ABSTRACT

This paper presents the concept of energy harvesting from uniaxially-aligned cardiomyocytes (CMs) on a flexible substrate for the first time. Experimentally, synchronously contracting neonatal rat ventricular cardiomyocytes (NRVCMs) at 0.5Hz have been found to cause the mechanical straining of a piezoelectric energy harvester to produce 87.5nA and 92.3mV of peak current and voltage, respectively. This work has been accomplished: (a) fabrication of a bio-hybrid energy harvester combining living cells, bio-compatible PDMS polymer substrate and piezoelectric PVDF films; (b) engineered living cell patterns on PDMS with uniaxially-aligned direction for enhanced mechanical actuation; and (c) up to one month of continuous synchronous contractions from NRVCMs for energy harvesting demonstration. This paper will detail the concept, design, fabrication, and experiments of the bio-hybrid energy harvester.

INTRODUCTION

With the combination and development of information technologies and medical sciences, more and more promising research on personal healthcare exists in implantable devices which enable for real-time in-body detection and treatment, diagnosis and therapy of major diseases, and organ transplantation and so on. Recent progress in microfabrication and bioengineering technologies are increasingly motivating the development of a variety of miniaturized implantable systems for sensing, health monitoring and deficiency treatments.

Among many diseases that may seriously impair health, some are extremely difficult to cure and only by means of implanted machines or the self-recovery mechanisms of the human body. Medical devices, some of which work in the body to help or replace the function of certain organs, are thus of great necessity. For example, a pacemaker is an electrical device that is implanted in a human body and issues regular electrical impulses to keep the heart beating. Nowadays it has been widely studied and used in clinical application [1]. Additionally, neural prosthesis [2], vascular grafts [3] and orthopedic devices [4], which are familiar to human life, are typically passive devices. The difference between passive devices and active devices is that latter ones need power supply.

Now the mainstream of power supply for implantable passive devices relies on primary or rechargeable lithium-ion batteries that may have a life of up to 5 to 7 years and after that should be replaced or recharged to continue to use [5]. In terms of applications in micro/nano devices, lithium-ion battery is hardly integrated to meet requirements of device sizes; in addition, there are a certain amount of battery safety and environmentally friendly issues. The optimized choice to feed implantable electronic devices is permanent power supplies or the batteries with a cycle life of no less than device lifetime. To achieve power supplies, many studies on the potential of view are carried out on energy conversion from chemistry or mechanical energy to electrical energy [6].

The current research focuses mainly on biofuel cells (BFCs), energy convertors and energy harvesters [7-8]. BFCs make use of in vivo biological substances (such as glucose) to achieve electrical output by electromechanical reaction [9-10]. D. Erickson et al. created a non-enzymatic glucose biofuel cell to achieve high power output, high output stability and great potential for integration [10]. But BFCs have strict requirements for the environment they exist, which causes challenges in packaging and design of fuel transportation. In the energy conversion field, Zhong Lin Wang et al. implanted a piezoelectric generator on a rat heart in 2010 for the first time and obtained steady power output [9], but such energy converter based on mechanical activity harvest vibration from the external environment. If the device works as long as needed, it is easier to make use of the in-body mechanical energy without invasive. To explore in-body usable mechanical vibrations, converting mechanical energy into electricity based on transducer materials is a good idea to power implantable electronic devices [11-12]. Kevin Kit Parker et al. conducted a fundamental study on CMs under the influence of the external environment like that single CM, cultured on various microstructures and shapes, can completely get access to the microstructural and shape constraints [13] and research on the corresponding frequencies of CMs [14]. They published their fancy work about microactuators based muscular thin film (MTF) in Science 2007. They achieved that in vitro experiments, the spatially ordered MTF can generate specific forces as high as 4 millinewtons per square millimeter, which inspires CMs practical applications in microactuators, microrobotics and microgenerators [15].

In this work we present the concept of energy harvesting from uniaxially aligned CMs on a flexible PDMS film. Based on sliding filament mechanism, CMs cultured in vitro convert biochemical energy into kinetic energy efficiently and beat themselves spontaneously. A bio-hybrid energy harvesting device combines living CMs, bio-compatible PDMS polymer substrate and piezoelectric PVDF films. The contractile force is transmitted to the piezoelectric film which is electromechanical coupling, and then the voltage and
current is generated by piezoelectric effect.

**DEVICE DESIGN AND FABRICATION**

**Working principle and device structure**

Muscle cells are micro linear actuators driven by activation of actin-myosin motors, coordinated in space and time through excitation-contraction (EC) coupling. Previously, researchers have demonstrated artificial muscular mechanical actuators [14] and tissue-engineered jellyfish [16] using CMs on a flexible substrate. This work advances these results by showing the feasibility to construct an energy harvester using CMs as the power source.

The principle of the bio-hybrid energy harvester is that uniaxially aligned myocardial cell sheet can generate mechanical stress to drive the piezoelectric film beneath to bend periodically. Figure 1A illustrates the conceptual drawing of the energy harvester, mainly consisting of a PDMS cantilever structure on top of a PVDF thin film coated with gold electrodes on both sides. The uniaxially aligned CMs are grown on top of the PDMS film. The contraction and relaxation of cells in Figure 1B generate mechanical stress on PDMS film and electrical outputs are collected from the PVDF thin film (Figure 1C).

![Figure 1: (A) Schematic of energy harvesting using uniaxially aligned cardiomyocytes, (B) stress of cell sheet varying periodically due to shortening of cardiomyocytes during synchronous contraction, (C) power generation collected from PVDF due to periodic mechanical strain from the contractions of cardiomyocytes.](image)

**Device fabrication**

Figure 2 describes the fabrication process of the bio-hybrid device. Firstly, glass coverslips were cleaned by sonicating for 60min in 95% ethanol and nitrogen dried. Next a sacrificial film was spin coated for 60s at 6,000 RPM using a solution with 10 wt% poly(N-isopropylacrylamide) (PIPAAm) in 99.4% 1-butanol. Sylgard 184 (Dow Corning) polydimethylsiloxane (PDMS) elastomer was mixed at 10:1 base to curing agent ratio and spin coated on top of the PIPAAm coated glass coverslip (step 2A). The PDMS film thickness is 28 um. A piece of PDMS film with the same shape and area as a metallized PVDF film (thick: 28um) was cut and replaced by the PVDF film (step 2B). Part of PDMS film was peeled off from step 2A and bonded on top of the device structure from step 2B (step 2C).

The next step is the key to the uniaxially aligned cell patterns. Microcontact printing (μCP) process is utilized to control the 2D cell patterns with alternating high- and low-density fibronectin (FN) lines for orientating cell bodies/sarcomeres and interconnecting adjacent cells, respectively. PDMS stamps were fabricated with 20um wide, 20um tall ridges separated by 20um spacing. Prior to use, the stamps were sonicated in 50% ethanol for 30min to sterilize and remove surface contaminants. Once dried, the stamps were inked with a droplet of 50ug/mL FN in DI water and incubated for 1 hour and then rinsed twice in DI water to remove excess protein and dried. High density FN lines were transferred from the stamps to the PDMS film by making conformal contact for 1min (step 2D). In order to create high and low density FN lines alternating, a droplet of 2.5ug/mL FN in DI water was spread over the patterned area and incubated on the PDMS surface for 15min (step 2E). Following the incubation period, the PDMS film was washed three times with DI water, air dried (step 2F). CMs were isolated from neonatal rats and seeded on the FN functionalized PDMS film with the concentration of 1 million per ml and cultured in a 37°C & 5% CO2 incubator for 3-4 days (step 2G). Finally, desired shape was cut out and peeled off PDMS film with bonded piezoelectric film (step H). Then the bio-hybrid film was fixed in a culture dish with DMEM media and connected with measuring setup.

![Figure 2: Bio-hybrid device design and fabrication: (step A-C) assemble piezoelectric film and PDMS film; (step D-F): fibronectin patterns using microcontact printing; (step G) isolate and seed neonatal rat ventricular cardiomyocytes; (step H) cut desired shape.](image)

**CELL ISOLATION AND CULTURE**

NRVCMs are isolated from two-day-old neonatal Sprague-Dawley rats based on published protocols [17]. All procedures are conducted in accordance with the guidelines
of the Institutional Animal Care and Use Committee at University of California, Berkeley. Ventricles were surgically isolated and homogenized by washing in Hank’s balanced salt solution (HBSS) followed by digestion with 0.1% trypsin overnight at 4 °C. After the supernatant discarded, the second digestion was started by adding digest enzyme (0.1% (v/v) collagenase type II in HBSS) at 37 °C water bath with stir bar for 5-8min and then was repeated for around eight times until the ventricle tissues became smaller. Subsequently, cells were re-suspended in DMEM culture medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), and 1% (v/v) Penicillin/Streptomycin. The isolated CMs were loaded on the aforementioned devices and incubated under standard conditions at 37 °C and 5% CO2. After 24 hours incubation the devices were washed three times with HBSS to remove non-adherent cells and then covered with media. Subsequently, every other day media was changed with maintenance media.

Images in Figure 3 show the microstructures of cell sheets on PDMS substrates without and with the alternating high- and low-density FN line patterns. In contrast to Figure 3A, the 3rd-day cell image in Figure 3B shows grown and uniaxially-aligned cardiomyocytes. In a recorded video clip, uniaxial contraction of a PDMS film at a frequency of 1 Hz has been observed, which validates the successful construction of confluent and interconnected cell lines.

**EXPERIMENTAL RESULTS**

The fabricated bio-hybrid film is tested under an optical microscope in a culture dish with DMEM media for deformation and bending measurements as illustrated in Figure 4. In the prototyped device of 6mm in length, the bio-hybrid film is found to deform hundreds of micrometers at the tip of the device during one contraction operation of a second in Figure 4A. Figure 4C recorded the specific magnitudes of deformation and bending angle from Figure 4A.

**CONCLUSION**

We succeed in electrical energy harvesting using bio energy of uniaxially aligned heart muscle cells for the first time. A bio-hybrid energy harvester combines living cardiomyocytes, bio-compatible PDMS polymer substrate and piezoelectric PVDF films, and was fabricated by patterning living cells on PDMS and PVDF films with uniaxially-aligned direction for enhanced mechanical actuation. Continuously synchronous contraction of NRVCs can last stable for up to one month for energy harvesting applications. The peak output voltage was measured at 92.3mV, the peak current at 87.5nA, and the calculated power output is 14.6μW/cm^3.

These results show the energy conversion happens from CMs movements to electrical energy for applications in
power supply. In the future, the prototyped energy harvester may be used to power miniaturized pacemakers or implanted micro/nano passive devices. The demonstrated device will probably be a stress sensor that the electrical power output can reflect the mechanical performance of CMs.

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REFERENCES


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