLG-T and OLG-C exhibited equivalent pharmaceutical properties, and we expect OLG-T to lead to more convenient preoperative skin preparation and further contribute to lowering SSI rates.
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Conflict of Interest

The authors are employees of Otsuka Pharmaceutical Factory, Inc. (Otsuka); M. Shiozaki and A. Hagi have related patent(s) (JP6622325, JP5268982, JP4526300) licensed to Otsuka.
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Table 1 Viability of challenge microorganisms after 30-s or 60-s exposure to various test antiseptic solutions, expressed in bacterial log₁₀ reduction.

| Test microorganism | OLG-T 30 s | OLG-T 60 s | OLG-C 30 s | OLG-C 60 s | PVP-I 30 s | PVP-I 60 s |
|--------------------|------------|------------|------------|------------|------------|------------|
| **Gram-positive bacteria** |            |            |            |            |            |            |
| *S. aureus* ATCC 29213 | >4.82      | >4.82      | >4.82      | >4.82      | >4.82      | >4.82      |
| *S. aureus* ATCC 33591 (MRSA) | >5.28      | >5.28      | >5.28      | >5.28      | >5.28      | >5.28      |
| *S. epidermidis* ATCC 12228 | >5.00      | >5.00      | >5.00      | >5.00      | >5.00      | >5.00      |
| *B. epidermidis* ATCC 35514 | >4.48      | >4.48      | >4.48      | >4.48      | >4.48      | >4.48      |
| *M. luteus* ATCC 4698 | >4.36      | >4.36      | >4.36      | >4.36      | >4.36      | >4.36      |
| *E. faecalis* ATCC 29212 | >5.14      | >5.14      | >5.14      | >5.14      | 0.20 ± 0.04 | 0.94 ± 0.03 |
| *E. faecalis* ATCC 51575 (VRE) | >5.19      | >5.19      | >5.19      | >5.19      | 0.05 ± 0.03 | 0.35 ± 0.07 |
| *C. minutissimum* ATCC 23348 | >4.82      | >4.82      | >4.82      | >4.82      | >4.82      | >4.82      |
| *C. acnes* ATCC 11827 | >5.06      | >5.06      | >5.06      | >5.06      | >5.06      | >5.06      |
| **Gram-negative bacteria** |            |            |            |            |            |            |
| *A. baumannii* ATCC 19606 | >5.13      | >5.13      | >5.13      | >5.13      | >5.13      | >5.13      |
| *E. coli* ATCC 25922 | >5.11      | >5.11      | >5.11      | >5.11      | >5.11      | >5.11      |
| *K. pneumoniae* ATCC 13883 | >5.10      | >5.10      | >5.10      | >5.10      | >5.10      | >5.10      |
| *P. aeruginosa* ATCC 15442 | >5.39      | >5.39      | >5.39      | >5.39      | >5.39      | >5.39      |
| *S. marcescens* ATCC 14756 | >5.53      | >5.53      | >5.53      | >5.53      | >5.53      | >5.53      |
| **Fungi** |            |            |            |            |            |            |
| *C. albicans* ATCC 90028 | >4.36      | >4.36      | >4.36      | >4.36      | >4.36      | >4.36      |
| *C. auris* JCM 15448 | >4.56      | >4.56      | >4.56      | >4.56      | >4.56      | >4.56      |
| *M. furfur* NBRC 0656 | >3.77      | >3.77      | >3.77      | >3.77      | >3.77      | >3.77      |

Values are represented as the mean ± SD (n = 3). Values with an inequality sign indicate that the value was calculated from the viable cell count, including values below the detection limit.
Fig. 1. A: colorless, olanexidine gluconate-containing antiseptic solution (OLG-C) launched in Japan. Left: bottle product, middle: applicator product, right: Photograph applied to artificial skin (BIOSKIN Plate, #BSC, Beaulax Co., Ltd., Saitama, Japan). B: orange-tinted, olanexidine gluconate-containing formulation (OLG-T) in the bottle (left) and filled to the applicator (middle). Right: Photograph applied to artificial skin.
Fig. 2. The bactericidal activity against vancomycin-resistant *E. faecalis* ATCC 51575 in the *ex vivo* skin model. The bactericidal activity of each antiseptic is indicated as a log_{10} reduction. A: fast-acting bactericidal activity for VRE-contaminated YMP skin piece was evaluated after exposure for 30 s or 3 min. B: The remaining bactericidal activity after rinsing of the test antiseptics. At 4 h after rinsing of the test antiseptic-treated YMP skin pieces, the VRE was inoculated, and the residual viable bacteria were counted after 1 min of inoculation. Each column and vertical bar represent the mean and SD values (n = 4 in A or n = 6 in B). Values not sharing a common letter at the same time point differ significantly at P < 0.05, as determined by Tukey’s test (A) or Steel-Dwass test (B).
**Fig. 3.** Adhesion force of incise drape (A) and the amount of stripped corneocytes on the peeled drape (B) in peel adhesion test. Each column and vertical bar represent the mean and SD values (n = 8 for OLG-T group or n = 6 for OLG-C and PVP-I groups). Values not sharing a common letter at the same time point differ significantly at P < 0.05, as determined by Tukey’s test.