Check for updates

Received: 28 November 2022 | Revised: 10 August 2023 | Accepted: 29 August 2023

DOI: 10.1111/jocd.15988

ORIGINAL ARTICLE

JCD Journal of Cosmetic Dermatology

WILEY

Effects of Schumann resonance on the proliferation and migration of normal human epidermal keratinocytes and the expression of *DEFB1* and *SIRT1*

Hidemi Sugiwaki[1] | Mayumi Kotani PhD[1] | Akihito Fujita[1] | Shinichi Moriwaki MD, PhD[2]

[1]Bloom Classic Co., Ltd., Ehime, Japan

[2]Department of Dermatology, Faculty of Medicine, Osaka Medical and Pharmaceutical University, Osaka, Japan

Correspondence
Shinichi Moriwaki, Department of Dermatology, Osaka Medical and Pharmaceutical University, 2-7 Daigaku-machi, Takatsuki, Osaka 569-8686, Japan.
Email: shinichi.moriwaki@ompu.ac.jp

Abstract

Background: When the skin is damaged and its barrier function is disrupted, the proliferation and migration of epidermal keratinocytes are vital for repairing the damaged area. The Schumann resonance at 7.8 Hz has been reported to protect rat cardiomyocytes against oxidative stress and inhibit the proliferation of B16 mouse melanoma cells. However, its effect on the skin is unknown.

Aims: In this study, we applied 7.8-Hz electromagnetic waves to normal human epidermal keratinocytes (NHEKs) and investigated its effects on cell proliferation and migration, β-defensin (*DEFB1*) and sirtuin 1 (*SIRT1*) expression.

Methods: We performed cell proliferation assay, cell migrationassay and gene expression analysis of *DEFB1* and *SIRT1*.

Results: We found that the application of 7.8-Hz electromagnetic waves caused a 2.8-fold increase in NHEK proliferation, enhanced cell migration, and increased the expression of *DEFB1* and *SIRT1* by 2.4-fold and 4.9-fold, respectively.

Conclusions: These results suggest that the application of 7.8-Hz electromagnetic waves may contribute to improving the skin barrier function and skin ulcer.

KEYWORDS
normal human epidermal keratinocytes, Schumann resonance, sirtuin 1, ultra-low-frequency waves, β-Defensin

1 | INTRODUCTION

Schumann resonances are electromagnetic wave patterns that resonate with the size of the Earth, forming standing waves on the Earth's surface. They occur at a number of different frequencies, including 7.8 Hz, 14.1 Hz, 20.3 Hz, 26.4 Hz, and 32.4 Hz. In particular, the Schumann resonance at the ultra-low frequency of 7.8 Hz resembles the waves that appear in the relaxed human brain (α waves, 8–14 Hz) and has been reported to protect rat cardiomyocytes against oxidative stress[1] and inhibit the proliferation of B16 mouse melanoma cells.[2] However, their effect on normal human keratinocytes (NHEKs) has not been reported.

Keratinocytes, ceramide, other stratum corneum intracellular lipids, and tight junctions in the stratum granulosum are all implicated in the maintenance of skin barrier function. Not only does this barrier function protect the skin against dryness, increased ultraviolet light exposure, chemical irritation from substances such as cleaning products, and physical irritation from pollen and

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2023 The Authors. *Journal of Cosmetic Dermatology* published by Wiley Periodicals LLC.

atmospheric pollutants such as PM 2.5, antimicrobial peptides produced by epidermal keratinocytes, such as β-defensin (*DEFB1)* and cathelicidin, also protect the skin against bacteria, viruses, and other pathogens, improving the skin barrier function. When the skin is damaged and its barrier function is disrupted, the proliferation and migration of epidermal keratinocytes are vital for repairing the damaged area, but substances derived from *Staphylococcus aureus* in the resident skin flora may be aggravating factors for inflammation.[3] Newly synthesized sirtuin 1 (SIRT1)-activating factors (piper amide derivatives (*E*)-3-(2,4-dichlorophenyl)-*N*-phenylacrylamide, NED416) upregulate SIRT1 expression and promote cell migration in NHEKs and fibroblasts,[4] indicating that SIRT1 may be a repair factor of damaged skin.

In this study, we investigated whether the application of electromagnetic waves at 7.8 Hz, a Schumann resonance, affects the proliferation and migration of NHEKs, as well as its effect on the expression of the antimicrobial peptide gene *DEFB1* and *SIRT1*, which is involved in cell proliferation.

2 | MATERIALS AND METHODS

2.1 | Cultivation of normal human epidermal keratinocytes

NHEKs from a single child donor (NHEK, PromoCell LLC, Heidelberg, Germany, 2–4 generations) were cultured in keratinocyte growth medium 2 (PromoCell LLC, Heidelberg, Germany) at 37°C in 5% CO_2.

2.2 | Cell proliferation assay

One hundred microliters of NHEKs at a concentration of 4×10^4 cells/mL was seeded into individual wells of a 96-well plate ($n=6$). After culturing for 24, 48, or 72 h, the plates were placed on top of a 7.8-Hz ultra-low-frequency pulse generator (CF-FM783-BA, Wal front, operating current 1 mV) (Figure 1) emitting block pulses of 7.8-Hz electromagnetic waves for 10 min. Plates were further incubated for 96 h, at which point the culture solution was removed and wells were washed with phosphate-buffered saline (PBS, Sigma-Aldrich Japan LLC). One hundred microliters of 50 μg/mL neutral red (NR, Nacalai Tesque Inc.) was added to each well, and the plates were incubated for 3 h. The NR solution was removed, and the cells were fixed with 200 μL of 1% formaldehyde. The fixation solution was removed, and 100 μL of 1% acetic acid/50% 2-propanol was added to each well to visualize the NR that was taken up intracellularly. Absorbance was measured at 540 nm using a microplate reader (Immuno Mini, NJ-2300, Nalge Nunc International Co.). Cell proliferation was evaluated by calculating the NR uptake in cells treated with 7.8-Hz electromagnetic waves relative to the mean NR uptake in untreated control cells.

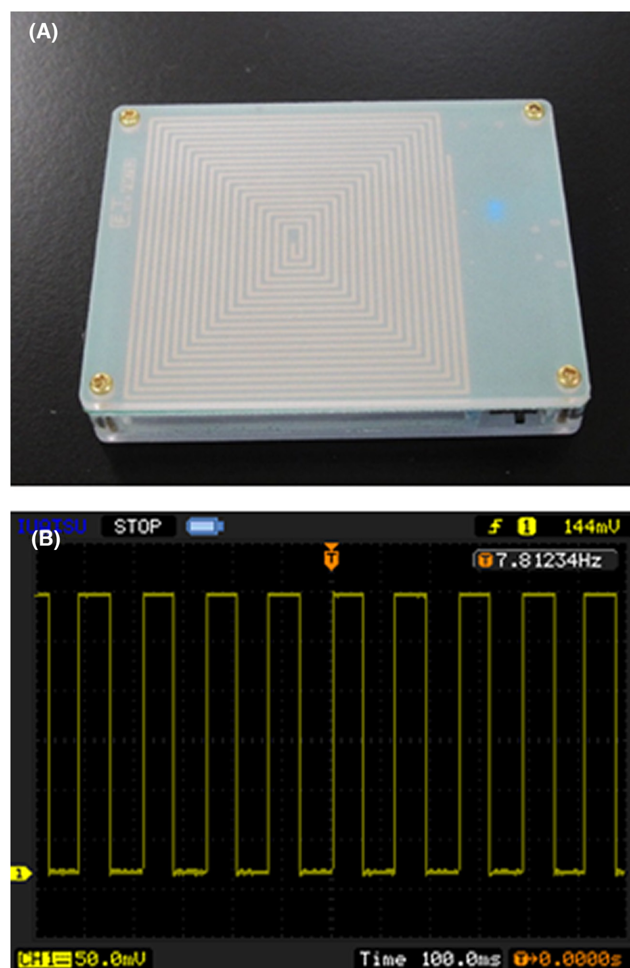

FIGURE 1 The 7.8-Hz ultra-low-frequency pulse generator and its waveform. (A) The 7.8-Hz ultra-low-frequency pulse generator (7.0 cm × 5.5 cm × 1.0 cm). (B) Waveform emitted by the 7.8-Hz ultra-low-frequency pulse generator, as measured by a digital oscilloscope (DS-5110B, Iwatsu Electric Co., Ltd) (block pulses, applied voltage 2.8 V).

2.3 | Cell migration assay

Two milliliters of NHEKs at a concentration of 12×10^4 cells/mL was seeded into 35-mm dishes ($n=3$). After the cells reached confluence, they were cultured for a further 24 h to ensure all of the cells were in G_0 phase. A 200-μL pipette tip was used to draw a cross on each dish, detaching the cells in the scratch, and the culture medium was changed. A mycoplasma-derived synthetic diacyl lipopeptide (fibroblast-stimulating lipopeptide, FSL-1, Adipogen Life Sciences Inc.)[5] was added at a concentration of 0.1 μg/mL, and the dishes were cultured for 24 h. Immediately after the scratch was made and 24 h later, the dishes were placed on top of a 7.8-Hz ultra-low-frequency pulse generator emitting 7.8-Hz electromagnetic waves for 10 min. After 24 h, the area around the scratch was examined under a microscope to assay cell migration into the wound.

14732165, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jocd.15988 by Osaka Med And Pharm Univ, Wiley Online Library on [12/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14732165, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jocd.15988 by Osaka Med And Pharm Univ, Wiley Online Library on [12/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.4 | *DEFB1* gene expression analysis

One milliliter of NHEKs at a concentration of 4×10^4 cells/mL was seeded into individual wells in a 24-well plate ($n = 5$–9). Cells were stimulated with 0.1 µg/mL FSL-1, and the plates were cultured for 24 or 48 h. The plates were placed on top of a 7.8-Hz ultra-low-frequency pulse generator emitting 7.8-Hz electromagnetic waves for 10 min. After 48 h of culture, the culture medium was removed and the cells were washed with PBS, at which point they were lysed using 1 mL of 1% sodium dodecyl sulfate (Nippon Gene Co.). We added 1 µL of 1% KOH (Nacalai Tesque Inc.) and 20 µL of 20 mg/mL proteinase K solution (Thermo Fisher Scientific Inc., Waltham MA, USA) for every 180 µL of cell suspension. The mixture was incubated at 37°C for 15 min, and RNA was extracted using the RNA Clean XP (Beckman Coulter Inc.).

The RNA was subjected to reverse transcription with a Super Script™ IV VILO™ Master Mix (Thermo Fisher Scientific Inc.) for 10 min at 25°C, 10 min at 50°C, and 5 min at 85°C to obtain cDNA. To quantify the expression of *DEFB1*, Taqman® Gene Expression Assays (ACTB Hs99999903_m1, DEFB4A/DEFB4B Hs00175474_m1, Thermo Fisher Scientific Inc.), TaqPath™ qPCR Master Mix, CG (Thermo Fisher Scientific Inc.), and cDNA solution were combined, and real-time polymerase chain reaction (PCR) was conducted at 25°C for 2 min and 40 cycles of 95°C for 20 s, 95°C for 3 s, and 60°C for 30 s (C1000 Touch™ Thermal Cycler, Bio-Rad Laboratories Inc.).

2.5 | *SIRT1* gene expression analysis

Five milliliters of NHEKs at a concentration of 3×10^4 cells/mL was seeded in T25 flasks ($n = 3$–5). After culturing for 24, 48, or 72 h, the flasks were placed on top of a 7.8-Hz ultra-low-frequency pulse generator emitting 7.8-Hz electromagnetic waves for 10 min. After 72 h of culture, the cells were recovered, RNA was extracted, and reverse transcription was performed as described for *DEFB1* gene expression analysis. PCR was conducted using Taqman® Gene Expression Assays (SIRT1 Hs01009006_m1, Thermo Fisher Scientific Inc.) as described for *DEFB1* gene expression analysis.

2.6 | Statistical analysis

Measured values are expressed as means and standard deviations. The statistical software EZR version 1.41 was used for statistical analysis,[6] and *t*-test and Dunnett's test were used for multiple comparisons with untreated controls. $p < 0.05$ was considered statistically significant.

3 | RESULTS

Cell proliferation was increased 2.8-fold by the application of 7.8-Hz electromagnetic waves (Figure 2). Cell migration was enhanced by FSL-1 stimulation and was further increased by the application of 7.8 Hz electromagnetic waves (Figure 3).

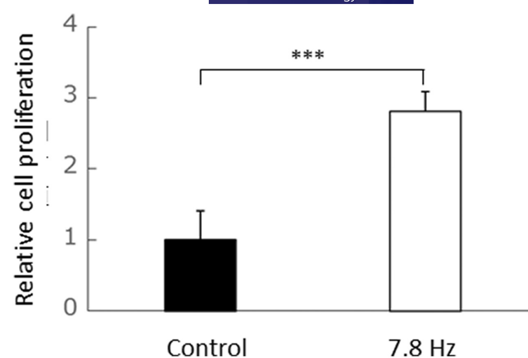

FIGURE 2 Effect of the application of 7.8-Hz electromagnetic waves on cell proliferation. The neutral red (NR) uptake during the application of 7.8-Hz electromagnetic waves increased 2.8-fold compared with the mean NR uptake of the untreated control. $n = 6$, values are presented as mean ± SD. *** $p < 0.001$, significant difference compared with control.

The *DEFB1* gene expression level was significantly increased 2.8-fold by FSL-1 stimulation ($p < 0.05$, Figure 4). *DEFB1* was also increased 2.4-fold by the application of 7.8-Hz electromagnetic waves ($p < 0.1$, Figure 4). The *SIRT1* expression level was also significantly increased 4.9-fold by the application of 7.8-Hz electromagnetic waves ($p < 0.05$, Figure 5).

4 | DISCUSSION

In this study, we investigated the effect of the application of electromagnetic waves at an ultra-low frequency (7.8 Hz) to NHEKs. The application of 7.8 Hz electromagnetic waves increased the proliferation and migration of NHEKs. We observed a 2.8-fold increase in the rate of NHEK proliferation as a result of 7.8-Hz wave pulse application. A study on the addition of recombinant human epidermal growth factor to the HaCaT human keratinocyte cell line found that it increased cell proliferation 1.2- to 1.3-fold, irrespective of the presence or absence of serum.[7] Therefore, the increase in proliferation we observed was extremely high. We also found that ultra-low-frequency electromagnetic waves increased the expression of *DEFB1*, which is an antimicrobial peptide gene, and *SIRT1*, which is involved in cellular senescence. These data suggest that 7.8 Hz electromagnetic waves can activate to repair a skin damage and a skin barrier dysfunction via inducing the expression of *DEFB1* and *SIRT1*.

Previous studies have examined the effects of the application of electromagnetic fields at frequencies other than 7.8 Hz. In human bone marrow mesenchymal stem cells, 50-Hz electromagnetic fields suppress proliferation and increase *SIRT1* expression,[8] and they increase the amount of melanin and tyrosinase activity in human melanocytes.[9] Another study found that the application of electromagnetic fields at 1 Hz enhances the migration of HaCaT cells, whereas 80-Hz fields decrease migration.[10] Thus, there is a

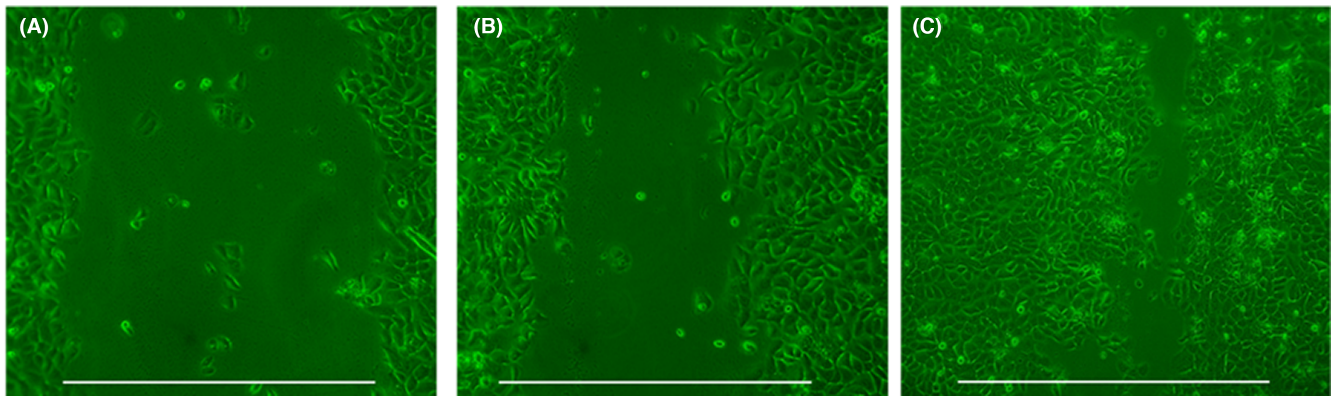

FIGURE 3 Effects of the application of 7.8-Hz electromagnetic waves and FSL-1 stimulation on cell migration. FSL-1 stimulation and the application of 7.8-Hz electromagnetic waves both increased cell migration. (A) Untreated control, (B) FSL-1 stimulation, (C) application of 7.8-Hz electromagnetic waves, —: 0.6 mm. FSL-1: fibroblast-stimulating lipopeptide 1.

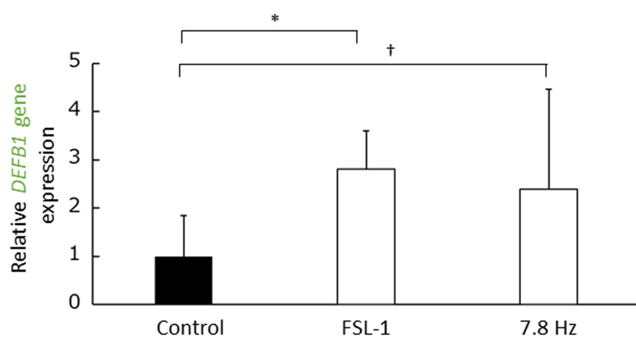

FIGURE 4 Effects of the application of 7.8-Hz electromagnetic waves and FSL-1 stimulation on *DEFB1* gene expression. The *DEFB1* gene expression level was increased 2.8-fold by FSL-1 stimulation (FSL-1) and 2.4-fold by the application of 7.8-Hz electromagnetic waves (7.8 Hz). $n=5-9$, values are presented as mean ± SD. $*p<0.05$, significant difference compared to the control. $\dagger p<0.1$, significant tendency compared to the control. *DEFB1*, β-defensin; FSL-1, fibroblast-stimulating lipopeptide 1.

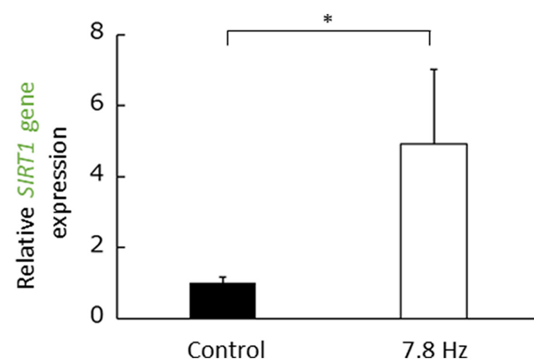

FIGURE 5 Effect of the application of 7.8-Hz electromagnetic waves on *SIRT1* gene expression. The *SIRT1* expression level was increased 4.9-fold after the application of 7.8-Hz electromagnetic waves. $n=3-5$, values are presented as mean ± SD. $*p<0.05$, significant difference compared to the control. *SIRT1*: sirtuin 1.

possibility that different electromagnetic wavelengths may exert different actins even on the skin.

We found that stimulation with FSL-1, which has the same N-terminal sequence as an *S. aureus*-derived diacyl lipopeptide,[3] promoted cell migration and upregulated the antimicrobial peptide gene *DEFB1*. The application of 7.8-Hz electromagnetic waves increased cell migration by a greater amount than FSL-1 stimulation and also tended to increase *DEFB1* gene expression. β-defensin is suppressed in patients with atopic dermatitis.[11] The application of 7.8-Hz electromagnetic waves to NHEKs may stimulate the expression of β-defensin in a manner equivalent to that of FLS-1 and may promote the function of antimicrobial peptides. From this point of view, 7.8-Hz electromagnetic waves may bring a good effect to a patients with atopic dermatitis.

As wound healing is delayed in *SIRT1*-knockout mice,[12] our data suggest that the application of 7.8-Hz electromagnetic waves may be useful to skin ulcer via activating *SIRT1* expression.

There are some limitations in the present study. First, we used human cultured cells to examine the effect of 7.8-Hz electromagnetic waves and all of the findings are from in vitro experiments. Second, we only used one cell strain of NHEKs and there may be an individual difference in the effect of 7.8-Hz electromagnetic waves to the cells. More additional studies to use different cell strains, molecular analysis and clinical study should be performed to conclude the effect of 7.8-Hz electromagnetic waves to skin barrier function.

In conclusion, our results suggest that the application of 7.8-Hz electromagnetic waves may contribute to the recovery of skin barrier function by upregulating the expression of several genes including *DEFB1* and *SIRT1*, orchestrating to maintain skin homeostasis. Further studies are required to ascertain the mechanism of action of the application of 7.8-Hz electromagnetic waves to the skin, with the aim of enabling the clinical administration of 7.8-Hz electromagnetic waves for disorders such as atopic dermatitis, intractable cutaneous ulcer and for skin damage due to laser exposure or similar causes in aesthetic dermatology.

14732165, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jocd.15988 by Osaka Med And Pharm Univ, Wiley Online Library on [12/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with respect to this report.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available in figshare at http://doi.org following an embargo from the date of publication to allow for commercialization of research findings.

ETHICAL APPROVAL

Authors declare human ethics approval was not needed for this study.

ORCID

Shinichi Moriwaki https://orcid.org/0000-0003-0803-9455

REFERENCES

1. Elhalel G, Price C, Fixler D, Shainberg A. Cardioprotection from stress conditions by weak magnetic fields in the Schumann resonance band. *Sci Rep*. 2019;9(1):1645. doi:10.1038/s41598-018-36341-z
2. Tang JY, Yeh TW, Huang YT, Wang MH, Jang LS. Effects of extremely low-frequency electromagnetic fields on B16F10 cancer cells. *Electromagn Biol Med*. 2019;38(2):149-157. doi:10.1080/15368378.2019.1591438
3. Tawaratsumida K, Furuyashiki M, Katsumoto M, et al. Characterization of N-terminal structure of TLR2-activating lipoprotein in Staphylococcus aureus. *J Biol Chem*. 2009;284(14):9147-9152. doi:10.1074/jbc.M900429200
4. Wahedi HM, Chae JK, Subedi L, et al. NED416, a novel synthetic Sirt1 activator, promotes cutaneous wound healing via the MAPK/rho pathway. *Int J Mol Med*. 2020;46(1):149-158. doi:10.3892/ijmm.2020.4564
5. Vu AT, Takai T, Le TA, et al. Proinflammatory response in human keratinocytes stimulated with ligands for TLR2, TLR1, and TLR6. *Juntendo Med J*. 2010;56(1):50-55. doi:10.14789/pjmj.56.50
6. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48(3):452-458. doi:10.1038/bmt.2012.244
7. Hardwicke J, Moseley R, Stephens P, Harding K, Duncan R, Thomas DW. Bioresponsive dextrin-rhEGF conjugates: in vitro evaluation in models relevant to its proposed use as a treatment for chronic wounds. *Mol Pharm*. 2010;7(3):699-707. doi:10.1021/mp9002656
8. Jeong WY, Kim JB, Kim HJ, Kim CW. Extremely low-frequency electromagnetic field promotes astrocytic differentiation of human bone marrow mesenchymal stem cells by modulating SIRT1 expression. *Biosci Biotechnol Biochem*. 2017;81(7):1356-1362. doi:10.1080/09168451.2017.1308243
9. Kim YM, Cho SE, Kim SC, Jang HJ, Seo YK. Effects of extremely low frequency electromagnetic fields on melanogenesis through p-ERK and p-SAPK/JNK pathways in human melanocytes. *Int J Mol Sci*. 2017;18(10):2120. doi:10.3390/ijms18102120
10. Mojena-Medina D, Martínez-Hernández M, de la Fuente M, et al. Design, implementation, and validation of a piezoelectric device to study the effects of dynamic mechanical stimulation on cell proliferation, migration and morphology. *Sensors (Basel)*. 2020;20(7):2155. doi:10.3390/s20072155
11. Hata TR, Kotol P, Boguniewicz M, et al. History of eczema herpeticum is associated with the inability to induce human β-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. *Br J Dermatol*. 2010;163(3):659-661. doi:10.1111/j.1365-2133.2010.09892.x
12. Qiang L, Sample A, Liu H, Wu X, He YY. Epidermal SIRT1 regulates inflammation, cell migration, and wound healing. *Sci Rep*. 2017;7(1):14110. doi:10.1038/s41598-017-14371-3

How to cite this article: Sugiwaki H, Kotani M, Fujita A, Moriwaki S. Effects of Schumann resonance on the proliferation and migration of normal human epidermal keratinocytes and the expression of *DEFB1* and *SIRT1*. *J Cosmet Dermatol*. 2023;00:1-5. doi:10.1111/jocd.15988

14732165, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jocd.15988 by Osaka Med And Pharm Univ, Wiley Online Library on [12/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License