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RESEARCH ARTICLE

Purification of pluripotent embryonic stem cells using dielectrophoresis and a flow control system

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Abstract

Pluripotent stem cells (PSCs) such as embryonic stem cells and induced PSCs can differentiate into all somatic cell types such as cardiomyocytes, nerve cells, and chondrocytes. However, PSCs can easily lose their pluripotency if the culture process is disturbed. Therefore, cell sorting methods for purifying PSCs with pluripotency are important for the establishment and expansion of PSCs. In this study, we focused on dielectrophoresis (DEP) to separate cells without fluorescent dyes or magnetic antibodies. The goal of this study was to establish a cell sorting method for the purification of PSCs based on their pluripotency using DEP and a flow control system. The dielectrophoretic properties of mouse embryonic stem cells (mESCs) with and without pluripotency were evaluated in detail, and mESCs exhibited varying frequency dependencies in the DEP response. Based on the variance in DEP properties, mixed cell suspensions of mESCs can be separated according to their pluripotency with an efficacy of approximately 90%.

KEYWORDS
cell sorting, dielectrophoresis, embryonic stem cell, pluripotent stem cell, pluripotency

1 | INTRODUCTION

Pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can self-renew and are pluripotent. In recent years, studies on regenerative medicine using ESCs and iPSCs have been actively conducted [1, 2]. For PSC culture, it is important to maintain the pluripotency of PSCs. Moreover, for therapeutic applications, numerous PSCs ($\sim 10^9$ cells per patient) are required in a clinical setting [3]. Therefore, the expanding culture of PSC without loss of pluripo-

tency is important. However, PSCs are easily affected by disturbances in the culture process and often lose their pluripotency during expansion. When expanding or maintaining the culture of PSC, the exclusion of cells lacking pluripotency from the PSC population is important for PSC-based research and therapies. Cell sorting technologies are required for PSC culture to resolve this problem. Conventionally, fluorescence-activated cell sorting (FACS) is employed in cell separation. FACS distinguishes cells based on the fluorescence of labeled cells to enable accurate cell sorting. However, this requires bulky and expensive instruments and fluorescent dyes for the target cells [4, 5]. Another conventional technology is magnetic-activated cell sorting (MACS), which distinguishes cells

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Abbreviations: DEP, dielectrophoresis; ESC, embryonic stem cell; PSC, pluripotent stem cell

based on magnetic labeling [6]. MACS enables high-throughput cell sorting and does not require expensive instruments. However, this technique requires a magnetic antibody or particle to label the target cells [6–8]. Fluorescent staining or magnetic antibodies can damage the cells and living bodies. Therefore, label-free cell sorting technology is required for PSC culture to enable the use PSCs at clinical stages.

Dielectrophoresis (DEP) is one of the most promising approaches for manipulating and separating cells. DEP is a phenomenon caused by a non-uniform electric field that induces dipoles within a cell suspended in a buffer. This phenomenon generates a nonzero Coulomb net force on the cell. This dielectrophoretic force can move cells toward high- or low-electric-field regions depending on their relative electric properties [9]. The difference in electrophoretic properties (that is, amplitude and direction of dielectric force) enables cell distinguishment and separation without a fluorescent dye or magnetic antibody. Recently, numerous studies have reported the use of DEP devices to separate and manipulate cells. Cell manipulation can be performed through DEP [10–12]. Therefore, living cells and other particles or live and dead cells can be separated through DEP [13–16]. Sorting of living cells according to cell properties (function, type, and size) through DEP has been studied in the past [17–21]. In our previous study, a novel simplified cell manipulating and sorting device using DEP and fluid-induced shear force was developed [22]. We also reported on continuous cell-sorting methods using DEP and fluid-induced shear forces to separate mouse embryonic stem cells (mESCs) from feeder cells [23]. However, these studies only highlighted the evaluation and separation of cells from varying origins or cells expressing distinctly different natures such as viability and cell types. Moreover, DEP cell sorting to analyze the pluripotency of PSCs and separate them according to pluripotency has not yet been developed.

The goal of this study was to develop a novel cell-sorting system to separate PSCs according to their pluripotency using DEP and a fluid flow control system. For the fundamental study, the dielectrophoretic properties of self-renewing and differentiated mESCs were characterized. Based on the measured DEP properties of mESCs, we established a DEP cell-sorting system to separate mESCs according to their pluripotency without employing fluorescent dyes or magnetic antibodies.

2 | MATERIALS AND METHODS

2.1 | mESC culture

The mESCs derived from the 129/Ola strain (EB3 cell line, Riken Bioresource Center, Japan) were used to evaluate

PRACTICAL APPLCATION

Previous research on dielectrophoretic (DEP) cell sorting could separate cells of varying origins or cells expressing distinctly different natures such as viability and cell types. In this study, we performed a detailed characterization of the DEP properties of pluripotent and nonpluripotent mouse embryonic stem cells (mESCs) that were denatured from similar mESCs. We also propose a cell sorting method using the DEP force and flow-control system to enable purification of mESCs according to their pluripotency with an efficacy of approximately 90%. Our DEP-cell sorting method is applicable to research and clinical applications requiring non-cell-labeled purification of pluripotent stem cells such as regenerative medicine.

the dielectrophoretic properties of self-renewing and differentiated mESCs. EB3 cells usually require gelatin coating on cell culture dishes instead of feeder cell layers to maintain their pluripotency and carry the blasticidin S-resistance gene activated by the Oct3/4 promoter. Blasticidin S-resistance gene expression enables the elimination of differentiated cells by culturing in blasticidin S-containing medium.

For the DEP experiments, the EB3 cells were thawed from cryovials and expanded for 3 days on gelatin-coated flasks in Glasgow Modified Essential Medium (GMEM) supplemented with 10% FBS, 1% antibiotic-antimycotic, 0.1 mM nonessential amino acid, 0.1 mM 2-mercaptoethanol, 0.1 mM sodium pyruvate, 10 μg/ml blasticidin S, and 1000 U/ml leukemia inhibitory factor (LIF). The cell cultures were maintained in a humidified tissue culture incubator at 37°C and 5% CO_2. After 3 days of culture, the cells were dissociated enzymatically with 0.25% trypsin and seeded in two 75 cm^2 flasks.

Typically, LIF is used to maintain cell pluripotency in mESC cultures. In this study, one of the flasks was cultured in a medium similar to the one described above to prepare cells with pluripotency [LIF(+) group]. In the LIF(+) groups, the EB3 cells lacking pluripotency were eliminated in blasticidin S-containing medium. The other flask was cultured for 3 days in the medium without LIF and blasticidin S to prepare the cells lacking pluripotency (LIF(−) group). To evaluate the pluripotency of cells from LIF(+) and LIF(−) groups, an alkaline phosphatase evaluation assay (AP assay) was performed using the Leukocyte Alkaline 187 Phosphatase Kit (#86R, Sigma-Aldrich) according to the manufacturer's instructions. Prior to the DEP
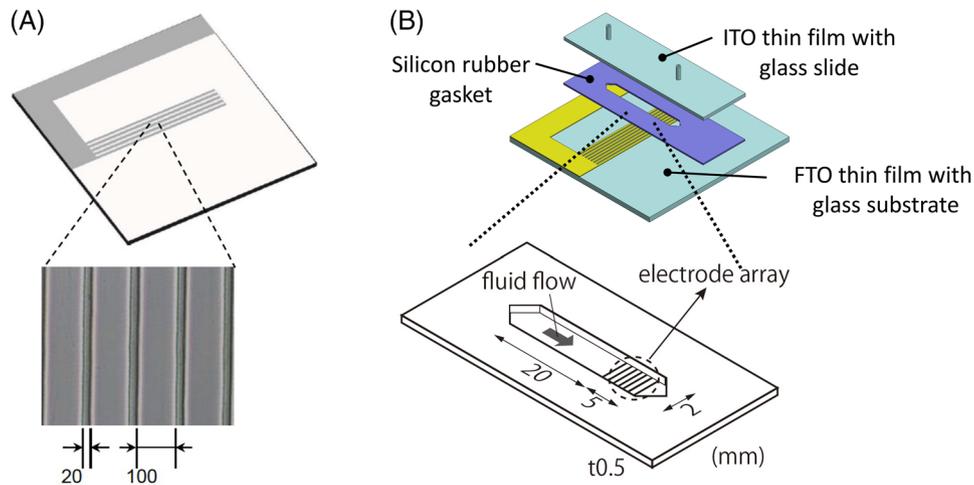
FIGURE 1 Schematic of dielectrophoretic chamber for characterization of cells. (A) Parallel line electrode array. (B) Flow channel made by PDMS gasket sandwiched with ITO-membrane coated and ITO-electrode array fabricated slide glasses

experiments, the cells of both experimental groups were suspended in a low-conductivity, osmotically balanced buffer (LC buffer: 10 mM HEPES, 0.1 mM $CaCl_2$, and 59 mM D-glucose in a sucrose solution [22–25]) at 1.0×10^6 cells/ml. In our previous studies, we confirmed that the immersion of mESCs in LC buffer during the experimental time (approximately 30 min) did not affect cell viability [24].

2.2 | DEP characterization of undifferentiated and differentiated mESCs

The DEP chamber for dielectrophoretic evaluation was constructed by sandwiching a silicon rubber gasket with a transparent parallel-line electrode array-fabricated glass and an ITO-coated glass (Geomatec, Kanagawa, Japan). The thickness of the ITO layer was 1500 Å, and the resistance was 5 Ω/sq The pattern of parallel-line electrodes was made using laser-etching techniques. Each electrode line had a width of 20 μm, and the lines were 80 μm apart. The parallel-line microelectrodes were designed to generate a highly nonequal electric field (Figure 1A) [22, 23]. The silicon rubber gasket determines the chamber height. The resulting geometry of the experimental volume of the DEP chamber was 15 mm in length, 5 mm in width, and 0.5 mm in height (Figure 1B).

The experimental setup used to evaluate the DEP properties of the cells is shown in Figure 2. A 1-ml disposable syringe containing the cell-suspended LC buffer was connected to the inlet of the microchannel. The cell-suspended buffer was injected using a syringe pump (Harvard 33 Twin Syringe Pump, Harvard Apparatus, Holliston, MA, USA). After the injection of the sample solution, DEP was performed to evaluate the DEP properties of mESCs
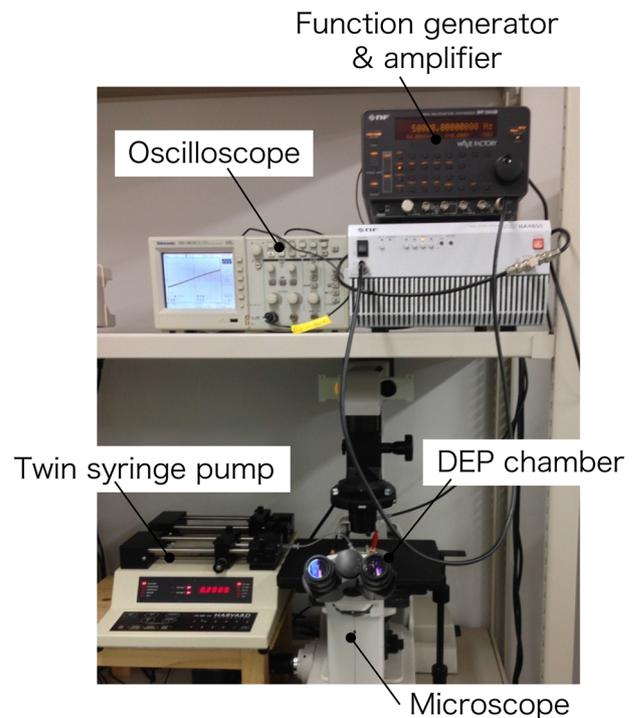

FIGURE 2 Experimental setup to evaluate dielectrophoretic properties of cells and cell sorting using DEP cell sorting

in the LIF(+) and LIF(−) groups. During DEP, the cells were moved toward the electrodes by positive DEP and between the electrodes by negative DEP in the DEP chamber (Figure 3A).

To evaluate the frequency dependency of the DEP properties, an AC electric field was applied between the parallel-line electrode array and bare ITO-coated glass using a function generator (WF1944B, NF Corp., Yokohama, Japan) and an amplifier (BA4850, NF Corp., Yokohama, Japan). The applied voltage was monitored using an
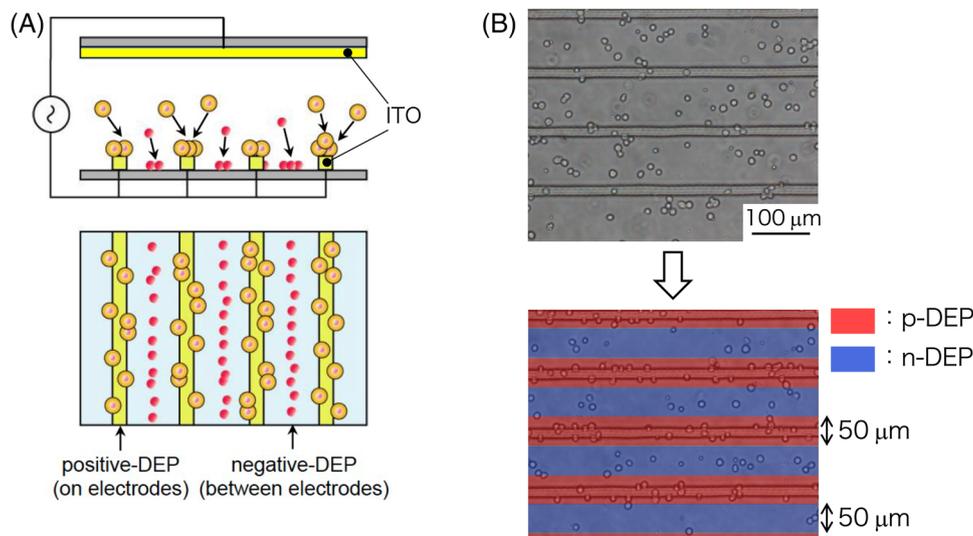
FIGURE 3 Schematic of positive and negative dielectrophoresis of living cells. (A) Cell movement during dielectrophoresis. (B) Evaluation of dielectrophoretic properties

oscilloscope (TDS1001B, Tektronix, Beaverton, OR, USA) connected in parallel to the amplifier. The movement of cells within the DEP chamber was observed using a phase-contrast microscope (Nikon Eclipse TE300, Nikon, Tokyo, Japan) with a digital video camera. Photomicrographs were captured at 0 and 180 s after the AC voltage was applied to evaluate the DEP properties.

The number of cells on the electrodes (positive DEP) and between the electrodes (negative DEP) were counted, as shown in Figure 3B. The frequency dependency of the DEP property was evaluated by the positive-DEP ratio R_p and negative-DEP ratio R_n, calculated as follows:

$$R_p = N_P/(N_P + N_n) \tag{1}$$

$$R_n = N_n/(N_P + N_n) \tag{2}$$

where N_p and N_n denote the numbers of cells under positive and negative DEP, respectively.

2.3 | Mouse ESC purification using DEP and fluid-induced shear forces

A DEP chamber with a fluid-flow control system was developed to sort mixed mESCs expressing pluripotent and non-pluripotent phenotypes. As mentioned above, ITO-coated glass (Geomatec, Kanagawa, Japan) was used as the conductive substrate to fabricate a transparent parallel-line microelectrode array on a glass slide. The pattern of the electrodes was also made using laser-etching techniques. The geometry of the parallel-line microelectrodes was similar for the DEP chamber to evaluate the DEP properties of the stem cells (Figure 4A).

The flow channel was made from a polydimethylsiloxane (PDMS) polymer to establish a rectangular volume. The DEP chamber was formed by contacting the PDMS flow channel on the electrode-fabricated glass (Figure 4B). The resulting geometry of the DEP chamber was 5.5 mm long from the inlet to the electrodes, 5 mm wide, and 100 μm high. The reservoir for the LC buffer was fabricated in a DEP chamber to sort the cells continuously.

The experimental setup for DEP cell sorting using a flow control system is shown in Figure 2. The flow rates of the cell-suspended solution and bulk LC buffer were controlled using a twin-syringe pump (Harvard 33 Twin Syringe Pump, Harvard Apparatus, Holliston, MA). The process of DEP cell sorting is illustrated in Figure 5. In the first step, 30 μl of the mixed cell solution was injected into the DEP chamber with LC buffer filled in the reservoir (Figure 5A). Subsequently, the mixed cell suspension was introduced into the chamber at 5 μl/min to trap the cells in the LIF(+) group only on the electrodes using the positive-DEP force, while cells in the LIF(−) group passed through the chamber to outlet B (Figure 5B). The whole process of DEP cell sorting takes for approximately three minutes.

Thereafter, the AC electric field was turned off to remove the trapped cells from the electrodes at a flow rate of 50 μl/min to collect them into outlet A (Figure 5C). This experiment was repeated 10 times to evaluate the efficacy of DEP cell sorting. An AC electric voltage (7 Vp-p, 130 kHz) was applied between the adjacent parallel-line electrodes using a function generator (WF1944B, NF, Kanagawa, Japan) and an amplifier (BA4850, NF, Kanagawa, Japan), and the voltage was monitored using an oscilloscope (TDS1001B, Tektronix, Beaverton, OR) connected in parallel. The movement of cells within the
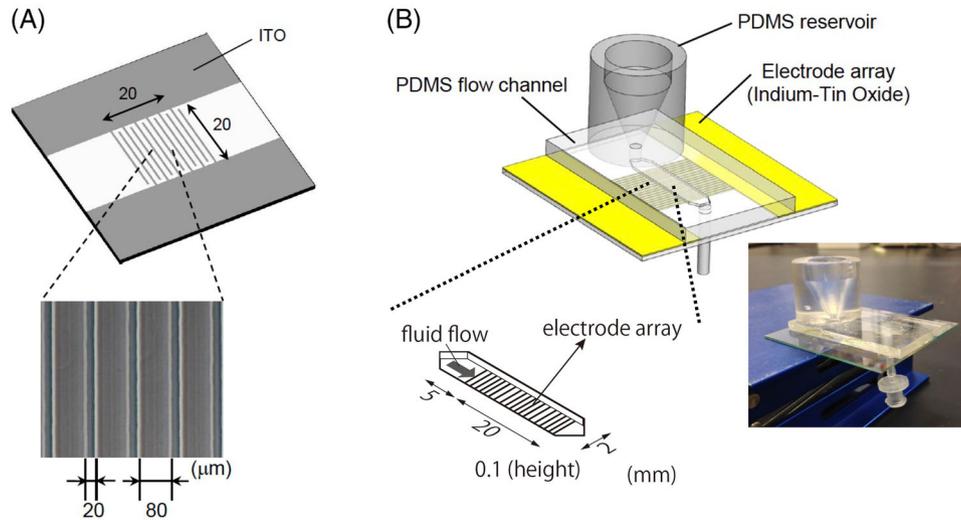
FIGURE 4 Schematic of dielectrophoretic chamber for dielectrophoretic cell sorting. (A) Opposing comb-shape electrode array. (B) Flow channel made by PDMS
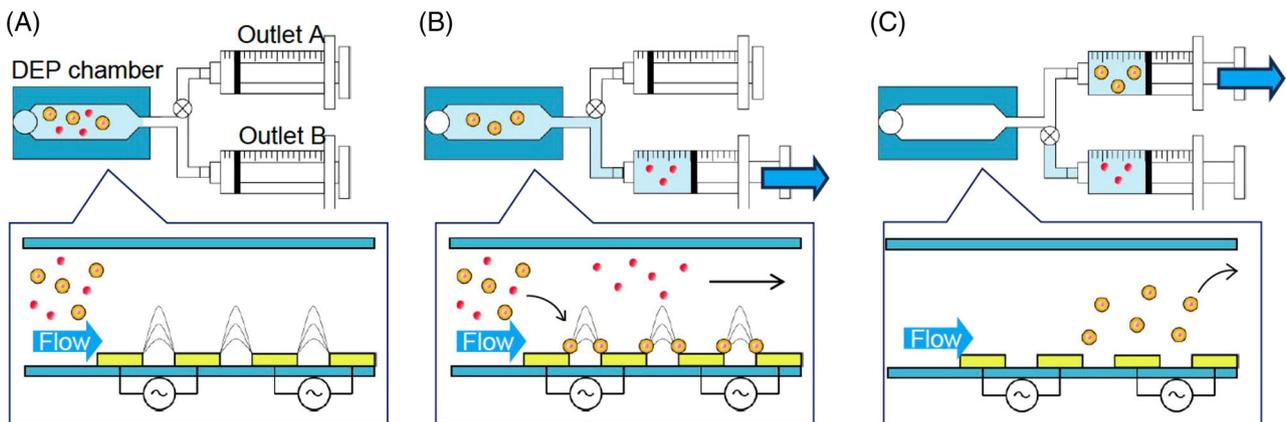

FIGURE 5 Sorting procedure of mESC suspension using positive dielectrophoresis and fluid-flow control. (A) A Mixed population of cells with and without pluripotency was injected into the DEP sorting chamber. (B) Cells belonging to the LIF (+) group are trapped on the electrode array by the p-DEP force, whereas cells belonging to the LIF(−) group pass through the electrode array and are collected at outlet B. (C) The p-DEP force is removed, and the cells of LIF(+) are collected at outlet A

DEP chamber was observed using a phase-contrast and fluorescence microscope (BX51, Olympus, Tokyo, Japan) equipped with a CCD camera.

Before the cell sorting experiment, the cells from LIF(+) and LIF(−) groups were collected from cell culture dishes and only the cells of the LIF(+) group were fluorescently stained with calcein AM to distinguish the cells of the LIF(+) group from those of the LIF(−) group. Fluorescence staining was performed as follows: Cells were suspended in LC buffer containing 20-μg/ml calcein AM and incubated for 30 min at 37°C, 5% CO_2, and 95% humidity. After fluorescent-staining of cells from the LIF(+) group, the cells of both groups were suspended in fresh LC buffer at 1.0×10^6 cells/ml and mixed for the DEP sorting experi-

ment. The mixed ratio of cells from the LIF(+) and LIF(−) groups was set at 5:5. The DEP chamber was thereafter degassed and sterilized. The flow channel was filled with 70% ethanol for 5 min to sterilize the chamber and washed twice with LC buffer. For the cell sorting experiment, the cell suspension mixed with cells from LIF(+) and LIF(−) groups was injected via the inlet of the DEP chamber, and the flow rate was controlled by the twin-syringe pump according to the DEP cell sorting process as described above. Three individual experiments were performed in the cell sorting experiments.

The efficacy of cell sorting was evaluated by counting the cell numbers from the LIF(+) and LIF(−) groups in the sorted cell suspension at outlets A and B. The cell
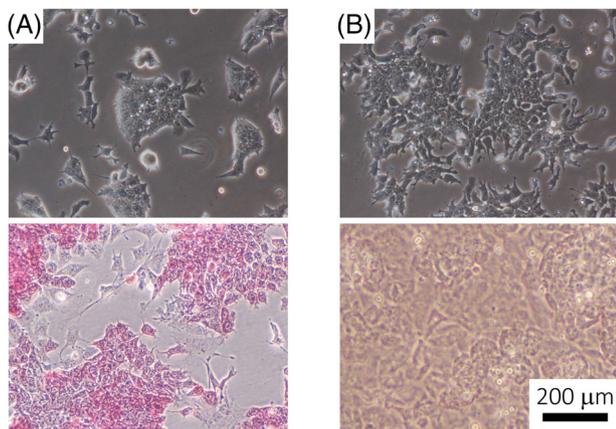
FIGURE 6 Mouse embryonic stem cells cultured (A) with LIF and (B) without LIF. The alkaline phosphatase activity was confirmed in the cells culture with LIF whereas that was not confirmed the cells cultured without LIF

counting procedure is shown in Figure S1. The fluorescent and phase-contrast images of cell suspensions collected at outlets A and B were obtained (Figure S1A). The number of cells in the phase-contrast image was counted to evaluate the total number of cells in both the LIF(+) and LIF(−) groups. The number of fluorescent cells from the LIF(+) group was also counted from the fluorescent images (Figure S1B). The purification ratio of cells from the LIF(−) group was determined using the total number of cells and the number of cells from the LIF(+) and LIF(−) groups (Figure S1C). To evaluate the pluripotency of the separated cells, cells collected from both outlets A and B were cultured for 48 h with culture medium containing LIF and blasticidin S. AP assay was performed as described in Section 2.1.

3 | RESULTS AND DISCUSSION

3.1 | DEP properties of mESCs expressing undifferentiated and differentiated phenotypes

EB3 cells from the LIF(+) and LIF(−) groups exhibited varying morphologies. The mESCs with pluripotency [LIF(+) group] formed densely packed three-dimensional colonies, whereas the cells lacking pluripotency [LIF(−) group] formed a flat monolayer and were dispersed (Figure 6). Alkaline phosphatase (AP) activity was also maintained in the cells from the LIF(+) group, whereas it was not observed in cells from the LIF(−) group. The cells from the LIF(+) and LIF(−) groups also exhibited varying DEP responses according to the imposed frequency of the AC voltage. Figure 7 shows photomicrographs of cells in the LIF(+) and LIF(−) groups under DEP at varying fre-

quencies. The cells in the LIF(+) group exhibited a negative DEP at 10 kHz, whereas a positive DEP was observed at 1000 kHz. Moreover, the cells of the LIF(+) group exhibited both negative and positive DEP at frequencies ranging from 30 to 50 kHz. The cells of the LIF(−) group exhibited a negative DEP at 10 kHz and a positive DEP at 1000 kHz. Moreover, these cells showed both negative and positive DEP responses at frequencies ranging from 110 to 130 kHz.

To evaluate the DEP frequency dependency of the cells, we defined R_p−R_n as the frequency-dependent parameter. Figure 8 shows the ratio of cells expressing positive and negative DEP responses (R_p−R_n) in the LIF(+) and LIF(−) groups. The cells in the LIF(+) group switched from positive to negative DEP between 30 and 50 kHz. In contrast, the cells in the LIF(−) group switched from positive to negative DEP between 110 and 130 kHz. These results indicate that the crossover frequencies of DEP were different for the LIF(+) and LIF(−) groups: the crossover frequency of cells in the LIF (+) group was between 30 and 50 kHz, whereas that of the LIF(−) group was between 110 and 130 kHz.

PSCs change the expression of membrane proteins and ion channels according to their phenotype (pluripotent or non-pluripotent). It has been reported that the DEP response of cells depends on the cell size, capacitance of the cell membrane, and the dielectric constant of the cytoplasm. Since the mESCs belonging to the LIF(+) and LIF(−) groups have a similar origin, there is almost no variance in cell size. Therefore, the difference in DEP response between LIF(+) and LIF(−) cells was considered to be owing to the change in capacitance of the cell membrane caused by the denaturation of membrane proteins and ion channels owing to LIF(−) cells losing their pluripotency. It was suggested that the crossover frequency changes depending on the difference in the pluripotency of mESCs. Based on the variance in crossover frequency, it is possible to distinguish between pluripotent and non-pluripotent mESCs using DEP.

3.2 | Dielectrophoretic cell sorting of mESCs expressing undifferentiated and differentiated phenotypes

In this study, we performed dielectrophoretic cell sorting based on differences in the crossover frequencies of mESCs with and without pluripotency. The results of DEP characterization demonstrated that the cells in the LIF(+) group exhibited a positive DEP response at 130 kHz, whereas those in the LIF(−) group exhibited weak positive or negative DEP responses because the frequency of 130 kHz was almost equal to the crossover frequency of DEP for the cells in the LIF(−) group (Figure 8). In our previous study, we reported the difference in the positive
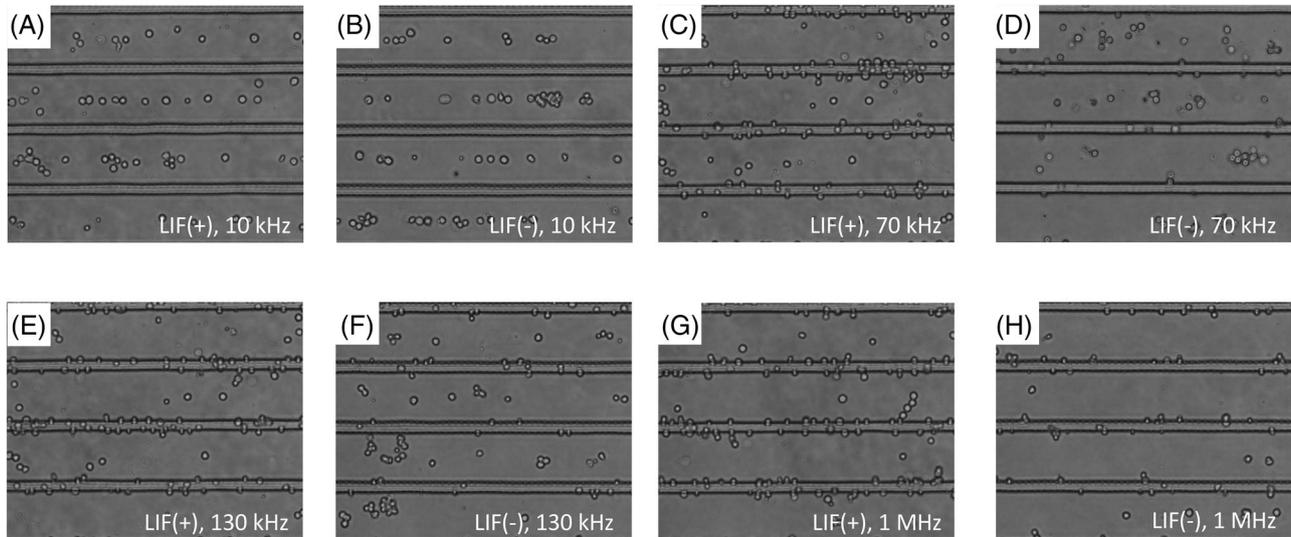
FIGURE 7 Dielectrophoresis of mESCs belonging to the LIF(+) and LIF(−) groups at frequencies of (A and B) 10; (C and D) 70; (E and F) 130; and (G and H) 1000 kHz
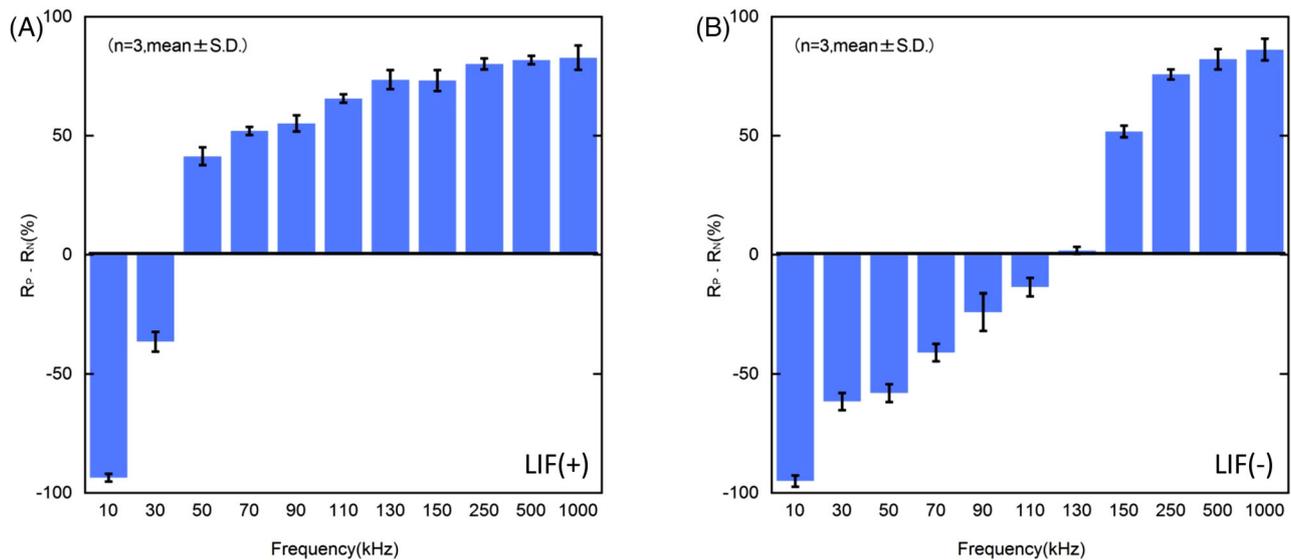

FIGURE 8 DEP frequency dependency parameter, Rp−Rn, of cells from the (A) LIF(+); (B) LIF(−) groups

and negative DEP forces of living cells and microparticles [22]. We also reported that the positive DEP forces were larger than the negative DEP forces. Therefore, only the cells in the LIF(+) group were trapped on the electrode array by positive DEP forces, whereas those in the LIF(−) group were flushed away by fluid flow because they were subjected to weak positive or negative DEP forces.

The merged images of fluorescent and phase-contrast images of cell suspensions collected at outlets A and B are shown in Figure 9. The number of cells stained by calcein AM was larger in outlet port A than in outlet port B. These results indicate that the number of cells from the LIF(+)

group was large in outlet port A because the cells from the LIF(+) group were only stained with calcein AM. To quantitatively evaluate the sorting efficiency, the purity of cells from the LIF(+) group in both outlets was calculated from the counted cell number (Figure 10). The percentage of cells in the LIF(+) group at outlet port A was 87%. At outlet port B, the percentage of cells in the LIF(+) group was less than 13%. This result indicates that our dielectrophoretic cell-sorting device was capable of purifying pluripotent mESCs from a mixed population of pluripotent and non-PSCs with a purity of approximately 87%. The pluripotency of the separated cells was also evaluated using the AP assay
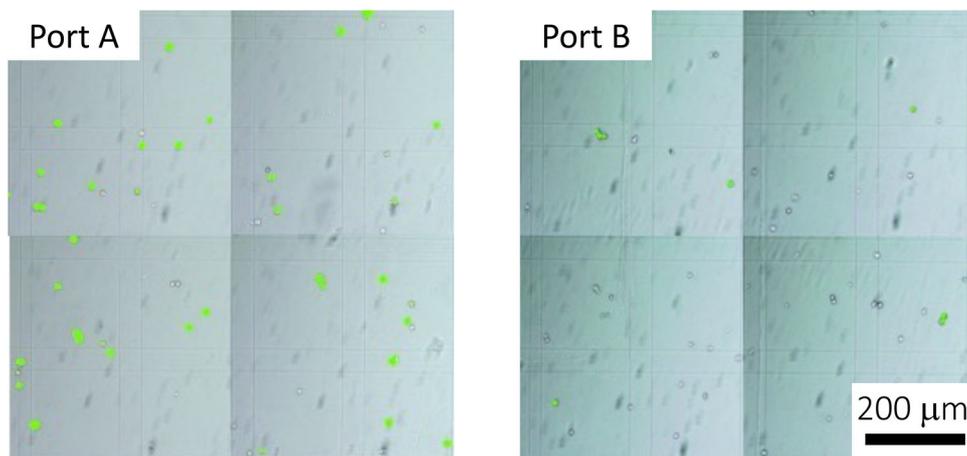
FIGURE 9 Merged images of phase-contrast and fluorescent images. The cells stained with calcein AM indicated those from LIF(+) group
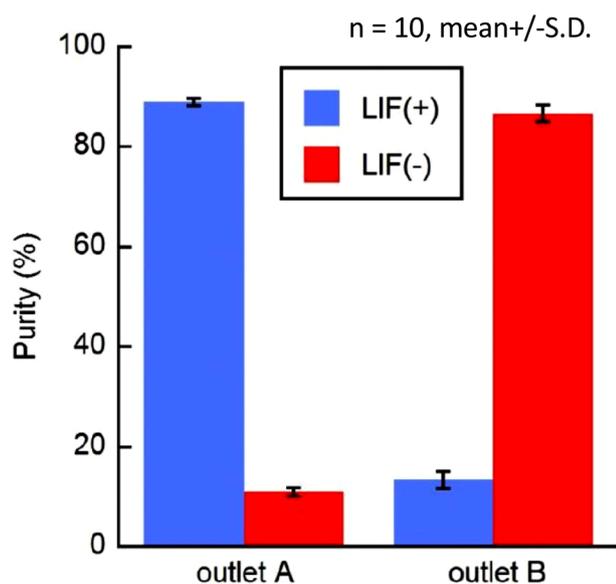

FIGURE 10 Proportion of sorted cells of the LIF(+) and LIF(−) groups in outlet ports A and B

(Figure 11). The cells collected from outlet port A exhibited higher AP activity, whereas those from outlet port B exhibited lower activities after 48-hour culture. The number of cells from outlet port B was insiginificant because the cells lacking pluripotency could not survive in the medium containing blasticidin S. From these results, the pluripotent mESCs could be collected in outlet port A and recovered after the DEP cell sorting.

There are superior conventional cell-sorting methods, such as FACS and MACS, for assessing the cell type or cell function quickly and accurately. The sorting efficacy of MACS was approximately 80%, and that of FACS was over 90%. Although these methods are effective for cell sorting, they require labeling of cells with antibodies or fluorescent dyes [6–8]. The DEP cell-sorting system does not require

antibodies, fluorescent dyes, or magnetic beads to reduce cell damage and loss. Considering these advantages, our DEP cell-sorting system achieves mESC separation according to pluripotency with efficiently high purity without the use of cell labeling.

Although there are cell sorting systems using DEP [26–28], these systems can separate cells of varying origins or cells expressing distinctly different natures such as viability. In this study, we performed a detailed characterization of the DEP properties of pluripotent and nonpluripotent mESCs. Based on a detailed analysis of the differences in the DEP properties of mESCs, our DEP cell-sorting system combined with a fluid-flow control system [23] could achieve separation of mESCs with similar origins, expressing pluripotency or lacking pluripotency. To validate our DEP-cell sorting for research and clinical applications requiring non-cell-labeled purification of PSCs, the evaluation of sorting efficacy under different mixed ratios of LIF(+) and LIF(−) cells, qRT-PCR or immunostaining of separated cells, and a pilot study using human PSCs would be required in future studies. From the results of this study, our DEP-cell sorting system is applicable to research and clinical applications requiring non-cell-labeled purification of PSCs.

4 | CONCLUDING REMARKS

In this study, the dielectrophoretic properties of mESCs expressing pluripotent and non-pluripotent phenotypes were characterized in detail. From the DEP characterization, mESCs expressing the pluripotent phenotype exhibited a different DEP behavior from that of cells expressing nonpluripotent phenotypes. Based on the variance in DEP properties and the fluid-flow control system, our DEP cell sorting system enabled the sorting of mESCs according to
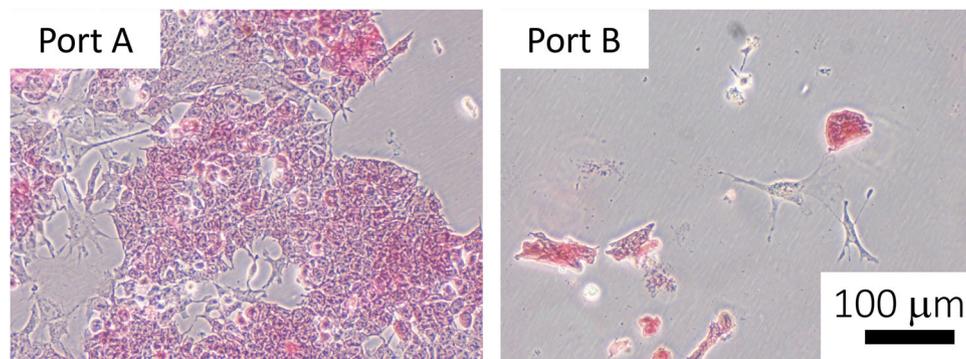
FIGURE 11 AP activity of mESCs collected in outlet port A and port B after 48-h culture

their pluripotency. This stem cell sorting technology was developed to separate undifferentiated and differentiated cells using DEP force and flow-induced shear force.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES
1. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007;448:313–317. https://doi.org/10.1038/nature05934
2. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292:154–156. https://doi.org/10.1038/292154a0
3. Segers VFM, Lee RT. Stem-cell therapy for cardiac disease. Nature. 2008;451:937–942. https://doi.org/10.1038/nature06800
4. Ibrahim SF, Van Den Engh G. High-speed cell sorting: Fundamentals and recent advances. Curr Opin Biotechnol. 2003;14:5–12.
5. Herzenberg LA, Parks D, Sahaf B, et al. The history and future of the fluorescence activated cell sorter and flow cytometry: a view from stanford. Clin Chem. 2002;48:1819–1827. https://doi.org/10.1093/CLINCHEM/48.10.1819
6. Makker K, Agarwal A, Sharma RK. Magnetic activated cell sorting (MACS): utility in assisted reproduction. Indian J Exp Biol. 2008;46:491–497.
7. Parks DR, Herzenberg LA. Fluorescence-activated cell sorting: theory, experimental optimization, and applications in lymphoid cell biology. Methods Enzymol. 1984;108:197–241. https://doi.org/10.1016/S0076-6879(84)08086-1
8. Krüger J, Singh K, O'Neill A, et al. Development of a microfluidic device for fluorescence activated cell sorting. J Micromech Microeng. 2002;12:486–494. https://doi.org/10.1088/0960-1317/12/4/324
9. Jones TB. Electromechanics of Particles. Cambridge University Press; 2005:34–81.
10. Rosenthal A, Voldman J. Dielectrophoretic traps for single-particle patterning. Biophys J. 2005;88:2193–2205. https://doi.org/10.1529/biophysj.104.049684
11. Punjiya M, Nejad HR, Mathews J, Levin M, Sonkusale S. A flow through device for simultaneous dielectrophoretic cell trapping and AC electroporation. Sci Rep. 2019;9:11988. https://doi.org/10.1038/s41598-019-48198-x
12. Li M, Anand RK. Cellular dielectrophoresis coupled with single-cell analysis. Anal Bioanal Chem. 2018;410:2499–2515. https://doi.org/10.1007/S00216-018-0896-Y
13. Lapizco-Encinas BH, Simmons BA, Cummings EB, Fintschenko Y. Insulator-based dielectrophoresis for the selective concentration and separation of live bacteria in water. Electrophoresis. 2004;25:1695–1704. https://doi.org/10.1002/elps.200405899
14. Kuczenski RS, Chang HC, Revzin A. Dielectrophoretic microfluidic device for the continuous sorting of Escherichia coli from blood cells. Biomicrofluidics. 2011;5:32005–3200515. https://doi.org/10.1063/1.3608135
15. Jung JY, Kwak HY. Separation of microparticles and biological cells inside an evaporating droplet using dielectrophoresis. Anal Chem. 2007;79:5087–5092. https://doi.org/10.1021/ac0702903
16. Zhang J, Song Z, Liu Q, Song Y. Recent advances in dielectrophoresis-based cell viability assessment. Electrophoresis. 2020;41:917–932. https://doi.org/10.1002/ELPS.201900340
17. Shafiee H, Sano MB, Henslee EA, Caldwell JL, Davalos RV. Selective isolation of live/dead cells using contactless dielectrophoresis (cDEP). Lab Chip. 2010;10:438–445. https://doi.org/10.1039/b920590j
18. Jubery TZ, Dutta P. A new design for efficient dielectrophoretic separation of cells in a microdevice. Electrophoresis. 2013;34:643–650. https://doi.org/10.1002/elps.201200560
19. Waheed W, Alazzam A, Mathew B, Christoforou N, Abu-Nada E. Lateral fluid flow fractionation using dielectrophoresis (LFFF-DEP) for size-independent, label-free isolation of circulating tumor cells. J Chromatogr B Analyt Technol Biomed Life

Sci. 2018;1087–1088:133–137. https://doi.org/10.1016/j.jchromb.2018.04.046

20. Piacentini N, Mernier G, Tornay R, Renaud P. Separation of platelets from other blood cells in continuous-flow by dielectrophoresis field-flow-fractionation. Biomicrofluidics. 2011;5:34122–341228. https://doi.org/10.1063/1.3640045

21. Ding J, Woolley C, Hayes MA. Biofluid pretreatment using gradient insulator-based dielectrophoresis: separating cells from biomarkers. Anal Bioanal Chem. 2017;409:6405–6414. https://doi.org/10.1007/s00216-017-0582-5

22. Ojima Y, Miyata S. Discrimination methodology of living-cells and microbeads using dielectrophoresis and fluid-induced shear force. J Biorheol. 2015;29:42–50. https://doi.org/10.17106/jbr.29.42

23. Takahashi Y, Miyata S. Continuous ES/feeder cell-sorting device using dielectrophoresis and controlled fluid flow. Micromachines. 2020;11:734. https://doi.org/10.3390/MI11080734

24. Takeuchi Y, Miyata S. Micro cell patterning technology by dielectrophoresis and application to regenerated cartilage, Trans, JSME (in Japanese). 2010;76:3015–3020.

25. Sugimoto Y, Miyata S. Multi-layered cell assembling technology using dielectrophoresis and construction of skin tissue microelement. Trans Jpn Soc Mech Eng C. 2017;83(848):16-00387. https://doi.org/10.1299/TRANSJSME.16-00387

26. Zhu J, Xuan X. Curvature-induced dielectrophoresis for continuous separation of particles by charge in spiral microchannels. Biomicrofluidics. 2011;5:24111. https://doi.org/10.1063/1.3599883

27. Wu Y, Ren Y, Tao Y, Hou L, Jiang H. High-throughput separation, trapping, and manipulation of single cells and particles by combined dielectrophoresis at a bipolar electrode array. Anal Chem. 2018;90:11461–11469. https://doi.org/10.1021/acs.analchem.8b02628

28. Rahmani A, Mohammadi A, Kalhor HR. A continuous flow microfluidic device based on contactless dielectrophoresis for bioparticles enrichment. Electrophoresis. 2018;39:445–455. https://doi.org/10.1002/elps.201700166

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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