

# Fatty acid potassium improves human dermal fibroblast viability and cytotoxicity, accelerating human epidermal keratinocyte wound healing in vitro and in human chronic wounds

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## Abstract

Effective cleaning of a wound promotes wound healing and favours wound care as it can prevent and control biofilms. The presence of biofilm is associated with prolonged wound healing, increased wound propensity to infection, and delayed wound closure. Anionic potassium salts of fatty acids are tested with commonly used anionic surfactants, such as sodium laureth sulphate (SLES) and sodium lauryl sulphate/sodium dodecyl sulphate (SLS/SDS). The normal human dermal cells demonstrated significantly greater viability in fatty acid potassium, including caprylic acid (C8), capric acid (C10), lauric acid (C12), oleic acid (C18:1), and linoleic acid (C18:2), than in SLES or SLS after a 24-hour incubation. Cytotoxicity by LDH assay in a 5-minute culture in fatty acid potassium was significantly lower than in SLES or SLS. *in vitro* wound healing of human epidermal keratinocytes during the scratch assay in 24-hour culture was more significantly improved by fatty acid treatment than by SLES or SLS/SDS. In a live/dead assay of human epidermal keratinocytes, C8K and C18:1K demonstrated only green fluorescence, indicating live cells, whereas synthetic surfactants, SLES and SLS, demonstrated red fluorescence on staining with propidium iodide, indicating dead cells after SLES and SLS/SDS treatment. Potassium salts of fatty acids are useful wound cleaning detergents that do not interfere with wound healing, as observed in the scratch assay using human epidermal keratinocytes. As potassium salts of fatty acids are major components of natural soap, which are produced by natural oil and caustic potash using a saponification method, this may be clinically important in wound and peri-wound skin cleaning. In human chronic wounds, natural soap containing fatty acid potassium increased tissue blood flow based on laser speckle flowgraphs after 2 weeks ( $P < .05$ ), in addition to removing the eschars and debris. Wound cleansing by natural soap of fatty acid potassium is beneficial for wound healing.

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## KEYWORDS

fatty acid potassium, human chronic wounds, laser speckle flowgraph, scratch assay, wound cleaning

## 1 | INTRODUCTION

Biological burden, particularly in the form of microbial 'biofilms', is a serious obstacle to wound healing.

Wound cleaning is the most important process when preparing wound beds for healing to start the subsequent healing process.

Wound cleaning is generally considered an important part of wound healing and wound care because it helps remove dead tissue, bacteria, and foreign bodies from the wound and peri-wounds. No specific cleansing fluid or technique has been demonstrated as the best,<sup>1</sup> but saline or tap water are considered appropriate.<sup>2</sup> However, saline and tap water are chemically limited for dissolving debris in wounds.

Effective cleaning of a wound promotes wound care as it can prevent and control biofilms. Biofilms have been identified in non-healing chronic wounds and are intractable to both the immune system and antimicrobials; their presence is associated with prolonged wound healing, increased wound propensity to infection, and delayed wound closure.<sup>3</sup> Even in acute wounds, the development of biofilms leads to chronic inflammation. This inflammation increases the levels of pro-inflammatory cytokines and matrix metalloproteinase production.

Wound cleaners are often used before and/or together with debridement agents to remove loose tissue debris, bacteria, and other materials.

Wound debridement is markedly effective, and has been widely adopted to remove necrotic tissue from a wound and its margin, to remove dead or infected tissue, and to promote healing.<sup>4-6</sup>

Necrotic tissue prolongs the inflammatory stage and may create the environmental conditions necessary for bacteria biofilms to form. Wound debridement may be performed by several different methods, including surgical, mechanical, autolytic, enzymatic, and electrical.<sup>7-10</sup>

Potassium oleate (C18:1K), a type of fatty acid potassium and most representative ingredient in natural soap, exerts bactericidal effects against numerous species, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Clostridium difficile*. It also removed significantly greater amounts of *S aureus* biofilm material than sodium laureth sulphate (SLES) and sodium lauryl sulphate/sodium dodecyl sulphate (SLS/SDS), and maintained fibroblast viability with lower cytotoxicity;

### Key Messages

- we compared the anionic fatty acid potassium components, ingredients of natural soap, with other anionic surfactants, such as SLES and SLS/SDS, common ingredients of detergents, in terms of cytotoxicity, cell viability and wound healing, and tested a natural soap containing fatty acid salts on chronic wounds for two weeks using non-contact and non-invasive measurement by laser speckle flowgraphs for clinical efficacy
- anionic potassium salts of fatty acids were compared and tested with commonly used anionic surfactants, such as sodium laureth sulphate (SLES) and sodium lauryl sulphate/sodium dodecyl sulphate (SLS/SDS). The normal human dermal cells demonstrated significantly greater viability in fatty acid potassium, including caprylic acid (C8), capric acid (C10), lauric acid (C12), oleic acid (C18:1), and linoleic acid (C18:2), than in SLES or SLS after a 24-hour incubation. Cytotoxicity by LDH assay in a 5-minute culture in fatty acid potassium was significantly lower than SLES or SLS
- in a live/dead assay of human epidermal keratinocytes, C8K and C18:1K are tested
- in results, in vitro, wound the potassium salts of fatty acids significantly improved than in SLES or SLS/SDS. In vivo, chronic wounds are tested with wound cleansing by natural soap of fatty acid potassium is beneficial for wound healing observed by laser speckle flowgraph after two weeks ( $P < .05$ )
- wound cleansing by natural soap of fatty acid potassium is beneficial for wound healing

therefore, it may be useful for wound cleaning and peri-wound skin healing.<sup>11</sup>

We compared the anionic fatty acid potassium components with other anionic surfactants, such as SLES and SLS/SDS, in terms of cytotoxicity, cell viability and wound healing, and tested a natural soap containing fatty acid salts on chronic wounds for 2 weeks using non-

contact and non-invasive measurement by laser speckle flowgraph.

## 2 | MATERIALS AND METHODS

### 2.1 | Reagents

Lauric acid (C12) and oleic acid (C18:1) were purchased from Tokyo Chemical Industry Co, Ltd (Tokyo, Japan). Caprylic acid (C8), capric acid (C10), linoleic acid (C18:2), SLS, potassium hydroxide (KOH), and Hank's balanced salt solution (HBSS) (+) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). SLES was purchased from the NOF Corporation (Tokyo, Japan).

### 2.2 | Preparation of surfactant solutions

A total of five different fatty acids, potassium caprylate (C8K), potassium caprate (C10K), potassium laurate (C12K), potassium oleate (C18:1K) and potassium linoleate (C18:2K), and two synthetic surfactants, SLES and SLS, were used in the assays. HBSS(+) buffer containing Ca and Mg was used to produce the fatty acid solutions. All HBSS(+) solutions of the fatty acid salts (C8K, C10K, C12K, C18:1K, and C18:2K; all 0.5 mM) were prepared by mixing the relevant fatty acid (C8, C10, C12, C18:1, and C18:2, respectively) with KOH that was solubilised with HBSS(+) at 50°C. Then, the pH of each fatty acid salt was adjusted to 10.4 by adding KOH. SLES and SLS (0.5 mM) were prepared by diluting them in HBSS(+). Both solutions had final pH values of 8.0. HBSS(+) was used as a positive control.

### 2.3 | Cell culture

Adult normal human dermal fibroblasts, NHDF-Ad cells (Lonza Japan Ltd, Tokyo, Japan, CC-2511), and adult normal human epidermal keratinocytes, NHEK-Ad cells (Lonza Japan Ltd, 00192627), were cultured in FGM™ – 2 BulletKit™ medium (Lonza Japan Ltd)<sup>12</sup> and KGM-Gold™ BulletKit™ medium (Lonza Japan Ltd),<sup>13</sup> respectively. In total,  $1 \times 10^4$  NHDF-Ad cells were added to each well of Nunc™ MicroWell™ 96-well microplates (Thermo Fisher Scientific K. K., Tokyo, Japan) before being pre-cultured for 24 hours in the cytotoxicity assay. In total,  $2 \times 10^5$  NHEK-Ad cells were added to each well of Nunc™ MicroWell™ 12-well microplates (Thermo Fisher Scientific K. K.) before being pre-cultured for 20 hours in the scratch assay and for 24 hours in the live/

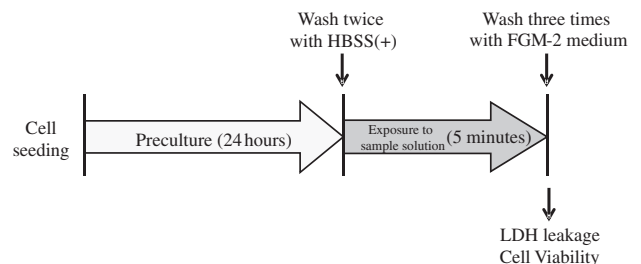
dead assay. All cells were cultured in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C.

### 2.4 | Cytotoxicity assay

LDH leakage and cell viability were evaluated as indices of cytotoxicity.<sup>11,14</sup> NHDF-Ad cells were pre-cultured for 24 hours and washed twice with HBSS(+) before treatment with 100 μL/well of 0.5 mM C8K, C10K, C12K, 18:1K, C18:2K, SLES, or SLS for 5 minutes at room temperature. HBSS(+)-treated cells were used as the control. Then, the solution was collected from each well and used for the LDH leakage assay. NHDF-Ad cells were washed three times with FGM-2 medium and fresh FGM-2 medium was added before their viability was evaluated. LDH leakage and cell viability were assessed in each well using a cytotoxicity LDH assay kit-WST (Dojindo Laboratories, Kumamoto, Japan) and cell counting kit-8 (Dojindo Laboratories), respectively (Figure 1).

### 2.5 | Scratch assay

NHEK-Ad cells were pre-cultured for 20 hours and washed twice with HBSS(+) before treatment with 1 mL/well of 0.5 mM C8K, C10K, C12K, C18:1K, C18:2K, SLES, or SLS for 5 minutes at room temperature. During the treatment, a linear scratch was made by a sterile P200 pipette tip. The cells were washed twice with KGM-Gold medium to remove debris and cultured with fresh KGM-Gold medium in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C for 24 hours.<sup>15</sup> Before and after the culture, the cells were photographed using a phase-contrast microscope (Bioevo BZ-9000, Keyence Corporation, Osaka, Japan) and the cell images were analysed using the ImageJ



**FIGURE 1** Cytotoxicity test procedure for NHDF-Ad cells. NHDF-Ad cells pre-cultured in FGM-2 medium for 24 hours were treated with 100 μL/well of each sample for 5 minutes. The solution was collected from each well and used for the LDH leakage assay. Then, their viability was assessed

software.<sup>12,13,16,17</sup> The wound healing rate after 24 hours was calculated according to the following equation<sup>17</sup>:

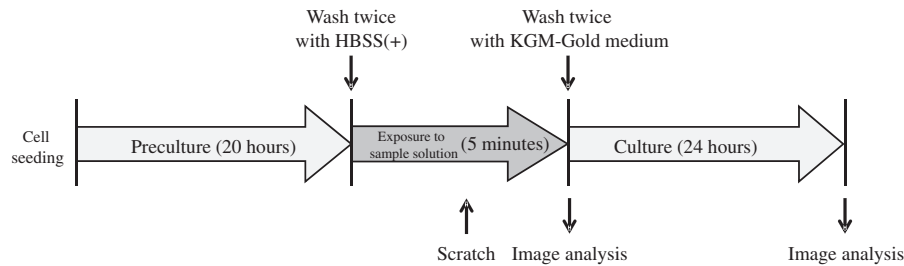
$$\text{Wound healing rate (\%)} = \frac{(\text{Scratch area at 0 hours}) - (\text{Scratch area after 24 hours})}{(\text{Scratch area at 0 hours})} \times 100$$

Each sample was assayed in triplicate and three time points in each assay were selected for taking

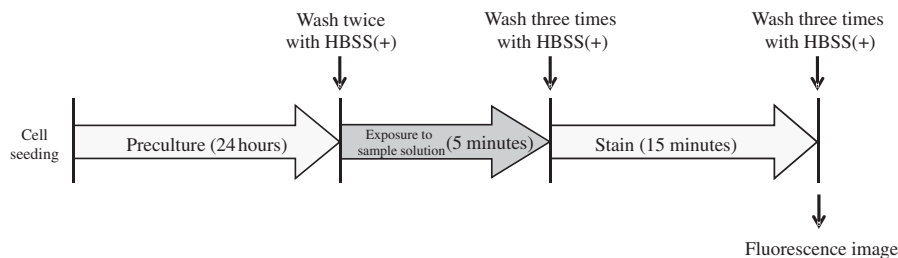
photographs. The wound healing rate was calculated as the mean value  $\pm$  standard deviation in  $n = 9$  (Figure 2).

## 2.6 | Live/dead assay

Calcein-AM and propidium iodide (PI) were used to identify live and dead cells, respectively (Cellstain Double Staining Kit; Dojindo Laboratories). An assay solution consisting of calcein-AM and PI was prepared by adding 10  $\mu\text{L}$  of 1 mmol/mL calcein-AM stock solution and 15  $\mu\text{L}$



**FIGURE 2** Scratch assay procedure for NHEK-Ad cells. NHEK-Ad cells pre-cultured in KGM-Gold medium for 20 hours were treated with 1 mL/well of each sample for 5 minutes. During the treatment, a linear scratch was made by a sterile P200 pipette tip. NHEK-Ad cells were washed twice with KGM-Gold medium and cultured. Just after and 24 hours after the treatment, the cells were photographed using a phase-contrast microscope



**FIGURE 3** Live/dead assay procedure for NHEK-Ad cells. NHEK-Ad cells pre-cultured in KGM-Gold medium for 24 hours were treated with 1 mL/well of each sample for 5 minutes. NHEK-Ad cells were washed three times with HBSS(+), and stained with calcein AM and propidium iodide for 15 minutes. After staining, the cells were washed three times with HBSS(+) and photographed using a fluorescence microscope

**TABLE 1** Patients in this study

	Location	Age	Sex	Outcome	Margin, pre-	Centre, pre-	Margin, 2 weeks	Centre, 2 weeks
1	Sacral	87	M	Improved	856.5	978.2	1450.3	1050.1
2	Lower ext	80	M	Improved	486.9	245.0	586.1	278.0
3	Trochanter	86	F	Healed	789.1	1043.2	1350.1	1134.5
4	Lower ext	66	F	Improved	521.3	456.3	621.5	601.1
5	Sacral	67	M	Healed	434.3	540.5	589.2	578.3
6	Lower ext (toe)	68	F	Improved	533.3	444.3	1000.6	1161.3
7	Sacral	81	M	Improved	452.3	645.7	521.5	701.5
8	Sacral	89	F	Healed	677.1	590.6	601.5	901.2
	Mean $\pm$ SD	78.0 $\pm$ 9.59			563.6 $\pm$ 201.01	648.2 $\pm$ 234.30	801.6 $\pm$ 419.43	839.3 $\pm$ 252.72

of 1.5 mmol/mL PI solution to 5 mL of HBSS(+). NHEK-Ad cells were pre-cultured for 24 hours and washed twice with HBSS(+) before treatment with 1 mL/well of 0.5 mM C8K, C18:1K, SLES or SLS for 5 minutes at room temperature. The cells were washed three times with HBSS(+) and stained with 0.5 mL of the assay solution for 15 minutes.<sup>18</sup> The cells were washed with HBSS(+) three times and fresh HBSS(+) was added to each well. The double-stained cells were assessed by fluorescence microscopy (Bioevo BZ-9000, Keyence Corporation) (Figure 3).

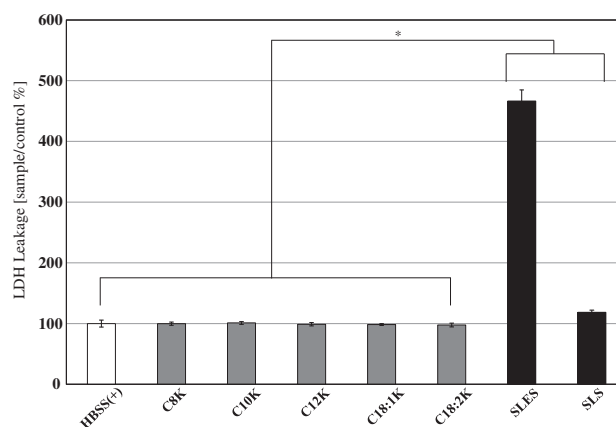
## 2.7 | Laser speckle flowgraphy of chronic wounds

Laser speckle flowgraphy (LSFG) (LSFG-PI, Softcare Co, Ltd, Fukuoka, Japan) was used to assess chronic wounds.



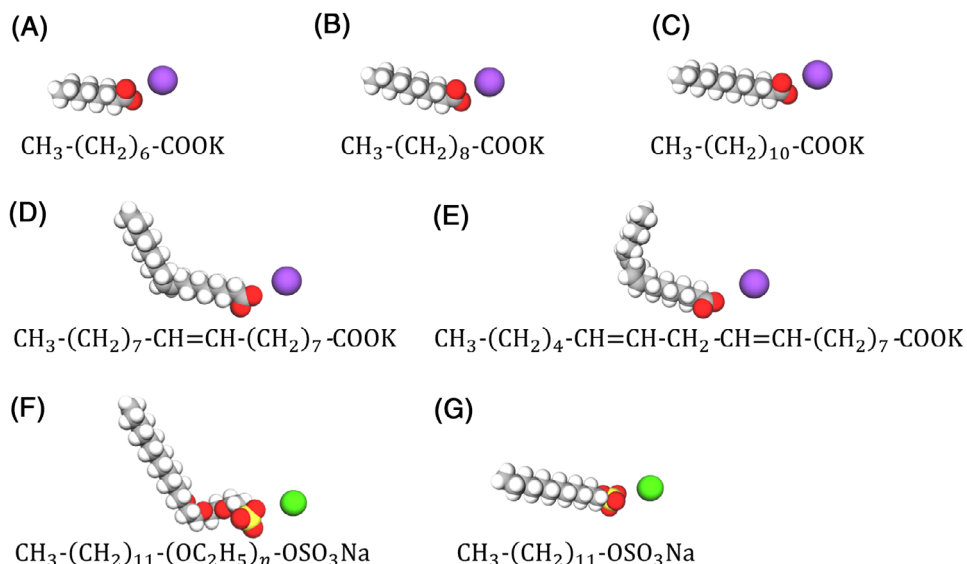
**FIGURE 4** Laser speckle flowgraph can measure wounds in a non-invasive and non-contact manner

Eight patients, four males and four females,  $78.0 \pm 9.59$  years, were included (Table 1). The study was approved by the internal review board of the hospital (Menoto Hospital, Nagasaki, Japan, MN-001-05) and consent to the study was received from all patients. Wounds consisted of four sacro-coccygeal injuries, three lower extremity injuries, and one trochanter pressure injury. The natural soap used in this study was Body Soap (Shabondama Soap Co, Ltd, Fukuoka, Japan). This soap is made only of natural ingredients, such as vegetable and animal oils with hydroxylate potassium; thus, the active ingredient of the soap is the fatty acid potassium. Assessment was performed before and 2 weeks after soap cleansing. The perimeters and centres of the wounds



**FIGURE 6** LDH leakage of NHEK-Ad cells treated with 0.5 mM fatty acid potassium, sodium laureth sulphate (SLES) or sodium lauryl sulphate (SLS) for 5 minutes. Cells treated with HBSS(+) were used as the control. The percent LDH leakage was calculated relative to the control value. Results are expressed as the mean  $\pm$  standard deviation (SD) ( $n = 6$ ). \* $P < .01$ . Control: white; fatty acid potassium: grey; synthetic detergent: black

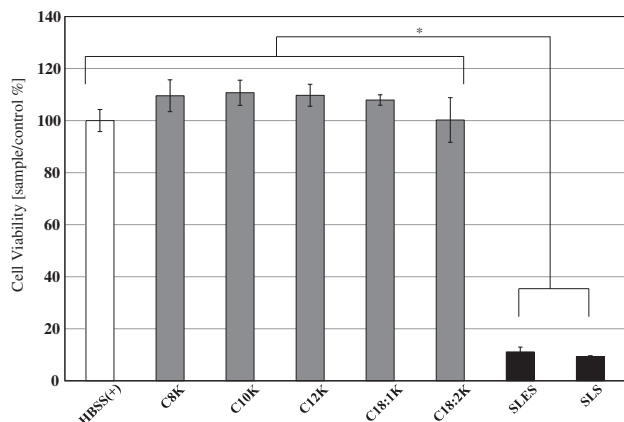
**FIGURE 5** Structures of the fatty acid potassium molecules and synthetic surfactant detergents. A, Potassium caprylate (C8K); B, potassium caprate (C10K); C, potassium laurate (C12K); D, potassium oleate (C18:1K); E, potassium linoleate (C18:2K); F, sodium laureth sulphate (SLES); and G, sodium lauryl sulphate (SLS). Carbon: grey; hydrogen: white; oxygen: red; sulphur: yellow; potassium ions: purple; and sodium ions: green





were measured three times each, and the mean value was calculated.

LSFG emits an 830-nm laser, LASER class 1, which can maximally detect a 50 × 35-cm area, with a measurement time of 4 seconds at a distance of 30 to 50 cm from the object.



**FIGURE 7** Viability of NHDF-Ad cells treated with 0.5 mM fatty acid potassium, sodium laureth sulphate (SLES) or sodium lauryl sulphate (SLS) for 5 minutes. The cells treated with HBSS(+) were used as the control. Percent cell viability was calculated relative to the control value. Results are expressed as the mean  $\pm$  SD (n = 6). \* $P < .01$ . Control: white; fatty acid potassium: grey; synthetic detergent: black

LSFG captures the speckle change over time, and the relative flow volume and region of interest (ROI) can be mapped two-dimensionally (Figure 4).

### 3 | STATISTICAL ANALYSIS

Data are presented as the mean  $\pm$  standard deviation. Statistical analyses were performed using repeated measures analysis of variance for the cytotoxicity assay and the scratch assay.  $P$ -values of  $<.05$  were considered significant.

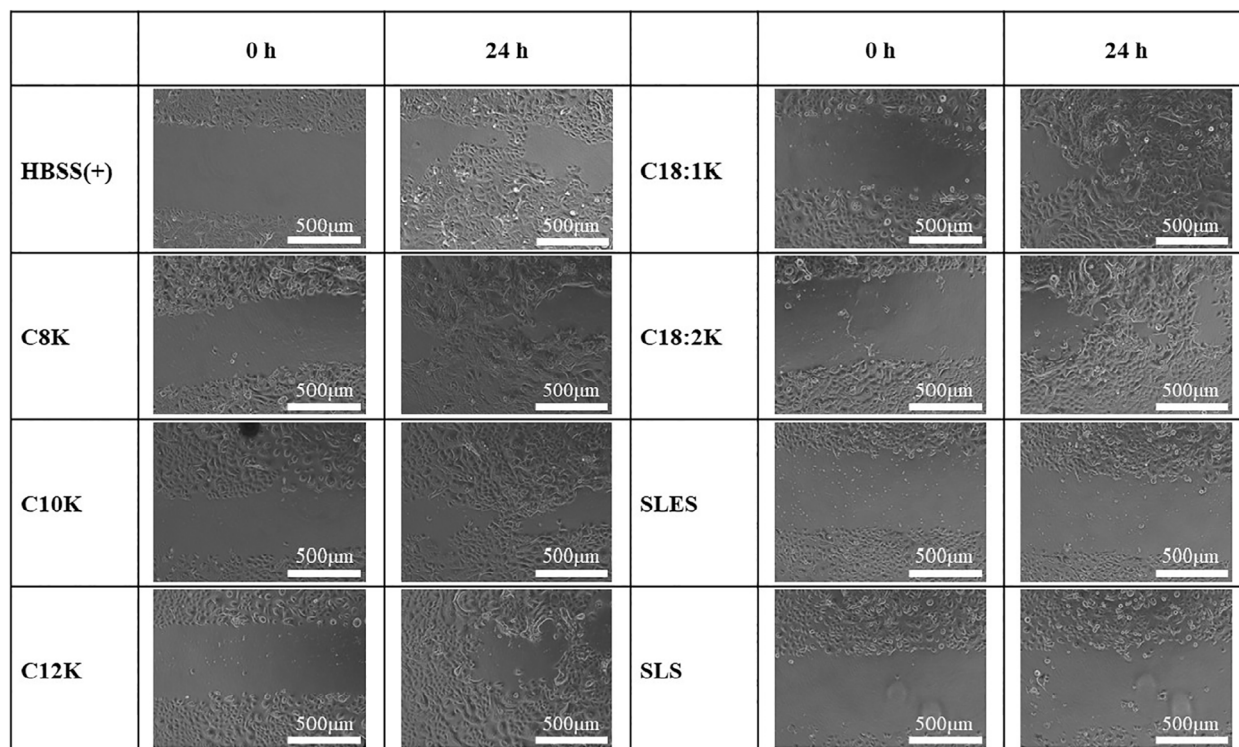
## 4 | RESULTS

### 4.1 | Structures of fatty acid potassium and synthetic surfactant detergents

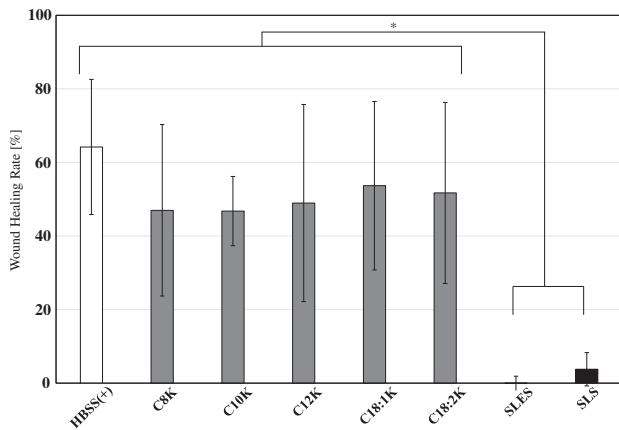
Five types of fatty acid potassium and two types of synthetic surfactant detergents were examined in the present study (Figure 5).

### 4.2 | Cytotoxicity assay

To evaluate the cytotoxicity of the test solution to NHDF-Ad cells, lactate dehydrogenase (LDH) leakage, and cell viability were measured.

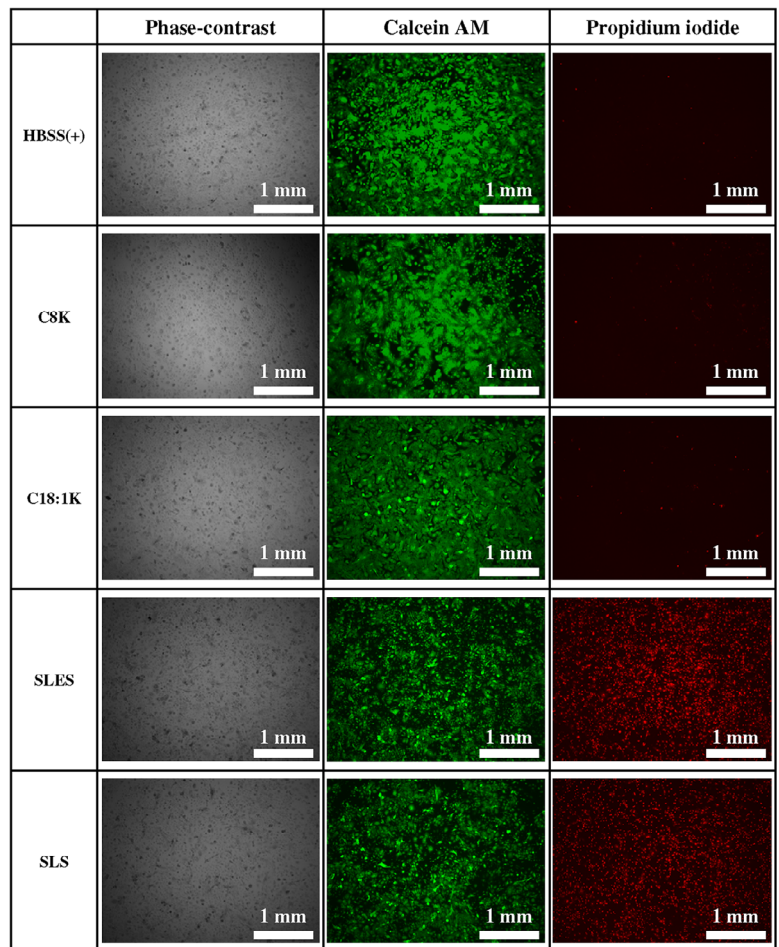


**FIGURE 8** Phase-contrast microscope images of NHEK-Ad cells just after and 24 hours after treatment with HBSS(+), C8K, C10K, C12K, C18:1K, C18:2K, sodium laureth sulphate (SLES) or sodium lauryl sulphate (SLS) (10x)



**FIGURE 9** Semi-quantitative results of Figure 4. The wound healing rate of NHEK-Ad cells treated with 0.5 mM fatty acid potassium, sodium laureth sulphate (SLES) or sodium lauryl sulphate (SLS) for 5 minutes. The cells treated with HBSS(+) were used as the control. Results are expressed as the mean ± SD (n = 9). \*P < .01. Control: white; fatty acid potassium: grey; synthetic detergent: black

**FIGURE 10** Phase-contrast and fluorescence images of NHEK-Ad cells treated with HBSS(+), C8K, C18:1K, sodium laureth sulphate (SLES) or sodium lauryl sulphate (SLS). The green and red fluorescence indicates the presence of living cells and dead cells, respectively (4x)



### 4.2.1 | LDH leakage

Five minutes after treatment, LDH leakage by the NHDF-Ad cells was quantified using a measurement kit and absorbance measurement. The cells treated with fatty acid potassium demonstrated significantly less LDH leakage than those treated with synthetic surfactants (percent relative to the cells treated with HBSS(+): [HBSS(+): 100.0% ± 5.7%; [C8K]: 99.8% ± 2.7%; [C10K]: 101.1% ± 2.2%; [C12K]: 98.8% ± 2.8%; [C18:1K]: 98.4% ± 1.6%; [C18:2K]: 97.6% ± 3.1%; [SLES]: 466.5% ± 18.0%; and [SLS]: 118.4% ± 3.8%; P < .01 for all types of fatty acid potassium versus SLES and SLS) (Figure 6).

### 4.2.2 | Cell viability

Five minutes after treatment, cell viability of the NHDF-Ad cells was quantified using a measurement kit and

absorbance measurement. The cells treated with fatty acid potassium demonstrated significantly higher cell viability than those treated with synthetic surfactants (percent relative to the cells treated with HBSS(+): [HBSS(+)]:  $100.0\% \pm 4.2\%$ ; [C8K]:  $109.5\% \pm 6.1\%$ ; [C10K]:  $110.7\% \pm 4.9\%$ ; [C12K]:  $109.7\% \pm 4.2\%$ ; [C18:1K]:  $107.9\% \pm 2.0\%$ ; [C18:2K]:  $100.2\% \pm 8.6\%$ ; [SLES]:  $11.1\% \pm 1.9\%$ ; and [SLS]:  $9.3\% \pm 0.4\%$ ;  $P < .01$  for all types of fatty acid potassium versus SLES and SLS) (Figure 7).

### 4.3 | Scratch assay

After 5 minutes of treatment followed by 24 hours of culture, the NHEK-Ad cells treated with sample solutions were photographed using a phase-contrast microscope (Figure 4). A monolayer of cells was scratched to create a cell-free zone with a width of approximately  $500\ \mu\text{m}$  in each well, and wound closure due to the proliferation and migration of cells was observed. The cells treated with HBSS(+) or fatty acid potassium demonstrated a significantly higher wound healing rate than those treated with synthetic surfactants (percent wound healing rate: [HBSS(+)]:  $64.2\% \pm 18.4\%$ ; [C8K]:  $47.0\% \pm 23.3\%$ ; [C10K]:

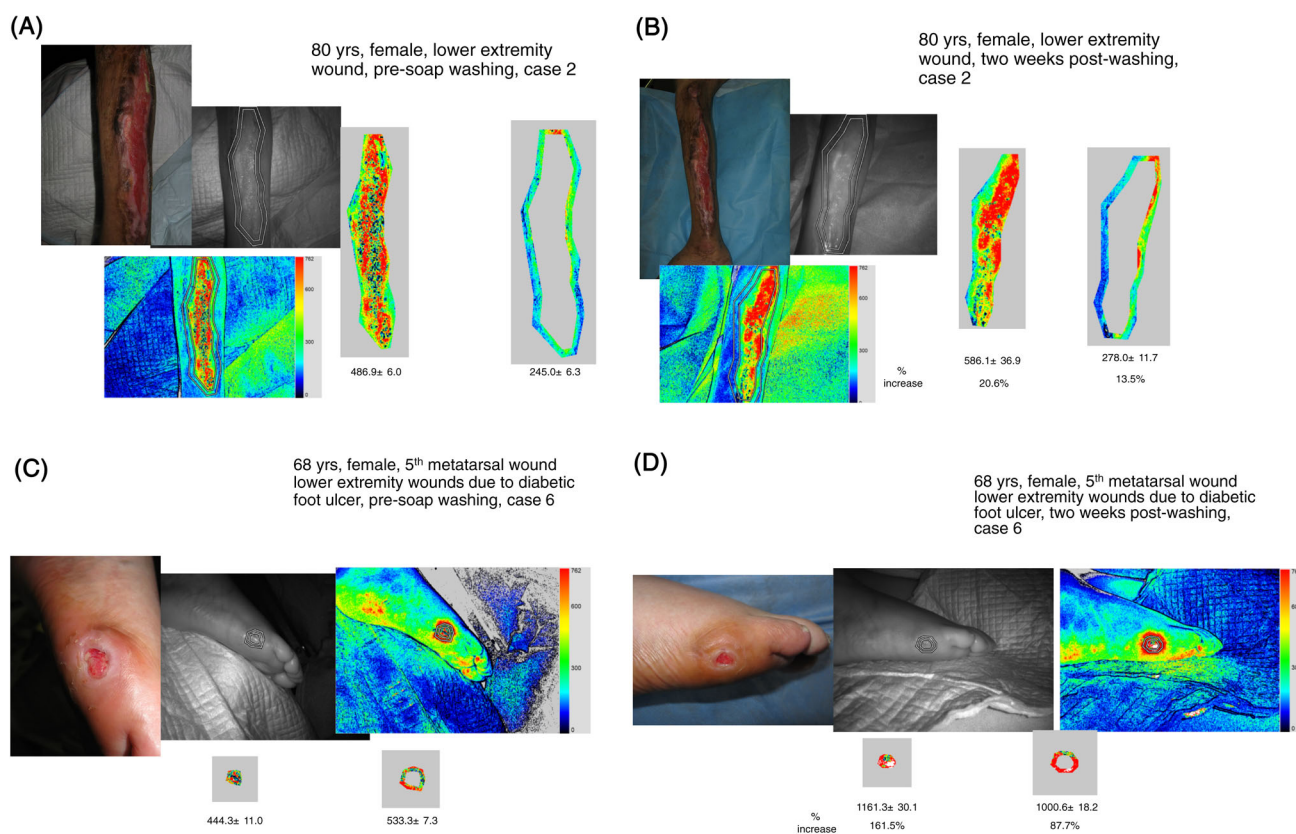
$46.8\% \pm 9.4\%$ ; [C12K]:  $49.0\% \pm 26.8\%$ ; [C18:1K]:  $53.7\% \pm 22.9\%$ ; [C18:2K]:  $51.7\% \pm 24.6\%$ ; [SLES]:  $0.0\% \pm 1.9\%$ ; and [SLS]:  $3.8\% \pm 4.5\%$ ;  $P < .01$  for all types of fatty acid potassium versus SLES and SLS) (Figures 8 and 9).

### 4.4 | Live/dead assay

After 5 minutes of treatment followed by 15 minutes of staining with calcein AM and PI, the NHEK-Ad cells were photographed using a fluorescence microscope. The cells treated with HBSS(+) and fatty acid potassium, C8K and C18:1K, exhibited only green fluorescence staining by calcein AM, indicating the presence of living cells, whereas the cells treated with synthetic surfactants, SLES and SLS, exhibited red and green fluorescence when stained by PI, suggesting the presence of dead cells (Figure 10).

### 4.5 | LSFG for chronic wounds

After 2 weeks of washing, five wounds improved and three healed. Sloughing and necrosis were removed by washing. The mean values in the ROI on LSFG for the



**FIGURE 11** A, LSFG of an 80-year-old female, lower leg wound, pre-washing. B, LSFG of an 80-year-old female, lower leg wound, 2 weeks after washing. C, LSFG of a 68-year-old female, 5th metatarsal wound due to diabetic foot ulcer, pre-washing. D, LSFG of a 68-year-old female, 5th metatarsal wound due to diabetic foot ulcer, 2 weeks after washing



wound perimeter and centre were  $563.6 \pm 201.01$  and  $648.2 \pm 234.30$ , respectively, and those at 2 weeks were  $801.6 \pm 419.43$  and  $839.3 \pm 252.72$ , respectively ( $P < .05$ ) (Figure 11; Table 1).

## 5 | DISCUSSION

In vitro tests using 17 commercially available cleaners and 3 liquid bath soaps with human infant dermal fibroblasts and epidermal keratinocytes showed that Dial antibacterial soap and Ivory Liqui-Gel were the most toxic to fibroblasts, whereas hydrogen peroxide, modified Dakin's solution and povidone (10%) were the most toxic to keratinocytes using a toxicity index.<sup>19</sup>

Surfactants are extensively used as detergents in the food and emollient industries. In wound care, many wound dressings may contain surfactants in a concentrated formula.<sup>20</sup> However, the roles of these surfactants in wound healing are not well known because they are generally considered to be of limited value to support wound cleaning, biofilm management and inflammation reduction or to improve cellular proliferation and regeneration. On the other hand, the use of poloxamer-based surfactants in wound healing was reported to play a significant role in biofilm management, MMP regulation, and cellular resuscitation/salvage. Poloxamer-based surfactants are non-ionic and are used as wound dressings or in gels to cover the wound.

SLES and SLS/SDS are detergents used in cleaning, and are often used in skin patch tests for skin irritants. SLS has been used in skin patch tests, and discussed in terms of skin atopic diathesis, dermatitis, rhinitis, and asthma, which do not depend on the atopic status of the patient's skin.<sup>21</sup> SLES is a minor irritant to skin compared with SLS.<sup>22</sup> In a soak test, however, cutaneous capacitance was significantly reduced in an SLES-treated region, even though SLES is used globally in personal care products because of its mildness and good foaming ability.<sup>23</sup>

Squamometry is a sensitive assessment technique to detect surfactant-induced subclinical skin surface alterations, and to differentiate surfactant effects in a washing model through as few as three washes using SLES and SLS.<sup>24</sup>

In pulse/chase experiments, the use of a mild detergent to solubilise normal and mucopolipidosis II human skin fibroblasts, which maintain insoluble aggregated latency-associated peptide (LAP), may account for the lack of intra-cellular LAP accumulation compared with normal human skin fibroblasts. The pool detected in whole SDS/SLS lysates in mucopolipidosis II skin fibroblasts may also represent protein that stably accumulated in the lysosome over an extended culture time.<sup>25</sup>

In contrast, fatty acid potassium, a major ingredient in natural soap, is also anionic and used when a wound and peri-wound skin are cleaned by rubbing.

In a previous report, both mouse embryonic fibroblasts and human keratinocytes demonstrated significantly increased cell viability and decreased cytotoxicity in the presence of fatty acid potassium and potassium oleate compared with SLES and SLS/SDS, whereas fatty acid potassium maintained comparable bactericidal effects on *E coli*, *S aureus*, and *B cereus*, and significantly increased bactericidal activity against *C difficile* compared with alcohol-based disinfectants. Potassium oleate can remove *S aureus* biofilms more effectively than SLES and SLS/SDS.<sup>11</sup>

In this experiment, normal human dermal cells demonstrated significantly greater viability in fatty acid potassium-like caprylic acid (C8), capric acid (C10), lauric acid (C12), oleic acid (C18:1), and linoleic acid (C18:2) than in SLES or SLS in a 24-hour incubation.

Cytotoxicity denoted by the LDH assay in a 5-minute culture in fatty acid potassium was significantly lower than in SLES or SLS.

In vitro wound healing of human epidermal keratinocytes by scratch assay in a 24-hour culture was more significantly increased after fatty acid treatment than with SLES or SLS/SDS.

In a live/dead assay of human epidermal keratinocytes, C8K and C18:1K demonstrated only green fluorescence when stained by calcein AM, indicating the presence of living cells. On the other hand, the cells treated with synthetic surfactants, SLES and SLS, exhibited red fluorescence staining with PI in addition to green, suggesting the presence of dead cells.

After washing and cleansing using natural soap containing fatty acid potassium for 2 weeks, increased wound healing was observed. As reported for critical limb ischemia, factors impairing topical tissue perfusion are important in wound healing,<sup>26,27</sup> and a proper wound cleanser is able to remove the biological burden and infection.<sup>28</sup>

In a randomised clinical trial of irrigation solutions to 400 open lower extremity patients, castile soap solution group was significantly less wound healing problem compared with bacitracin solution group.<sup>29</sup> Also, a randomised clinical trial of wound irrigation comparing soap and normal saline did not affect health-related quality of life after open fractures.<sup>30</sup> In the treatment of foot wounds in patients affected by critical limb ischemia, CLI, the vacuum-assisted closure, VAC, improve tissue perfusion and suctioning the exudates, and facilitating the removal of bacteria from the wound by causing vacuum.<sup>31</sup> Soap may also remove wound debris and provide better healing condition such as tissue perfusion.

LSFG is frequently used in ophthalmic fields to evaluate the microvasculature as a rapid and non-invasive

method to assess basal blood flow and neuronal activity-dependent alterations in ocular diseases. LSFSG was also suggested to be a useful biomarker not only for detection, but also for monitoring postoperative visual outcomes in patients with retinal and choroidal vascular diseases.<sup>32</sup> LSFSG was also used as a neuromonitor of cerebral circulation for retrograde femoral artery perfusion.<sup>33</sup>

In our wound assessment, the relative flow volume significantly increased in the perimeter and centre of the wounds after 2 weeks, and all wounds improved by washing with a natural soap containing fatty acid salts. Cleansing with fatty acid salts may aid in wound bed preparation by reducing the infectious biological burden.<sup>11</sup>

Therefore, fatty acid potassium is a useful detergent for wound cleaning that does not interfere with wound healing, as noted in the scratch assay using human epidermal keratinocytes.

As fatty acid potassium is a major component of natural soap, which is produced by natural oil and caustic potash using a saponification method, this may be clinically important for peri-wound skin cleaning.

## CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

## AUTHOR CONTRIBUTIONS

Akihiro Masunaga: Conceptualization, methodology, validation, investigation, writing—original draft preparation, visualisation, project administration. Takayoshi Kawahara: Conceptualization, validation, writing—original draft preparation, project administration. Hayato Morita: resources, supervision, funding acquisition. Kohji Nakazawa: methodology, formal analysis, investigation, supervision. Yuto Tokunaga: methodology, investigation. Sadanori Akita: Conceptualization, validation, data curation, writing—original draft preparation, writing—review and editing. All authors read and agreed to the published version of the manuscript.

## DATA AVAILABILITY STATEMENT

Yes

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