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MEA-ON-CANTILEVER – A NOVEL MULTIFUNCTIONAL DEVICE FOR DRUG TOXICITY SCREENING IN CARDIOMYOCYTES

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ABSTRACT

This paper demonstrates an easy-to-use dual-function biosensor for real-time monitoring of drug-induced cardiac toxicity screening, by measuring both electrophysiology and mechanophysiology of drug-treated cardiomyocytes simultaneously. The proposed biosensor consists of a photosensitive polymer cantilever on which microelectrodes are patterned. Electrophysiology is measured by recording field potential using microelectrode array (MEA), and contractile motion is measured using cantilever deflection upon culturing of cardiomyocytes. This dual-function biosensor has been tested for its reliability for drug screening by adding Verapamil to the cell culture and assessing the outputs in synchrony. Field potential duration (FPD) reduced on increasing concentration (IC_{50}=166.2 nM), as did contraction force (IC_{50}=242.9 nM). This biosensor is a reliable next-generation device of drug toxicity screening.

KEYWORDS

Cardiomyocytes, Electrophysiology, Microelectrode array, Contraction force, Cantilever

INTRODUCTION

Microelectrode array (MEA) is an established method to understand the electrophysiology of electrogenic cells such as cardiomyocytes [1-3]. MEAs measure the extracellular action potential, commonly known as field potential, of the cells at the tissue level. Intracellular action potential is traditionally measured with the use of patch clamp technique. However, patch clamp being an invasive and low-throughput technique that requires high amount of expertise, measurement of field potential using MEA is a more widely used method currently. MEAs are used to measure the ion channel activity of cardiomyocytes and understand their response to various drugs and determine drug toxicity [2].

In order to get the entire representation of intracellular processes, electrophysiological responses of cardiomyocytes have been studied in synchrony with their mechanophysiological response. For this, MEAs have been combined with techniques such atomic force microscopy (AFM) [4]. However, this technique suffers from the limitation that they are invasive to the cells during measurement. Some non-invasive techniques for contraction measurements, such as high-frequency impedance and phase-contrast video microscopy, have also been combined with MEA [5-6]. Although these methods are non-invasive, the amount of force exerted by the cells during contraction cannot be quantified. Hence, a superior yet straightforward method to measure the contraction force of cardiomyocytes is by the use of cantilevers.

Contraction force and drug toxicity analyses of cardiomyocytes using cantilever have been studied extensively till date [7-8]. Flexible miniature cantilevers using SU-8 and polydimethylsiloxane (PDMS) have been developed to understand the contractile response of cardiomyocytes at the tissue level [8-9]. Cantilever has also been combined with strain sensor to study the strain changes of cantilever due to drug toxicity, and has been combined with MEA in a simplified geometrical array earlier in our lab and impedance has been analyzed [10-11].

In our present work, we designed an MEA pattern on cantilever that has higher throughput as compared to the earlier design, in such a way that major areas of the tissue could be studied, and throughput could be increased. Field potential was recorded by patterning 18 nos. of microelectrodes, in sets of 6 each, in 3 different locations of a photosensitive SU-8 based polymer cantilever. This was done so that information regarding electrical activity from most of the device could be recorded, in conjunction with contractile activity. Neonatal rats' ventricular myocytes (NRVM) were seeded and electro-mechano physiology was measured simultaneously. Further, in order to test the device for its response to drug toxicity, Verapamil, which is a calcium ion channel blocker and known for its toxic effects on cardiomyocytes, was added to the cell culture and simultaneous responses were recorded and analyzed.

METHODS AND MATERIALS

Working principle of the device

This dual-function biosensor simultaneously measures electrophysiology as well contractile response of cardiomyocytes, both of which are essential to understand cardiotoxicity in-depth. Contractile response is measured by fabricating an SU-8 based polymer cantilever. Thickness of the cantilever is kept low to enhance sensitivity. Upon culturing cardiomyocytes on the cantilever, measurement of the corresponding cantilever deflection upon their contraction and relaxation give the estimate of the amount of contraction force exerted by the cardiac tissue. Furthermore, microelectrodes made of Cr/Au are patterned on top of the cantilever that simultaneously measure field potential of the cardiomyocytes. 6 nos. of MEAs have been placed each on (1) free end of cantilever, (2) fixed end of cantilever and (3) on the device body, so that overall field potential trend is understood. Figure 1(a) shows the schematic of the proposed device, and figure 1(b) shows working principle of measurement of contraction force.

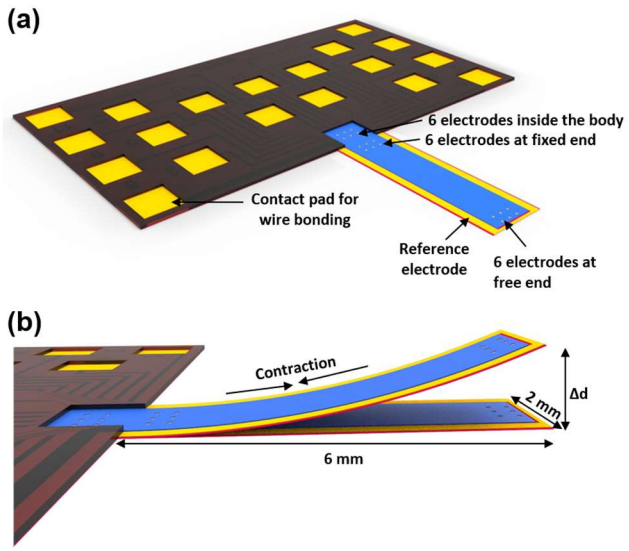

Figure 1: (a) Schematic of the proposed dual-function biosensor denoting positions of the microelectrodes on the cantilever. Diameter of MEAs = 50 µm and pitch = 200 µm, (b) working principle of measurement of contraction force by cantilever displacement.

Cell culture

Cardiomyocytes isolated from the heart of one-day-old neonatal rats (approved by the Animal Ethics Committee of Chonnam National University) were used and its suspension solution, whose culture medium was supplemented with 10% fetal bovine serum, was cultured at 37 ºC in an atmosphere with 5% CO_2. Before seeding cardiomyocytes with a density of 1000 cells/mm^2 onto cantilever surface, the cantilever was treated by fibronectin which was used as an extra cellular matrix in advance. Here, the culture solution comprised of 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).

Simultaneous recording and data acquisition of electrophysiology and contraction force for drug toxicity screening

Field potential was measured with the help of fabricated MEAs using a 64-channel amplifier (Intan Technologies RHD2164) connected to the Intan acquisition system (RHD2000). Sampling rate was kept at 20 kHz and the bandwidth of recorded measurement was 0.1 Hz – 7.5 kHz.

For measurement of contraction force, a laser vibrometer (Polytec GmbH) based measurement setup was used. The laser vibrometer, that measures displacement in nanoscale, is placed vertically facing a home-made tabletop incubator, in which the cell cultured sample is placed for drug toxicity screening. Atmospheric temperature in the incubator is controlled at 37 ºC and CO_2 level at 5%.

On the 8th day after neonatal rats' ventricular myocytes (NRVM) cell culture, the device was tested for its response to drug toxicity using a calcium ion channel blocker drug, Verapamil. Several concentrations of Verapamil, ranging from 0.1 nM to 1 µM, were added sequentially in the culture medium. Simultaneous measurements were recorded after a stabilization period of 5 min.

RESULTS AND DISCUSSION

Figure 2(a) shows the optical image of the dual-function device in cell culture medium. Cells are uniformly distributed across the surface of the cantilever.

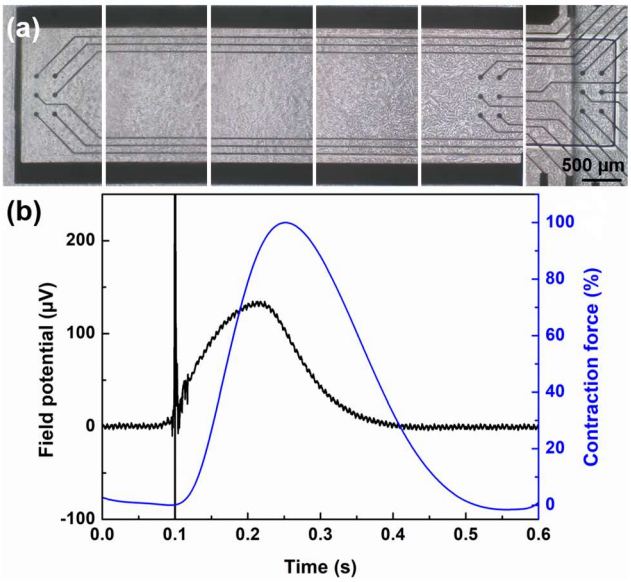

Figure 2: (a) Optical image of the cantilever in cell culture medium, (b) simultaneous measurement of field potential and contraction force from the biosensor.

Figure 2(b) shows a representative figure of contraction force and field potential measured together. Field potential duration is smaller than the contractile motion duration, which is in agreement with results by Hayakawa et al [6]. This dual function biosensor can be said to be a reliable tool for drug toxicity analysis.

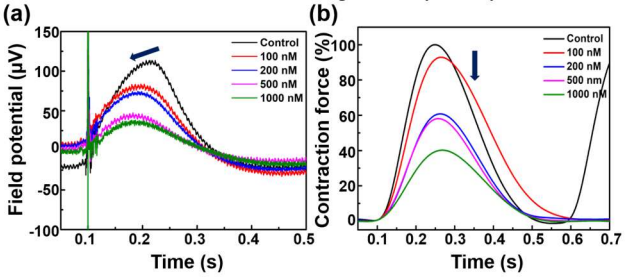

Figure 3: (a) Field potential and (b) contraction force measured simultaneously from the dual-function biosensor.

Figure 3 shows the response of the device on addition on Verapamil. Verapamil is a calcium ion channel blocker and is used to treat cardiac arrhythmia, hypertension and vasodilator during cryopreservation of blood vessels. Figure 3(a) and 3(b) show simultaneous measurement of field potential and contraction force recorded for different concentrations. Field potential duration decreased on increasing contraction, as can be seen from the left-shift of the last peak in figure 3(a). Along with the left-shifting of the peak, the peak amplitude is also decreasing in a concentration-dependent manner. Negative inotropic effect of Verapamil is evident from figure 3(b), as contraction

force is decreasing on increasing concentration. Based on the measurement done from 0.1 nM – 1 μM and fitting with Hill's equation, IC_{50} of field potential duration was calculated to be 166.2 nM, and that of contraction force was calculated to be 242.9 nM.

CONCLUSION

In this study, we have successfully demonstrated a high-throughput dual function biosensor that can simultaneously measure electrophysiology and mechanophysiology of cardiomyocytes. The capabilities of the proposed biosensor have been verified by the addition of calcium ion channel blocker Verapamil drug. Our results confirmed with the earlier reported data, that proves the reliability of our biosensor. This biosensor can become a versatile device for preclinical drug screening applications of cardiomyocytes.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No.2017R1E1A1A01074550).

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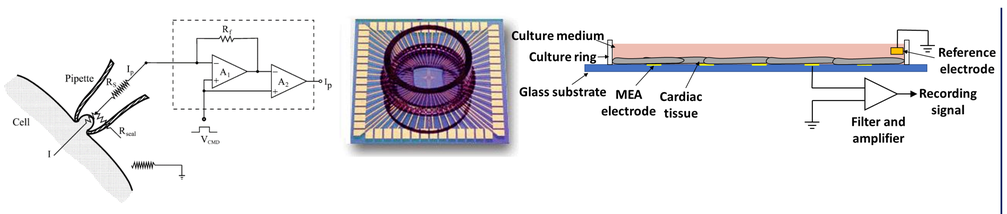
ABSTRACT

This work demonstrates an easy-to-use dual-function biosensor for real-time monitoring of drug-induced cardiac toxicity screening, by measuring both electrophysiology and mechanophysiology of drug-treated cardiomyocytes simultaneously. The proposed biosensor consists of a photosensitive polymer cantilever on which microelectrodes are patterned. Electrophysiology is measured by recording field potential using microelectrode array (MEA), and contractile motion is measured using cantilever deflection upon culturing of cardiomyocytes. This dual-function biosensor has been tested for its reliability for drug screening by adding Verapamil to the cell culture and assessing the outputs in synchrony. Field potential duration (FPD) reduced on increasing concentration (IC_{50}=166.2 nM), as did contraction force (IC_{50}=242.9 nM). This biosensor is a reliable next-generation device of drug toxicity screening.

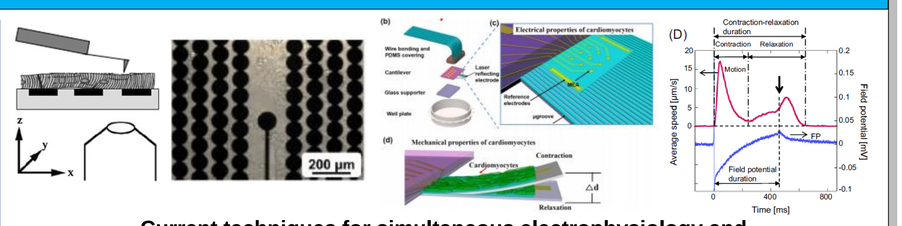
◆Keywords : Microelectrode arrays, Cantilever, Cardiomyocytes, Electrophysiology, Contraction force

INTRODUCTION



Current techniques for electrophysiology measurement

These techniques measure only the electrophysiology of cardiomyocytes and not mechanophysiology



Current techniques for simultaneous electrophysiology and contraction force measurement

Invasive, low-throughput and elaborate techniques have been developed so far

Motivation

- Cardiac cells show chronotropy but no significant inotropy in response to β-adrenoceptor agonist such as isoproterenol and nifedipine; i.e. several drugs only show changes in contraction force but not electrophysiology of cardiac cells, and vice versa.
- Simultaneous electrophysiology and mechanical recordings not analyzed extensively till date
- Hence, there is a need of an integrated platform to measure simultaneous electrophysiological and mechanical response.

Objective

- To develop a high throughput integrated cardiac sensing drug screening platform for simultaneous measurement of electrophysiological and mechanical properties of cardiomyocytes
- This dual function biosensing platform is to be realized using a cantilever-based non-invasive mechanical device that has microelectrodes patterned on it.

DEVICE CONCEPT

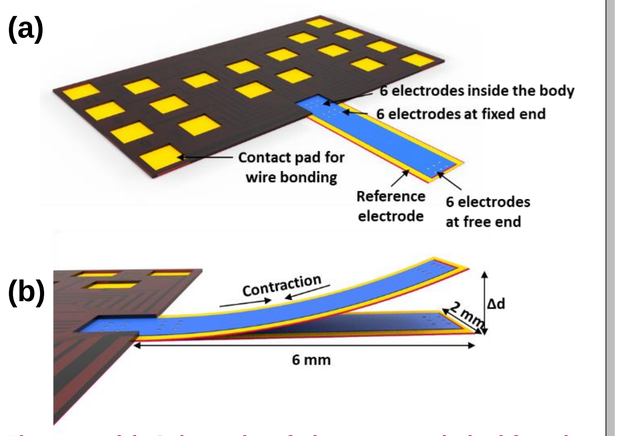


Figure 1. (a) Schematic of the proposed dual-function biosensor denoting positions of the microelectrodes on the cantilever. Diameter of MEAs = 50 µm and pitch = 200 µm, (b) working principle of measurement of contraction force by cantilever displacement.

DEVICE FABRICATION

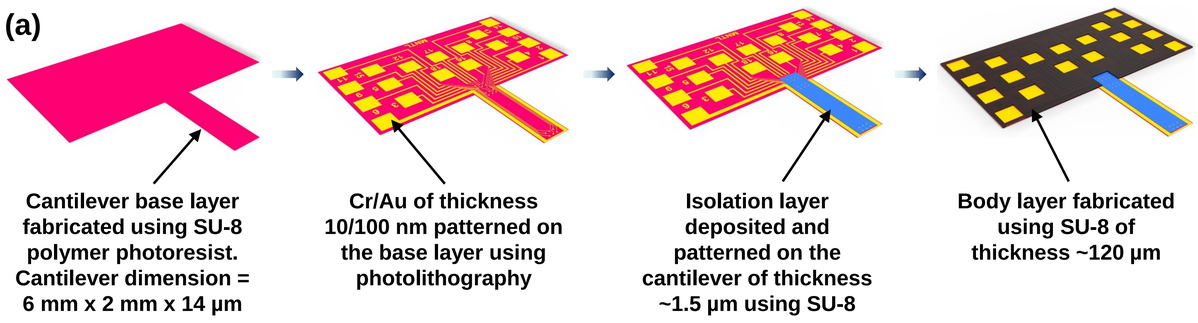


Figure 2. (a) Fabrication process flow of the proposed dual-function biosensor. The cantilever layer is deposited first using photolithography using a photosensitive polymer, then microelectrodes of Cr/Au, then isolation layer and, lastly, body layer of the device is fabricated, (b) optical image of the final dual-function biosensor showing positions of microelectrodes and reference electrode on the cantilever. Scale bar = 200 µm.

EXPERIMENTAL RESULTS

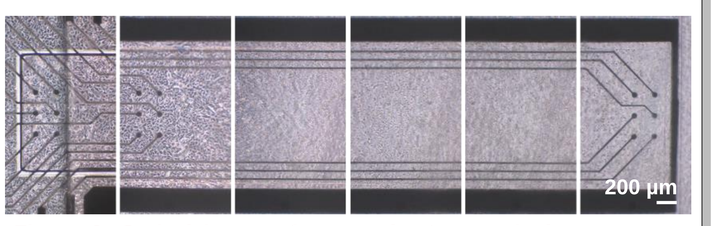


Figure 3. Optical image of neonatal rats' ventricular myocytes (NRVM) distributed on the entire cantilever.

- Shape of the extracellular field potential signal is similar in all electrodes of cantilever, including field potential duration – with a small propagation delay in each area.

EXPERIMENTAL RESULTS

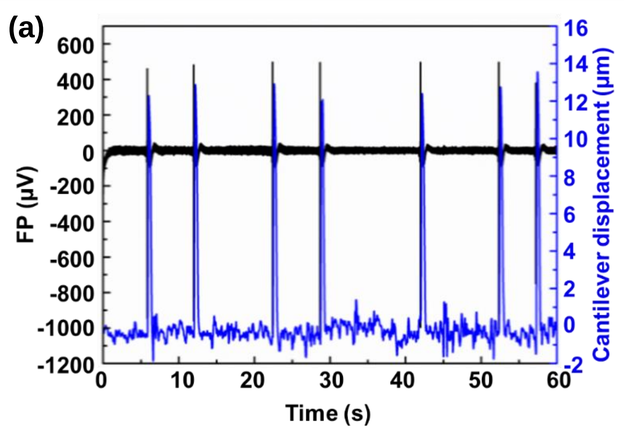


Figure 4. (a) Field potential and cantilever displacement measured simultaneously from the fabricated dual-function biosensor, (b) overlapped curve of field potential and contraction of one waveform.

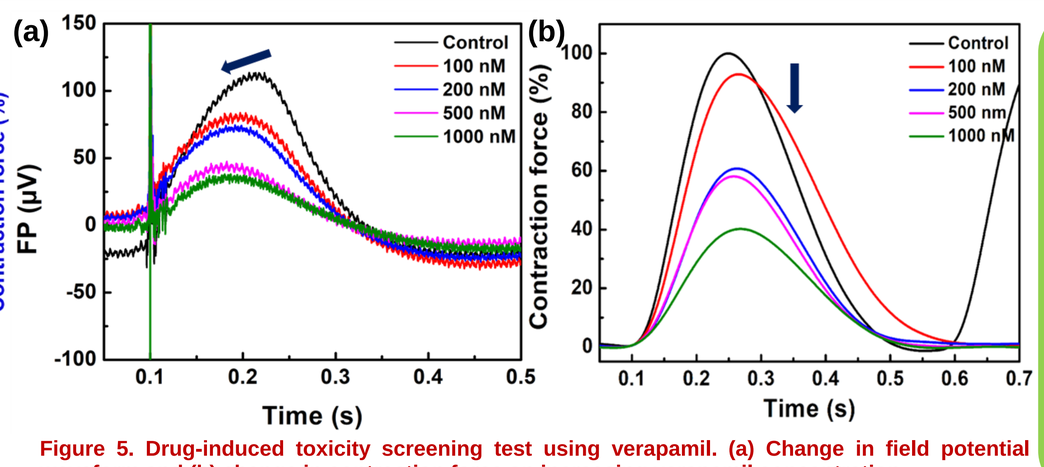


Figure 5. Drug-induced toxicity screening test using verapamil. (a) Change in field potential waveform and (b) change in contraction force on increasing verapamil concentration.

- Field potential duration is decreasing with increasing concentration of verapamil.
- Contraction force is also decreasing with increasing verapamil concentration.
- Results from our device show the expected negative chronotropic and inotropic effects of verapamil.

CONCLUSION

We have successfully demonstrated a high-throughput dual function biosensor that can simultaneously measure electrophysiology and mechanophysiology of cardiomyocytes. The capabilities of the proposed biosensor have been verified by the addition of calcium ion channel blocker Verapamil drug. This biosensor can become a versatile device for preclinical drug screening applications of cardiomyocytes.

ACKNOWLEDGMENT

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