A Polymer-based Piezoelectric Transducer for Real-time Monitoring Contractile Behavior of Cardiomyocytes

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ABSTRACT
In this paper, we present an intelligent piezoelectric transducer that can automatically monitor the contractile behavior of cardiomyocytes for the application of drug screening. By integrating a biomimetic substrate and a piezoelectric smart structure, neonatal rat cardiomyocytes could adhere onto the surface of the piezoelectric transducer, and their mechanical contractions could be measured in real time. Preliminary studies demonstrated that spontaneous beating cardiomyocytes could be induced after 6-day culture, and mechanical contractions of cells were detected by the piezoelectric transducer at day-12. This piezoelectric transducer can easily be scaled up to a large piezoelectric transducer array for parallel studies of multiple 2-D cardiac microtissues. Mechanical information of cardiac micro-tissues like beating frequency, contraction rate, contractile force and profile can be massively and quantitatively monitored and be used to screen drug effects and identify their cardiotoxicity.

Categories and Subject Descriptors
A.0 [General]:Conference proceedings;
B.0 [General]

General Terms
Design

Keywords
Drug screening, piezoelectric transducer, cardiomyocyte, Lab-on-a-chip.

1. INTRODUCTION
Current challenge in the screening of the function and cardiotoxicity of cardiac or non-cardiac drugs is the necessity to quantitatively and massively study the biomechanical responses of cardiac muscles, such as, force profile, systolic and diastolic forces, beating frequency, contraction rate, and electro-physiological responses.[1-8] However, due to complex and dynamic behaviors of cardiac tissue, this requirement sets a high barrier to the development of new technologies for cardiovascular drug discovery and screening. Thus, unexpected side effects and cardiotoxicity found at latter stage of drug development and clinical trials became the major cause of attrition during drug development.

To overcome this barrier, both BioMEMS and Microfluidic technologies have been applied to develop Lab-on-a-chip system to study the drug effects on cardiomyocytes. The detecting methods can be separated into two categories. One is to use a flexible structure to serve as the substrate for cardiomyocytes, and the mechanical contraction of anchored cells was measured by monitoring deformations of microstructures. For example, the contractile profile of spontaneous beating cardiomyocytes was monitored through the bending deformation of a 15~20 μm thick poly(dimethylsiloxane) (PDMS) cantilever[1-2] or a 1 μm thick circular silicone membrane,[3] and deflections of a micropost array.[4] However, the detected contractile force and profile were indirect measurements since it is based on optical images of the deformed flexible structures. Further, due to the need to process optical images, these methods could not be fully automatic since they need an experienced personnel to process images. The number of devices can be processed is also limited by the size of the image field of the optical system. The second category is to study the electrophysiological behavior and response of cardiomyocytes under electrical stimulation.[5-8] This method could potentially become an automatic system, but it could not infer the mechanical force generated by the cardiac muscle.

In this paper, we present a novel polymer-based piezoelectric transducer that combines the technologies of smart structures and tissue engineering to develop a cardiovascular drug screening microplatform.[9] The piezoelectric transducer can automatically converts contractile force of cardiomyocytes into an electrical signal. The biomechanical response of cardiomyocytes could be measured automatically. The function and toxicity of drugs under study can be analyzed through electrical signal, which is direct related to the force profile. The success of this platform could potentially realize the goal of massive, quantitative, and automatic drug screening.

2. PIEZOELECTRIC TRANSDUCER
The concept of using a piezoelectric transducer to detect the surface traction exerted by adherent cells have been reported by Hsu and Tang in 2009.[9] It was achieved by using a ZnO piezoelectric thin-film and thermal oxide with Ti/Au electrodes to form the transducer core. Finite element analysis suggested that the cellular behavior of adherent cells could be monitored through the impedance response of the piezoelectric transducer. The surface traction applied by cells correlates to its cellular event can be analyzed through the resonant and anti-resonant frequencies of the piezoelectric transducer.

BICT 2014, December 01-03, Boston, United States

DOI 10.4108/icst.bict.2014.258051

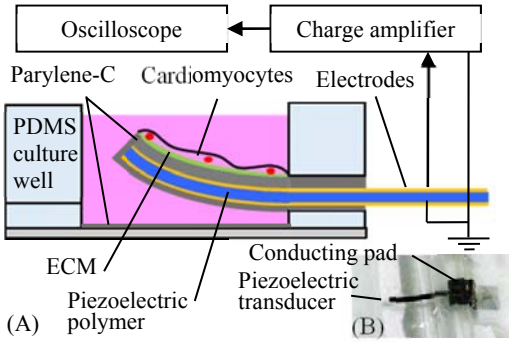

Figure 1. Illustrations of (A) the design of the piezoelectric transducer and (B) an image of the fabricated platform.

Follow the same concept, the present study develops a polymer-based piezoelectric transducer that can real-time monitor the mechanical force and profile generated by spontaneous beating cardiomyocytes. The basic design of the thin-film piezoelectric transducer is illustrated in Fig. 1(A) and implemented platform is shown in Fig. 1(B). Biocompatible piezoelectric PVDF thin film and parylene-C were chosen as the main structural materials for the piezoelectric transducer. Since the beating frequency of cardiomyocytes was below a couple Hz, the transducer structure was designed to be a 10 mm long by 1mm wide by 18μm thick 1-D cantilever plate so that the resonant frequency of the first bending mode was close to this range to increase sensitivity.

The piezoelectric transducer was constructed by a 9 μm thick PVDF piezoelectric thin-film and an 8 μm and 1 μm thick parylene-C coated on each side to form a unimorph structure. This design moved the neutral axis of the 1-D cantilever plate to the interface between 9 μm thick PVDF thin-film and coated 8 μm thick Parylene-C. Thus, the piezoelectric PVDF could detect the bending deformation induced by the contractile forces of the adhered cardiomyocytes on the transducer surface. Theoretical derivation of the interaction between cardiomyocytes and the piezoelectric transducer will be discussed in a separated paper. The edges of the piezoelectric transducer were also coated with a 1 μm thick parylene-C to serve as insulating layer to prevent shortage when soaking in the culture media. Multiple piezoelectric transducers were embedded in a PDMS culture well for parallel processing. To facilitate cardiomyocytes to adhere onto the parylene-C coated transducer surface, fibronectin was chosen as the cell-adhesion promoter. It was coated on the parylene-C coated transducer surface and also on the bottom of parylene-C coated culture well to mimic in-vivo extracellular matrix. Seeded cardiomyocytes were allowed to settle down and develop into spontaneously beating cardiac microtissues. A charge amplifier was served as the interface circuit. The detected mechanical force induced piezoelectric surface charges were monitored and recorded by an oscilloscope. The measured amplitude and profile were direct proportional to the spontaneous contractile force and profile of beating cardiomyocytes.

3. MATERALS AND METHODS

3.1 Microfabrication of the Piezoelectric Transducer Platform

The piezoelectric layer was made by using a commercialized 9 μm thick piezoelectric PVDF thin-film coated with Cu/Ni electrodes (40 nm/15 nm). It was purchased from Measurement Specialties, Inc. Because PVDF has an excellent chemical resistance, it is not easy to pattern it into a complex geometry with wet or dry etching processes. To bypass this limitation, we used a commercialized xurographic machine (Graphtec Corp.) with 10 μm resolution to cut out the structure of the piezoelectric transducer. The developed microfabrication process is summarized in the following: The structure of the piezoelectric transducer was first designed by using AutoCAD software. Then, a 10 mm long by 1 mm wide piezoelectric cantilever plate with 15 mm by 5 mm conducting pad connected to one end was cut out from a letter size PVDF thin-film (Fig. 1(B)). The cutout piezoelectric cantilever plates were placed on a glass slide with a layer of 7 μm thick parylene-C film coated on the exposed side. These one-side coated piezoelectric films were then sandwiched between two casted PDMS rectangular well by using O2 plasma. The thickness of the top and bottom PDMS layers were 2 mm and 1 mm, respectively. These PDMS rectangular wells were made by using standard 10:1 polydimenthylsiloxane elastomer and curing agent mixture, and casted in CO2 laser shaped acrylic molds. The casted PDMS wells were degased and cured in a 60°C oven overnight. A 1 mm thick glass slide was O2 plasma bonded to the bottom layer to seal the culture well. The assembled platform was then coated with a 1 μm thick parylene-C to reach 8 μm and 1 μm thick on each side of the transducer surfaces to complete the piezoelectric transducer core. The sidewalls of the transducer and the bottom of the culture well were also coated with 1 μm thick parylene-C in this process.

3.2 Cardiomyocyte Seeding and Culture

The fabricated piezoelectric transducer platform was sterilized under UV light for 12 hours. To promote cardiomycytes anchoring, a 5 μg/ml concentrated fibronectin (Merck) in sterile DI-water was coated on the transducer surface and on the bottom of the culture well for 1 hr. Excess fibronectin was washed away by 1X PBS and the platform was ready for seeding cells.

Neonatal rat cardiomyocytes and medium-RCGM BulletKit™ supplemented with rat cardiac myocyte basal medium (RCBM) were purchased from Lonza for this study. We followed the standard thawing and initiation of culture processes provided by Lonza instruction manual.[11] Cardiomyocytes were seeded into the piezoelectric transducer platform with 1 x 10^5 cells/cm^2 and 2 x 10^5 cells/cm^2 concentrations.[1,12] Seeded cells were allowed to settle down in a 37 ^oC incubator with 5% CO_2 for four hours. Access cells were removed and washed with PBS followed by changing media every other day. Finally, the cardiomyocyts were fixed and stained with anti-F-actin antibody and anti-α-actinin antibody by using immunostaining method. Cardiomyocytes were then studied by using inverted microscopes (Olympus CKX41 & ZEISS Axio Scope.A1) and a stereoscope (Zeiss SteREO Discovery.V12)

3.3 EXPERIMENTAL

A charge amplifier (Kistler 5011) was connected to the piezoelectric transducer to serve as the interface circuit. The amplified signal was monitored and recorded on a digital phosphor oscilloscope (Tektronix MSO 3014) (Fig. 1). Detected signal was then analyzed in frequency domain by using Fast Fourier Transform (FFT) on the MATLAB software.

4. RESULTS

4.1 Substrate Evaluation

Parylene-C is known to be a biocompatible material. The interaction between native, fibronectin coated or O_2 plasma treated parylene-C surfaces and cells were evaluated by using NIH-3T3 fibroblast and AML-12 hepatocyte.[13] Experimental finding suggested that parylene-C was a biocompatible material, but it was

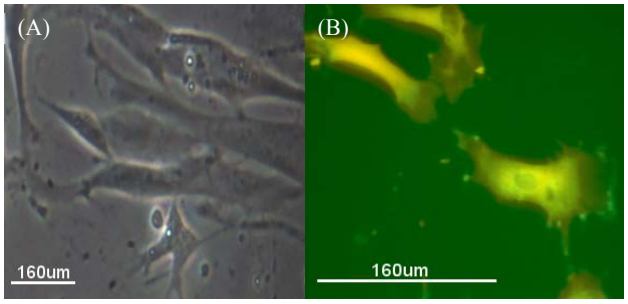

Figure 2. Micrographs of cardiomyocytes cultured on fibronectin coated parylene-C surface at (A) day-6 and (B) day-13, where F-actin and α-actinin were immunostained in red and green, respectively.

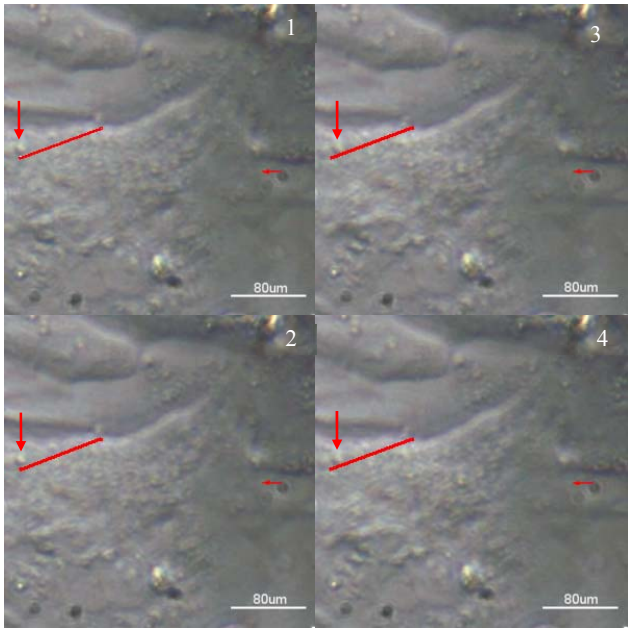

Figure 3. Time lapse images of beating cardiomyocye 2-D like microtissue in two cycles.

not a proper substrate for culturing adherent cells. It was also demonstrated that cells spread well on fibronectin coated parylene-C surface, but had a relatively poor adhesion and morphology on plasma treated surface on the other hand.

To verify that a fibronectin coated parylene-C surface can also provide a biomimetic surface for neonatal rat cardiomyocytes, we monitored cell morphologies for two weeks. We used a loading cell density of 2 x 10^5 cells/cm^2 for this study. Figure 2(A) and (B) show micrographs of cultured cardiomyocytes at day-6 and day-13. The fluorescent signal of α-actinin found on 13^{th} day suggested the maturation of seeded neonatal rat cardiomyocytes on fibronectin coated parylene-C substrate. Multiple synchronous and asynchronous beating cardiomyocytes in 2D-like microtissues were also observed after 6-day culture. Figure 3 shows time-lapse images of one spontaneous beating 2-D like cardiac microtissue in two of the beating cycles. Red lines and arrows are added in the images to demonstrate the motion of beating. The average beating frequency was 2.85 Hz with a standard deviation of 0.21 Hz. The rate of contraction was 0.56 Hz with a standard deviation of 0.01 Hz.

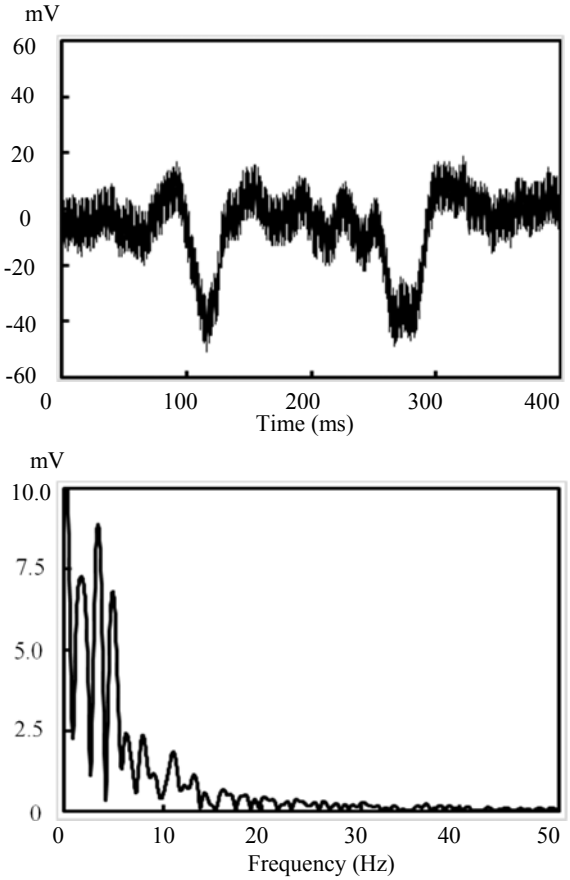

Figure 4. (A) Time trace of the contraction profile of cardiomyocytes cultured on and detected by a piezoelectric transducer, and (B) its frequency response by using Fast Fourier Transform.

4.2 Detection of Spontaneous Beating Cardiomyocytes

To evaluate the capability of the piezoelectric transducer for the detection of anchored cardiomyocytes, the time trace signal of the transducer was monitored. Cell density at 1 x 10^5 cells/cm^2 was loaded in the piezoelectric transducer platform, and electrical signal were monitored and studied after 12-day culture. The transducer sensitivity and scale in mechanical units of the charge amplifier were 7.5 (pC/M.U) and 10 (M.U./V), respectively. A one order low pass filter with corner frequency set at 10 Hz were added to minimize high frequency noise. Figure 4(A) shows one of the time trace signal of a piezoelectric transducer during cardiac microtissue beating. Repeating negative pules were observed. Since the piezoelectric signal is direct related to the mechanical force applied on the transducer surface, this time trace profile could potentially be used to study the contractile force and profile of the corresponding cardiac microtissue. Transforming this time-domain signal into frequency domain by using Fast Fourier Transform, multiple peaks were found below 5 Hz (Fig. 4(B)). It suggested that there might be multiple cardiac microtissues were beating asynchronous on the transducers. Note that the signal of cardiomyocyte contractions were below the 10 Hz corner frequency of the low-pass filter. The influence of the low pass filter should be minimal. Peaks of noises were also found at 60 Hz and 120 Hz (not show), which were far from the frequency domain of the

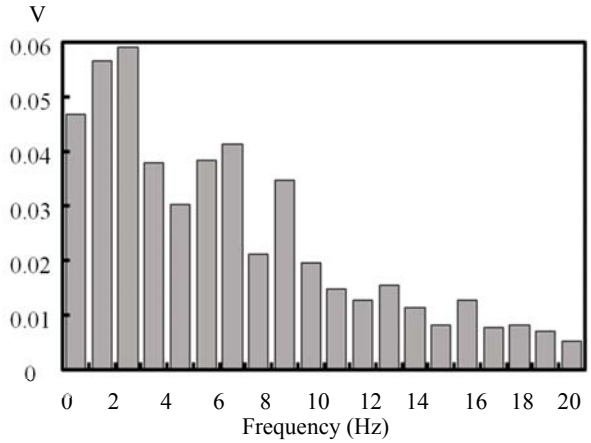

Figure 5. Superposed frequency responses of multiple time tracesignals of two piezoelectric transducers

cardiomyocyte beating frequency. Similar signal responses were also identified on different time traces and also on other piezoelectric transducers. More complicated time trace signals were also observed when multiple cardiac microtissues were beating nearly at the same time. We analyzed 11 FFT results of time traces of two piezoelectric transducers, and added their peak values between every 1 Hz. Figure 5 shows the results of superposed frequency response. It clearly demonstrated that the beating frequency is in the range of several Hz, which is close to reported value for carduiomyocytes cultured in vitro.[14]

5. CONCLUSIONS

We present a piezoelectric transducer platform that can detect the mechanical force and profile of spontaneous beating cardiomyocytes. By using biocompatible piezoelectric PVDF thin-film and parylene-C to construct a 1-D cantilever plate, the piezoelectric transducer provided a good sensitivity to measure the contractile forces exerted by cardiac microtissue in real time. Experimental results demonstrated that the mechanical information of the 2-D cardiac mictotissues could automatically be converted into electrical signals by the piezoelectric transducer. Once the seeded cardiomyocytes can be developed into one continuous 2-D cardiac microtissue and synchronized, this piezoelectric transducer platform can serve as a fully automatic detection platform for drug screening applications. The goal of developing a microplatform for massively, quantitatively, and automatically screening of drugs can be realized.

6. ACKNOWLEDGMENTS

This research was supported by the Ministry of Science and Technology (NSC 102-2218-E-002 -021 -.

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