Sensors and Materials, Vol. 34, No. 9 (2022) 3383–3396
MYU Tokyo

S & M 3047

Device to Collect Skin Cells Using Ultrasound Irradiation and Aspiration

Yang Ye,[1*] Tatsuya Uechi,[1] Noriko Tsuruoka,[2] Makoto Akashi,[3] and Yoichi Haga[1,2]

[1]Graduate School of Biomedical Engineering, Tohoku University,
6-6 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan
[2]Graduate School of Engineering, Tohoku University,
6-6 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan
[3]The Research Institute for Time Studies, Yamaguchi University,
1677-1 Yoshida, Yamaguchi City, Yamaguchi 753-8511, Japan

(Received June 29, 2022; accepted July 27, 2022)

Keywords: ultrasound, cavitation, cell collection, fine needle, minimally invasive

Dynamic cell activity can be examined by collecting and analyzing cells every few hours during the day. However, current techniques to extract and analyze skin cells are deemed ineffective and/or invasive. Thus, in this study, we designed and fabricated a device that collects skin cells using ultrasound irradiation. An ultrasonic horn amplifies a Langevin transducer, which then generates ultrasound and transmits it to a fine needle. Ultrasound is irradiated from the needle to the body surface, and ultrasonic oscillation and cavitation cut or weaken intercellular binding. We successfully aspirated 30 cells from pig skin using our minimally invasive device.

1. Introduction

The human body has 37.2 trillion cells.(1) All the cells in a human body have deoxyribonucleic acid (DNA), whose instructions enable human development, survival, and reproduction. Recent studies have linked specific genes to human health and lifespan. One of these genes is the clock gene, which is known to control the circadian rhythm. Circadian clocks regulate diverse functions in our bodies, such as the sleep-wake cycle, body temperature, hormone production, brain wave activity, cell regeneration, and other biological activities.

Subepidermal cells can be used to examine detailed cell activity, such as dynamic gene expression levels, by collecting and analyzing them every few hours during the day.(2) Easier cell extraction and analysis of proteins translated from DNA could offer more reliable data for such diagnoses. Living cells must first be collected from tissue to extract their genetic material, such as DNA and RNA. Human oral epithelial cells are constantly peeling from and mixing with saliva; genetic material can be easily collected by rubbing the inside of the cheek with a cotton swab or disposable chopsticks. Although living cells can be harvested rapidly and noninvasively, samples are difficult to analyze because saliva contains ribonuclease, which decomposes RNA and negatively affects the genetic analysis.(3)

*Corresponding author: e-mail: yang.ye.t1@dc.tohoku.ac.jp
https://doi.org/10.18494/SAM3983

ISSN 0914-4935 © MYU K.K.
https://myukk.org/

Another method to collect cells is a skin biopsy. This procedure uses a sample skin specimen to diagnose skin disorders. Skin biopsy can collect many cells, but its invasive nature often leaves scars.[4] The high invasiveness restricts this method from collecting cells multiple times a day for the dynamic analysis of the production level of mRNA or protein. By using hair follicle cells, dynamic changes in gene expression can be analyzed by plucking 20 hairs every 3 h.[5]

Ultrasound therapy has historically been used in medicine for diagnostic imaging applications. Therapeutic ultrasound provides a clinical effect within the body without damaging the intervening tissue. Ultrasound has been widely used to treat several diseases—stroke, cancer, cardiovascular diseases, glaucoma, nerve damage, skin wounds, and bone fractures—because it is safe, noninvasive, painless, and versatile.[6]

Ultrasound transmits pressure waves with frequencies above 20 kHz through a medium such as air or water. This type of mechanical energy can physically interact with tissues and cells. The resulting periodic pressure oscillation has two downstream effects: elevated temperature due to the absorption of sound waves and nucleation due to the formation of gas bubbles, or cavitation. The vapor phase forms when 20 to 100 kHz ultrasonic vibration is applied to a liquid. Alternating positive and negative pressure on the liquid can cause its expansion and collapse, generating instantaneous negative pressure and shock waves. Cavitation phenomena can be used to disrupt cell junctions and separate intercellular binding in tissue.[7] Here, ultrasound was selected to noninvasively collect living cells from tissue. Compared with a skin biopsy, the sample size obtained by this method is small and cannot be used for morphological analysis. However, owing to its low invasiveness and ease of use, cells can be collected multiple times a day for analysis. The number of cells required for analysis is around 100. Thus, we believe that we can measure the dynamic changes of some specific mRNA and protein by this method.

Cells were collected from the skin, which is the body's largest organ. There are three layers of skin: the epidermis, dermis, and subcutaneous tissue. In the epidermis, 95% of the cells are keratinocytes, which are dead squamous cells that no longer multiply in the stratum corneum; therefore, the epidermis was not chosen for this study. The thickness of the dermis is approximately 1–3 mm and consists of fibroblasts, macrophages, mast cells, and plasma cells. Subcutaneous tissue consists of fibroblasts, adipose cells, and macrophages.[8] The loose connective tissue found within the subcutaneous tissue, the shallow layer of the dermis, and the mucous membrane is one of the most widely distributed connective tissues, whose fibrous components and cells randomly extend and are embedded in a semi-liquid matrix. A needle can access the depth of the epidermis and dermis from the surface of the skin, and the collected cells can then be used to isolate genetic material and proteins. In this study, we developed and fabricated a device that collects cells from skin tissue every few hours in a day with minimal invasiveness using ultrasonic waves.

2. Fabrication and Characteristics

There are four components in the proposed ultrasonic cell collection device: a Langevin transducer, ultrasonic horn, jig, and two hollow needles (Fig. 1). The Langevin transducer generates ultrasonic irradiation. The ultrasonic oscillation is amplified and efficiently

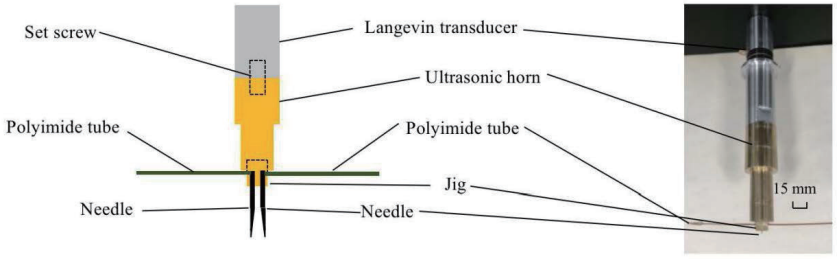

Fig. 1. (Color online) Structure of proposed ultrasonic cell collection device.

transmitted to the jig by using an ultrasonic horn. The jig is used to transmit the ultrasonic oscillation from the horn to the needles, providing a flow path for both the injected phosphate-buffered saline and the cells aspirated by the needle. Small-diameter needles (30 G, DN3021-KL) are used for cell aspiration, which can minimize invasiveness.

The Langevin transducer (HEC-1540P2BF, Honda Electronics Co., Ltd.) generates high-power ultrasonic vibrations that are widely used in ultrasonic cleaning equipment, underwater sonar, and ultrasonic scalpels. The vibrator has two piezoelectric ceramic discs, which are composed of porous lead zirconate titanate (PZT) to alternately adjust the polarization direction and a piezoelectric structure whose piezoelectric element is sandwiched between two metal blocks. The Langevin transducer used in this study has a resonance frequency of 40 kHz and a maximum allowable voltage of 38.35 Vp-p.

The ultrasonic horn transmits the ultrasonic vibration energy generated from the Langevin transducer to the needles, thus maximizing the amplitude. The amplitude expansion ratio of a simple step-type ultrasonic horn depends on the area ratio of the two end faces. The areas of the large and small end faces of the ultrasonic horn are determined by the size of the jig and the diameter of the Langevin transducer. A set screw then connects the Langevin transducer and the ultrasonic horn. The design dimensions and style of ultrasonic horn are shown in Fig. 2. The ultrasonic horn in our proposed device satisfies the following equation.

$$L1 = L2 = \lambda / 4 \tag{1}$$

$L1$: length of large-diameter step
$L2$: length of small-diameter step
λ: wavelength

The amplitude expansion ratio M is 2.5 if the amplitude is maximum at both ends and 0 at the step. We used brass to fabricate the ultrasonic horn by cutting owing to its good conductivity of sound waves, high energy transfer efficiency, easy processing, and low price. The speed of sound c in brass is 3400 m/s, the frequency f of ultrasonic waves from the Langevin transducer is 40 kHz, and the wavelength λ of ultrasonic waves is

$$\lambda = c / f = 85 \text{ (mm)}. \tag{2}$$

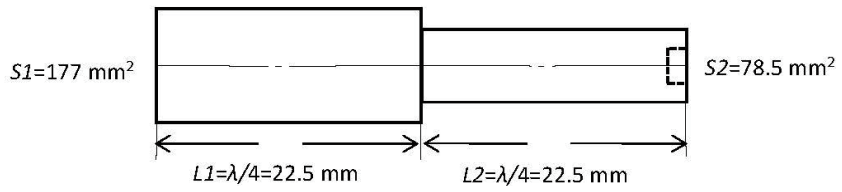

Fig. 2. Dimensions of fabricated step-type horn. *S*1: large cross-sectional area. *S*2: small cross-sectional area.

We also used brass to create the jig, ensuring high ultrasonic transmission efficiency. The jig was designed to effectively transfer ultrasonic energy from the Langevin transducer to the fine hollow needles; two flow paths were fabricated on the jig by cutting: one for delivering buffer solution to skin tissue and the other for cell extraction (Fig. 3).

30G hollow needles (DN-3021KL, Terumo Co., Ltd.) with outer and inner diameters of 0.3 and 0.13 mm, respectively, were selected to minimize invasiveness while accommodating the size of adipose cells, whose diameters are approximately 0.1 mm. Needles with 1.5 mm effective length were used to collect cells from dermal or subcutaneous tissue, based on the depth of the tissues from the epidermal surface. The needles were fabricated with 4 mm length because they were inserted 2.5 mm inside the jigs. The opposite sides of the needle tips were then designed to have a 45° angle to improve the flow of aspirated cells. The needle was inserted into the groove of the jig and fixed with glue, and the jig surface was covered with polyimide tape. The openings of the two needles were opposite each other to ensure a smooth flow path. The distance between the two hollow needles ranged from 0.1 to 0.3 mm depending on the thickness of the polyimide tapes. The two jigs were combined, glued, and inserted into the groove on the front end of the horn to complete the assembly (Fig.4).

The device's ultrasonic intensity was measured to select the appropriate voltage and frequency for disrupting intercellular binding and emulsifying subepidermal tissue. The output ultrasound intensity from the hollow needle was measured at different frequencies using a needle-type hydrophone (TC4013-1, TOYO Co., Ltd.) to verify its function. The output voltage of the hydrophone was measured and converted to the ultrasonic intensity of the device. Various sine waves with frequencies from 20 to 80 kHz were also generated using a function generator (WF1946B, NF Co., Ltd.) to evaluate the resonance frequency of the entire device. The function generator was connected to an amplifier (7058/10, Yokogawa Co., Ltd.) to amplify the voltage to 30 Vp-p, nearly the maximum input voltage of the Langevin transducer. An amplified sine wave was applied to the device. An oscilloscope (DLM2054, Yokogawa Co., Ltd.) was then connected to the amplifier to adjust it to a specific input voltage. The oscilloscope was also used to measure the output voltage from the hydrophone in response to the sound waves from the device. When the hydrophone was 1 mm away from the needle tip and sine waves of frequency from 20 to 80 kHz were applied to the device, the resonance frequency of the device and the maximum output ultrasound intensity were measured as 43.1 kHz and 18.25 W/cm^2, respectively (Fig. 5).

The input voltage was changed to 30, 20, and 10 Vp-p while maintaining a resonant frequency of 40 kHz to measure the relationship between the input voltage and ultrasonic intensity. The output ultrasonic intensity was found to be proportional to the square of the input voltage (Fig. 6).

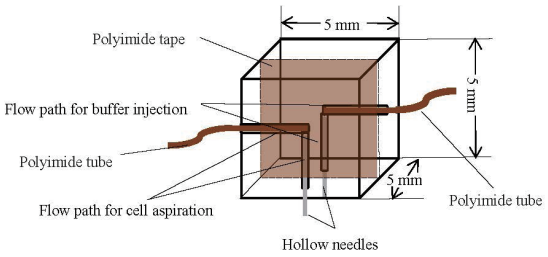

Fig. 3. (Color online) Brass jig with two flow paths.

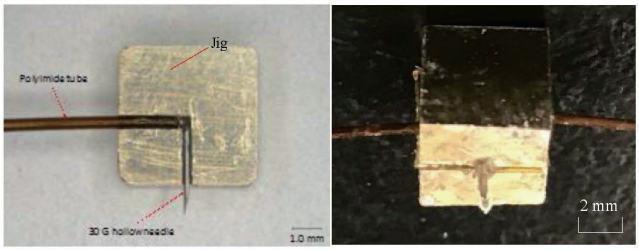

Fig. 4. (Color online) Fabricated brass jig with two flow paths.

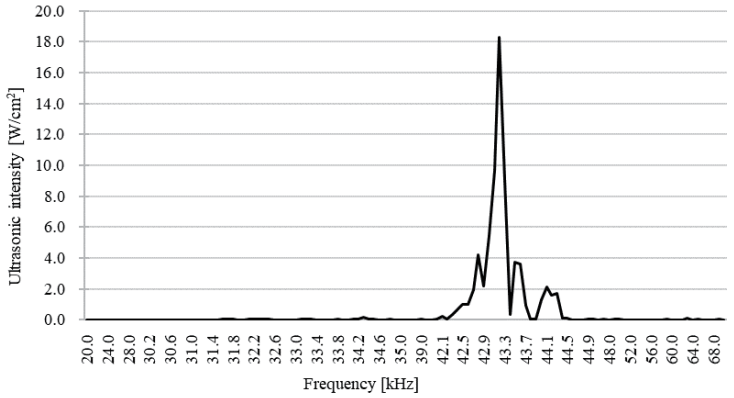

Fig. 5. Relationship between frequency and ultrasonic intensity.

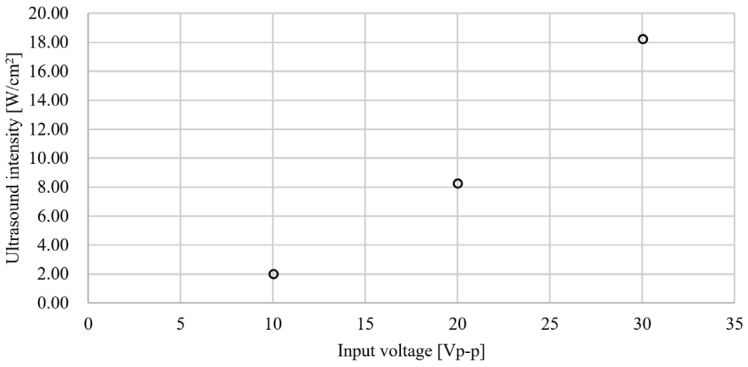

Fig. 6. Output ultrasonic intensity for different input voltages.

The output ultrasonic intensity was measured while varying the distance between the needle tip and the hydrophone to identify where ultrasonic cavitation occurred. A 30 Vp-p sine wave was applied to the device at 43.1 kHz. The hydrophone was placed 1, 2, and 3 mm from the needle tip and measured (Fig. 7).

3. Procedure

The efficacy of the proposed subcutaneous cell collection mechanism was tested using the fabricated device and skin from a porcine nose purchased from a meat processing plant (Tokyo Shibaura Zouki Co., Ltd.). The pig was slaughtered the day before the sample was received or in the early hours of the same day. The device was attached to a stage, and the needle was inserted to a depth of 1.7 mm. The porcine nose was marked with a marker pen to indicate the point of insertion. One of the polyimide tubes of the device was connected to the syringe containing phosphate buffer to start infusion. To maintain the balance of intracellular and extracellular osmotic pressure, phosphate buffered saline (PBS) was used as a buffer in this experiment. The porcine nose was irradiated with ultrasound for 5 min before cell aspiration. The conditions for ultrasonic irradiation are specified in later sections. A Live/Dead Cell Staining Kit II (Takara Bio Inc. Co., Ltd.) was used to stain the collected cells, which were observed with a fluorescence microscope (FLUOVIEW FV1000, Olympus Co., Ltd.). In this experiment, the nose of a pig was used as the collection object, and the tissue had left the living body for more than 10 hours when the cell collection experiment was carried out, thus the cells collected in this experiment were dead cells. Owing to the small number of cells collected in this experiment, they were counted one by one in the field of view of the fluorescence microscope. The setup for the cell collection experiment is shown in Fig. 8.

3.1 Effect of changing ultrasonic frequency at same voltage

The device was powered by a 43.1 kHz sine wave with 30 Vp-p (18.26 W/cm^2) to examine the effect of the ultrasonic frequency on cell collection; the buffer was injected at a rate of 15 mL/h for 20 min and aspirated at a rate of 20 mL/h for 15 min using a syringe pump. Figure 9 shows

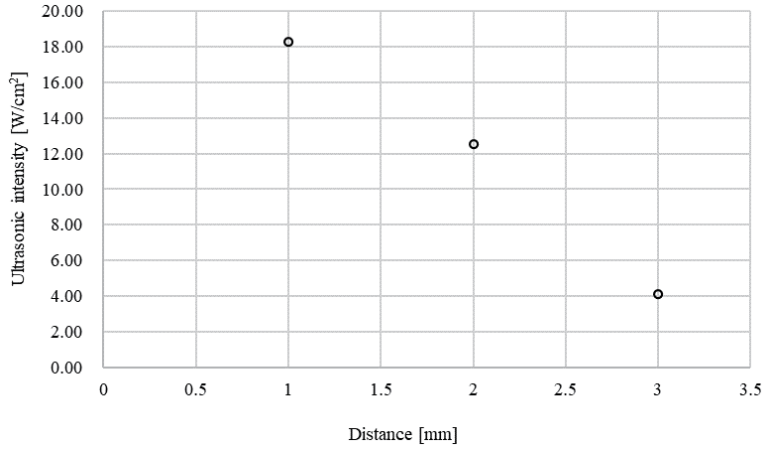

Fig. 7. Ultrasonic intensity at various distances from the needle tip.

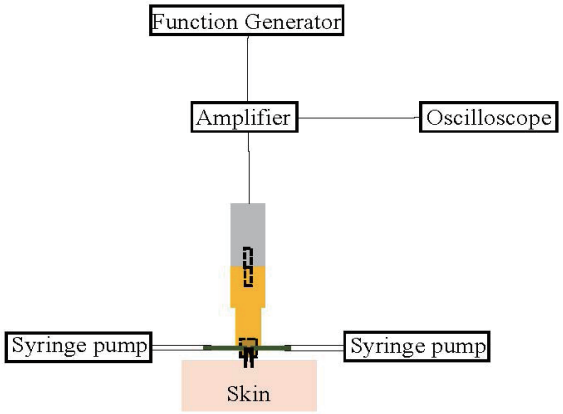

Fig. 8. (Color online) Setup for cell collection experiment.

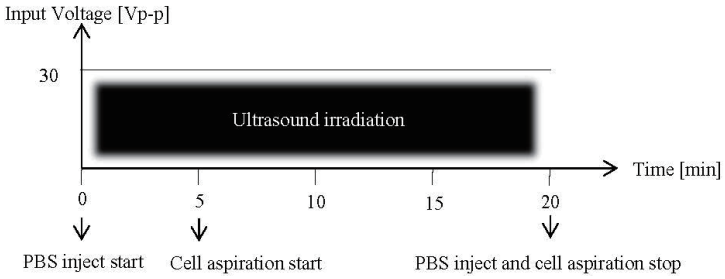

Fig. 9. Experimental protocol.

the experimental protocol. The same procedure was followed using a 32.1 kHz sine wave with 30 Vp-p (0.04 W/cm^2), using a 20.3 kHz sine wave with 30 Vp-p (0.01 W/cm^2), and without ultrasound as the control. The cavitation thresholds at ultrasonic frequencies of 43.2, 32.1, and 20.3 kHz were previously reported to be 6.3, 3.2, and 2.1 W/cm^2, respectively.[9]

3.2 Effect of changing voltage at resonance frequency

The device was powered by a 43.1 kHz sine wave with 30, 20, and 10 Vp-p to examine the effect of the voltage on cell collection. The buffer was then injected at a rate of 15 mL/h for 20 min and aspirated at a rate of 20 mL/h for 15 min using a syringe pump. The theoretical minimum voltage required for ultrasonic cavitation at the resonant frequency is 20.5 Vp-p.

3.3 Effect of changing aspiration time

Three groups of control experiments were conducted with various aspiration times at the same aspiration speed to examine the impact of the ultrasonic irradiation time on the test results (Table 1). Previous experiments indicated that a voltage of 30 Vp-p applied at the resonance frequency maximizes the number of cells collected; our device was powered by a 43.1 kHz sine wave with 30 Vp-p. The buffer was injected and aspirated at a rate of 20 mL/h for 5, 10, or 15 min using a syringe pump.

Table 1
Conditions for cell aspiration experiments using various aspiration times.

Condition	Amount of buffer (mL)
Aspiration speed: 20 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	5
Aspiration speed: 20 ml/h Aspiration time: 10 min Ultrasonic irradiation time: 15 min	3.33
Aspiration speed: 20 ml/h Aspiration time: 5 min Ultrasonic irradiation time: 10 min	1.67

3.4 Effect of changing aspiration speed

Conversely, three groups of control experiments were conducted with various aspiration speeds while keeping the aspiration time constant (Table 2). The device was powered by a 43.1 kHz sine wave with 30 Vp-p, and the buffer was injected and aspirated at a rate of 13.3, 20, or 30 mL/h for 15 min using a syringe pump.

4. Results

Cells observed from fluorescence microscopy are shown in Fig. 10. The photos of skin tissue before and after the cell collection experiment are shown in Fig. 11.

4.1 Result of changing ultrasonic frequency

Three experiments were performed under the same conditions to reduce the influence of incidental factors. The cell aspiration results are summarized in Table 3 and Fig. 12.

4.2 Result of changing voltage at resonance frequency

As before, three experiments were carried out under the same conditions to reduce the influence of incidental factors. Table 4 and Fig. 13 show the cell aspiration results.

4.3 Result of changing aspiration time

Three groups of experiments were carried out at a constant frequency, voltage, and aspiration speed but using various aspiration times. Table 5 and Fig. 14 present the cell aspiration results.

4.4 Result of changing aspiration speed

The results of cell aspiration are shown in Table 6 and Fig. 15.

Table 2
Conditions for cell aspiration experiments at various aspiration speeds.

Condition	Amount of buffer (mL)
43.1 kHz, 30 Vp-p Aspiration speed: 13.3 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	3.33
43.1 kHz, 30 Vp-p Aspiration speed: 20 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	5
43.1 kHz, 30 Vp-p Aspiration speed: 30 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	7.5

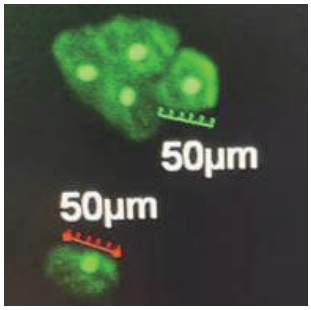

Fig. 10. (Color online) Cells observed in fluorescence microscopy

(a) (b)

Fig. 11. (Color online) Photos of skin tissue before and after the cell collection experiment. (a) Before the cell collection experiment. (b) After the cell collection experiment.

Table 3
Average number of cells collected at various ultrasonic frequencies.

Condition	Cavitation thresholds	Average number of cells
43.1 kHz, 30 Vp-p	Exceed	34
32.1 kHz, 30 Vp-p	Not exceed	15
20.3 kHz, 30 Vp-p	Not exceed	12
Without ultrasound	Not exceed	4

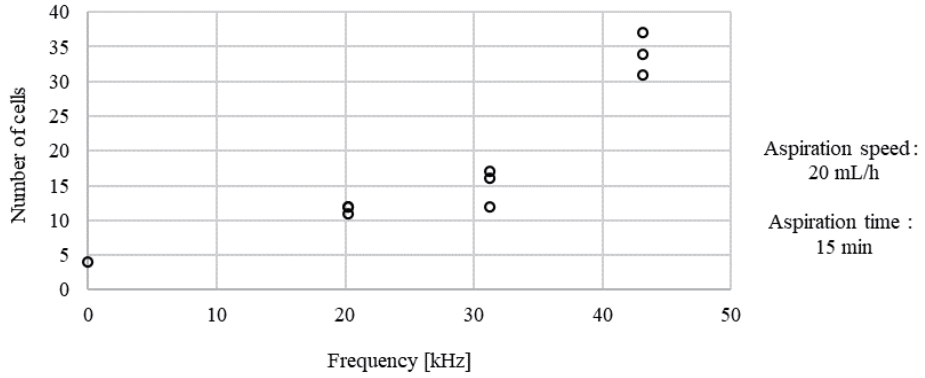

Fig. 12. Number of collected cells at different frequencies.

Table 4
Average number of cells collected at various voltages.

Condition	Cavitation thresholds	Average number of cells
43.1 kHz, 30 Vp-p	Exceed	34
43.1 kHz, 20 Vp-p	Not exceed	16
43.1 kHz, 10 Vp-p	Not exceed	18
Without ultrasound	Not exceed	4

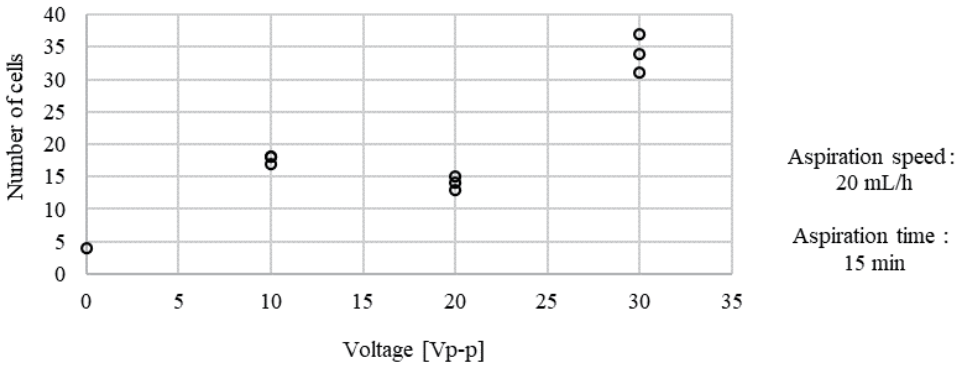

Fig. 13. Number of collected cells at different voltages at the same resonance frequency.

Table 5
Average number of cells collected at various aspiration times.

Condition	Cavitation thresholds	Average number of cells
43.1 kHz, 30 Vp-p Aspiration speed: 20 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	Exceed	34
43.1 kHz, 30 Vp-p Aspiration speed: 20 ml/h Aspiration time: 10 min Ultrasonic irradiation time: 15 min	Exceed	23
43.1 kHz, 30 Vp-p Aspiration speed: 20 ml/h Aspiration time: 5 min Ultrasonic irradiation time: 10 min	Exceed	12

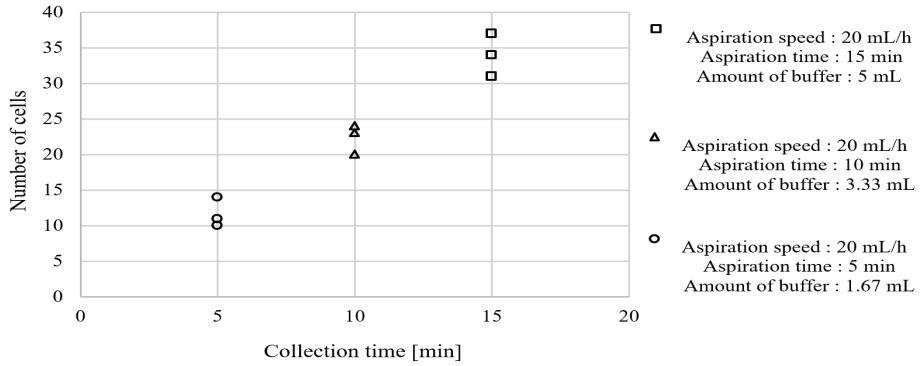

Fig. 14. Number of collected cells at different aspiration times with the same voltage and frequency.

Table 6
Average number of cells collected at various aspiration speeds.

Condition	Cavitation thresholds	Average number of cells
43.1 kHz, 30 Vp-p Aspiration speed: 30 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	Exceed	46
43.1 kHz, 30 Vp-p Aspiration speed: 20 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	Exceed	34
43.1 kHz, 30 Vp-p Aspiration speed: 13.3 ml/h Aspiration time: 15 min Ultrasonic irradiation time: 20 min	Exceed	22

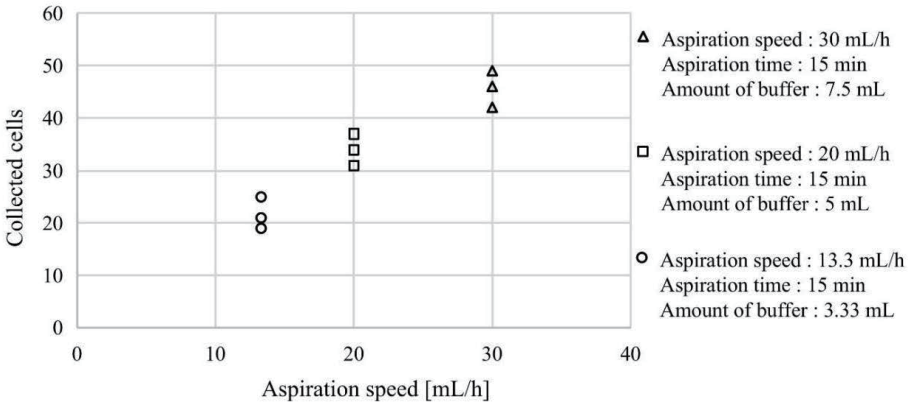

Fig. 15. Number of collected cells at various aspiration speeds and constant voltage (30 Vp-p) and frequency (43.1 kHz).

5. Discussion

In this study, we fabricated a device that collects cells from the skin using ultrasonic irradiation and aspiration. The size of cells in the dermis is about 10–50 μm. As shown in Fig. 10, the device successfully collected cells with a size of about 40–50 μm through the *in vitro* cell collection experiment. As shown in Fig. 11, the skin tissue did not change before and after ultrasonic irradiation, so the device was considered to be minimally invasive. Factors that affect the final number of cells aspirated can be divided into two categories. The first is the efficiency of weakening the intercellular binding because intercellular connections formed by membrane proteins tightly hold cells together within tissues. The second is the efficiency of extracting free cells from the body after the intercellular binding is severed, which is affected by the buffer recovery, buffer aspiration speed, and other factors.

We performed various cell collection experiments, as shown in Sects. 3.1 and 3.2, to examine how the degree of weakened intercellular binding affects the number of cells collected. A small number of cells could be collected even without ultrasonic irradiation because they remained inside the needles upon insertion and were recovered with the buffer.

There were four experimental groups with different voltages and frequencies. Compared with the group without ultrasound irradiation, the number of cells collected after ultrasound irradiation increased significantly. Approximately 15 cells were collected, but the ultrasonic intensity generated did not reach the ultrasonic threshold at the current frequency. However, when the ultrasonic intensity exceeded the cavitation threshold, the number of collected cells further increased, reaching about 35 cells.

Compared with the group without ultrasonic irradiation, the number of collected cells also increased under ultrasonic irradiation whose ultrasonic intensity did not exceed the cavitation threshold; this is because the needles generated a small range of vibrations due to an ultrasonic wave, and the generated energy weakened intercellular binding, even partially severing them. A small number of free cells in the originally tightly bonded tissue were collected together with the buffer solution. When the ultrasonic intensity is greater than the cavitation threshold, the number of collected cells further increases, which may be due to the cavitation effect, which makes bubbles in the buffer burst under negative pressure to produce a shock wave, breaking down an intercellular binding and yielding more free cells. As shown in Sec. 2, the maximum allowable voltage of the Langevin transducer used in this experiment is 38.35 Vp-p, and excessive ultrasonic intensity may also cause damage to cells, such as cell membrane rupture and other adverse effects. Thus, voltages higher than 30 Vp-p were not used in cell collection experiments.

The number of cells collected was proportional to the aspiration time; more cells were collected when the aspiration speed was increased from 13.3 to 20 and 30 mL/h while keeping the aspiration time constant at 15 min. However, the duration of ultrasound irradiation also varied among different groups: it is possible that longer ultrasound irradiation times further weaken intercellular binding and thus increase the number of collected cells. Figure 16 integrates the results from all cell collection experiments; different aspiration times and speeds change the same variable (buffer volume). The aspiration speed multiplied by time is the volume of the buffer.

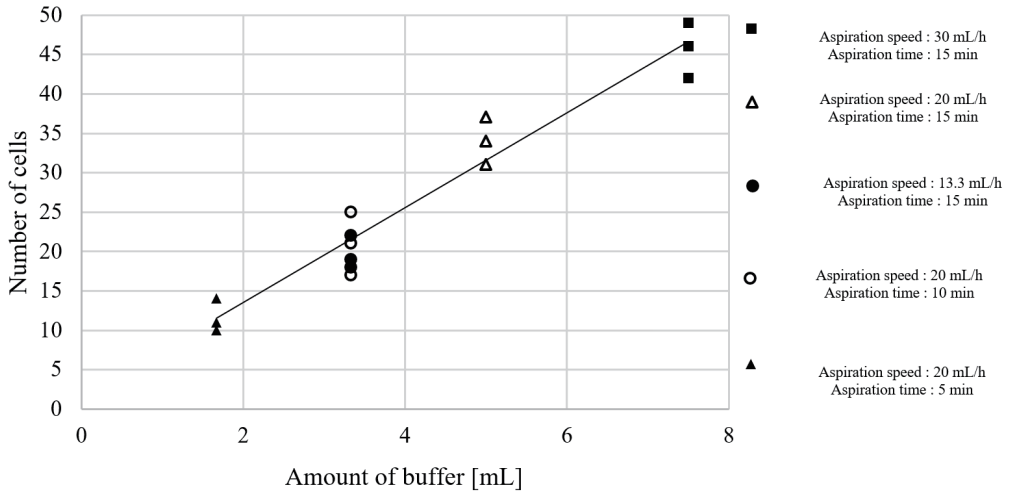

Fig. 16. Number of collected cells under various aspiration conditions with constant voltage and frequency.

The number of cells collected in the two experiments (13.3 mL/h, 15 min and 20 mL/h, 10 min) was roughly the same. Therefore, the buffer volume was the dominant factor in this study. Some of the buffer overflowed from the pinhole to the skin surface when the aspiration speed was too high, reducing the efficiency of cell collection. A small amount of buffer overflowed from the surface of the skin tissue when the aspiration speed was 40 mL/h, and even more overflowed when the aspiration speed was 50 ml/h. Here, 30 mL/h was the highest possible aspiration speed that optimized cell collection.

6. Conclusion

In this study, a minimally invasive cell aspiration device was fabricated to extract skin cells using ultrasound irradiation, saline injection, and aspiration. Various ultrasonic tests were carried out using the device, and the voltages needed to induce ultrasonic cavitation at different frequencies were calculated. The favorable condition for cell collection was 30 Vp-p with 43.1 kHz, according to the *in vitro* cell aspiration experiment. The buffer volume largely determined the number of cells collected. The maximum number of cells collected in this experiment was 49, which did not reach the target value of 100. However, it is possible to obtain more than 100 cells in a single collection by increasing the flow rate or replacing the buffer with an enzyme solution for cell separation. In future work, we will optimize cell extraction from a mouse.

Acknowledgments

We would like to thank Associate Professor Makoto Kanzaki and Ms. YuQing Li of Tohoku University Graduate School of Medical Engineering for teaching us how to count cells using a fluorescence microscope in this research.

References

1 E. Bianconi, A. Piovesan, F. Facchin, A. Beraudi, R. Casadei, F. Frabetti, L. Vitale, M. Chiara Pelleri, S. Tassani, F. Piva, S. Perez-Amodio, P. Strippoli, and S. Canaider: Ann. Hum. Biol. **40** (2013) 463. https://doi.org/10.3109/03014460.2013.807878

2 L. S. Mure, H. D. Le, G. Benegiamo, M. W. Chang, L. Rios, N. Jillani, M. Ngotho, T. Kariuki, O. D. Benyahya, H. M. Cooper, and M. Panda: Science **359** (2018) 6381. https://www.science.org/doi/10.1126/science.aao0318

3 M. R. Bailey, V. A. Khokhlova, O. A. Sapozhnikov, S. G. Kargl, and L. A. Crum: Acoust. Phys. **49** (2003) 369. https://doi.org/10.1134/1.1591291

4 Z. Seia, L. Musso, P. Stefania, and M. Bertero: InTech (2011). https://doi.org/10.5772/25300

5 M. Akashi, H. Soma, T. Yamamoto, A. Tsugitomi, S. Yamashita, T. Yamamoto, E. Nishida, A. Yasuda, J. K. Liao, and K. Node: Proc. Natl. Acad. Sci. U.S.A. **107** (2010) 15643. https://doi.org/10.1073/pnas.1003878107

6 S. Mitragotri: Nat. Rev. Drug Discov. **4** (2005) 255. https://doi.org/10.1038/nrd1662

7 W. G. Pitt, G. A. Husseini, and B. J. Staples: Expert Opin. Drug Delivery **1** (2004) 37. https://doi.org/10.1517/17425247.1.1.37

8 A. Firooz, A. Rajabi-Estarabadi, H. Zartab, N. Pazhohi, F. Fanian, and L. Janani: Skin Res. Technol. **23** (2017) 13. https://doi.org/10.1111/srt.12294

9 L. A. Frizzell: Ultrasound: Its Chemical, Physical, and Biological Effects (VCH, New York, 1988) p. 38.