Contractile Force Measurements of Cardiac Myocytes Using a Micro-manipulation System

Sukho Park*, **Seokkyu Ryu**, **Seokchang Ryu**, **Deokho Kim**
Microsystem Research Center, Korea Institute of Science and Technology, Seoul, Korea
Byungkyu Kim
School of Aerospace & Mechanical Engineering, Hankuk Aviation University, Kyonggi-do, Korea

In order to develop a cell based robot, we present a micro-mechanical force measurement system for the biological muscle actuators, which utilize glucose as a power source. The proposed measurement system is composed of a micro-manipulator, a force transducer with a glass probe, a signal processor, an inverted microscope and video recording system. Using this measurement system, the contractile force and frequency of the cardiac myocytes were measured in real time and the magnitudes of the contractile force of each cardiac myocyte under different conditions were compared. From the quantitative experimental results, we could estimate that the force of cardiac myocytes is about 20~40 μN, and show that there are differences between the control cells and the micro-patterned cells.

Key Words : Cardiac Myocytes, Cell Force Measurement, Micro-Manipulation, Piezo Resistive Sensor

1. Introduction

Over the last ten years, novel concepts of the microrobot (or nanorobot) have been introduced (http://www.foresight.org ; Phee et al., 2002 ; Jung et al., 2003). Because of the size limitation of the robot with bio-mimetic actuation, however, the selection of the proper actuator and the realization of the robot are very difficult. In general, the electrostatic, electromagnetic, pneumatic, piezoelectric and thermal forces are mostly used as a micro actuator. However, these actuators need an external power source and have some limitations in the application to human body, such as locomotion in human digestive organ or blood vessels.

As an alternative, cell based actuators have been proposed and the miniature robot has been also introduced by Xi et al. (2004). The cell based actuator is fuelled by a simple glucose nutrient in physiological fluids as an energy source and transforms the chemical energy into mechanical energy. In the presented micro-robots (Xi et al., 2004), as a structural backbone, an arch of silicon 50 μm wide is used and a cord of rat cardiac muscle fibers has been grown. Contraction and relaxation of the cardiac muscle makes the arch bend and stretch to produce a crawling motion.

For the design of the micro robot powered by living cardiac muscle, the estimation of the contractile force is very important. From the measured contraction force, the shear force generated by living and cultured cells is estimated and thus is utilized in the design of the microrobot backbone. By using the measured force, the motion of the microrobot structure can be simulated and

* Corresponding Author,
E-mail : shpark@kist.re.kr
TEL : +82-2-958-5780; **FAX** : +82-2-958-6910
Microsystem Research Center, Korea Institute of Science and Technology, Seoul, Korea. (Manuscript **Received** December 13, 2005; **Revised** February 28, 2006)

thus the microrobot can be re-designed. The measurement methods of the cell's contractile force are as follows.

First of all, van der Velden et al.(1998) executed a feasibility test of isometric force development in single cardiac myocytes from human ventricular muscle tissue obtained from small biopsies taken during open heart surgery. The authors proposed a measurement method for the force of isolated cardiac myocytes with silicon glue to a sensitive force transducer and a piezoelectric motor. It was reported that the average isometric force at saturating calcium concentration obtained on 20 myocytes is about 51 kN/m^2 and mechanical properties of myocytes are correlated with the protein composition.

Yin et al.(2004) presented a new method of a single cardiac myocyte contractile force measurement using a magnetic bead. A magnetic bead is attached on one end of the myocyte and adjusted magnetic field is applied on the magnetic bead. Using an inverted microscope with edge detection, the myocyte contractile force can be derived by measuring of the maximal displacement of cell contraction and the magnetic field loading force on the bead. From this report, the estimated contraction force is about 10 μN.

Lin et al.(2001) used microelectromechanical system (MEMS) technology for cell force measurement. By using MEMS processes, strain gauge is attached to one end of two beams fabricated with a clamper and a hinge at each end. The heart cell ends are attached to the transducer beams using clamps. Thus, when the cell is contracted, the strain is activated. The average measured maximal force is about 5.77 μN.

Balaba et al.(2001) and Zaho et al.(2005) proposed micro patterned elastic substrates for cardiac myocyte force measurements in real time and in living cells. The elastic micro posts are bent and the displacements of the posts are transformed to cell forces which can be calculated by a simple beam theory. The cell force is estimated about 600 nN from this method.

Finally, Wojcikiewicz et al.(2004) and van Vilet et al.(2003) proposed cell force measurements on living cells using atomic force microscopy (AFM) and other methods. Although higher resolution can be achieved using AFM, a complex transmit-receive setup is required and the accuracy of the cellular force measurement may be sacrificed considerably due to the reflection and refraction of the transmitted light in aqueous medium (Yu and Nelson, 2004).

In general, we need to measure the contractile force of cardiac myocytes not only in control but also in micro patterned cell, which is used in microrobot's structure. However, the above mentioned methods cannot directly apply to control cell and micro patterned cell. Therefore, we propose to use a micro-manipulator for contractile force measurements of cardiac myocytes. By a micro manipulator, the probe tip of a force transducer can approach to the cells under different conditions. The measurements are carried out in real time and the video images are captured. The experimental results show not only the magnitude of the cell force but also the frequency of the cell movement. In addition, this study makes it possible to evaluate the performance of such cell based actuators with regard to force and displacement by controlling beating activity of patterned cardiac myocytes (Park et al., 2005). The micro-patterned cardiac myocytes unit can be also applied as a flexible platform for drug screening, cell-based sensors, and self-organizing cell motors.

2. Cell Force Measurement System

Figures 1 and 2 illustrate the overall schematic diagram and photograph of the force measurement system. The system consists of a micro-manipulator, a force transducer integrated with a glass probe, an inverted microscope, a signal amplifier, a force data acquisition system, and a video image recording system. When the end of glass probe approaches a cardiac cell, the beating force is transferred to the probe and is sensed by the piezo-resistive force transducer.

The proposed micro-manipulation system consists of a micro-manipulator (Model : MM3A of Nanotechnik (http ://www.nanotechnik.com)) controlled by its own controller and two micro stages operated manually. For the cell force measure-

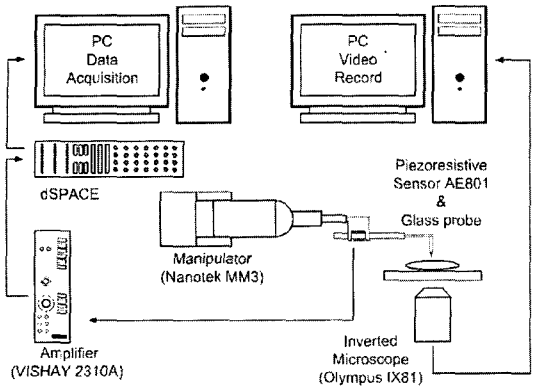


Fig. 1 Schematic diagram of cell force measurement system

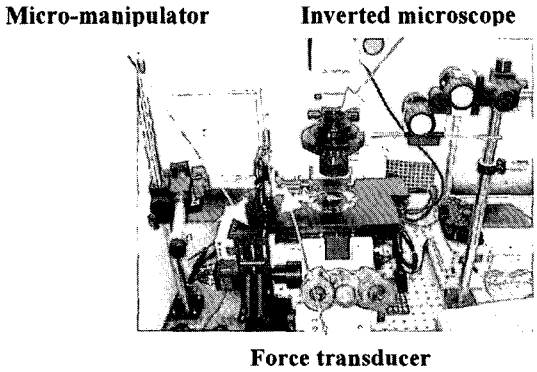


Fig. 2 Photograph of our cell force measurement system

ment, a piezo-resistive type sensor (Model AE801 of SensorOne (http://www.sensorone.com)) with a glass probe is used. Calibration was preformed with a precision load cell (Model: GSO-10 of Transducer techniques Inc., Max. measurement range: 100 mN, resolution: 10 μN (http://www.transducertechniques.com)), yielding the calibration factor of about 841 μN/V and a good linearity.

The force signal from the transducer is amplified by the signal amp (Model: 2301A of VISHAY (http://www.vishay.com)) and the amplified force signal is A/D converted and recorded by dSPACE data acquisition system (http://www.dspaceinc.com). The ratio of amplification in the signal amp is about 10000, and the A/D converter of dSPACE has 16bit resolution and ± 10 voltage range. And thus, the resolution of the force measurement system is about 0.257 μN. The reported values of the contractile force of the cardiac cell is about $10 \sim 20$ μN. Therefore, the resolution of the force measurement system is sufficiently small.

3. Experimental Procedures and Results

3.1 Cell preparation

Primary cardiac myocytes were isolated from postnatal day 1 Sprague-Dawley rat and cultured in Dulbecco's modified Eagles' medium (DMEM) (Gibco Invitrogen Co., Grand Island, NY, USA (http://www.invitrogen.com)) supplemented with 10% Fetal Bovine Serum (Gibco Invitrogen) at 37℃ in 5% CO_2. The isolated cardiac myocytes were directly seeded on the surface at a concentration of 1×10^5 cells/cm^2 and maintained for 7 days.

3.2 Experimental procedure

The procedures for the cell force measurement are as follows:

(1) First of all, focus the inverted microscope on the end of the glass probe and place the tip at the center of the frame using the micro-manipulator.

(2) While adjusting the focus on the control dish of the cardiac cell, choose the target cell to measure the contractile force and place the target cell at the same place as the tip using the microscope stage. At this time, the tip is out of focus.

(3) After adjusting the focus of microscope up and down to find a new plane in the sample, make the tip close to the target cell using the micro-manipulator.

(4) When the tip is adjacent to the target cardiac cells, the contractile force signals are generated in the force transducer. It is possible to make the tip touch the target cell repeating the second and third step in order.

To get the force signal from the cell, it is very important that the glass probe is aligned with the contraction direction of the target cell. If the glass probe is not aligned with the contractile direction, the contractile force is not transferred to the force

transducer. In order for the probe to approach to the myocyte, the glass probe is kinematically inclined and aligned with the contractile direction of the target cell. The effect of kinematical tilting can be calibrated from the measuring force signal.

3.3 Cell patterning into microwells

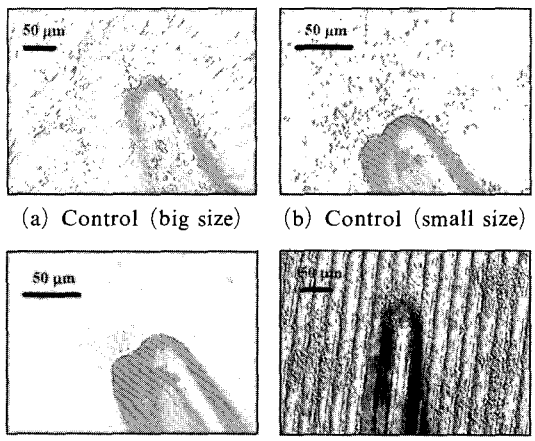
We developed a simple method for patterning cardiac myocytes confined within microwells that contain a collagen-coated surface and a poly (ethylene glycol) (PEG) copolymer barrier. Capillary lithography (Khademhosseini et al., 2003) was used to coat the PEG copolymer on glass substrate and to generate micro arrays for patterning cardiac myocytes. A few drops of 10% (w/v) PEG copolymer [poly (TMSMA-r-PEGMA)] (Jon et al., 2003) solution in ethanol were placed on a glass substrate. A thin film of the PEG polymer was achieved by spin coating (Model CB 15, Headway Research, Inc., USA (http://www.headway-research.com)) at 1000 rpm for 10 s. To make conformal contact, patterned PDMS molds were carefully placed onto the surface and then the molds were peeled off using a sharp tweezers after complete evaporation of the solvent for 30 min at room temperature. Prior to cell plating, collagen (Type I, Sigma Chemical Co., St. Louis, MO, USA (http://www.sigmaaldrich.com)) was coated for 3 hours within the microwells on the PEG layer prepared on the glass substrate to enhance cell attachment to and survival in the microwell patterns.

In these steps, collagen was selectively absorbed on the exposed glass substrate to produce pre-patterned microwells. Cardiac myocytes suspended in medium were then plated onto the surfaces and the cell cultures were analyzed at intervals. After 20h, non-adhered cells present on the PEG layer were washed away with Hank's balanced salt solution (HBSS, pH 7.4) (Gibco Invitrogen). Finally, cells were selectively attached on collagen-coated microwell surfaces due to an adhesion- resistant PEG surface.

3.4 Experimental results

The experiments are preformed in four conditions: (a) big size control myocytes, (b) small size control myocytes, (c) microwell-patterned myocytes, and (d) microgroove-patterned myocytes. First of all, among control cardiac myocytes groups, we choose the largest beating displacement group. In this group, many cardiac myocytes are aggregated and have a vivid beating feature. Then, the small size myocytes group is selected and its contractile force is also measured. By the proposed cell patterning method, the force measurements of microwell-patterned cardiac myocytes are performed. Finally, in the microgroove-patterned myocytes, 15 μm groove pattern with about 3~4 μm height is used.

Figures 3 shows that the glass tip is adjacent to the target cardiac myocytes. With the inverted microscope, we observe that each myocyte become synchronous and beat simultaneously. The contractile force signals of the cardiac myocytes are shown in Fig. 4 and the statistics and the comparisons of the force magnitudes are presented in Fig. 5 and Table 1. First of all, (a) big size control myocytes have a contractile force about 35~40 μN and a beating frequency about 28~30 pulses/min. In (b) small size control myocyte, a contractile force is about 15 μN and a beating frequency is about 40~42 pulses/min. Because the big size control myocytes are three dimensionally aggregated and strongly synchronized, the higher contractile force can be measured. The microwell-patterned myocytes show a contractile

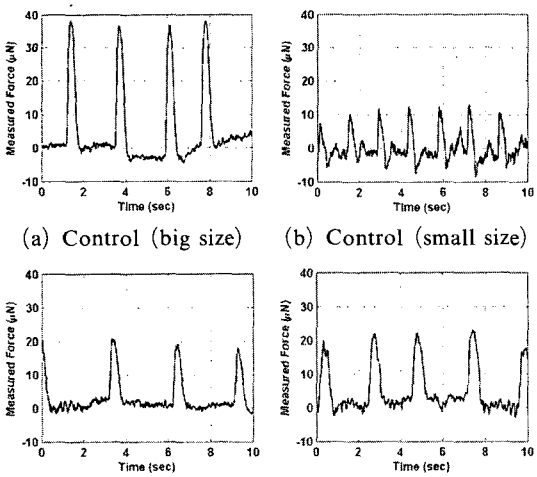


(a) Control (big size) (b) Control (small size)

(c) Microwell-patterned (d) Microgroove-patterned

Fig. 3 Photograph of cell contractile force measurement

force about 20 μN and a beating frequency is about 19~20 pulses/min. Assuming that the myocytes in Fig. 4(c) are covered with a half of the well area, the force per unit area can be estimated as about 9.05 kN/m^2, where the diameter of the micro well is 75 μm. Finally, the microgroove-patterned myocytes in Fig. 4(d) has a contractile force about 20~23 μN and a beating frequency about 25~30 pulses/min.



(a) Control (big size) (b) Control (small size)

(c) Microwell-patterned (d) Microgroove-patterned

Fig. 4 Contractile force signals of cardiac myocytes

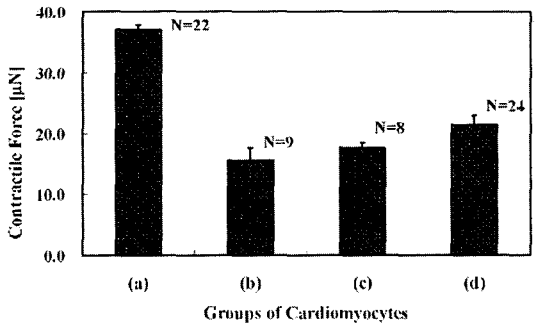


Fig. 5 Results of cell contractile force measurement (a) control (big size), (b) control (small size), (c) microwell-patterned, and (d) microgroove-patterned

Table 1 Statistics of cell contractile force measurement

	Average (μN)	Standard Deviation (μN)
(a) control (big size)	36.94	0.85
(b) control (small size)	15.47	2.21
(c) microwell-patterned	17.58	0.88
(d) microgroove-patterned	21.47	1.56

Our quantitative experimental results indicate that the contractile force of cardiac cells is about 20-40 μN, and the control cells and the micro-patterned cells exhibit differences in the magnitude of the contractile force. The differences are caused by the cell number and the three dimensional aggregation of the cardiac myocytes.

3.5 Immunostaining

Cardiomyocytes on the microgroove were briefly washed with phosphate buffered saline (PBS, pH 7.4) and then fixed in 4% (w/v) paraformaldehyde (Sigma) for 30 min. To analyze distribution of α-sarcomeric actins of cardiomyocytes, the cells were extracted with 0.2% Triton X-100 in PBS, and then incubated with anti-α-sarcomeric actin antibody (Sigma), followed by goat anti-mouse antibody conjugated to fluorescein isothiocyanate (FITC, Sigma). The cells were analyzed under a Zeiss Axoplan Fluorescence Microscope (Zeiss, Germany (http://www.zeiss.com)), and their images were recorded by a Nikon digital camera (Nikon Co, Japan (http://www.nikon.com)).

Immunostaining was used to evaluate the pro-

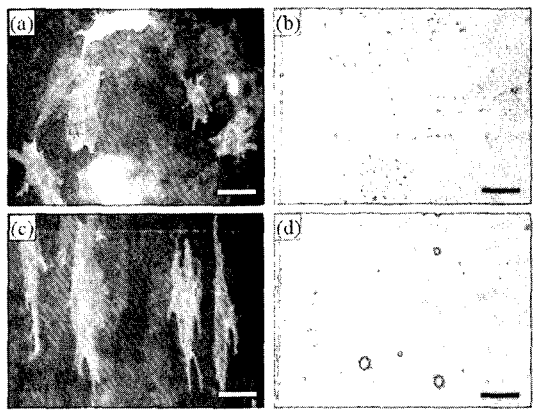


Fig. 6 Immunostaining result of cardiomyocytes cultured on the control (a, b) and the patterned microgroove (c, d) along with the corresponding bright field image (scale bar in all figures is 30 μm)

perties and organization of the adhered cardiomyocytes on the patterned microgroove. Figure 6 suggests that the microgroove induced the directed arrangement of cytoskeletal bundles which are parallel to the underlying microgroove. The polarity of the adhered cardiomyocytes is responsible for the directional movement of aggregated muscle cells. It would potentially contribute to the actuation force in a desirable direction for muscle-powered microrobot.

3.6 Discussion

In these experiments, we measure the contractile force under two different conditions, namely the control and the micropatterned cells. The results show the magnitude of the cell force measured in real time and can be used for the dynamic analysis of the cell mobility. In the view of the quantitative analysis, we believe that the microwell-patterned myocytes can be used as a proper tool for the quantification of the contractile force of cardiac myocytes. This is why the number of cells in the microwell has small variations compared with a control myocytes group. In addition, when the micro patterns were not a well type but a line groove type, the cardiac myocytes are aligned along the longitudinal direction. On the other hand, control myocytes randomly spread and have the various contractile directions. These aligned myocytes by the groove micro pattern contract with the longitudinal direction of the pattern. Therefore, these aligned myocytes will be applied to the design of the cell based microrobot. The contractile forces of the aligned cells will be accumulated with the longitudinal direction and thus it will be expected that the microrobot which is powered by the aligned cardiac myocytes can have large deformation and actuation forces.

4. Conclusions

We proposed the micro-manipulation system for the contractile force measurements of cardiac myocytes. The force measurements system consists of a micro-manipulator, a force transducer, a glass probe, an inverted microscope, a signal amplifier, a force data acquisition system, and a video image recording system. By using this measuring system, the contractile force for control cardiac cells and micro-patterned cardiac myocytes can be measured in real time. From the experimental results, we can observe that the contractile force of cardiac cells is about 20-40 μN and there are differences between the control cells and the micro-patterned cells. Especially, the aligned cardiac myocytes by the grooved micro pattern have the contractile force which is accumulated with the longitudinal direction and can be applied to the microrobot. Finally, the engineered cell motor using patterned cardiac myocytes will be fabricated and evaluated, which does not depend on any external power supply such as electricity or magnetic forces but biochemical activation such as calcium ion stimulus or calcium ion blocker.

Acknowledgments

This research has been supported by the Intelligent Microsystem Center (IMC; http://www.microsystem.re.kr), which carries out one of the 21st century's Frontier R&D Projects sponsored by the Korea Ministry Of Commerce, Industry and Energy.

References

Balaban, N. et al., 2001, "Force and Focal Adhesion Assembly: a Close Relationship Studied Using Elastic Micro Patterned Substrates," *Nature Cell Biology*, Vol. 3, pp. 466~472.

Jon, S., Seong, J., Khademhosseini, A., Tran, T. T., Laibinis, P. and Langer, R., 2003, "Construction of Nonbiofouling Surfaces by Polymeric Self-Assembled Monolayers," *Langmuir*, Vol. 19, pp. 9989~9993.

Jung, J., Kim, B., Tak, Y. and Park, J., 2003, "Undulatory Tadpole Robot (TadRob) Using Ionic Polymer Metal Composite (IPMC) Actuator," *Int. Conf. Intelligent Robots and Systems*, Vol. 3, pp. 2133~2138.

Jungyul Park, et al., 2005, "Real-Time Measurement of the Contractile Forces of Self-Organized Cardiomyocytes on Hybrid Biopolymer Microcantilevers," *Analytical Chemistry*, Vol. 10,

No. 5, pp. 601~606.

Khademhosseini, A., Jon, S., Suh, K. Y., Tran, T. T., Eng, G., Yeh, J., Seong, J. and Langer, R., 2003, "Direct Patterning of Protein- and Cell-Resistant Polymeric Monolayers and Microstructures," *Advanced Materials*, Vol. 15, pp. 1995~2000.

Lin, G., Palmer, E., Pister, K. and Roos, K., 2001, "Miniature Heart Cell Force Transducer System Implemented in MEMS Technology," *IEEE Trans. Biomedical Engineering*, Vol. 48, No. 9, pp. 996~1006

Phee, L., Accoto, D., Menciassi, A., Stefanini, C., Carrozza, M. C. and Dario, P., 2002, "Analysis and Development of Locomotion Devices for the Gastrointestinal Tract," *IEEE Trans. Biomedical Engineering*, Vol. 49, No. 6, pp. 613~616.

Wojcikiewicz, E., Zhang, X. and Moy, V., 2004, "Force and Compliance Measurements on Living Cells Using Atomic Force Microscopy (AFM)," *Biological Procedures Online*, Vol. 6, No. 1, pp. 1~9.

Xi, J., Schmidt, J. and Montemagno, C., 2004, "First Self-Assembled Micro-Robots Powered by Muscle," SPIE Nanotechnology e-bulletin, 6.

Yin, S., Zhang, X., Zhan, C., Wu, J. and Cheung, J., 2004, "Measuring Single Cardiac Myocyte Contractile Force Via Moving a Magnetic Bead," *Biophys J. BioFAST*.

Yu Sun and Bradley J. Nelson, 2004, "MEMS for Cellular Force Measurements and Molecular Detection," *International Journal of Information Acquisition*, Vol. 1, No. 1, pp. 23~32.

Zhao, Y., Yu, H. and Zhang, X., 2005, "Creating Polymer-based Microstructures with Various Aspect Ratios from a Single Template for Cellular Force Measurements," *Proceeding of the 18th IEEE International Conference on Micro Electro Mechanical Systems (MEMS '05)*, Miami Beach, FL, USA, January 30-February 3, in press.

http://www.foresight.org, Foresight Institute, USA.

http://www.nanotechnik.com

http://www.sensorone.com

http://www.transducertechniques.com

http://www.vishay.com

http://www.dspaceinc.com

http://www.invitrogen.com

http://www.headwayresearch.com

http://www.sigmaaldrich.com

http://www.zeiss.com

http://www.nikon.com

van Vilet, J., Bao, G. and Suresh, S., 2003, "The Biomechanics Toolbox; Experimental Approaches for Living Cells and Biomolecules," *Acta Materialia*, 51, pp. 5881~5905.

van der Velden, J. et al., 1998, "Force Production in Mechanically Isolated Cardiac Myocytes from Human Ventricular Muscle Tissue," *Cardiovascular Research*, 38, pp. 414~423.