ASPEN

ASPEN 2017

The 7th International Conference of Asian Society for Precision Engineering and Nanotechnology Program

November 14 Tue.
- 17 Fri., 2017

COEX, Seoul, Korea

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Novemver 15 Wed.

Time	Paper ID	Title / Speaker / Affiliation

Nano/Bio Technology — Room 4(E4)

Chair Ming Chang(Chung Yuan Christian University)

09:30~09:50	NBT-O-01	***A Mathematical Investigation for the Estimation of Piezoelectric Actuator Displacement for High Speed Motion Sensorless Control*** Tien-Fu Lu(University of Adelaide)
09:50~10:10	NBT-O-02	***DLC-Based Nanocomposites for Bio Applications*** Oleksiy V. Penkov(Yonsei University), Mehdi Kheradmandfard(University of Tehran), Jung-Seung Lee(Yonsei University), Seung-Woo Cho(Yonsei University), Dae-Eun Kim (Yonsei University)
10:10~10:30	NBT-O-03	***High-throughput Cardiac Toxicity Screening System*** Jong Yun Kim(Chonnam National University), Nomin-Erdene Oyunbaatar(Chonnam National University), Dong-Weon Lee(Chonnam National University)

Molding and Forming Technology — Room 4(E4)

Chair Keun Park(Seoul National University of Science and Technology)

13:50~14:10	MFT-O-01	***Metal-polymer Injection Molded Direct Joining Using Electrolyte Jet Machining*** Xiaoyan Lyu(The University of Tokyo), Fuminobu Kimura(The University of Tokyo), Yonghua Zhao(The University of Tokyo), Masanori Kunieda(The University of Tokyo), Yusuke Kajihara(The University of Tokyo)
14:10~14:30	MFT-O-02	***Effect of Vibration Transmission Direction in Ultrasonic Thermoforming*** Hojin Bae(Seoul National University), Hyun-Joong Lee(Seoul National University), Keun Park(Seoul National University of Science and Technology)
14:30~14:50	MFT-O-03	***Development of the Micro-molding Technology using Ultrasonic Vibration Energy*** Keun Park(Seoul National University of Science and Technology), hyun-Joong Lee (Seoul National University of Science and Technology)

High-throughput Cardiac Toxicity Screening System

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KEYWORDS: Cardiomyocytes, Cantilever array, Contraction force, Heart Rate, Drug screening, Groove

This paper describes a biocompatible polymer cantilever arrays for drug-induced cardiac toxicity screening applications, which analyze the changes of the contraction force and the beating frequency of cardiomyocytes. The bending displacement of the cantilever was precisely evaluated by using a laser vibrometer and a motorized XY stage system. Minimum detectable sensitivity of the cantilever displacement caused by cardiac contraction is below 100nm. The proposed system has a great potential to systematically investigate the contraction force change of cardiac cells in different drug environments. The experimental results are very useful to assist the toxicity screening results of a patch clamp method (gold standard method).

1. Introduction

Cardiac diseases are one of the biggest problem in worldwide. According to the American Heart Association, 40 % of heart disease was caused by drugs side effect such as arrhythmias. Electric physiological measurement patch-clamp [1], is considered as an efficient method to detect drug-induced cardiac toxicity by measuring the changes in ions of cardiomyocytes. However, it is not convenient method to analyze the side-effect of drugs. Therefore, measuring the changes of the contraction force of cardiomyocytes, one of the measurement method micro post [2] and cantilever [3-4] were proposed. Using polymer structures to measure bending displacement caused by contraction force. However, analyzes displacement using optical microscope images are difficult to measure in real time due to the small amount of displacement less than micrometers. Furthermore, using Polydimethylsiloxane (PDMS) material to make structure is difficult to miniaturization and mass product. Precise measurement is highly desirable for the next generation system in drug toxicity screening.

2. Materials and Methods

2.1 Fabrication of proposed cantilever

Fig. 1 shows the SU-8 cantilever fabrication process flow. In the process, N-type (100) silicon wafer with a diameter of four inches is used as a substrate (a). Next, a 300 nm-thick SiO_2 layer formed through wet air oxidation is used as a sacrificial layer to separate the SU-8 cantilever from the silicon wafer (b). Following patterning of the cantilever shape using a 2 μm-thick SU-8 layer (c), a 100 nm-thick Au reflective plate is fabricated through a lift-off process (d). In order to form uniform μgrooves over the upper part of the fabricated SU-8 cantilever, μgrooves with a pitch of 3 μm are fabricated (e). In this process, an SU-8 cantilever body approximately 120 μm-thick is fabricated (f). Finally, SiO_2 is wet-etched over the body-formed substrate using a BHF (6:1) solution, and the SU-8 cantilever fabricated from the silicon wafer is separated (g). Subsequently, through an additional process such as post-baking, SU-8 toxicity is completely removed. To improve the adhesiveness of cardiomyocytes on the SU-8 cantilever surface, 50 μg / ml concentration fibronectin (Corning) solution was coated onto the SU-8 cantilever surface for one hour. Then, the SU-8 cantilever surface was washed three times with phosphate-buffered saline (PBS, Takara).

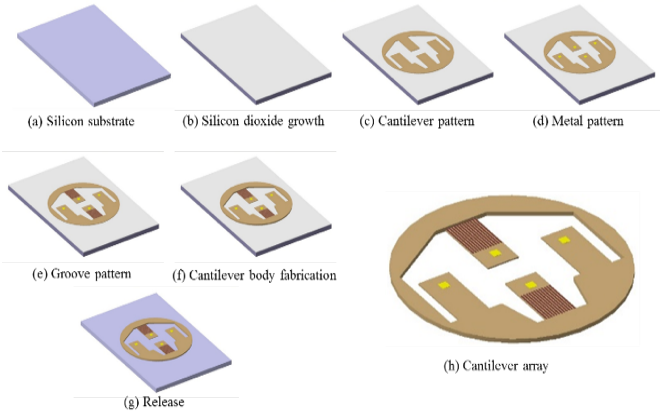


Fig. 1 Fabrication process of cantilever

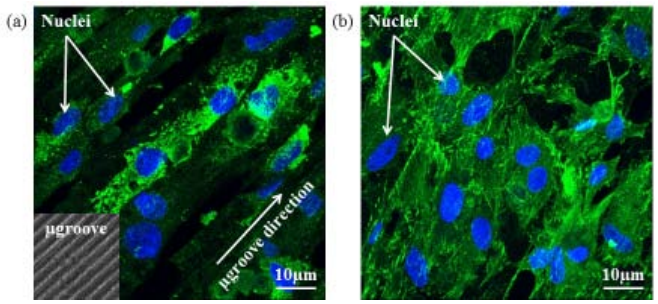
2.2 Cell culture

Ventricles were isolated from one-day old neonatal Sprague-Dawley rats (SD). Cardiomyocytes were digested with 0.4 $mgmL^{-1}$ of collagenase and 0.6 $mgmL^{-1}$ of pancreatin mixed with 1 x ADS solution from ventricular tissue. The digested solution was separated into cardiomyocytes and fibroblast layers using percol solution. The separated layers were pre-plated to prepare high purity cardiomyocytes. Cardiomyocytes were cultured on the SU-8 cantilever with a density of $1000/mm^2$ and media consisting was 67 % Dulbecco's modified Eagle medium (DMEM, LONZA), 17 % Heparin sodium salt from porcine intestinal mucosa (M199, Sigma-

Aldrich), 10 % Horse serum(HS, Sigma-Aldrich), 5 % fetal bovine serum (FBS, Sigma-Aldrich), and 1 % penicillin streptomycin (P/S, Sigma-Aldrich). Cardiomyocytes were cultured in an incubator that maintained 37 ℃ and CO_2 at 5 %, and the culture solution was replaced every three days. Cardiomyocytes start beating after 24 hours and synchronization proceed after 48 hours.

2.3 Immunocytochemical staining

In order to identify the grown of cardiomyocytes, immunocytochemical staining was processed. First, the cardiomyocytes were fixed using formalin solution (3.7%, 15 min, RT) and washed with PBS 3 times. Permeabilization was then accomplished with 0.1% Triton-X (Sigma-Aldrich) in PBS for 10 min at room temperature. To prevent non-specific binding of the antibody, cardiomyocytes were cultivated at room temperature for 30 min by adding 1% bovine serum albumin (1% BSA, Sigma-Aldrich). The primary antibody (monoclonal anti-actin (α-actinin)) was mixed with 1% BSA in a ratio of 1:100, and the cardiomyocytes cultivated at room temperature for 120 min. Then, a secondary antibody (Alexa-Flour 488 goat anti-mouse IgG conjugate) was mixed with 1% BSA in a ratio of 1:200, and the cardiomyocytes cultivated at room temperature for 90 min. Finally, DAPI solution (4′, 6-Diamidino-2-phenylindole) was added and the cardiomyocytes cultured for 15 min at room temperature to conduct nuclear staining. Fig. 2 shows the fluorescence staining image of the cardiomyocytes cultured on proposed SU-8 cantilever with and without micro grooves. As shown in the image that merges stained α-actinin and nuclei in fig. 2, the



cardiomyocytes were aligned along to the micro groove direction.

Fig. 2. Staining images of cardiomyocytes; (a) cantilever with μgrooves, (b) cantilever without μgrooves. Nuclei (blue), α-sarcomere actin (green)

2.4 Drug test

Contraction and relaxation of cardiomyocytes generates bending displacement of cantilevers. Laser vibrometer based measurement system was used to precisely measure bending displacement of cantilever. The system consists laser vibrometer that can measure displacement in nanoscale, honeycomb board for anti-vibration, motorized stage that can to quickly measure samples and stage-top incubator to avoid external environment. Several drugs were used to measure the changes of the contraction force and the beating frequency of cardiomyocytes. Contraction force of cardiomyocytes was directly changed in calcium ions. Beating frequency of cardiomyocytes were changed by every drug treatment. Fig. 3 shows contraction property changes due to the drug treatment. Without treatment of drugs, it shows regular beating (fig. 3 (a)) and after drug treatment, the contraction of cardiomyocytes were changed as shown in fig. 3 (b). After all drug experiments, fresh media was exchanged to monitor permanent damage of the cardiomyocytes. Even though the concentration of the drug increased, calcium and sodium ion related drug were fully recovered to initial condition of the cardiac cells. However, potassium ion related drugs could not be recovered once cardiac cells were damaged by the drug toxicity. Also, potassium channel blocker, E-4031 and Astemizole significantly decreased beating. This drug has a great potential to induce a heart disease. The critical values (IC 50) in drug concentration which influences the cardiac cells were very close to that of the gold standard method.

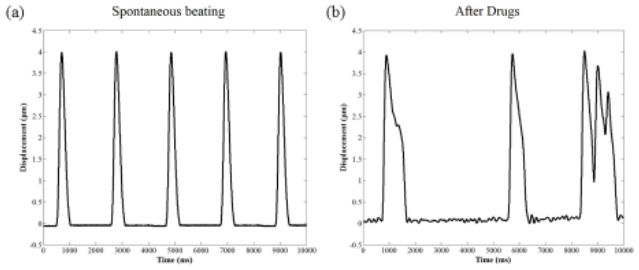


Fig. 3. Contraction property changed due to drugs; (a) before treatment of drugs, (b) after treatment of drugs.

3. Conclusions

In this paper we used SU-8 cantilever with μgroove patterns and laser vibrometer system to measure cardiomyocytes contraction force change due to drug treatment *in vitro*. Various SU-8 cantilever with and without μgrooves were produced to confirmed the effect on contraction force of the three-dimensional surface structures on cardiomyocytes, it optimized the structure of μgrooves to maximize the contraction force of cardiomyocytes. The cardiomyocytes were cultured on SU-8 cantilever that groove had been integrated; various concentrations of E-4031 and Astemizole, we analyzed the characteristics of cardiomyocytes with drug treatment. The contraction force was not changed due to concentration of drugs, but heart rate did decrease due to concentration of drug increased. The proposed SU-8 cantilever arrays with a laser vibrometer based measurement systems can be expected to the novel drug toxicity screening system in future.

ACKNOWLEDGEMENT

This study was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP)(No.2015R1A4A1041746)

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