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DEMONSTRATION OF MUSCLE-POWERED AUTONOMOUS MICRO MOBILE GEL

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***Abstract*— A three-dimensional cardiomyocyte gel was reconstructed using an extracellular matrix gel formed from Matrigel and collagen I. The autonomous beating of the cardiomyocyte gel was synchronized with contraction of cardiomyocytes in the gel. The gel beating frequency was increased by increasing the concentration of adrenaline in the cardiomyocyte culturing medium. Drug stimulation to modulate the beating frequency could be used for control of a pumping application. A micropump driven by the cardiomyocyte gel was demonstrated. The micropump consisted of two check valves and the cardiomyocyte gel actuator. The volume changes of the channel in the micropump, synchronized with the autonomous gel beating, were seen.**

I. Introduction

Miniaturized chemical systems, which are fabricated by MEMS technologies, have attracted much interest because they offer experimental systems that save energy, resources and space. There are many microfluidic devices, such as micropumps which generate flows are very important. Presently, most mechanical actuators have driving sources that use shape memory alloys, piezoelectric materials, heat, or electrostatic force [1]. It is difficult to miniaturize a whole system because external power sources are needed. On the other hand, actuators constructed from muscle cells have many advantages; they are small volume, flexible, and self-regenerative, and they can be used as a chemical energy-driven mechanical component without an electrical energy supply [2][3]. Cardiomyocytes especially have the characteristic that they beats autonomously only by chemical energy. However, the force generated by a single cell is small [4]. Previous studies have tried to overcome that problem by reconstructing cells into a cell sheet [5][6]. However, this approach has its own problem that actuators are limited by the shape of the cell sheet when the sheet is applied as a micro bioactuator. As an alternative, cardiomyocytes reconstructed three-dimensional structure using an extracellular matrix, such as collagen I and Matrigel, has attracted some interest (Fig.1) [7]. Three-dimensional reconstructed cardiomyocyte gels are expected to be able to generate larger force than two-dimensional reconstructed sheets and to be able to be fabricated into a variety of shapes because the shape of gels are dependent on the mold shape.

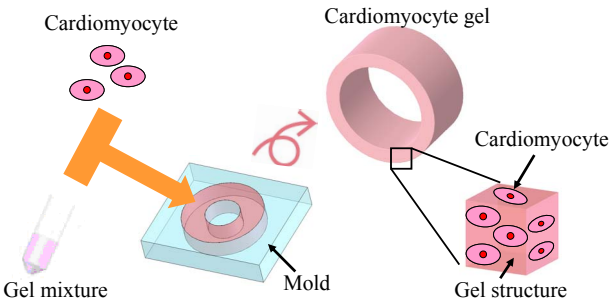

Fig.1 Three-dimensional cardiomyocyte gel constructed from an extracellular matrix

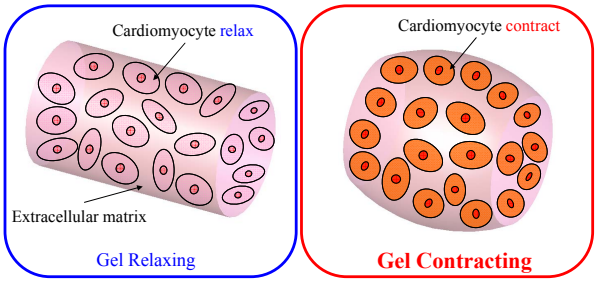

Fig.2 Principle of the beating of the gel. The autonomous beating of the gel was synchronized with contraction of cardiomyocytes in the gel.

In this study, a micro gel was prepared that could be fabricated into various shapes, and its movement was synchronized with contraction of cardiomyocytes in the gel and it could beat autonomously. Additionally, the drug stimulation to control gel behavior was evaluated. Adrenaline was used to excite them. Finally, a micro bioactuator driven by this gel was demonstrated.

II. Principle of Autonomous Beating of Cardiomyocyte Gel

Autonomous beating of the cardiomyocyte gel is generated by the movement of cardiomyocytes in the gel (Fig.2). They contracted autonomously using chemical energy from a culture medium at 37 °C and 5 % CO_2. Cells

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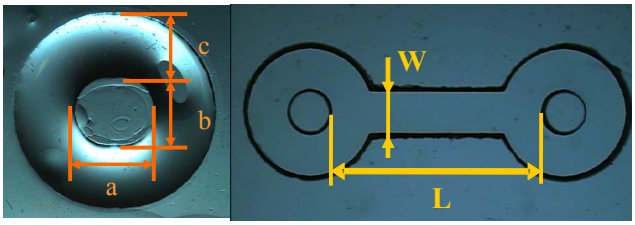

Fig.3 Photos of molds. Left: circular shape, right: beam shape

Table 1: Dimensions of molds (a×b×c: circular mold, W×L: beam mold)

a×b×c(mm)	2.4×2×2		
W × L(mm)	1 × 5	2 × 5	3 × 5

grew using the extracellular matrix as scaffold. So beating of the cardiomyocyte gel was synchronized with the contraction of cells in the gel without an external power source.

III. EXPERIMENT

A. Fabrication of cardiomyocyte gel

Various kinds of shapes of cardiomyocyte gel could be fabricated by pouring a gelating cell suspension into a PDMS (polydimethylsiloxane) mold which had been made by soft lithography. PDMS is a highly biocompatible material and it has a hydrophobic characteristic, so all molds were given a hydrophilic treatment by plasma-ion irradiation. Photos and dimensions of two mold shapes, such as beam and circular, are shown in Fig.3 and Table 1. The circular shape was developed to fabricate the gel for the micro actuator. Both molds were 500μm depth. To study the relationship between the width and shape of the gel, beam shape gels were fabricated with widths of 1mm, 2mm and 3mm. The cell suspension was prepared by mixing isolated cardiomyocytes from neonatal Wistar rats (cell density: 1.0×10^7cells/ml), collagen I (Rat Tail Collagen, Type I, BD Bioscience Inc.), a basement membrane protein mixture (Matrigel, BD Bioscience) and concentrated Dulbecco's Modified Eagle medium (DMEM; Gibco Invitrogen Co.) supplemented with 20 % inactivated horse serum (HS; Gibco Invitrogen Co.), 200 U/ml penicillin and 200 μg/ml streptomycin. The liquid mixture was gelated by incubating it for 45 to 60 min at 37 °C and 5 % CO_2, then DMEM supplemented with 10 % inactivated horse serum, 100 U/ml penicillin and 100 μg/ml streptomycin, was added to the culture dish.(Fig.1) The culture medium was changed once every three days.

B. Drug stimulation with adrenaline

Beating of the cardiomyocyte gel is generated by contraction of cardiomyocytes in the gel. Movement of the gel will change if contraction of cardiomyocytes in the gel changes. To control behavior of the gel, adrenaline

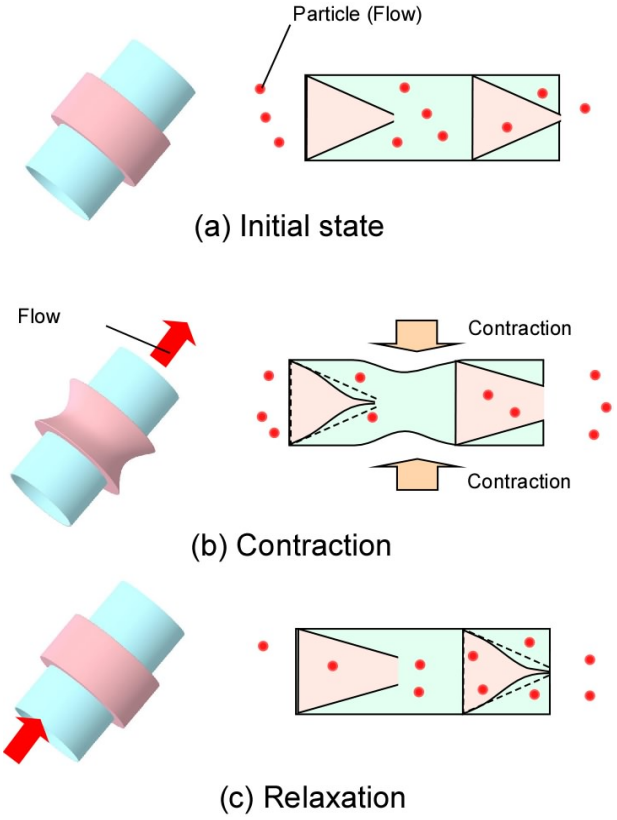

Fig.4 Principle of the micropump

((±)-Epinephrine, WAKO), which excites cells, was added to the culture medium containing the gel after calibration of its concentration at 10^{-6}M, 10^{-5}M and 10^{-4}M. The displacement of the gel was analyzed by image analysis.

C. Demonstration of micropump driven by cardiomyocyte gel

Micropumps are very important devices to drive and control fluids. However, the downsizing of existing micropumps is difficult because they have external power sources. Application of cardiomyocyte gels for the driving source is expected to allow downsizing of micropumps. An attempt was made to realize a micropump which was driven by the cardiomyocyte gel.

D. Principle of the micropump

Fig.4 shows the principle of the micropump. The cardiomyocyte gel is around the side of the micropump channel which has two check valves (Fig.4(a)). When the micropump is deformed by the contraction of the gel, liquid in the micropump is pumped out (Fig.4(b)). But, back flow of liquid does not take place because the left check valve is closed. When the shape of the micropump is restored by relaxation of the gel (Fig.4(c)), liquid flow to the inside takes place. However, back flow does not take place because the right check valve closes. In this way, the pump is driven by

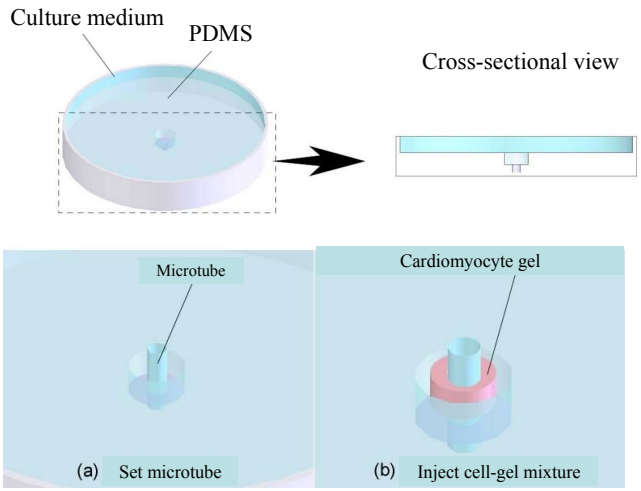

Fig.5 Assembly process of the micropump and the gel

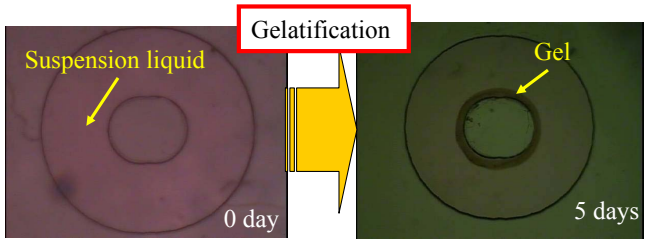

Fig.6 The cell suspension became the cardiomyocyte gel

contraction and relaxation of the gel.

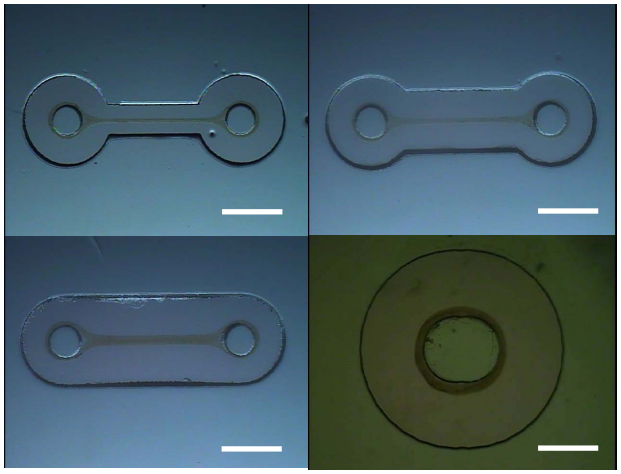

Fig.7 (a) Gel of 1×5 beam mold, (b) gel of 2×5 beam mold, (c) gel of 3×5 beam mold, (d) gel of circular mold (all scale bars:2mm)

E. Fabrication of the micropump

Two structures, the tube (external diameter: 2mm, inside diameter of outlet: 0.7mm, thickness of sidewall: 30μm) and the channel which has two check valves (diameter of check valve: 200μm, diameter of check valve outlet: 2mm, height of

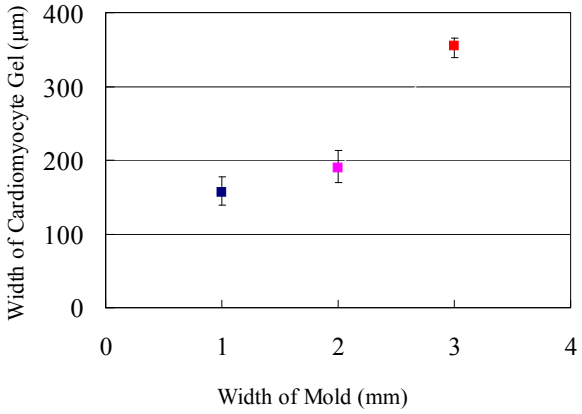

Fig.8 Relationship between widths of the cardiomyocyte gels and molds

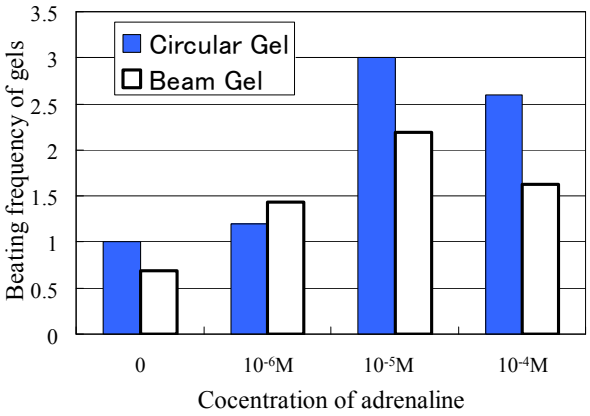

Fig.9 Relationship between concentration of adrenaline and beating frequency of cardiomyocyte gel shapes.

check valve: 3.4mm, length: 7.8mm), were fabricated from an ultraviolet cured resin (TSR-510, CMET). The minimum thickness of the fabrication is 30μm. Then the structures were put into the mold for fabricating the gel and this was placed on a culturing dish (Fig.5). Cell suspension was poured into the mold, and the gel was coupled with the structures.

F. Evaluation of micropump's performance

To confirm that the structures are deformed by the cardiomyocyte gel, the displacement of liquid level in the tube was evaluated using the tube combined with the gel. The flow rate was estimated by using movement of fluorescent beads (Fluospheres F13081, Molecular Probes) which were injected into the micropump channel with a micro pipette. The gel was stimulated by adrenalin.

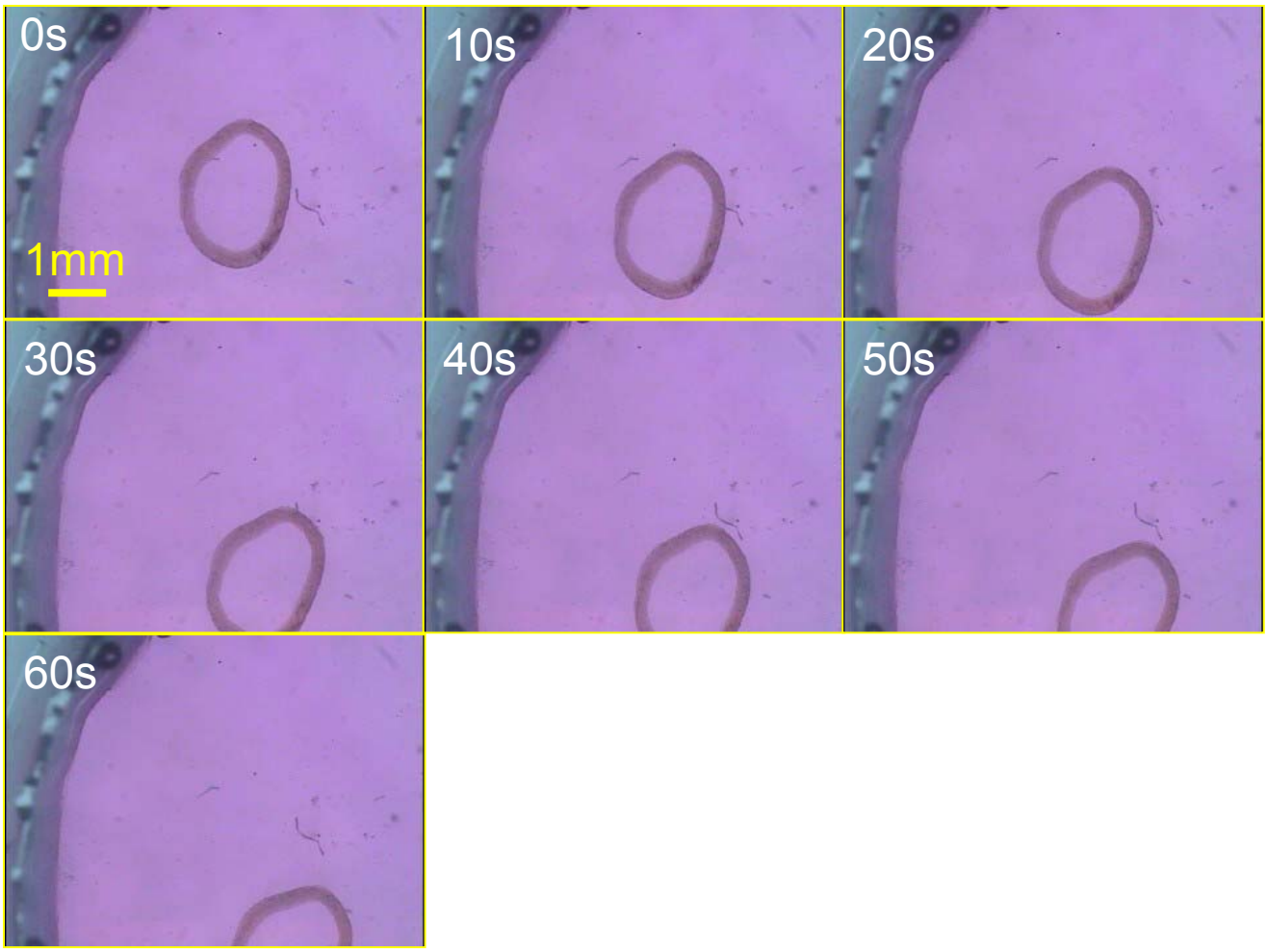

Fig.10 The circular shaped gel moved in the culture medium. This gel was stimulated by adrenaline.

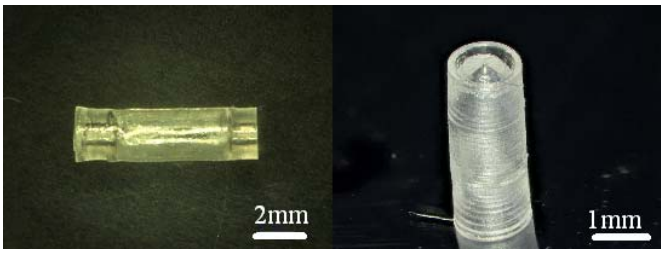

Fig.11 Left: the tube used to study deformation generated by the gel, right: the channel used to develop the micropump.

IV. Results and discussion

A. Fabrication of cardiomyocyte gel

The circular shaped cardiomyocyte gel is shown in Fig.6. Contractions of a single cardiomyocyte in the gel were confirmed 1day after the start of culturing. Then after 5 days, the cell suspension gelated and shrunk (Fig.6). Fig.7 shows other shapes of gels. Width of gels was measured (Fig.8). The width of the gel became a tenth of the width of the mold.

B. Drug stimulation with adrenaline

Fig.9 shows the relationship between the concentration of the adrenaline and the beating frequency of two shapes for the cardiomyocyte gel. The frequency increased with the concentration of the adrenaline. However, the frequency of both gel shapes decreased when the high adrenaline dose (10^{-4}M) was administered. Heart function of cardiomyocyte in the gel diminished because the concentration of adrenaline increased rapidly.

One circular shaped gel, excited by adrenaline after being detached from the mold, moved in the culture dish (Fig.10). A previous study [8] has described the mechanism

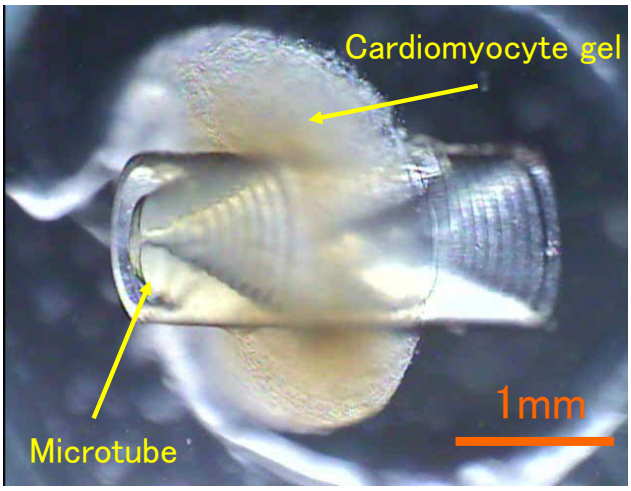

Fig.12 Side view of the micropump integrated cardiomyocyte gel

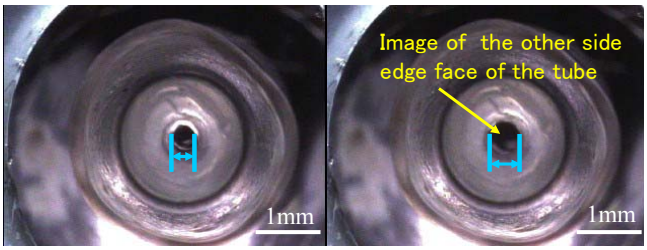

Fig.13 Views of the edge face of the tube with integrated cardiomyocyte gels. Left: relaxed cardiomyocyte gel, right: contracted gel

for contractions of cardiomyocytes generated by action potential propagation. Because of the propagation speed or path, a phase difference of the gel beating would be generated.

C. Demonstration of micropump driven by cardiomyocyte gel

Fig.11 shows two structures, the tube and the channel, which were fabricated from ultraviolet cured resin. The cardiomyocyte gel was around the structure (Fig.12). Fig.13 shows views of the edge face of the tube integrated cardiomyocyte gel. The liquid level in the tube was a curved surface due to the surface tension. So the edge face of the tube appeared as smaller than the actual size due to the effect of the lens when looking through the liquid level. The tube was deformed by the gel contract and the curvature of the liquid level changed because the liquid level was pushed up. As a result, the observed size of the cross section of the tube image changed. It was confirmed from the observation movie that the changes were generated in synchronization with beating of the cardiomyocyte gel. The size of the tube image was 320μm when the gel relaxed and it was 500μm when the gel contracted.

From the observation of the fluid level, it was apparent that cardiomyocyte gel could deform the channel and generate volume changes.

V. CONCLUSION

A cardiomyocyte gel was reconstructed using the extracellular matrix of collagen I and Matrigel. The gel was confirmed to autonomously beating in synchronization with contractions of cardiomyocytes in the gel. Different gel shapes could be obtained by changing the size of the fabrication mold. The cardiomyocytes were excited by drug stimulation, and the gel beating frequency was changed by adding adrenalin to the culture medium. Moreover one of the gel samples which were stimulated by adrenaline moved in the culturing dish. It should be possible to control this movement by optimizing the propagation speed or the propagation path of the action potential. Finally, the cardiomyocyte gel could be incorporated into a microstructure consisting of a tube and a channel, and deformation of the channel occurred by beating of the gel. Optimizing the shapes of the gels and microstructures should lead to development of novel micro devices.

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