바닥면상태에 따른 심장세포의 수축력 변화

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The dependence of contractile force for the cardiomyocytes on a different engineered surface

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Abstract

We present a microfabricated three-dimensional (3-D) hybrid biopolymer micro cantilever which can measure the contractile force of cardiomyocytes and analyze the force dependence on different types of surface. To make different conditions of the cell seeding surface, we fabricated the specific type of cantilever which was grooved on the surface. The presented cantilever was facilitated to measure bending of the cantilever and to calculate the contractile force of cardiomyocytes. Also, we demonstrate the dependence of the morphology for the cardiomyocytes seeded on a plain surface and a grooved surface. Finally, we show the dependence of force for the cardiomyocytes on a different surface.

I. INTRODUCTION

A heart attack is the most dreaded heart disease. And chronic heart attacks, even slow-going, are the most common and fatal. Heart muscles weaken slowly and its cells simply can't contract. In this case, each heart muscle cell has longer pulsation and loses its force. Therefore, the information on contractile force of the heart cell is expected to play an important role in finding out the basic solution of the remedy and substitution of the cells with heart disease.[1]

Although force measurements at the cellular tissue and whole organ levels have been performed (Kawai et al., 1993; Roos, 1997),[2,3] influence from the outside factor was not entirely excluded and the force of heart cells was not measured exactly. Therefore, there have been several approaches proposed and methods developed to measure the contractile force of cardiomyocytes either in direct or indirect ways.

In the first approach, a force transducer was made using micro fabrication technology to reduce the size of the device for measuring the contractile force of cardiomyocytes.[4] In this system, two micro clamps hold each end of a cardiomyocytes cell to measure the contractile force of the cell. However, this approach involves cell manipulations which may have unknown effects on the cells and their functions.

In the second approach, an array of micro-scale elastic posts was developed. The attached cells bend each post independently because the forces of cell for adhesion were used locally. Poly-(dimethylsiloxane) (PDMS) pillar arrays were used as elastic posts.[5] However, contact between the cell and

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the heads of pillars may have some effects on cell membranes and cardiomyocytes functions.

In this paper, we tried to measure the difference of the force of cardiomyocytes cells due to the surface change of cantilevers. We used a PDMS as a material of biopolymer cantilever. Over previous contractile force measurement techniques, the important feature in our system is that our system can quantitatively measure contractile forces on a specific area in real time. This can give opportunities for better understanding of the mechanisms of heart failure and develop further design of optimal micro scale hybrid biopolymer actuators and microdevices.

II. MATERIALS AND METHODS

A. *Fabrication of a Microcantiler*

To make the hybrid biopolymer microcantilever, we used a sandwich molding process. Briefly, a master was made on a silicon wafer using a thick negative photoresist (PR) (KMPR-1050, Micro-Chem). After the PR mold master process, the whole wafer was rinsed immediately with isopropyl alcohol to clean residue from the wafer. Before the PDMS mixture was poured onto the fabricated master, the master was silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane (Sigma Chemical Co., St. Louis, MO) for easier detaching of the PDMS after curing.

The PDMS mixture was poured onto the master, and then a rubber film was placed on the PDMS mixture. After an extra wafer had been placed on the top of the rubber film to generate an even PDMS surface, the stack comprising the fabricated master, PDMS, a rubber sheet and an extra wafer were placed between two Al plates and clamped as shown in Figure 1.

B. *Surface Treatment*

The initial PDMS surface is hydrophobic. It prevents adhesion of bio molecules and cells. Therefore, reactant ion etcher (RIE) treatment was applied to increase adhesion forces between the PDMS surface, extracellular matrix, and cardiac cells. The PDMS surface can be changed to hydrophilic by exposure to oxide plasma in an RIE. Then, the plasma-treated PDMS surface was coated with a 0.5% fibronectin (Sigma Chemical Co).

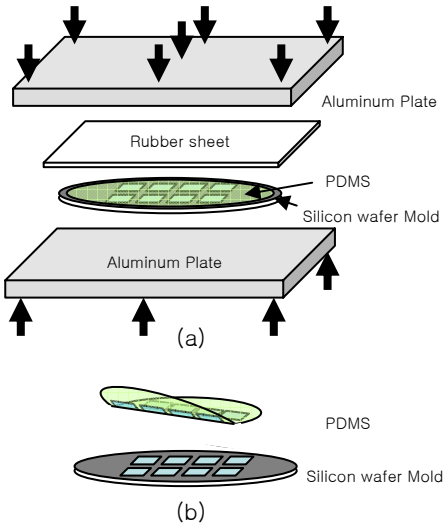


Fig. 1: Schematic diagram of the fabrication processes for hybrid biopolymer microcantilever. (a) A sandwich molding process for 3-D structure made of PDMS (b) Detaching PDMS molded structure from Si wafer mold

C. *Cell Culture*

A heart was aseptically isolated from a neonatal Sprague-Dawley rat at day 1 and briefly washed with Hank's balanced salt solution (Gibco Invitrogen Co., Grand Island, NY). After removing of the ventricles, the remaining tissues were minced and incubated in a 0.3 mg/mL collagenase solution containing 0.6 mg/mL pancretin (Sigma Chemical Co.). Isolated cardiomyocytes were directly seeded on the hybrid biopolymer cantilever at a cell density of 5x103 cells/mm2 and cultured in Dulbecco's modified Eagles' medium (Gibco Invitrogen) containing 10% fetal bovine serum (Sigma), 50 íg/mL streptomycin, and 50 íg/mL penicillin (Gibco Invitrogen) at 37 °C in 5% CO2 in air. The medium was changed at 48-h intervals in order to maintain continuous beating.

III. RUSULTS

A. *Fabrication Results of the Hybrid Biopolymer Cantilever*

The hybrid biopolymer microcantilever array consisted of five different sizes of microcantilevers that were 50, 100, 150, 200, and 300μm wide and, respectively, five times longer with each width. However, all cantilevers were 20μm thick. It was found that cantilevers longer than 1 mm were frequently stuck onto the substrate or bent, probably due to both the flexibility of the PDMS microcantilever and the cell mass formed by the

self organizing cells. Therefore, we eliminated all data obtained from cantilevers longer than 1 mm.

B. *Simulation of cantilever stucture*

As the difference occurred in thickness in a grooved cantilever unlike a surface flat cantilever as shown in Fig.2, we verified the structural deflection change ratio on the same force before culturing the cardiomyocytes cell.

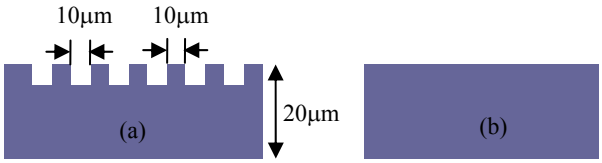
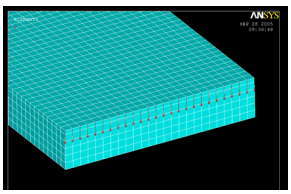


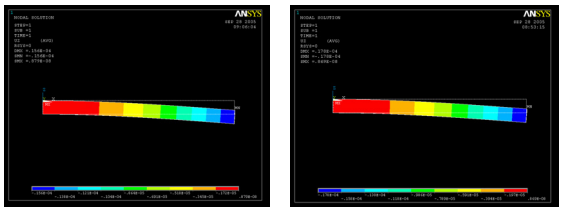
Fig. 2: Cross section of molded PDMS cantilever structure (a) Microgrooved cantilever (b) Plain cantilever.

Table 1. Comparison between grooved and plane cantilever

ITEM		Grooved	Plane	Remarks
Structure	Length	700 μm	700 μm	
	Width	250 μm	250 μm	
	Thickness	50 μm	50 μm	
	Groove Depth	5 μm	-	
Force	At the end of cantilever	$260x10^{-9}$	$260x10^{-9}$	(Pressure Type)
Result	Deflection	17.8 %	15.6 %	Grooved 14.1% up



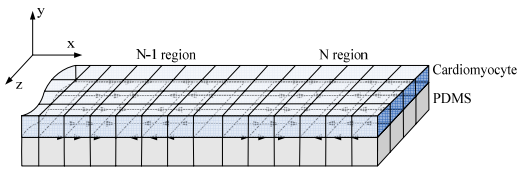
(a)



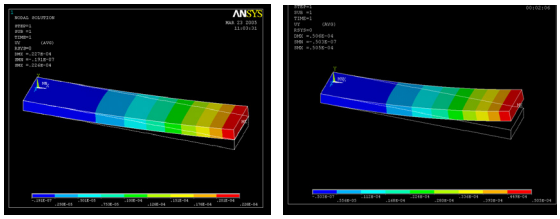
(b) (c)

Fig. 3: Comparison between two types of cantilever due to the point pressure at the end of the cantilever. (a)Applied point pressure at the end of the cantilever (b) on surface grooved cantilever (c) on surface flat cantilever

A grooved cantilever was expected to bend much more than a surface flat cantilever in the small force structurally as shown in Fig.3. As we expected, from the result of the simulation, we were able to confirm that a grooved cantilever could bend as much as 14% more than a surface flat cantilever as shown in Table 1 and Fig.3. This result shows the deflection difference of two cantilevers with the same dimension, one with a grooved surface, and the other, a flat surface, in the same force.



(a)



(b) (c)

Fig. 4: Simulation of the PDMS cantilever using measured contraction force of cardiomyocytes: Schematic of the distribution of contractile force (a) on plain cantilever, Bending motion (b) on plain cantilever (c) on microgrooved cantilever

C. *Simulation of cardiomyocytes cell*

We applied the gradient force distributions, varied the size of arrows in Figure 4(a), on each region in the intersectional area of cardiomyocytes and PDMS, which represents the continuously varied cellular force in a single cardiomyocytes cell to verify the contractile force of the cardiomyocytes cell. We assumed the contractile force of cardiomyocytes on micro-grooved surfaces were larger than on plain surfaces. The reason why the force can be merged in one direction is the aligned cardiomyocytes through the micro-grooves. Figure 4(b),(c) shows the bending motion of microcantilever by the contractile force of cardiomyocytes on the flat surface and micro-grooved surface.

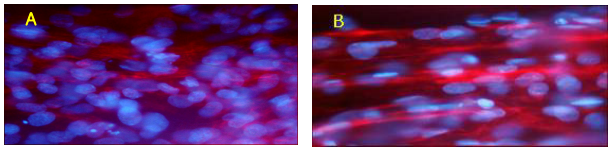
D. *Simulation of contractile force*

From the simulation, we noticed the structural difference between a surface grooved cantilever and a surface flat cantilever. Further, we analyzed the transformational difference of them due to the contractile force of the cardiomyocytes cell on the surface. The transformation of the cantilever by cardiomyocytes cells aligned by the surface groove was about 71%, which exceeded 14% of the structural transformation difference in Table2.

Table 2. Comparison of analysis due to the contractile force of cardiomyocytes cell

ITEM		Grooved	Flat	Remarks
Deflection		251 μm	146μm	Grooved 71% up
Force	Fx	-0.358×10^{-14}	-0.617×10^{-15}	
	Fy	0.902×10^{-15}	0.469×10^{-15}	
	Fz	0.809×10^{-15}	0.469×10^{-15}	

The cardiomyocytes cell is influenced by the structure of a cantilever on a flat surface cantilever and aligned to the lengthwise direction which is somewhat in the same direction, and it does not show the perfect radiative morphology like the cardiomyocytes cell under control. This is the reason why the force of Fx, in the lengthwise direction of the surface flat cantilever is bigger than that of Fy and Fz as shown above in Table2. In a surface grooved cantilever, Fx is much larger than Fy and Fz. As shown in Fig.5, the arrangement of actin filament, which is in the charge of contractile and ground adhesion of cardiomyocytes cell, is in one direction, and the nucleus itself is transformed to a long oval shape. Therefore, we analyzed the contractile force in one direction using the suggested beating model of a cardiomyocytes cell.



(a) (b)

Fig. 5: Fluorescence image of nucleus (blue) and actin filament (red) of cardiomyocytes cell (a) on plain cantilever (c) on microgrooved cantilever

E. *Bio-Hybrid Cantilever Force Sensor*

We measured the force after seeding a cardiomyocytes cell both on a surface grooved cantilever and on a surface flat cantilever. As the result from the previous simulation, you can see the deflection difference between a surface grooved cantilever and a surface flat cantilever due to the structural transformation ratio difference in Fig.6. To maintain the same condition, we compared two types of cantilevers in the same seeding condition with the same dimension, and confirmed the occurrence of the deflection difference.

We confirmed the transformation due to the cardiomyocytes cell of a surface grooved cantilever and a surface flat cantilever expected from the simulation rose almost to 400%, which exceeded 71%. It seems to be caused not only by the influence of the ground adhesion force of a cardiomyocytes cell but also by the contraction of a cell aligned in the lengthwise direction of the Fx cantilever during the drying process after fixing a cell.

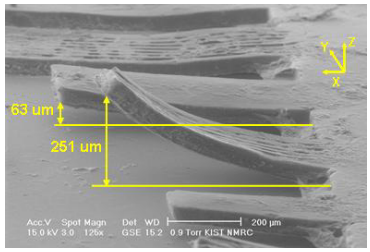


Fig. 6: Deflection difference between surface grooved cantilever and surface flat cantilever due to the cardiomyocytes cell

IV. CONCLUSIONS

We expected the contractile force of cardiomyocytes cell on microgrooved cantilevers to be larger than the contractile force of plain cantilevers because the morphology of cardiomyocytes cell on microgrooved surfaces shows aligned through the microgroove while cytoskeleton of cardiomyocytes has polygonal shape on flat surfaces. There was additional transformation ratio difference in the seeding of a cardiomyocytes cell than the structural transformation ratio difference between a grooved surface cantilever and a flat surface cantilever.

We assume the reason is that the cell aligned to the lengthwise direction of a grooved cantilever contracted because it had more displacement during the drying process. As a result, we confirmed that a cantilever with 14% of structural transformation ratio difference practically had almost 400% transformation ratio difference due to the seeding of a cell, different from our expectation of 71%.

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