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PDMS Cantilever Integrated with a Strain Sensor for Contraction Force Measurement of Cardiomyocyte

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ABSTRACT

This study focuses on the fabrication and characterization of a polydimethylsiloxane (PDMS) cantilever integrated with a piezoresistive strain sensor for real-time measurement of contraction forces of cardiomyocytes. A pattern of microgrooves with various sizes was formed on the surface of the PDMS cantilever to optimize the anisotropic alignment of cardiomyocytes. The contraction force of the anisotropically aligned cardiomyocytes was maximized with a PDMS cantilever in which microgroove arrays precisely dimensioned at a pitch of 2 μm. An increase in the contraction force enhanced the sensitivity of the integrated piezoresistive strain sensors. The cardiomyocytes incubated on the cantilever start to begin beat on day 3 and the contraction force of the cardiomyocytes was at the maximum on day 7 in which the PDMS cantilever deflection was approximately 600 μm. The PDMS cantilever integrated with a piezoresistive sensor is expected to be used in a high-speed drug toxicity screening appliction owing to its ability to detect real-time contraction forces of cardiomyocytes.

KEYWORDS: PDMS cantilever, Metal piezoresistive sensor, Contraction force, Cardiomyocytes

INTRODUCTION

The heart is a crucial organ in the human body; it supplies blood to tissues through the repetition of the systole and diastole cycle. Frequent abnormal pulses in the heart cause heart systole disorder and cardiac dysfunction. The causes of abnormal pulses in the heart include drug toxicity and arrhythmia. Analysis on the heart behavior in relation to drugs or blood ion concentration is made possible using a patch clamp, which is an electrophysiological method. The patch-clamp method is a technique in which a micropipette, whose tip diameter is 1 μm or less, is inserted into the surface of a cell membrane to measure minute ion currents, by which various mobility characteristics of the ions on the surface of cells can be evaluated [1]. However, this process suffers from disadvantages in that it requires a long time to align the micropipette and the cell ion channel, and the resolution can decrease depending on the material used in manufacturing the micropipette. Various methods have been attempted to solve these problems, and in particular, the micropost or cantilever method, which uses the mechanical characteristics of cardiomyocytes, have gained attention. The micropost array method utilizes mechanical deformation of the elastic column. This was generated by the contraction force of cardiomyocytes that grow at the top of the column. Image processing techniques and an optical microscope are often used to analyze the characteristics of the deformed micropost, and thus, the magnitude and direction of the force generated by the cardiomyocytes are measured [2]. However, this technique suffers from the drawbacks of low resolution due to the limitations of the optical microscope used to measure the deformations in the micropost and the difficulty in cultivating cardiomyocytes on an elastic column. The cantilever method measures the displacements caused by the contraction force of cardiomyocytes grown on the cantilever surface using an optical microscope [3]. Because the cantilever generates a greater displacement compared with the existing micropost, using the optical microscope becomes easy; however, it still suffers from a drawback because of its difficulty in measuring micro displacements of 1 μm or less. In particular, making real-time measurements in the parallel cantilever system is difficult for high throughput drug analyses.

In this paper, we propose a PDMS cantilever integrated with a piezoresistive sensor to measure the contraction force of cardiomyocytes without using a microscope. The piezoresistive sensor can measure

the cantilever displacement in real time using electrical signals. The sensitivity can be further increased by the use of a different material with a high gauge factor. In addition, the utilization of a parallel cantilever in which a displacement sensor is being integrated offers potential application in high-speed multiple-drug screening.

EXPERIMENTAL RESULTS

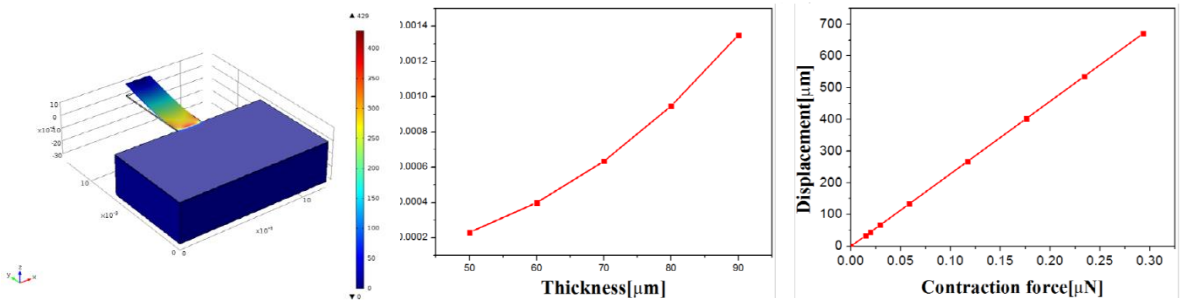

Figure 1. PDMS cantilever analysis using the COMSOL program.

Figure 1 shows the COMSOL analysis results of the PDMS cantilever design. The cantilever, which was designed based on the simulation results, had dimensions of 6,000 μm (l) × 2,000 μm (w) × 70 μm (h) and a spring constant of approximately 0.7 mN/m. Figure 2(a) shows the fabrication process of the PDMS cantilever with an integrated piezoresistive sensor for a high-throughput drug screening application. The details of the fabrication process of the PDMS cantilever are described as follows: (1) a 1-μm-thick photoresist (PR) layer was formed after AZ5214 was spin-coated (5,000 rpm, 40 s) onto a p-type silicon wafer. (2) Microgrooves with a 1-μm thick line and spaces were formed using a conventional photolithography process. (3) PDMS was spin-coated (1,200 rpm, 40 s) onto the microgroove pattern to form a cantilever layer and cured in an oven (65 ℃, 4 h), forming a 60-μm-thick PDMS layer. (4) Ti/Au with 5 nm/100 nm thickness was deposited on the PDMS layer using an e-beam evaporator. The sensor structures were defined by AZ5214 patterning, and a wet etchant was then used for selective etching of the metal layers. (5) To electrically isolate the piezoresistive sensor of the PDMS cantilever used in the culture fluid, PDMS was spin-coated (2,000 rpm, 40 s) again onto the PDMS layer with electrodes. (6) A 70-μm-thick PDMS layer was cut using a blade along the cutting mark to form the cantilever. (7) The PDMS cantilever and PDMS body were bonded by O_2 plasma-bonding method. (8) The fabrication of the PDMS cantilever was completed by removing the PR sacrificial layer, which was used as a mold, using an AZ remover. Figure 2(b) shows the optical image of the fabricated PDMS cantilever with an integrated piezoresistive sensor.

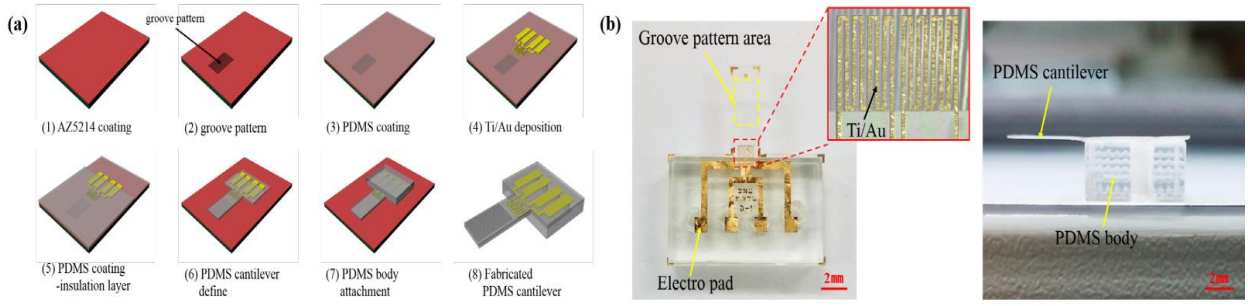

Figure 2. (a) Fabrication process and (b) optical images of the fabricated PDMS cantilever with integrated sensor.

The initial resistance of the strain sensor was about 100 Ω, and to evaluate the strain sensor properties with respect to the allowed displacement, the measurement system shown in Fig. 3(a) was prepared. When the PDMS cantilever was allowed a maximum displacement of 500 μm, the change in the sensor resistance was approximately 0.03 Ω, and the minimum displacement that could be detected by the sensor was approximately 20 μm. When the displacement was increased by 20 μm at each step, the linearity in the resistance change could be observed as shown in Fig. 3(b). The cantilever displacement caused by the contraction force of cardiomyocytes was more than 100 μm; thus, the contraction forces of cardiomyocytes could be analyzed in real time using the fabricated PDMS cantilever with integrated piezoresistive sensor.

The purity of the cardiomyocytes obtained by neonatal rat ventricular myocyte isolation was increased through various post processes. Subsequently, the contraction force of the cardiomyocytes was maximized by comparative analysis of the growth characteristics of the cardiomyocytes using the PDMS substrate whose surface was treated under various conditions. The process used in this experiment is described as follows: the hydrophobic characteristics of the fabricated PDMS cantilever surface was changed to a hydrophilic interface by O_2 plasma treatment (Femto Science, 80 W, 30 s). Next, 200 μL of aliquoted fibronectin was spread over the entire cantilever surface and was kept at room temperature for 1 h for even coating. After spreading 300 μL of cardiomyocytes mixed in the culture fluid onto the cantilever, it was then incubated (37 ℃, CO_2 5%) for three days before observing the cardiomyocytes beating characteristics using an optical microscope. The differences in the cardiomyocytes alignment and growth in the presence and absence of microgrooves are shown in Fig. 3(c). For the ordinary PDMS cantilever, the cardiomyocytes isotropically grew in the radial directions. Thus, they did not align in a single direction. However, when microgrooves were formed at the surface of the cantilever, the cardiomyocytes aligned and grew in a certain direction along the grooves. Cardiomyocytes were found to anisotropically grow regardless of the groove width, but the cantilever displacement showed large variations with respect to the contraction force. Figure 4(a) shows the cantilever device for the characterization of contraction force of cardiomyocytes. Water contact angles of PDMS cantilevers under various surface treatment conditions are shown in Fig. 4 (b). Figure 4(b) shows that all samples displayed the largest displacement around the seventh day after the cardiomyocytes seeding. The cantilever displacements were between a minimum of 200 μm (at a pitch of 6 μm) and a maximum of 655 μm (at a pitch of 2 μm). This difference was caused by the different adhesion force of cardiomyocytes in relation to the groove width. Because the cantilever displacement changes with the number of cardiomyocytes attached to the surface, further examination on reproducibility is required through several repetitions of the experiments.

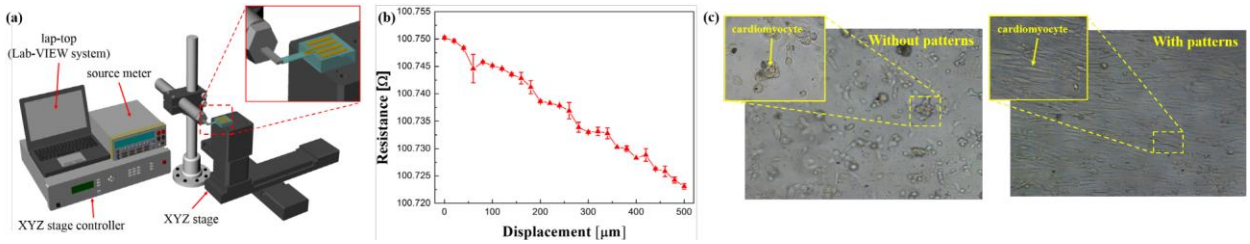

Figure 3. (a) Measurement system for the cantilever displacement. (b) Voltage output of the Wheatstone bridge circuit formed on the PDMS cantilever. (c) Alignment of cardiomyocytes in the presence and absence of microgroove patterns.

CONCLUSION

In this study, a PDMS cantilever with an integrated piezoresistive sensor was proposed and characterized to evaluate the contraction force of cardiomyocytes. The contraction force of cardiomyocytes was maximized by forming a groove pattern on the PDMS cantilever surface, which was

employed for anisotropic alignment and growth of cardiomyocytes. The contraction force of the cardiomyocytes was successfully evaluated using the PDMS cantilever device whose design was based on the basic experimental results. In addition, the potential use of the PDMS cantilever with an integrated piezoresistive sensor was characterized as a drug toxicity testing application through injection of a drug that can increase or decrease the contraction forces of cardiomyocytes.

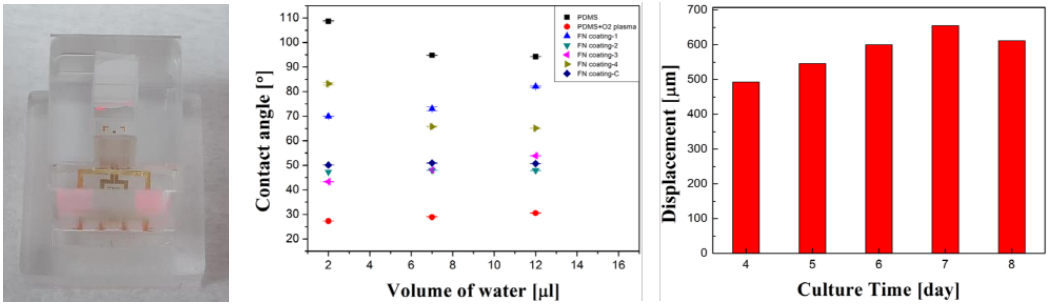

Figure 4. (a) Optical image of cantilever device for the evaluation of the contraction force of cardiomyocytes, (b)water contact angle measurement on various PDMS substrates and (c) cantilever displacement as a function of the microgroove width.

ACKNOWLEDGEMENTS

This work was supported by International Collaborative R&D Program through KIAT grant (N0000894) and the National Research Foundation (NRF) grant (No.2015R1A2A2A05001405) funded by the Korea government.

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PDMS Cantilever Integrated with a Strain Sensor for Contraction Force Measurement of Cardiomyocyte

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Abstract

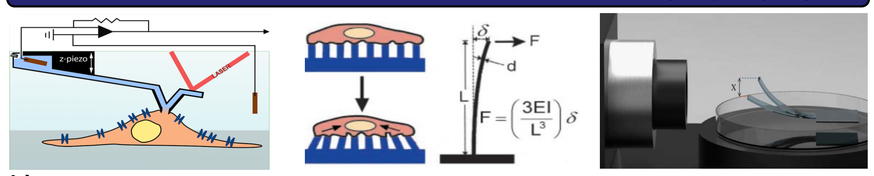
This study focused on the fabrication and evaluation of a polydimethylsiloxane (PDMS) cantilever integrated with a metal piezoresistive sensor for real-time measurement of contraction forces in cardiomyocytes. A pattern of microgrooves with various sizes was formed on the top surface of the cantilever to optimize the arrangement of cardiomyocytes, which helped optimize the cardiomyocyte contraction force. The contraction force of the cardiomyocytes was maximized by forming a pattern of approximately 1 μm wide grooves, and an increase in the contraction force increased the sensitivity of the integrated piezoresistive sensors. The contraction force of the cardiomyocytes cultured on the cantilever began to increase on day 3 and was at a maximum on day 7 when the cantilever displacement was approximately 600 μm. The PDMS cantilever with the integrated metal piezoresistive sensor proposed in this paper is expected to be used in areas such as a high-speed drug toxicity test systems owing to its capability to detect real-time contraction forces of cardiomyocytes.

◆ Keywords : PDMS cantilever, Metal piezoresistive sensor, Contraction force, Cardiomyocytes

Introduction

◆ Diversity of cardiomyocytes contraction force measurement method

How to measure the contraction force of cardiomyocyte (CM)



Advantage

- Measurement by the electrical signal
- Measurement of the μpost or cantilever deformation caused by contraction of cardiomyocytes

Disadvantage

- Fabrication process is complex, Cells of the channel and alignment of the pipette is required
- Small deflection is difficult to be observed
- Low throughput optical microscope

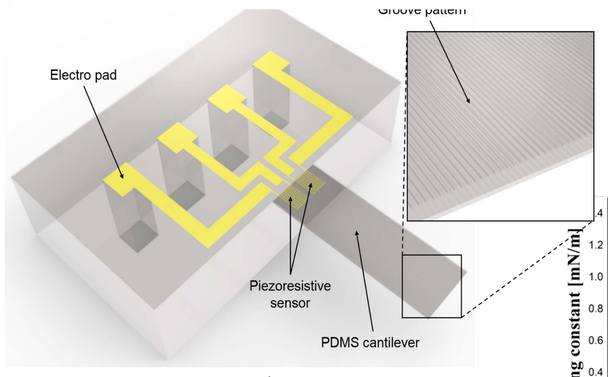
Design concept and FEM Simulation result of PDMS cantilever

◆ Strain sensor and Micro-groove integrated on PDMS cantilever – taking advantage of half bridge and flexibility

◆ Specification

1) Cantilever size (L×W×H) 6mm×2mm×70μm

2) Body size (L×W×H) 7mm×12mm×30mm



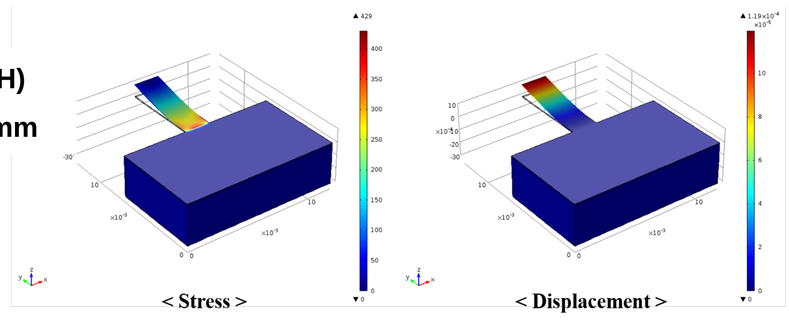


Fig. 1 Schematic diagram for piezoresistive sensor and surface-patterned PDMS cantilever

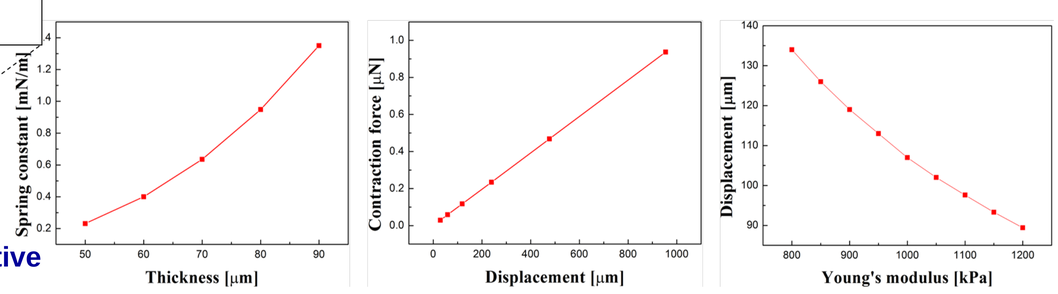


Fig. 2 PDMS cantilever analysis using COMSOL multiphysics software

Fabrication of PDMS cantilever

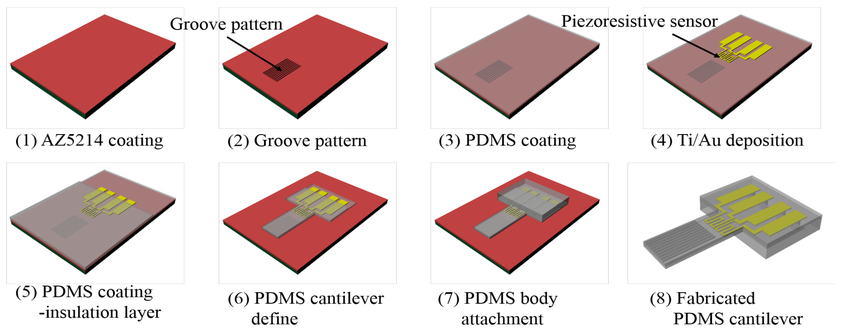


Fig. 3 Fabrication process using MEMS and PDMS casting

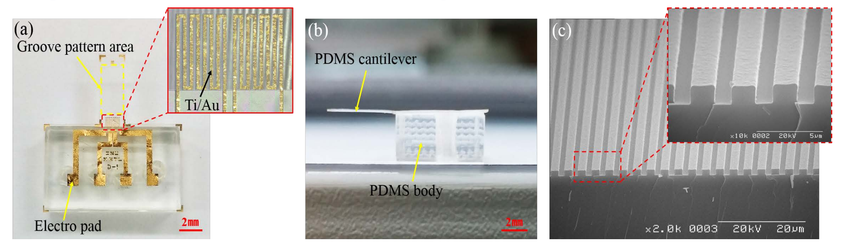


Fig. 4 Optical image (a)Top view, (b) side view and (c) SEM image of micro groove

Cardiomyocytes observe

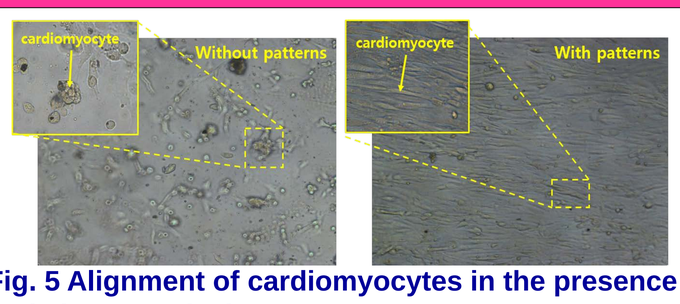


Fig. 5 Alignment of cardiomyocytes in the presence and absence of microgroove patterns

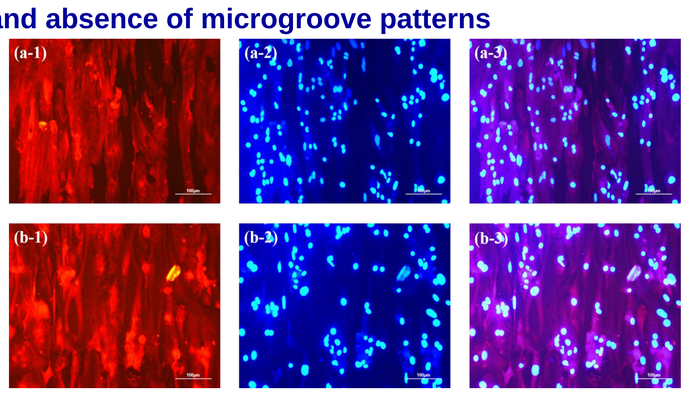
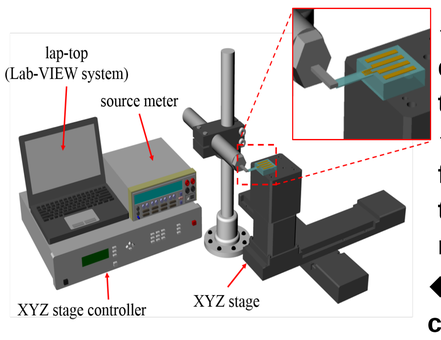


Fig. 6 Staining image (a-1, b-1) sarcomere actin, (a-2, b-2) DAPI, (a-3, b-3) merged image

PDMS cantilever measuring system



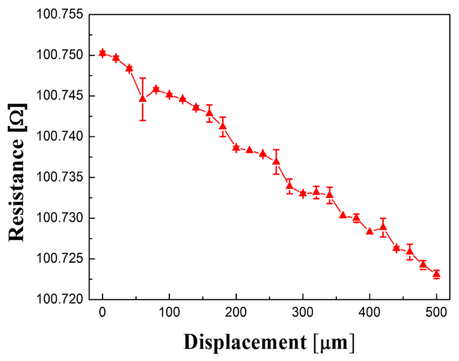
◆ A minimum change of 20μm in displacement can be detected by the sensor

◆ In the experiment, concentrate force is applied on the cantilever end to achieve various displacement. The resistance of sensor is measured

◆ The resistance exhibits a linear characteristic with the displacement

Fig. 7 Measurement system for the cantilever displacement (based on Lab-VIEW software)

Fig. 8 Resistance change according to the applied displacement



Characterization

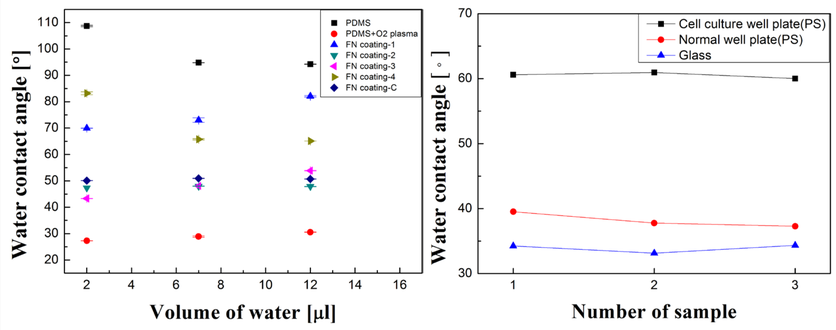
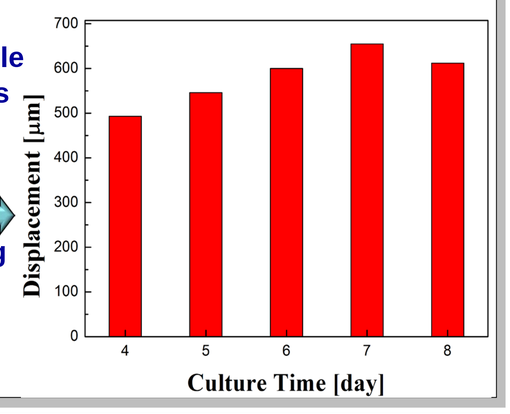


Fig. 9 Water contact angle measurement on various PDMS substrates

Fig. 10 Cantilever displacement depending on cell culturing day



Sensing of displacement and drug toxicity test

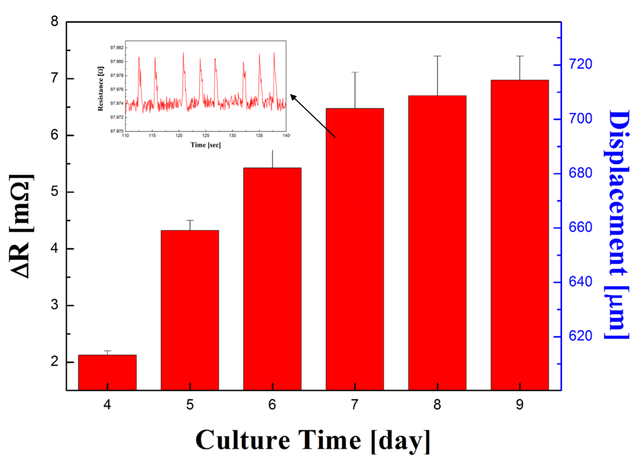


Fig. 11 The resistance change of the sensor and the cantilever displacement caused by cardiomyocyte contraction versus culture time.

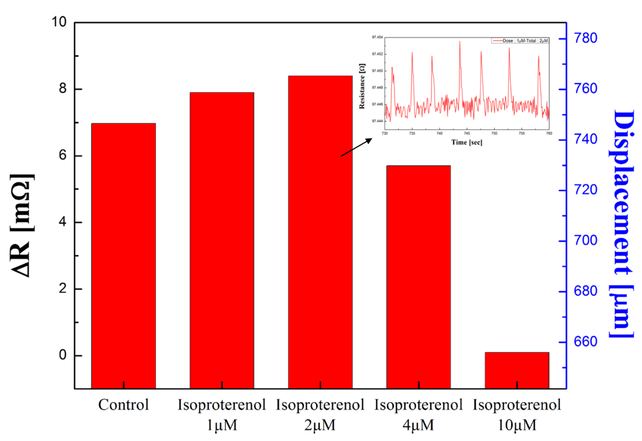


Fig. 12 The influence of Isoproterenol concentration on the beating frequency and contractile force of the cardiomyocytes was characterized

Conclusion

In this study, a PDMS cantilever with an integrated piezoresistive sensor was proposed and characterized to evaluate the contraction force of cardiomyocytes. The contraction force of cardiomyocytes was maximized by forming a groove pattern on the PDMS cantilever surface, which was employed for anisotropic alignment and growth of cardiomyocytes. The contraction force of the cardiomyocytes was successfully evaluated using the PDMS cantilever device whose design was based on the basic experimental results. In addition, the potential use of the PDMS cantilever with an integrated piezoresistive sensor was characterized as a drug toxicity testing application through injection of a drug that can increase or decrease the contraction forces of cardiomyocytes.

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