Bacterial infection of skin wounds, as one of the most important and potentially serious conditions (Appelgren et al., 2002), is of increasing public health concern and causes an enormous medical and financial burden due to the rapid emergence of drug-resistant bacteria (Irwansyah et al., 2015; Mahmoudi and Serpooshan, 2012). Systemically and topically administered antibiotics do not effectively decrease the level of bacteria, due to resistance to antibiotics exhibited by some bacteria, such as multidrug-resistant Pseudomonas aeruginosa (MDRP) and methicillin-resistant Staphylococcus aureus (MRSA), and formation of biofilms attached on various surfaces (Zhou et al., 2018).

Water droplets in a high-velocity steam-air micromist jet spray (HVS-AMJS) (SGM-MX; Aqua Science Co., Yokohama, Japan) have a much stronger impact on a surface than droplets in air, since the kinetic energy of water droplets in steam is much higher than that of droplets in air (Fukuda et al., 2017). Furthermore, the generation and propagation of the shock wave upon impact of the droplets play a significant role in cleansing. Vapour steam cleaners or steam vapour systems are cleansing devices that use steam to quickly dry, cleanse, and sanitize inanimate surfaces (Fukuda et al., 2017).

In this study, improved HVS-AMJS installed scattering prevention cover with tap water effectively removed normal bacterial flora (total viable count and coliform bacteria) from contaminated skin pieces and pig skin wounds. Furthermore, the cleansing efficacy increased by advanced-treating with bio-shell calcium oxide (BiSCaO), hypochlorous acid (HClO), sodium hypochlorite (NaClO), povidone iodine, or chlorhexidine gluconate. Especially, the improved HVS-AMJS combined with BiSCaO had higher bactericidal activity than when combined with other disinfectants. This study suggests that application of the HVS-AMJS with disinfectants, especially BiSCaO, may be useful for skin cleansing to prevent infection.

Key words: Cleansing technique / Steam-air micromist / Jet spray / Disinfectant / Bactericidal activity.
to the cellular components for wound healing (Wiegand et al., 2015), since higher concentrations are required for bactericidal activity (Kinoda et al., 2016; McCauley et al., 1989). This has led to the current use of various disinfectants such as silver nanoparticles (Ishihara et al., 2015; Nguyen et al., 2014; Mori et al., 2011), bio-shell calcium oxides (BiSCaO) powder, hypochlorous acid (HClO), and sodium hypochlorite (NaClO), based on their bactericidal properties with relatively low concentrations. (Sato et al., 2019, Kuwabara et al., 2018; Ishihara et al., 2017; Sawai, 2011). The purpose of this study was to propose a novel cleansing technique using the improved HVS-AMJS installed scattering prevention cover prototype combined with disinfectants such as BiSCaO.

The capability of the improved HVS-AMJS to remove bacteria from two contaminated materials applied with various disinfectants was evaluated (Fukuda et al., 2017). Figure 1 shows a schema of the experiment. Contaminated pig skin pieces and skin wounds created with a diameter of 1 cm and a depth of 3 mm with an 8-mm dermal punch (Kai Industries, Gifu, Japan) on a surface of pig abdomen skin (5 cm × 5 cm × 1 cm) were prepared using the contaminated suspension with normal bacterial flora which was prepared by incubating the remaining water in a bath with 10% Dulbecco’s Modified Eagle’s Medium (DMEM) at 37 ºC for 24 h (Ishihara et al., 2017; Sato et al., 2018).

Each disinfectant and their concentrations used in this study was determined according to our previous study (Sato et al. 2019). For evaluation using pig skin pieces and skin wounds, 900 ppm, 300 ppm, and 100 ppm of BiSCaO (Bio Shell Co. Ltd., Tokyo, Japan), NaClO (pH 9), and HClO (pH 6), and 9000 ppm, 3000 ppm, and 1000 ppm of povidone iodine (70 mg/mL; Iodine gargle solution 7%®, Meiji Seika Pharma Co., Ltd.) and chlorhexidine gluconate (CHG 4w/v%; Hibiscrub®, Sumitomo Dainippon Pharma Co. Ltd. Osaka, Japan) using pure water as a diluent were applied. The NaClO (pH 9) and HClO (pH 6) were prepared from 5000 ppm NaClO (pH 10.2) (Yoshida Pharmaceutical Corp., Tokyo, Japan) by dilution with distilled water and neutralization with 1N HCl. After a treatment of the improved HVS-AMJS installed scattering prevention cover combined with those disinfectants, cleansed pig skin pieces and wound skin were gently rinsed with 10 mL of pure water. The skin pieces and cotton swabs that had wiped pig skin wounds were added to 10 mL of pure water and vortexed hard for 2 min, and then the number of colony forming units (CFU) of bacterial flora per 1 mL was counted using Compact Dry TC for the total viable count (TC) and Compact Dry CF for coliform bacteria (CF) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) (Ishihara et al., 2017; Sato et al., 2018; Sato et al., 2019). Plating and counting were performed as a set of 4 technical replicates (n = 4).

The average CFU values of a contaminated pig skin piece, and wound skin were about 2 × 10⁷ (TC) and 8 × 10⁶/mL (CF), and about 9 × 10⁵ (TC) and 4 × 10⁵/mL (CF), respectively. The HVS-AMJS’s ability to remove normal bacterial flora from a contaminated pig skin piece (Figure 2), and skin wound (Figure 3) was evaluated after treatment with various disinfectants. With all contaminated materials, HVS-AMJS cleansing after rinsing with each disinfectant showed a higher additional disinfectant effect. Notably, even when rinsing with tap water or saline, bacteria flora of more than 2 log (CFU/mL) were removed from all contaminated materials.

The improved HVS-AMJS cleansing after rinsing pig skin pieces with 900 ppm of BiSCaO suspension and NaClO solutions resulted in decreases to below (near) detection limit of both TC and CF (Figure 2). It suggested that BiSCaO suspension has equal to or higher disinfection activity to skin materials than HClO and NaClO for both TC and CF. Povidone iodine and chlorhexidine gluconate required about 10-fold higher concentrations for disinfection activity comparable to BiSCaO suspension (Figure 2). At applying the improved HVS-AMJS to pig skin wounds, we found the difference of the disinfection activity between 900 ppm of BiSCaO and HClO (Figure 3). We also found that the disinfection activity of 300 ppm BiSCaO suspension was equal to 900 ppm of NaClO and HClO. It suggested that BiSCaO suspension has higher disinfection activity to skin wound than HClO and NaClO for both TC and CF. Povidone iodine and chlorhexidine gluconate with 10-fold higher concentrations than BiSCaO did not remove bacteria.
A cleansing technique using a HVS-AMJS with tap water or saline has been proposed to remove residue and microbes from skin wounds without using any chemical disinfectants or detergents (Fukuda et al., 2017). Although the HVS-AMJS can effectively remove microbes, it is difficult to completely disinfect with the HVS-AMJS alone, without combining it with any disinfectant.

Various solutions have been recommended for cleansing skins and wounds. Normal saline is favoured because it is an isotonic solution and is less likely to interfere with the normal healing process (Fernandez et al., 2007). If water is used as a cleansing solution, it should be at body temperature. Wound healing occurs at a normal core body temperature and at a body surface temperature above 33°C and below 43°C (Moufarrij et al., 2014). Maintaining the optimal wound temperature helps increase blood flow to the wound bed, enhances the rate of gain of wound tensile strength, and increases oxygen tension, which aids wound repair. The optimal wound temperature (approximately 42-45°C) also helps prevent uncontrolled proliferation of bacteria, thereby reducing the risk of infection (Moufarrij et al., 2014). In this study, the temperature on the sprayed skin surface treated with the improved HVS-AMJS installed scattering prevention cover was adjusted to approximately 42-45 °C.

Disinfectants used in the clinical setting, such as povidone iodine and chlorhexidine gluconate, have been demonstrated to be cytotoxic to the cellular components of skin.
of wound healing and with the higher concentrations required for disinfectant activity, compared to BiSCaO, NaClO, and HClO (Kinoda et al., 2016; McCauley et al., 1989; Wiegand et al., 2015). Therefore, a topical disinfectant that can decrease the bacterial bioburden of chronic wounds without inhibiting the wound healing process is a therapeutic imperative.

The hydration reaction of CaO generates strong alkaline condition, which is the primary mechanism for the disinfection action of BiSCaO. On the other hand, we found that the disinfection activity of BiSCaO for both TC and CF was much higher than that of NaOH or Ca(OH)_2 at the same pH (data not shown). This suggests BiSCaO has another mechanism for showing a high disinfection activity, for example, the OH\(^{-}\) concentration of the thin water layer formed around BiSCaO micro-particles might be higher than that of the equilibrated solution (Kubo et al., 2013). Furthermore, several researches reported that heated shell powders have higher disinfection activity against biofilms of *E. coli* (Kubo et al., 2013) and *Listeria* sp. (Shimamura et al., 2015) for deactivation and removal. Now we hypothesized that the higher concentration of OH\(^{-}\) groups might cause damage to bacterial cells in the thin aqueous surface layer, remove, and kill various bacterial cells, when BiSCaO particles contact the contaminated surfaces of pig skin pieces and pig skin wound. Furthermore, it has been reported that heated scallop-shell powder can generate active oxygen species which may also contribute to the strong disinfection activity (Kubo et al., 2013; Shimamura et al., 2015).

It has been reported that weakly acidic HClO solution has excellent *in vitro* bactericidal properties for Gram-positive organisms such as *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis*, as well as Gram-negative bacteria such as *P. aeruginosa* with lower concentrations (50-200 ppm) than basic NaClO solution (Ono et al., 2012; Sakarya et al., 2014). However, it has been reported that HClO reacts readily with various NH\(_3\)\(^{-}\) or CHO-containing organic compounds (e.g., protein, amino acid, and carbohydrate), which can result in rapid consumption of HClO molecules in environment of the infected wound (Ishihara et al., 2017; Sato et al., 2018; Sato et al., 2019). A literature demonstrated that HClO interacts with primary amino-groups (-NH\(_2\)) in organic compounds such as amino acids, thereby generating chloramine groups (-NH\(_2\)Cl or -NHCl\(_2\)) that are known to show oxidizing properties and antimicrobial activity (Gottardi et al., 2013).

This *ex vivo* study was still preliminary stage to evaluate the improved HVS-AMJS combined with disinfectants. It is remained to perform *in vivo* study using infected wound animal model. Although the improved HVS-AMJS installed scattering prevention cover combined with disinfectants had higher bactericidal activity, it is necessary to perform those disinfections using the further improved HVS-AMJS cleansing system which can simultaneously spray with disinfectants to improve convenience.

BiSCaO and HClO have not been approved by the Pharmaceuticals and Medical Devices Agency for use as a pharmaceutical or medical device, despite the approval of NaClO for this purpose. Additional systemic studies of BiSCaO and HClO are also required to establish their efficacy, safety, and stability for medical uses.

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