Controllable Bio-Microactuator Powered by Muscle Cells

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Abstract— **This paper reports a novel autonomous bio-microactuator powered by primary cultured cardiomyocytes and the control and evaluation of the contraction of skeletal muscle cell line C2C12 cells by means of electrical stimulation. To exploit contractions of cardiomyocytes, we fabricated a PDMS bulb–shaped dispenser and allowed cardiomyocytes to directly adhere to it. The displacement of the bulb-shaped dispenser by cardiomyocytes was larger than previously reported. Then, we employed electrical stimulation to control the contraction of muscle cells. The differentiated C2C12 cells (myotubes) were stimulated electrically, which was analyzed digitally. We succeeded in regulating contractions of C2C12 myotubes by electrical stimulations and manifested the relationship between the contraction and the pulse frequency.**

I. Introduction

Much attention has been directed to micro-total analysis systems (μ-TAS) and lab-on-a-chip, devices of which are fabricated by microfabrication technology such as micro electro mechanical systems (MEMS). Several efficient bioreactors, biosensors and bioassay systems have been produced by fusion of cell biology and various technologies. Those devices utilize only chemical or bio-chemical properties of cells.

Our group has focused on kinetic energy generated by cells and already reported that the micro bio-actuator, utilizing spontaneous contractions of primary cultured cardiomyocytes, can be driven by only chemical energy of biological reaction in living cells without electrical or mechanical energy[1,2,3]. This heart muscle cell based micro bio-actuator has three issues for practical use; the first is low conversion efficiency of myocardial contractions to drive flow, the second is less controllability of the actuators, and the final is inability to proliferate of cardiomyoctes.

To improve the first issue, we propose that isolated cardiomyoctes from the heart of a neonatal rat are plated on a polydimethylsiloxane (PDMS) thin film and adhere tightly to it. In the previous studies, the cell-sheet engineering using temperature-responsive polymer was utilized. It enables us to collect a cultured cell-sheet by only temperature reduction without any damage of the cell-cell junction. However, it is difficult to mount and fix the cell-sheet on the PDMS structure of a micro actuator. For this reason, the contractile force of cardiomyocytes was unable to bring out maximally for fluid drive.

For the second issue, we propose the use of skeletal muscle cells, such as the C2C12 mouse myoblast cell line, for a regenerative transducer which is capable of proliferating infinitely, fusing and differentiating into myotubes. Cardiomyocytes that can neither divide nor proliferate any further means the sacrifice of many laboratory animals are required as primary culture cells whenever the bio-microactuator is fabricated. The cell line can replace the laboratory animal. If the bio-actuator comes to be mass-produced, the cell line will play a very important role.

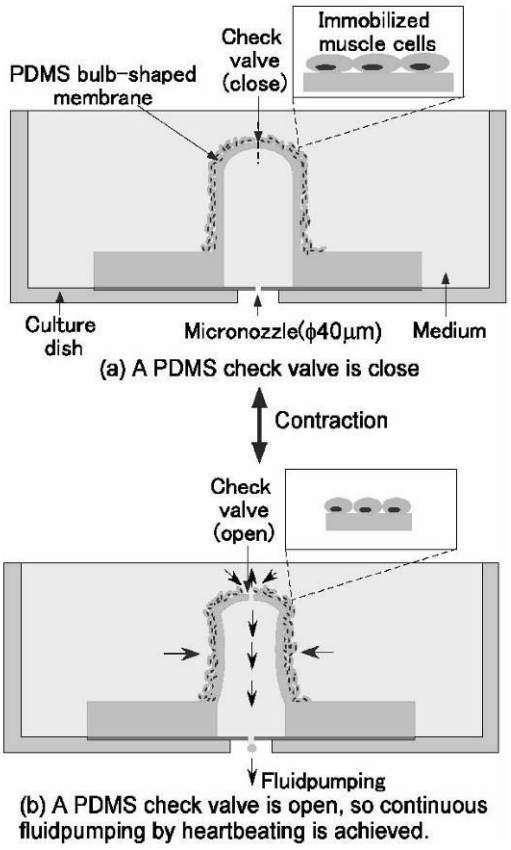

Fig.1 A principle of fluid pumping of bio-actuated bulb-shaped fluidic dispenser. A PDMS check valve is actuated by heart cell pulsating synchronously.

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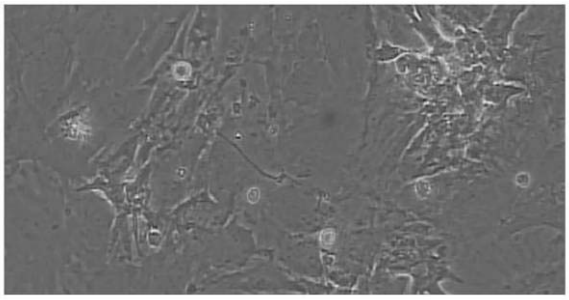

Fig. 2 Phase-contrast micrographs of cardiomyocytes one week in culture.

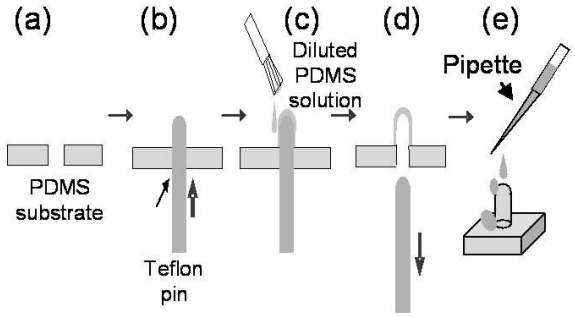

Fig.3 A schematic diagram of fabrication method of PDMS based bulb-shaped membrane structure and assembly process of a muscle-powered fluidic dispenser. (a), (b) Teflon pin was inserted through a hole on PDMS substrate. (c) Diluted PDMS solution was thin-layer coated. (d) PDMS thin membrane was baked at oven, and then Teflon pin was pulled out. (e) Primary neonatal rat cardiomyocytes were directly seeded on PDMS bulb-shaped membrane structure.

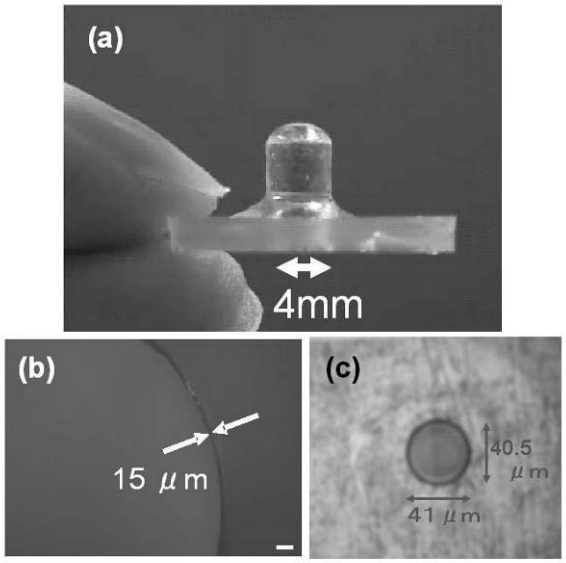

Fig.4 Photo of a prototype of PDMS bulb-shaped fluidic dispenser. (a) a whole view. (b) a cross sectional image of bulbed-shaped membrane(15μm thickness). (c) a micronozzle fabricated by micro discharge machining.

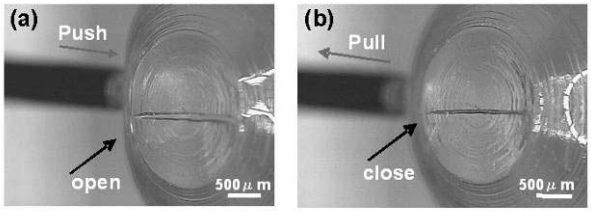

Fig.5 Photo of test of a PDMS check valve by pushing & pulling. A very small slit window was opened (a) and closed (b).

For the final issue, we propose to use electrical stimulation to control the contractions of muscle cells. Skeletal muscle cells are generally incapable to contract spontaneously without electrical stimulation. In addition, it is difficult to control the pulse rate of cardiomyocytes though temperature changes and cardiac agents affecting it. Electrical stimulation can make many kinds of muscle cells contract without any damage [4].

This paper describes a design and fabrication of a novel bio hybrid micro fluidic dispenser using cardiomyocytes and micromachined mechanical components, and to apply electrical stimulation to control technique for the contractions of muscle cells toward a controllable bio-microactuator.

II. Material and Method

A. Design of Micro Fluidic Dispenser

The principle of the proposed bio hybrid micro fluidic dispenser is shown in Figure 1. Cardiomyocytes are immobilized on a PDMS bulb-shaped thin membrane (15μm thickness). The check valve is closed while cardiomyocytes are relaxing; it is open autonomously while they are contracting spontaneously. A micromachined nozzle allows the dispenser to pump out a small amount of volume of fluid synchronously.

B. Preparation and Culture of Neonatal Rat Cardiomyocytes

Primary neonatal rat cardiomyocytes were prepared according to the following procedures. Cardiac ventricles of 1-day-old Wistar rats were digested at 37°C with collagenase in Dulbecco's phosphate buffered saline (DPBS, Ca^{2+} and Mg^{2+} free). Isolated cells were suspended in the culture medium, DMEM/F-12 supplemented with 10% FBS and 0.2% penicillin-streptomycin solution (Figure 2). The primary cell suspensions were prepared at above 1×10^6 cells / ml.

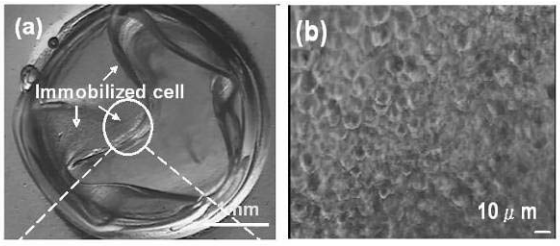

Fig.6 Microscopic image of cell-immobilized PDMS based bulb-shaped membrane structure from top view.

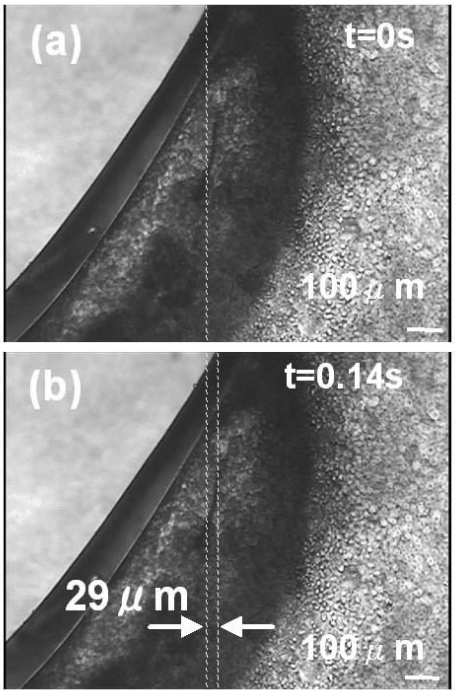

Fig.7 Microscopic image of pulsating cell-immobilized PDMS based bulb-shaped membrane and displacement of membrane per one pulse was 29μm.

C. *Microfabrication and cell assembly*

A bulb-shaped dispenser was made of PDMS without photolithography. The procedure is shown in Figure 3. Finally the primary cell suspensions were seeded on a bulb-shaped thin membrane coated with fibronectin.

D. *A bulb-shaped dispenser*

Figure 4 shows a photo of a prototype of PDMS bulb-shaped fluidic dispenser. The thickness of the bulb-shaped membrane was 15μm around a whole structure to realize the highly efficient mechanical energy conversion. A micronozzle, 40 μm in diameter, was fabricated by micro discharge machining for fluid dispensing. Figure 5 shows a photo of the test of a PDMS check valve by pushing & pulling. We succeeded in fabricating and testing a very small slit window, which was opened and closed. A microscopic image of cell-immobilized PDMS based bulb-shaped membrane structure from top view, is shown in Figure 6. The whole structure was filled with medium and was incubated at 37 degrees centigrade, CO_2 5%. A group of cardiomyocytes pulsated synchronously. Next, displacement of a thin membrane structure by cardiomyocytes was measured. A microscopic image of pulsating cell-immobilized PDMS based bulb-shaped membrane is shown in Figure 7. Result of displacement of membrane per single twitch was 29μm. Compared previous results [2, 3], the displacement of a thin membrane structure was relatively large.

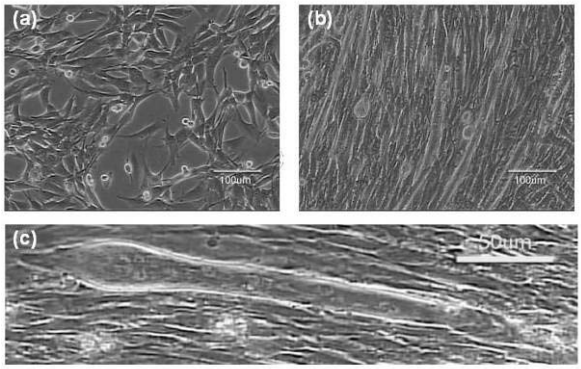

Fig.8 Microscopic image of pulsating cell-immobilized PDMS based bulb-shaped membrane and displacement of membrane per one pulse was 29μm.

E. *C2C12 Cell Culture*

A mouse myoblast cell line C2C12 (obtained from RIKEN Cell Bank) was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) until confluent. The medium was then changed with DMEM supplemented with a 7% house serum (HS) and was replaced every two days [5]. After 2-3 weeks culture, C2C12 myoblasts were partly fused and differentiated into myotubes (Figure 8).

F. *Electrical Stimulation*

Figure 9 shows a schematic diagram of electrical stimulation. Through all of the experiments, the stimulating

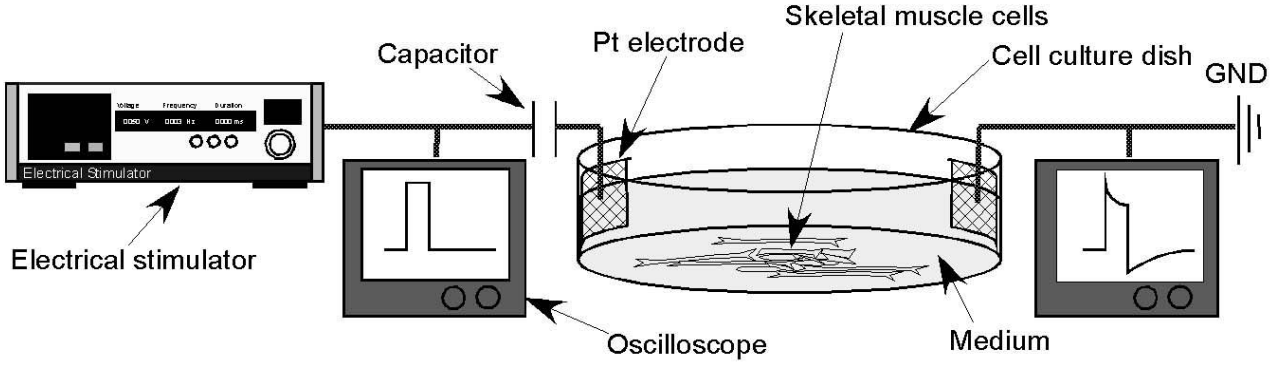

Fig. 9 Schematic diagram of electric stimulation. We used a capacitor of 220 μF to avoid medium electrolysis. The stimuli were applied to skeletal muscle cells through platinum electrodes.

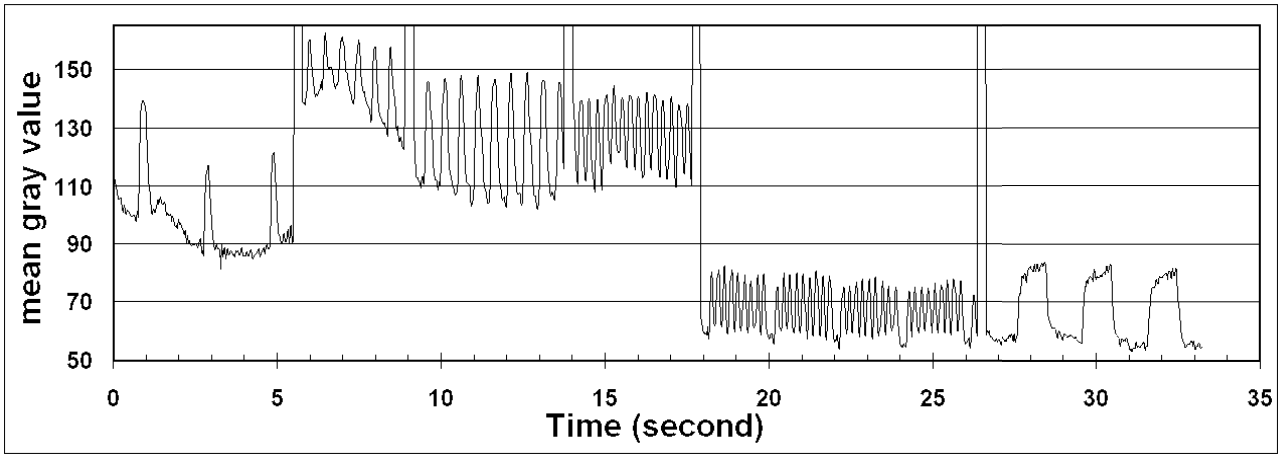

Fig. 10 Frequency-contraction diagram for a myotube. The contraction amount on the ordinate is expressed as a mean gray value of the selected pixels. The stimulation conditions were pulse amplitude of 50 volts, pulse width of 30 ms and various frequencies from 0.5 to 5 Hz.

voltage and the pulse duration were 50V and 30ms respectively (determined in preliminary experiments.) To avoid electrolysis, a 220 μF aluminum electrolytic capacitor was connected between the stimulator and the anode electrode in the cell culture dish.

G. Image Analysis

To clarify the relationship between the pulse frequency and the contraction, a C2C12 myotube was observed under microscope with phase contrast while stimulated at the frequencies of 0.5, 1, 2, 4, 5 and 10Hz. The microscopic animations were captured by a CCD camera into a PC and digitally analyzed with 'Scion Image' software (Scion Corporation, MD, US). The C2C12 myotube contracted synchronously with pulse stimulations at 0.5 to 5Hz and the contractions fused together at 10Hz. The amounts of contractions decreased with increasing stimulation frequencies above 5Hz (Figure 10).

III. CONCLUSION

We have confirmed that it is possible to achieve much more efficient force just when plating on the PDMS thin membrane directly without using cell-sheet engineering. These results show that fluid dispensing power and efficiency by heart muscle cells are large enough to deliver a small amount of volume of fluid in a microspace. We will improve the patterning method of the cardiomyocytes on PDMS membrane and further study on a point of view of mechanobiology using a prototype device. The result of electrical stimulation of skeletal muscle cells also indicates that it is possible to apply muscle cells as mechanical actuated components toward a controllable bio-micro actuation in future.

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