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Biohybrid Microsystems Actuated by Cardiomyocytes: Microcantilever, Microrobot, and Micropump

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Abstract— **This paper introduces biohybrid microsystems actuated by cardiomyocytes, such as microcantilever, microrobot, and micropump. The microfabicated biohybrid microcantilever can measure the contractile force of self-organized cardiomyocytes. The microcantilever is made of a biocompatible PDMS substrate, using a simple microfabrication technique and a specially designed 3D micromolding aligner. The contractile force of the cardiomyocytes makes a bending displacement of the microcantilever. Conversely, from the displacement, we can estimate the contractile of the cardiomyocytes on the microcantilever. The bending motion of the microcantilever can be applied as a biohybrid actuator of microrobot. We have developed a novel method to fabricate a crab-like microrobot that can actuate for a long period under a physiological condition. The microrobot consists of three separate front and rear legs which have a shape of the microcantilever. The performance of our crab-like microrobot was measured at an average velocity of 100 μ m/s, and the estimated total distance it traveled was 50 m for a one-week period. Finally, a micropump actuated by the self-beating cardiomyocytes is fabricated and the pumping performance is demonstrated.**

I. Introduction

BILLIONS of years of "natural R&D" have resulted in effective, optimized biological solutions that really work. By studying and mimicking nature's processes and structures, scientists and engineers can develop nature inspired solutions that are far more effective than solutions conceived and developed exclusively by man. Biomimetic artificial machines built with hybrid components (materials partly synthetic and partly biological in origin) offer the opportunity of combining enhanced sensitivity with robustness and the possibility to extend their application to diverse environmental conditions [1].

As examples of micro/nano machine using hybridization between organic and inorganic components, a few reports of adenosine triphosphate (ATP) biomolecular motors [2], [3], a microorganism carrier in microchannel [4], a walking microdevice driven by micromuscle [5], and a pump actuated by cultured cardiomyocytes [6] are reported. Although bimolecular motors are interesting, they can generate only between 5-60 pN forces [7] and are not robust to actuate microstructure. Micromuscles can be alternate microactuator for micro-sized biomimetic system. Previous studies using cell based actuators [5], [6] are very attractive, but for advanced biomimetic systems, understanding and exploiting higher-order assemblies for micromuscles are key points of today's quest [1].

Structure and functional changes ensue in cardiac cell networks when cells are guided by three-dimensional scaffold topography, such as enhanced actin cytoskeleton organization, higher nuclear eccentricity [8] and altering gene expression, protein localization [9], cell signaling [10] and the intracellular calcium dynamics [11]. These topology-induced changes are expected to enhance the mechanical activity of cells [12].

We demonstrated some applications of biohybrid microsystems actuated by cardiomyocytes: microcantilever [13], microrobot [14] and micropump [15]. The microcantilever is made by a biocompatible PDMS material using a simple microfabrication technique and a specially designed 3D micromolding aligner. The contractile force of the cardiomyocytes makes a bending displacement of the microcantilever. From the displacement of the microcantilever, we can estimate the contractile force of the cardiomyocytes. Therefore, the biohybrid microcantilever can be used as the measuring tools for the contractile force of the cardiomyocytes. The bending motion of the microcantilever can be applied as a biohybrid actuator of microrobot. We designed the crab-like microrobot with several legs which is similar to the microcantilever. The legs show the bending motions of the microcantilever and the synchronized bending motion of the legs makes the walking motion of the crab-like microrobot. Finally, the

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dome-shaped micropump actuated by the cardiomyocytes was also proposed and fabricated and the performance of the micropump is demonstrated.

This paper introduces the brief fabrication methods and the performances of the microsystems. In addition, we concern the limitations of the microsystems and give several suggestions for the biohybrid microsystems.

II. Biohybrid Microcantilever

A. Cell Culture

A heart was aseptically isolated from a neonatal Sprague-Dawley rat on day 1 and washed with Hank's balanced salt solution (HBSS, Gibco Invitrogen Co., Grand Island, NY, USA). After separating the ventricles, the tissues were minced and incubated in a 0.3 mg/ml collagenase solution containing 0.6 mg/ml of pancretin (Sigma Chemical Co., St. Louis, MO, USA). The isolated cardiomyocytes were seeded directly onto the PDMS microcantilever at a cell density of 1.65 × 104 cells/mm2 and cultured in Dulbecco's modified Eagles' medium (DMEM, Gibco Invitrogen) containing 10% fetal bovine serum (FBS, Sigma), 50 μg/ml streptomycin, and 50 μg/ml penicillin (Gibco Invitrogen) at 37 °C in 5% CO2 air. The medium was changed at 48-h intervals in order to maintain a continuous pulsation. The overall cell culturing processes are simply described in Fig. 1.

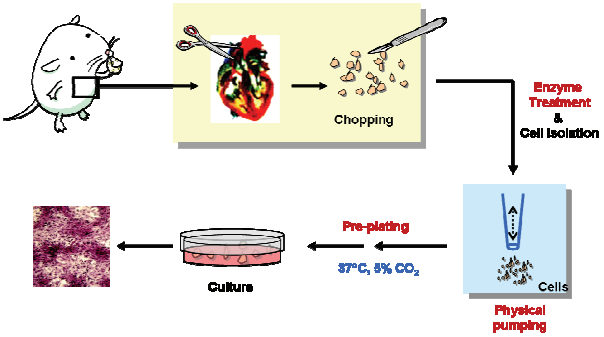


Fig. 1. Simplified Procedure for Cardiomyocytes Culturing

B. 3D Molding Aligner

To make the complex 3D polymer structures such as the microcantilever, we proposed a 3D-micromolding aligner, which can make two molding masters be aligned within 2 μm and allows applying high pressure to construct thin membranes of less than 20 μm. Using this system, wafer-level fabrication of thin polymer structures with complex 3D geometries was realized successfully. The proposed 3D micromolding aligner is shown in Fig. 2.

Firstly, the upper and lower molding master were fabricated by using conventional MEMS procedure, such as photolithography, wet or dry etching, and cleaning. Secondly, the lower molding master was placed on the bottom wafer chuck and the body material of microcantilever, PDMS, was poured on the lower molding master. Thirdly, the upper molding master was placed on the top wafer chuck and the upper and the lower molding masters could be aligned by the aligning part. And the two molding masters were stacked and clamped by the molding part in Fig. 2. Finally, the clamped molding part was cured for 2 hours at 120°C in an oven. After curing, the PDMS thin microcantilever could be peeled form the masters.

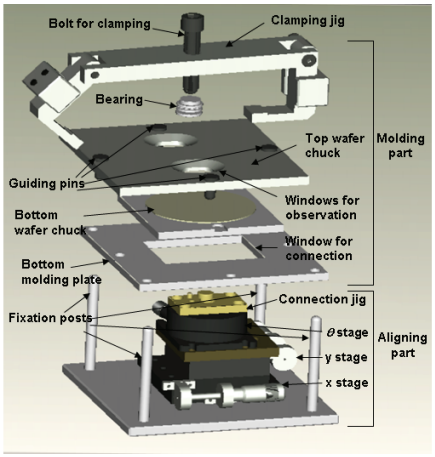


Fig. 2. Schematic diagram of the 3D-micromolding aligner

C. Molding Master and Microcantilever Fabrication

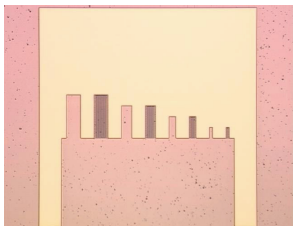
The bottom molding master was fabricated on the Si wafer using a deep silicon etching process and a thick negative photoresist (PR). The bottom molding master could define and form the structure of the microcantilever. The microcantilever body was fabricated from the top glass master. For the fabrication of the top glass molding master, a Cr/Au layer was coated and the microcantilever body was patterned by etching process. Finally, the top master fabrication was completed by removing the Cr/Au layer from the etched glass wafer. The fabricated polymer microcantilevers are shown in Fig. 3. The microcantilevers have various dimensions and different surface such flat surface and grooved surface. The grooved surface is expected to enhance the mechanical activity of cardiomyocytes.

D. Preparation of Microcantilever

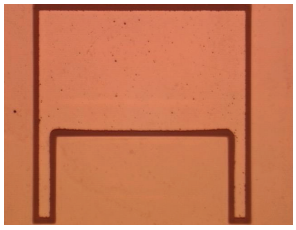
It is essential to make careful preparations before developing a primary culture of cardiomyocytes on the microcantilever manufactured using a PDMS molding process. The PDMS microcantilever was first detached from the mold master and it was washed and immersed in a solution of 70% ethanol to remove impurities and sterilize. Ethanol was removed and dried with UV lights on a clean

bench. The fresh PDMS surface was in a hydrophobic condition to prevent the adhesion of proteins and cells. Therefore, an O2 plasma treatment was next applied to increase the adhesion between the PDMS surface and ECM(Extra Cellular Matrix). The device was then coated by immersing it in the ECM fibronectin solution overnight. Following immersion it was dried on a clean bench under UV light. Once all the described steps were completed, the microcantilevers were ready to culture the primary cells.

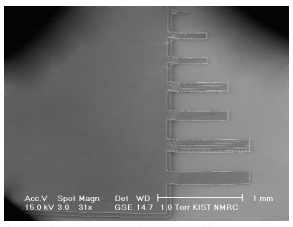
The cells were seeded on the microcantilevers and five days later the microcantilevers were able to bend by the contractile force of the cardiomyocytes.



(a) Silicon Wafer Molding Master (Bottom)



(b) Glass Wafer Molding Master (Upper)



(c) Fabricated PDMS Microcantilever Structure

Fig. 3. Fabrication Results of Microcantilever

E. Experimental Setup and Results

We measured the motion of the microcantilever using two microscopes, as shown in Fig. 4. The lateral motion of the microcantilever was measured by an inverted microscope (Olympus IX81, Olympus) and the vertical motion was observed by a camscope (ICS 305B, Sometech). The position of the camscope was controlled with the microstage and fixed to focus on the side of the microcantilever. The lateral and vertical motions were captured to PC.

Figure 5 shows the still images of the lateral motion of the microcantilevers and demonstrates the actuations of the flat microcantilever and the groove microcantilever. In figure 4 (a) and (b), the left side image and the right side image are at the relaxation state and the contraction state of cardiomyocytes, respectively. To assess the contractile forces, the displacements at the edge of the microcantilevers were measured by the proposed experimental setup in Fig. 4.

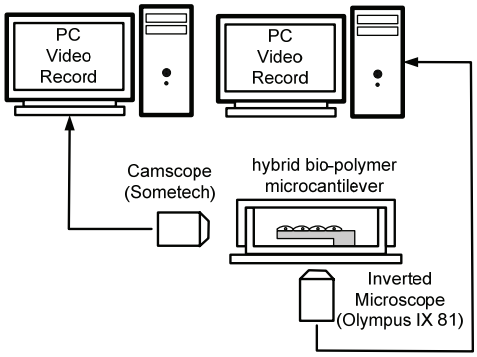
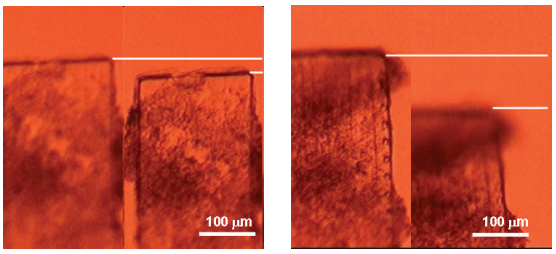


Fig. 4. Experimental Setup for Microcantilever

As shown in Figure 3, a flat and a grooved microcantilever were fabricated in single microcantilever arrays, so that the conditions for the two types of microcantilevers in cell culture would be the same. As shown in Fig. 5, the displacement of the grooved microcantilevers was larger than of the flat microcantilevers. Compared with the flat microcantilever, therefore, we could estimate that the mechanical activity of cardiomyocytes on the grooved surface microcantilever is enhanced. From the bending displacement of the microcantilever, we could calculate the contractile force of the cardiomyocytes and the contractile force could be described as a shear stress [16]. In the case of the flat microcantilevers, the calibrated shear stress is within 2-5 nN/μm^2. In the case of grooved microcantilevers, the calibrated shear stress is within 4-7 nN/μm^2.



(a) Flat Microcantilever (b) Grooved Microcantilever

Fig. 5. Experimental Results of Biohybrid Microcantilevers
(Still Image of the Lateral motions of the Microcantilevers)

F. Discussion

The biohybrid microcantilever was proposed as a biohybrid actuator which is combined with organic cardiac cells and inorganic polymer. In addition, the biohybrid

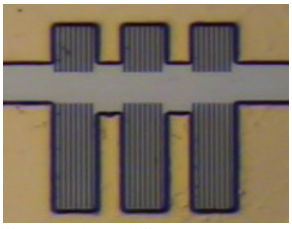
microcantilever was used as the sensor for the quantization of the contractile force of the cardiomyocytes. Therefore, it is expected that the biohybrid microcantilever will be used as sensors for the monitoring of the differentiation into heart muscle cells and for the observing of drug's effect on cardiomyocytes.

III. Crab-like Microrobot

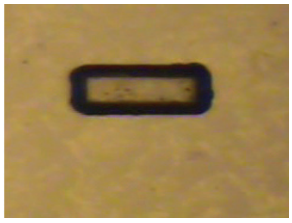
A. Fabrication & Preparation of Microrobot

The crab-like microrobot was designed and fabricated to facilitate a walking motion. It was designed as an asymmetric structure with three front and rear legs of different lengths. The multiple legs were joined to the middle of the robot body allowing connection of the cardiomyocytes on the robot body which were shown to develop synchronized beating.

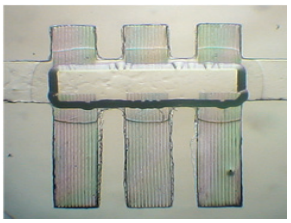
Similar with the microcantilever, using 3D micromolding aligner, the microrobot was fabricated by PDMS molding process. The bottom molding masters for the microrobot could define the front legs and the rear legs and the upper master could make the middle of the microrobot. The fabricated microrobot is shown in Fig. 6. To facilitate a high concentration of cardiomyocytes force, a grooved pattern was engraved on the surface of the legs.



(a) Silicon Wafer Molding Master (Bottom)



(b) Glass Wafer Molding Master (Upper)



(c) Fabricated PDMS Microrobot Structure

Fig. 6. Fabrication Results of Microrobot

For the primary cell culture, the polymer microrobot structure was prepared through the same procedure in the microcantilever. In addition, the cells were seeded on the device and five days later the microrobot was detached at the bottom and the hinge on which it hung was cut. At this point the microrobot device was able to move freely. Before observing it with an optical microscope, the device was turned over using a pipette so that the walking motion of the microrobot could be observed.

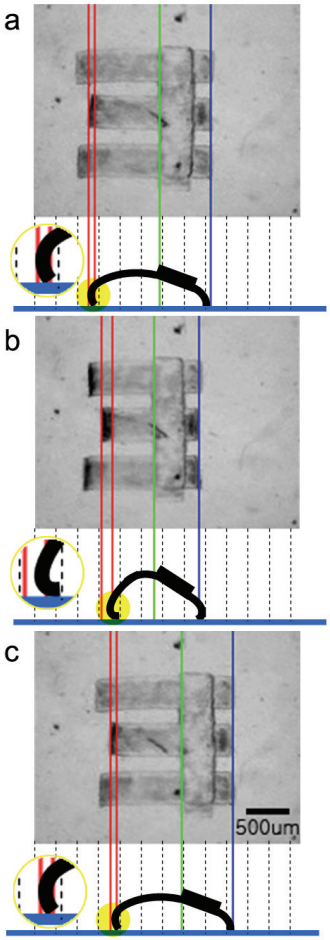


Fig. 7. Still images showing the sequential movement of the microrobot during one step.

B. Experimental Setup and Results

The motion of the microrobot was measured using an inverted microscope (Olympus IX 81, Olympus) and recorded using a digital camcorder (DCR-PC350, Sony Corp., Japan). The recordings were transferred into a digital movie file at the rate of 30 fps which enabled analysis of the walking motion of the microrobot.

After about 72 to 96 hours of culture, cardiomyocytes

began to beat in a synchronous manner and all legs show vertical displacement from the contractile force of cardiomyocytes. Finally, the microrobot activated by the contractile force of cardiomyocytes shows movement, as shown in Fig. 7. Figure 7 shows still images of the sequential movement of the microrobot during one step. The proposed microrobot has 3 front legs and 3 rear legs with different lengths. From the contractile force of the cardiomyocytes cultured on the microrobot, the deflection of the longer rear legs is much larger than that of the front legs. At the end of the legs in contact with the substrate, the asymmetric design of the microrobot produces a difference in the surface area of contact and thus causes an unbalanced frictional force between the legs and the substrate. Therefore, the microrobot moves in the direction of the shorter front legs.

The average beating frequency was 1.3 Hz and its average step stroke was 100 μm, thus the calculated speed was about 130 μm/s. Also, the average speed of the biohybrid micromachine was measured to be about 125 μm/s.

C. Discussion

Our microrobot was a self-assembled hybrid consisting of biotic muscle cells and a PDMS backbone—a well-known biocompatible material. The surface of the PDMS backbone was engineered via a 3D grooved pattern that led to high-order cell concentrations and enabled a high generative force from the muscle cells. The PDMS was easily fabricated by using a micromolding procedure.

As a potential application, the microrobot developed here may work for certain period of time in smaller lumen or vessel and probably removing various types of blockages accumulated in the ducts with dissolving agent in the microrobot.

IV. Dome-shaped Micropump

A. Fabrication of Micropump

The detailed fabrication process appears in our previous work [15], [17]. Briefly, a Si_3N_4 layer on a silicon substrate was deposited by LPCVD, and a micro-well was formed with the Si_3N_4 thin film remaining at the top by anisotropic backside etching. The thin PDMS was spin-coated on top of the Si_3N_4. After removing the supporting Si_3N_4 layer with RIE, the backside of the membrane was coated with Cr/Au to form the dome shaped membrane. This Cr/Au layers was also used as a seed layer on which cells adhere via self assembled monolayers (SAMs) treatment. The PDMS microchannel, which has a diffuser/nozzle pump shape, was fabricated by molding after photolithography with a negative photo resist. PDMS was poured over the fabricated master. The PDMS was cured in an oven at 90 °C for 2 hours and peeled off from the master. The top PDMS channel and the bottom silicon structures were aligned under an optical microscopy and permanently bonded after oxygen plasma treatment. The fabricated micropump is shown in Fig. 8.

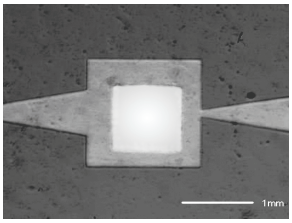


Fig. 8. Fabrication Results of Micropump

B. Preparation of Micropump

The fabricated micropump was immersed in ethanolic alkanethiol solution (1 mM in absolute ethanol) for 12 hours, and a SAM (Self Assembly Monolayer) was formed on the gold surface. Before cell seeding, the device was rinsed in ethanol, and dried with N_2. The surface was then coated with 0.001% of fibronectin (Sigma Chemical) and 0.02 % of gelatin mixture (Becton Dickinson, MA, USA) for cell adhesion.

C. Experimental Setup and Results

To visualize the motion of microflow in the micropump, we dispersed 2 μm spherical polystyrene particles (Polybead® Polystyrene Microspheres, Polysciences, Inc., USA) in the PDMS microchannel through microsyringe. After injecting polystyrene particles, we kept the pump staying at an incubator until particles were not influenced by the initial injection. Then, the motion of particles was monitored by a microscope (LSM5 PASCAL, Carl Zeiss, Germany) and captured by digital camcorder (DCR-PC350, Sony Corp., Japan) as shown in Fig. 9. The captured movies were transferred into digital movie files, and then we calculated the velocity of the particles using sequential video frames. Cardiomyocytes were well-spread growth on the dome shaped polymer membrane and the membrane has up and down motion by the contraction of the cardiomyocytes.

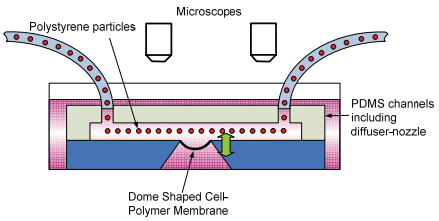


Fig. 9. Experimental Setup for Micropump

Fig. 10 shows a still image of polystyrene beads in the micropump. The flow rate of the micropump was monitored by tracking the polystyrene beads. As the cell-polymer membrane pulsated, the beads moved back and forth in the

microchannel. The net flow rate was formed in the direction intended by the diffuser-nozzle configuration. The net flow rate of our device was approximately 0.226 nl/min which was estimated by tracking the motion of the microbeads and considering the shape and the size of the microchannel. The average speed of the beads was 3.7815 μm/sec, and the size of the channel cross section was 1000 μm^2 at the point of observation.

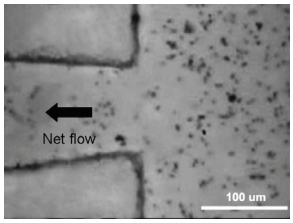


Fig. 10. Micropumping Result

D. Discussion

We presented a hybrid micropump driven by the self-beating of cardiomyoctes. The micropump was fabricated with cost-effective and noninvansive micro fabrication techniques. The performance was evaluated through the real-time measurement of the motion of polystyrene beads in the flow. The proposed device does not require any external power source and the pumped solution is not exposed to an electrical or a heat shock. Due to these advantages, this approach will find applications in various microfluidic systems such as point of care diagnostics and portable platform, especially in the area of in-vivo systems.

V. CONCLUSION

This paper demonstrated some applications of biohybrid microsystems actuated by cardiomyocytes: microcantilever, microrobot and micropump. Firstly, the biohybrid microcantilever was bended by the contractile force of the cardiomyocytes on it and could be used as the measuring tools for the contractile force of the cardiomyocytes. Secondly, the bending motion of the microcantilever could be applied as a biohybrid actuator of the proposed crab-like microrobot. The microrobot has several legs and the legs showed the bending motions of the microcantilever and the synchronized bending motion of the legs made the walking motion of the crab-like microrobot. Finally, the dome-shaped micropump actuated by the cardiomyocytes was also proposed and fabricated and the performance of the micropump is demonstrated.

ACKNOWLEDGMENT

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