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Fluid driving system for a micropump by differentiating iPS cells into cardiomyocytes on a tent-like structure

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ABSTRACT

A number of recent studies have exploited the sizes and functional properties of microdevices and cellular mechanical components to construct bio-microactuators. We previously developed bio-micropumps powered by cardiomyocytes that utilizes glucose in the medium as chemical energy. To fabricate the pump, however, primary neonatal rat cardiomyocytes are indispensable. The operation of harvesting primary cells is inconvenient and ethically not adequate due to the need for animals sacrifice. In contrast, induced pluripotent stem (iPS) cells are obtained from subcutaneous tissue. Their most significant properties are that they proliferate indefinitely and can be differentiated into many kinds of cells, including cardiomyocytes, and also have no ethical issue differently from ES cells. By exploiting these properties of iPS cells, the above issues will be addressed. Based on this concept, we constructed a system for driving fluids as a principal component of a micropump by differentiating iPS cells into spontaneously beating cardiomyocytes. Cellular contractile force was transmitted to fluid in a microchannel by a tent-like thin membrane. The microchip was irradiated with O_2 plasma and coated with gelatin to attach the cells. Embryoid bodies (EBs) of mouse-derived iPS cells were seeded on the microchip and incubated at 37 °C without Leukemia Inhibitory Factor (LIF) to differentiate them into cardiomyocytes. About 2 weeks later, EB beating and periodical oscillation of fluid in a microchannel connected to a diaphragm chamber was observed. The theoretical flow rate assuming the use of ideal check valves (Q) was 6.9 nL/min. Our device presents a reasonable alternative to normal cardiomyocytes for preliminary investigations requiring bio-actuating pumps.

1. Introduction

Micro total analysis systems (μ-TAS), or lab-on-a-chip, is a promising field that relies on microelectromechanical system (MEMS) technologies and creates extremely efficient and novel conceptual devices that exploit the advantages of microfluidics [1–4]. One of the most significant characteristics of these devices is that their scale can accommodate different cell sizes and processing capabilities. Based on this fact, a number of efficient bioreactors and bioassay systems using cellular functions have been produced [5]. To date, the main focus of these devices has been the analysis of cellular chemical functions.

Another application involving fusing of microdevices and cells is the use of cellular mechanical functions to realize novel actuators [6–8]. For example, actuators have been developed using microorganisms such as bacteria or protozoa. From the viewpoint of actuation function, various functions such as linear transferring [9–12], rotation [13] or both motion [14–16] of micro artificial objects and fluid control [17,18] were realized. Although these are effective for moving small objects, muscle cells or tissue are more suited for dynamic actuation. Bio-actuators powered by skeletal muscle tissue [19–21] and microstructure actuation systems using vascular smooth cells [22] have both been reported. These devices are attractive due to their controllability, but they do not actuate spontaneously.

On the other hand, cardiomyocytes, which use chemical energy input, actuate spontaneously and simultaneously if the cells are connected to each other, making larger motions feasible. Recently, various cardiomyocyte devices including actuation systems of microcantilevers [23], micropillars [24,25], ring structures [26,27],

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walking robots [28–31], swimming robots [32], and jelly fish-like actuators [33] powered by cardiomyocyte tissue have been developed. Among these, we have focused on micropumps powered by cardiomyocytes and have reported bio-microactuators that utilize spontaneously and synchronously beating cardiomyocytes such as an on-chip pump [34], a heart-like pump [35], and a previously frozen cardiomyocytes-based pump [36]. To improve mechanical transduction and fluid dynamic performance, we utilized a cardiomyocyte sheet shown to exhibit cooperative contractile forces produced by collectively synchronous, pulsatile properties across larger-scale dimensions (i.e., cm^2) [37,38]. However, to fabricate these cardiomyocyte devices, primary cardiomyocytes are required. The operation of harvesting primary cells is inconvenient because the experiments can only be carried out when rats are obtained, and the need for animal sacrifice brings up ethical issues. To avoid these problems, the novel system described herein utilizes induced pluripotent stem (iPS) cells [39].

Here, we utilize iPS cells obtained from subcutaneous tissue. Significantly, iPS cells proliferate indefinitely and can be differentiated into many kinds of cells, including cardiomyocytes [40].

The objective of this study is to construct a system for driving fluids as a principal component of a micropump by differentiating iPS cells into spontaneously beating cardiomyocytes. A technical challenge to achieve this is that, when using iPS cells that have already been differentiated into cardiomyocytes and recovering the cells by trypsinization, the actuation becomes small and not adequate to generate an oscillating fluid. Moreover, it is difficult to prepare a cardiomyocyte sheet that can generate large forces using iPS cells, because unlike primary cells, iPS cells do not spread and form a contiguous cell sheet. Therefore, it is necessary to seed embryoid bodies (EBs), which are groups of iPS cells, on the device before differentiation. After differentiation, EBs beat in a largely synchronous fashion that is adequate to generate fluid oscillation. Treatments of the device to optimize its structure and surface are required to efficiently utilize the contractile force of EBs. In this report, we first designed and fabricated a microchip to demonstrate fluid oscillations by differentiated cardiomyocytes. Fluid in microchannels was then observed to confirm that oscillations were generated by the cardiomyocytes.

Fig. 1 shows the structure and working principle of the micropump that was designed by modifying the previous pump design [36]. A flexible, thin membrane that serves as a scaffold for EBs is assembled as a tent-like structure to transmit cardiomyocyte forces. EBs are directly seeded on the membrane and then differentiated to cardiomyocytes on the device. Contractile forces of cultured cardiomyocytes are collected and transmitted to fluid within a chamber and a microchannel by a small, cylindrical block and a diaphragm. The diameter and height of the cylinder are 1.5 mm and 0.5 mm, respectively. All components are made of an elastomeric material, polydimethylsiloxane (PDMS). There are no check valves because this study focuses only on the generation of fluid oscillations.

2. Experimental

2.1. PDMS treatment for cell attachment

To investigate surface treatment for EB attachment, we prepared 4 conditions; A: Plastic cell culture dish (positive control), B: non-treated PDMS, C: PDMS coated with 0.1% gelatin (Millipore) for 1 h at 37 °C, D: PDMS treated with O_2 plasma for 30 s using a plasma exposure device (Femto, Diener Electronic) and coated with 0.1% gelatin for 1 h at 37 °C. PDMS were prepared on a 35 mm dish, cured overnight and UV sterilized for 30 min. Gelatin coated PDMS and O_2 plasma-treated PDMS coated with 0.1% gelatin were air dried and UV sterilized for 30 min before use.

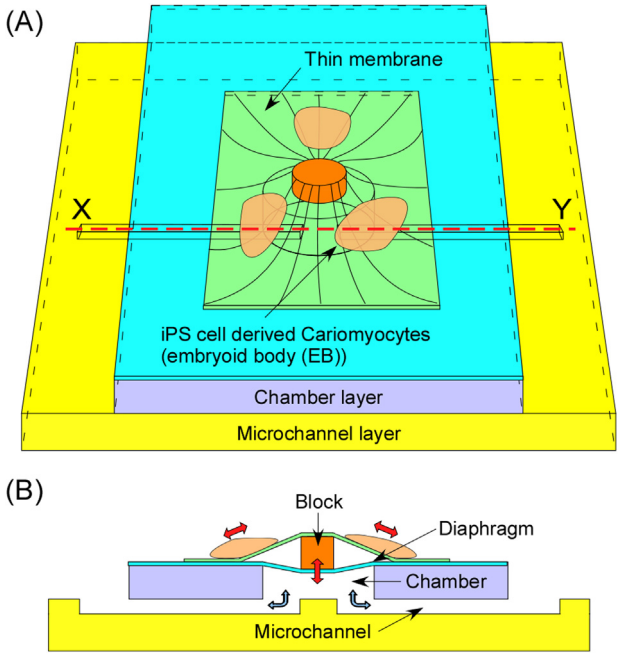

Fig. 1. Design of a micropump powered by differentiation of iPS cells into cardiomyocytes on a tent-like structure. (A) Schematic view. (B) Cross-sectional view along line *X*–*Y*.

EBs prepared by the method described in the following section were seeded (4 EBs per dish), and cultured for 14 days until observation.

2.2. Preparation and culture of iPS cells

Mouse iPS cells were purchased from CiRA and cultured on mitomycin-c treated mouse embryonic fibroblasts (MEF, Millipore) in high glucose DMEM (Gibco) containing 10% FBS (Gibco), 1% penicillin (Sigma–Aldrich), 1% streptomycin (Sigma–Aldrich), 1% GlutaMAX-1 (Gibco), 1% non-essential amino acids (Gibco), 1% nucleosides (Millipore), 1% sodium pyruvate (Sigma–Aldrich), 0.1% 2-mercaptoethanol (Sigma–Aldrich), and 0.1% leukemia inhibitory factor (LIF) (Sigma–Aldrich). EB were prepared by the hanging drop method (400 cells/drop, 2 days), transferred to a non-cell-attachment dish and further cultured for another 4 days. EBs were then transferred to the device or culture dish and LIF were withdrawn. Medium was exchanged every 2 days. Beating of cardiomyocytes was observed after 7–14 days.

2.3. Immunofluoresent staining

Cells were fixed and permeablized with PBS containing 4% paraformaldehyde and 0.4% Triton-X100 for 20 min at 4 °C. Cells were then washed with PBS, blocked in PBS containing 1% bovine serum albumin and stained against cardiac myosin heavy chain and cardiac troponin I antibodies (abcam) for 1 h. Cells were further stained with secondary antibodies for 1 h and observed under a confocal microscope (Olympus, FV1000).

2.4. Fabrication of a microchip

The microchip consists of four components: a microchannel layer, a chamber layer with a diaphragm membrane, and a cylindrical block on the diaphragm. The microchannel was fabricated using the replica molding method [41].

The basic fabrication process to produce this type of microchip is described in our previous report [36]. However, some of the

methods were revised, and the process is described below emphasizing the revised parts. The master templates for microchannel layers were fabricated by standard photolithography using photoresist. Microchip components of PDMS curable elastomer were then molded from the master template. A cylindrical block to be assembled on the diaphragm was fabricated by hollowing out a 1 mm thickness PDMS sheet using a biopsy punch (1.5 mm diameter). Chamber layers were fabricated by removing 3 mm diameter circles, also using a biopsy punch (3 mm diameter), from a 500 μm thickness PDMS sheet (2.0 cm × 1.5 cm rectangular). Importantly, a 1 mm height cubic block was used as a force transducer in the previous report [36], but this created tent membranes with a high angle slope making it difficult to attach the heavy EBs, which tended to slip down it. Therefore, a 500 μm height block was used in the revised procedure.

A microchip was then assembled from the different PDMS components. A diaphragm membrane was first attached to the chamber layer. The prepared prepolymer was poured over a silicon wafer, and it was spin-coated with a 10 μm layer of the prepolymer (7000 rpm, 1 min). Next, it was cut into a small piece (1 cm × 1 cm square), peeled from the silicon wafer in ethanol using a pair of tweezers and transferred onto a chamber layer to cover a chamber and create a diaphragm. After that, the chamber layer was attached to the microchannel layer, and then the prepared PDMS block was assembled on the diaphragm in the center of the chamber. Finally, another thin membrane (1 cm × 1 cm square) as a scaffold for cardiomyocytes was assembled on the block, to create another diaphragm. The membrane was carefully manipulated to make a tent-like structure. After the ethanol had evaporated, the positioned PDMS membrane spontaneously attached to the PDMS sheet, thus completing the assembly of the PDMS microchip.

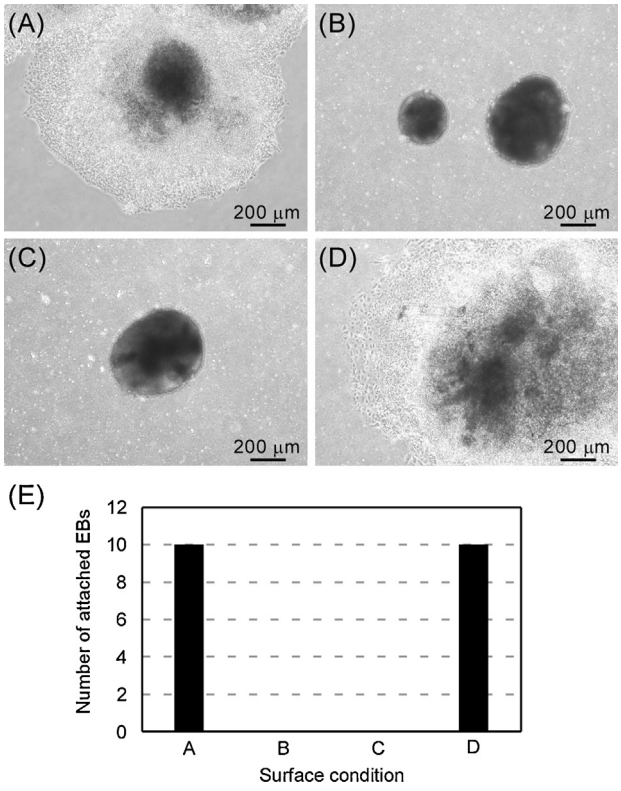

Fig. 2. Pictures of EBs cultured under different conditions a day after seeding. (A) EBs on a polystyrene dish. (B) EBs on non-treated PDMS. (C) EBs on PDMS modified only with gelatin. (D) EBs on PDMS modified with gelatin after O_2 plasma exposure. (E) The number of attached EBs under 4 conditions after seeding 10 EBs.

3. Results and discussion

3.1. Investigation of PDMS surface treatment for EB attachment

EB attachment to the device made with PDMS is critically important for the Bio-MEMS device to work as a pump. Thus, we surveyed the optimum surface treatment of the PDMS for EB attachment. Because cardiomyocyte differentiation of EBs is usually performed on a gelatin coated surface [42], we evaluated an efficient gelatin coating method. Although fibronectin was used as a coating material in previous reports because it is appropriate for culturing primary cardiomyocytes [34–36], it is not needed to attach EBs to PDMS before differentiation. Therefore, only gelatin was used as a coating material for these devices.

To assess the PDMS surface treatment for cell attachment, 10 EBs were seeded on a 35 mm dish coated with PDMS that had received various surface treatments, and the attached EBs were counted the next day. No EBs were attached on PDMS without any surface treatment or on gelatin coated PDMS (Fig. 2B and C). On the other hand, all the EBs were attached to PDMS treated with O_2 plasma and gelatin, in a similar manner to that used for the plastic cell culture dish (Fig. 2A and D). Attachment of EBs can be confirmed by the spreading of cells from the main EB bodies. The number of attached EBs under each condition is summarized in Fig. 2E. From this result, we concluded that treatment with O_2 plasma and gelatin was the optimal condition to attach EBs on PDMS.

3.2. EB seeding and differentiation on the microchip

As described above, a microchip device was treated with O_2 plasma and gelatin before cell culture. The microchip treatment and EB seeding protocols are shown in Fig. 3A. Pictures of the microchip are shown in Fig. 3B–D. A photograph of the assembled PDMS microchip is shown in Fig. 3B. The microchip was square (2 cm × 2 cm). The chamber had a 3 mm diameter and 500 μm depth. Depth and width of the microchannel were both 200 μm.

The microchip was immobilized on a cell culture dish using enamel, and a well made of PDMS having a diameter of 6 mm and a depth of 4 mm was placed at the middle of the microchip so that the tent structure was at the bottom of the well (Fig. 3C). A well structure was coated with a fluorine coating reagent (INT-332VE, Kanden Engineering) several times so that cell attachment to the well was minimal. This well structure is very important to prevent EBs from rolling down before they could become attached. EBs were placed in the well and cultured in a CO_2 incubator. One day after the placement of EBs, they successfully attached at the middle of the microchip (Fig. 3D–F). The well structure was removed the next day and the culture continued with medium changes every other day. After 1–2 weeks from seeding, beating cells were observed (see Supplementary multimedia file: Movie 1), indicating the presence of differentiated cardiomyocytes. To further confirm whether spontaneous differentiation of EBs on the device induced cardiomyocyte differentiation, we performed immunofluorescent analysis and found that some cells expressed cardiac myosin heavy chain and cardiac troponin-I (Fig. 3G and H), thus confirming the presence of differentiated cardiomyocytes. Approximately 40% of the cultured EBs showed spontaneous beating.

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We next optimized the structure and number of seeded EBs. Almost no EBs attached to the structure described in our previous report, which had a tent-like membrane with a 1 mm height block (force transducer) and no well structures [36]. This is because EBs fell down the steep slope of the tent-like membrane before they were able to attach to the surface. By using a 500 μm height block

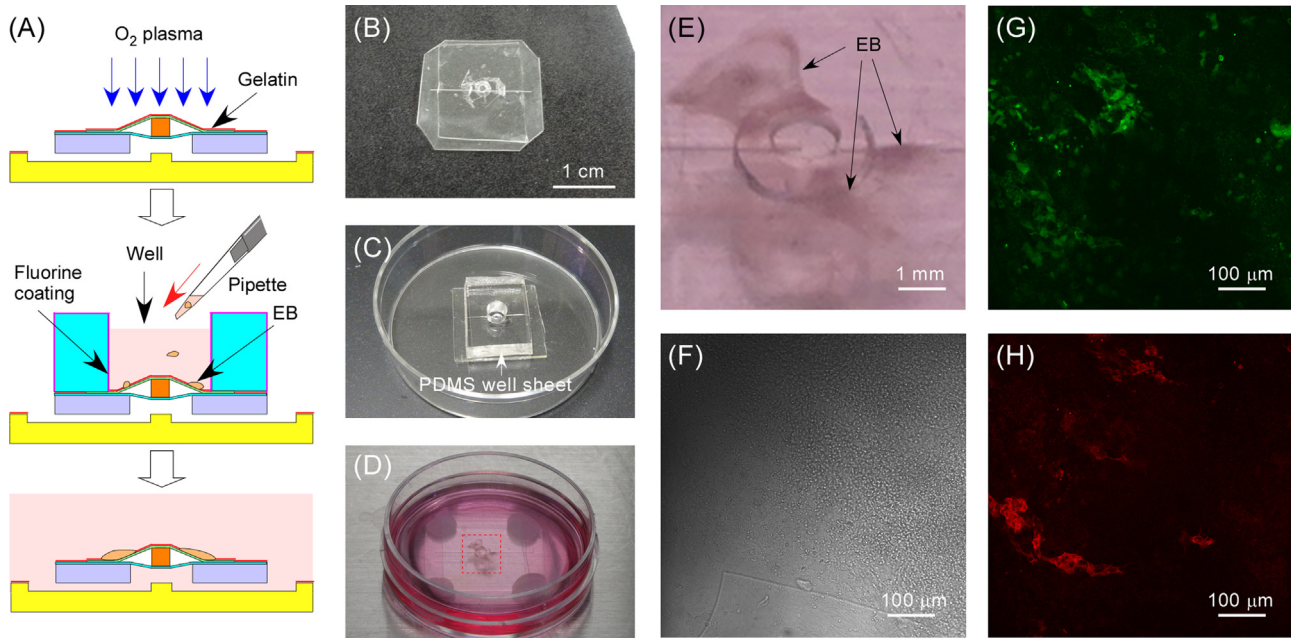

Fig. 3. Experimental protocol and pictures of devices and cells. (A) Method for attaching cells onto the device. A microchip was O_2 plasma treated and then gelatin treated, a PDMS well was equipped and EBs were seeded, and the well was removed after attachment of EBs. (B) Overview of the microchip. (C) A microchip equipped with a PDMS well. (D) A microchip 2 weeks after seeding. (E) An enlarged picture around a broken rectangle in D. (F) A bright field microscopic picture showing EBs differentiated into cardiomyocytes. (G and H) Fluorescent pictures of cardiomyocyte marker (G: anti-cardiac myosin heavy chain antibody, H: anti-cardiac troponin I antibody) stained corresponding to the F area.

Table 1
Property and performance comparison of the demonstrated pump with other cardiomyocyte pumps. Q (theoretical flow rate) is reported to two significant digits.

Reference number	Cardiomyocyte type	Pump shape	Pump driver	Q (nL/min)
This study	iPS cell derived	On-chip	Cell cultured membrane	6.9
[34]	Primary	On-chip	Cell sheet	240
[35]	Primary	Spherical	Cell sheet	47
[36]	Primary	On-chip	Cell cultured membrane	6.3
[44]	Primary	On-chip	Cell cultured membrane	7.1
[32]	Primary	Tubular	Cell cultured membrane	N.A.

and a well structure, the probability of adhesion became about 3/4. On the other hand, it is difficult to culture over 4 EBs per well because of the lack of medium in the wells. Therefore, we seeded exactly 4 EBs/well to achieve the expected value of at least 1 beating EB per well.

3.3. Observation of fluid actuation in a microchannel

After confirming cell beating, fluid in the microchannel was observed 2 weeks after EB seeding. To visualize the resulting fluid behavior in the microchannels, spherical polystyrene tracking particles (Fluoro Spheres, 1 μm diameter, Invitrogen) were dispersed in cell culture media within the microchannel, and the fluid behavior was observed directly using a phase contrast microscope (CKX-41, Olympus) with an objective lens (40×) (Fig. 4A). The microscope was focused on the center of the microchannel and the image was recorded using interfaced software (30 fps time resolution, EO-0413M, Edmund Optics). From our previous work, we knew that displacement is larger when the temperature is lower [34]. Therefore, in this experiment we measured particle displacement at room temperature in order to clearly observe the particle motion. Fig. 4B (device #1) shows the observed image in a microchannel. Spontaneous, oscillating fluid motion in the microchannel was produced from the repeated, regular pulsatile stroke movements of the cardiomyocytes attached to the membrane, as monitored by the movement of fluorescent polystyrene tracking particles (see Supplementary multimedia file: Movie 2). Beating motions of all particles were synchronized. This means that fluid in the microchannel oscillated. The displacement time-course trajectory for a selected particle near the center of the channel (the circled particle in Fig. 4B and in Movie 2) as directly observed by video is plotted in Fig. 4C. Particle displacement to the right (x) was measured directly from sequential video frames of the microscopic video image every 0.033 s. Displacement at $t = 0$ s was defined as $x = 0$ μm. Although the background line fluctuated a bit, the periodical oscillation was clear. The fluid oscillating frequency was 0.5 Hz and the maximum linear displacement was about 6 μm. Device actuation continued for at least 3 days, consistent with previous reports [34–36].

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Regarding the device performance stability, we confirmed reproducibility of this beating motion, and the motion and performance of the cells were quite similar. In this experiment, 10 devices were fabricated under the same conditions. Simultaneous periodic particle beating was observed in 2 of them, specifically devices #1 described above and #2. Fluid oscillation data for device #2 is shown as an insert in Fig. 4C (the circled particle in Fig. 4B and in Supplementary multimedia file: Movie 3). The background slope of device #2 was corrected to be the same as that of device #1 to compare the frequencies and displacements of the moving particles. Although the particle motion in device #2 fluctuated

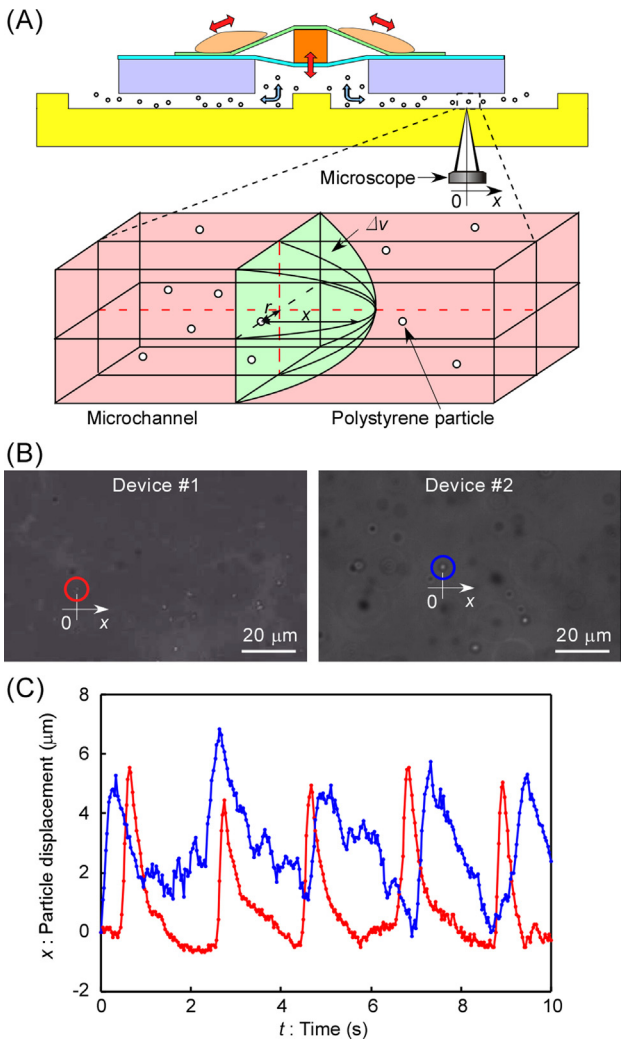

Fig. 4. Observations of a microchannel. (A) Measurement method and parameters for performance evaluation of the fluid oscillation. (B) Observation of particles in the microchannels (0.2 mm width and depth) of devices #1 and #2. The trajectory of the circled fluorescent polystyrene particles was traced for performance evaluation. (C) Displacement time-courses over 10 s of the particle near the center of the channel in the circles of B. Red and blue lines indicate samples in devices #1 and #2, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

compared with that of device #1, the displacement (about 5 μm) and frequency (0.5 Hz) were almost the same. In the negative control experiment, periodical and simultaneous particle beating was not observed after replacing the medium with ethanol (see Supplementary multimedia file: Movie 4).

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Since achieving confluence of EBs on a device is difficult compared to previous cardiomyocyte devices, the beat of the EBs that had not achieved confluence was not always synchronized. Nevertheless, constant, stable fluid beating was observed. We speculate that this is because the most actively beating or well-positioned EBs dominate the fluid actuation, although it is difficult to conclude which EBs mainly contribute most of the energy to deform the membrane. Membrane deformation was demonstrated by just fluid oscillations in a microchannel. To increase the success rate under stable conditions, the device size should be smaller and the EBs should be positioned more precisely, which would allow actuation by as little as one EB.

3.4. Estimation of potential performance

From the displacement of a selected particle near the center of the channel and the microchip dimensions, we estimated the following parameters regarding the performance of the fluid bio-actuation: volume change in the chamber per displacement (ΔV) and the theoretical flow rate assuming the use of ideal check valves to regulate the flow direction of the actuator without loss (Q) as discussed in previous reports [34,43]. The following analysis applies to device #1.

In this rough estimation, we assumed that the stress on the membrane was uniform. The relation between the volume change in the chamber (ΔV) and in the microchannel (Δv) is approximated as follows because the microchip is symmetric:

$$\Delta v = \frac{1}{2}\Delta V \tag{1}$$

From the measured displacement of one particle near the center of the channel (x), Δv is estimated using the following approximation for volume change in a square object:

$$\Delta v = \frac{0.47xwd}{1-(2r/w)^2} \tag{2}$$

where w is the width of the microchannel, d is the depth of the microchannel, and r is the distance of the selected particle from the central axis of the microchannel (Fig. 4A). The following values were used: $w = d = 200$ μm, $r = 8$ μm and $x = 6$ μm. From Eqs. (1) and (2), ΔV was calculated as 0.23 nL. The theoretical flow rate, assuming use of ideal check valves, was estimated from the following equation on the assumption that both resistance of check valves and reflux were negligible:

$$Q = f\,\Delta V \tag{3}$$

where f is the oscillation frequency. Since f was measured as 0.5 Hz and $\Delta V = 0.22$ nL, Q is calculated as 6.9 nL/min.

3.5. Evaluation of the actuation performance

Here, the calculated performance is compared with that of our previous pump [34–36] and also with cardiomyocyte pumps reported by other researchers [32,44]. The properties and performance of these pumps are summarized in Table 1. Although a pumping device in Reference [32] has no reported pumping rate, it was also included. Compared with cell sheet driven pumps [34,35], the theoretical flow rate (Q) was decreased in this study. However, Q was almost the same as that of similar types of cardiomyocyte pumps using cardiomyocytes directly cultured on PDMS thin membranes. Therefore, the current system using iPS cell derived cardiomyocytes presents a reasonable alternative to primary cardiomyocytes for substantial or preliminary investigations requiring bio-actuating pumps, for example in carrying out a drug response assay.

4. Conclusions

In this report, a system for driving fluids as a principal component of a micropump by differentiating iPS cells into spontaneously beating cardiomyocytes was constructed. First, we designed and fabricated a microchip to demonstrate fluid oscillations by differentiated cardiomyocytes. A novel method for iPS cell (EB) attachment on a tent-like structure by well structures and surface treatment was developed. Then, we observed fluid in microchannels to confirm the oscillations induced by cardiomyocytes. The theoretical flow rate Q was calculated as 6.9 nL/min, which was approximately the same as that reported previously for similar types of cardiomyocyte pumps.

A significant advantage of this method is that the use of animals for experiments can be reduced by relying on stored iPS cells. We hope that this new method will contribute to further research in the field of bio-microdevices.

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References

[1] C.T. Culbertson, T.G. Mickleburgh, S.A. Stewart-James, K.A. Sellens, M. Pressnall, Micro total analysis systems: fundamental advances and biological applications, Anal. Chem. 86 (2014) 95–118.
[2] M.L. Kovarik, D.M. Ornoff, A.T. Melvin, N.C. Dobes, Y. Wang, A.J. Dickinson, P.C. Gach, P.K. Shah, N.L. Allbritton, Micro total analysis systems: fundamental advances and applications in the laboratory, clinic, and field, Anal. Chem. 85 (2013) 451–472.
[3] G.M. Whitesides, The origins and the future of microfluidics, Nature 442 (2006) 368–373.
[4] T. Kitamori, M. Tokeshi, A. Hibara, K. Sato, Thermal lens microscopy and microchip chemistry, Anal. Chem. 76 (2004) 52A–60A.
[5] M.L. Kovarik, P.C. Gach, D.M. Ornoff, Y. Wang, J. Balowski, L. Farrag, N.L. Allbritton, Micro total analysis systems for cell biology and biochemical assays, Anal. Chem. 84 (2012) 516–540.
[6] V. Chan, H.H. Asada, R. Bashir, Utilization and control of bioactuators across multiple length scales, Lab Chip 14 (2014) 653–670.
[7] M. Pilareka, P. Neubauer, U. Marx, Biological cardio-micro-pumps for microbioreactors and analytical micro-systems, Sens. Actuators B: Chem. 156 (2011) 517–526.
[8] Y. Tanaka, K. Sato, T. Shimizu, M. Yamato, T. Okano, T. Kitamori, Biological cells on microchips: new technologies and applications, Biosens. Bioelectron. 23 (2007) 449–458.
[9] D.B. Weibel, P. Garstecki, D. Ryan, W.R. DiLuzio, M. Mayer, J.E. Seto, G.M. Whitesides, Microoxen: Microorganisms to move microscale loads, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 11963–11967.
[10] B. Behkam, M. Sitti, Effect of quantity and configuration of attached bacteria on bacterial propulsion of microbeads, Appl. Phys. Lett. 93 (2008) 223901.
[11] S. Martel, M. Mohammadi, O. Felfoul, Z. Lu, P. Pouponneau, Flagellated magnetotactic bacteria as controlled MRI-trackable propulsion and steering systems for medical nanorobots operating in the human microvasculature, Int. J. Robot. Res. 28 (2009) 571–582.
[12] S. Cho, S.J. Park, S.Y. Ko, J.O. Park, S. Park, Development of bacteria-based microrobot using biocompatible poly(ethylene glycol), Biomed. Microdevices 14 (2012) 1019–1025.
[13] Y. Hiratsuka, M. Miyata, T. Tada, T.Q.P. Uyeda, A microrotary motor powered by bacteria, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 13618–13623.
[14] A. Itoh, Motion control of protozoa for bio MEMS, IEEE/ASME Trans. Mechatron. 5 (2000) 181–188.
[15] E. Steager, C.B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, M.J. Kim, Control of microfabricated structures powered by flagellated bacteria using phototaxis, Appl. Phys. Lett. 90 (2007) 263901.
[16] M.S. Sakar, E.B. Steager, D.H. Kim, A.A. Julius, M.J. Kim, V. Kumar, G.J. Pappas, Modeling, control and experimental characterization of microbiorobots, Int. J. Robot. Res. 30 (2011) 647–658.
[17] N. Darnton, L. Turner, K. Breuer, H.C. Berg, Moving fluid with bacterial carpets, Biophys. J. 86 (2004) 1863–1870.
[18] M. Nagai, Y. Hayasaka, K. Kato, T. Kawashima, T. Shibata, Mixing of solutions by coordinated ciliary motion in Vorticella convallaria and patterning method for microfluidic applications, Sens. Actuators B: Chem. 188 (2013) 1255–1262.
[19] H. Herr, R.G. Dennis, A swimming robot actuated by living muscle tissue, J. Neuroeng. Rehabil. 1 (2004) 6.
[20] H. Fujita, K. Shimizu, E. Nagamori, Novel method for measuring active tension generation by C2C12 myotube using UV-crosslinked collagen film, Biotechnol. Bioeng. 106 (2010) 482–489.
[21] Y. Morimoto, M. Kato-Negishi, H. Onoe, S. Takeuchi, Three-dimensional neuronemuscle constructs with neuromuscular junctions, Biomaterials 34 (2013) 9413–9419.
[22] Y. Tanaka, K. Sato, T. Shimizu, M. Yamato, T. Okano, I. Manabe, R. Nagai, T. Kitamori, Demonstration of a bio-microactuator powered by vascular smooth muscle cells coupled to polymer micropillars, Lab Chip 8 (2008) 58–61.
[23] J. Park, J. Ryu, S.K. Choi, E. Seo, J.M. Cha, S. Ryu, J. Kim, B. Kim, S.H. Lee, Real-time measurement of the contractile forces of self-organized cardiomyocytes on hybrid biopolymer microcantilevers, Anal. Chem. 77 (2005) 6571–6580.
[24] Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, Demonstration of a PDMS-based bio-microactuator using cultured cardiomyocytes to drive polymer micropillars, Lab Chip 6 (2006) 230–235.
[25] Y. Zhao, X. Zhang, Cellular mechanics study in cardiac myocytes using PDMS pillars array, Sens. Actuators A: Phys. 125 (2006) 398–404.
[26] H. Horiguchi, K. Imagawa, T. Hoshino, Y. Akiyama, K. Morishima, Fabrication and evaluation of reconstructed cardiac tissue and its application to bio-actuated microdevices, IEEE Trans. Nanobiosci. 8 (2009) 349–355.
[27] Y. Akiyama, T. Sakuma, K. Funakoshi, T. Hoshino, K. Iwabuchi, K. Morishima, Atmospheric-operable bioactuator powered by insect muscle packaged with medium, Lab Chip 13 (2013) 4870–4880.
[28] J. Xi, J.J. Schmidt, C.D. Montemagno, Self-assembled microdevices driven by muscle, Nat. Mater. 4 (2005) 180–184.
[29] J. Kim, J. Park, S. Yang, J. Baek, B. Kim, S.H. Lee, E.S. Yoon, K. Chun, S. Park, Establishment of a fabrication method for a long-term actuated hybrid cell robot, Lab Chip 7 (2007) 1504–1508.
[30] V. Chan, K. Park, M.B. Collens, H. Kong, T.A. Saif, R. Bashir, Development of miniaturized walking biological machines, Sci. Rep. 2 (2012) 857.
[31] V. Chan, J.H. Jeong, P. Bajaj, M.B. Collens, H. Kong, R. Bashir, Multi-material biofabrication of hydrogel cantilevers and actuators with stereolithography, Lab Chip 12 (2012) 88–98.
[32] A.W. Feinberg, A. Feigel, S.S. Shevkoplyas, S. Sheehy, G.M. Whitesides, K.K. Parker, Muscular thin films for building actuators and powering devices, Science 317 (2007) 1366–1370.
[33] J.C. Nawroth, H. Lee, A.W. Feinberg, C.M. Ripplinger, M.L. McCain, A. Grosberg, J.O. Dabiri, K.K. Parker, A tissue-engineered jellyfish with biomimetic propulsion, Nat. Biotechnol. 30 (2012) 792–797.
[34] Y. Tanaka, K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, T. Kitamori, An actuated pump on-chip powered by cultured cardiomyocytes, Lab Chip 6 (2006) 362–368.
[35] Y. Tanaka, K. Sato, T. Shimizu, M. Yamato, T. Okano, T. Kitamori, A microspherical heart pump powered by cultured cardiomyocytes, Lab Chip 7 (2007) 207–212.
[36] Y. Tanaka, Y. Yanagisawa, T. Kitamori, Fluid actuation for a bio-micropump powered by previously frozen cardiomyocytes directly seeded on a diagonally stretched thin membrane, Sens. Actuators B: Chem. 156 (2011) 494–498.
[37] T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu, T. Okano, Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces, Circ. Res. 90 (2002) e40–e48.
[38] T. Shimizu, M. Yamato, A. Kikuchi, T. Okano, Cell sheet engineering for myocardial tissue reconstruction, Biomaterials 24 (2003) 2309–2316.
[39] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[40] L. Zwi, O. Caspi, G. Arbel, I. Huber, A. Gepstein, I.H. Park, L. Gepstein, Cardiomyocyte differentiation of human induced pluripotent stem cells, Circulation 120 (2006) 1513–1523.
[41] Y.N. Xia, G.M. Whitesides, Soft lithography, Angew. Chem. Int. Ed. Engl. 37 (1998) 550–575.
[42] J. Zhang, G.F. Wilson, A.G. Soerens, C.H. Koonce, J. Yu, S.P. Palecek, J.A. Thomson, T.J. Kamp, Functional cardiomyocytes derived from human induced pluripotent stem cells, Circ. Res. 104 (2010) e30–e41.
[43] C.T. Loy, S.C. Pradhan, T.Y. Ng, K.Y. Lam, A series solution approach to ananalytical load-deflection relation forthe measurement of mechanicalproperties of thin films, J. Micromech. Microeng. 9 (1999) 341–575.
[44] J. Park, I.C. Kim, J. Baek, M. Cha, J. Kim, S. Park, J. Lee, B. Kim, Micro pumping with cardiomyocyte–polymer hybrid, Lab Chip 7 (2007) 1367–1370.

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