Myocardial Cell Pattern on Piezoelectric Nanofiber Mats for Energy Harvesting

X Liu¹,², X Wang¹,², H Zhao³, Y Du¹
¹Institute of Microelectronics, Tsinghua University, Beijing, China
²Tsinghua National Laboratory for Information Science and Technology, Beijing, China
³Department of Biomedical Engineering, Tsinghua University, Beijing, China

E-mail: wxh-ime@tsinghua.edu.cn

Abstract. The paper presents in vitro contractile myocardial cell pattern on piezoelectric nanofiber mats with applications in energy harvesting. The cell-based energy harvester consists of myocardial cell sheet and a PDMS substrate with a PVDF nanofiber mat on. Experimentally, cultured on specifically distributed nanofiber mats, neonatal rat ventricular cardiomyocytes are characterized with the related morphology and contraction. Previously, we have come up with the concept of energy harvesting from heart beating using piezoelectric material. A bio-hybrid energy harvester combined living cardiomyocytes, PDMS polymer substrate and piezoelectric PVDF film with the electrical output of peak current 87.5nA and peak voltage 92.3mV. However, the thickness of the cardiomyocyte cultured on a two-dimensional substrate is much less than that of the piezoelectric film. The Micro Contact Printing (μCP) method used in cell pattern on the PDMS thin film has tough requirement for the film surface. As such, in this paper we fabricated nanofiber-constructed PDMS thin film to realize cell pattern due to PVDF nanofibers with better piezolectricity and microstructures of nanofiber mats guiding cell distribution. Living cardiomyocytes patterned on those distributed piezoelectric nanofibers with the result of the same distribution as the nanofiber pattern.

1. Introduction
Making good use of advanced smart materials in the field of biological and biomedical applications is promising to construct various biological robotics or “bio-bots” enabling sensing [1], drug delivery [2], tissue engineering [3] and energy transduction [4]. Combining biological entities such as DNA, cells or tissues with soft materials can yield soft bio-bots with the ability to dynamically sense and adapt to environmental cues or themselves cues with or without external stimuli triggered. Mechanical interactions are fundamental to cellular biology and physiology. Recent advances in cardiac muscle tissue engineering have yielded dense tissues that form a syncytium, with the coordinated propagation of electrical signals and synchronous contraction of engineered muscle [5]. This advantageous property has helped to produce machines that include self-assembling microelectromechanical-system-based cantilevers [6], 2D biohybrid “muscular thin films” [7], and “crab-like” robots [8]. These systems were powered by applied electric field stimulation or spontaneous contraction of engineered muscle.
cardiac muscle, which have also been used as power sources for loco-motive machines or implanted microdevices.

Previously, we have come up with the concept of energy harvesting from heart beating using piezoelectric material [9]. Figure 1A illustrates the potential applications of cell-based energy harvesting which tends to adhere to the heart tissue to supply the pacemaker or other implanted microdevices. According to the filament sliding mechanism, the cardiomyocyte drives the piezoelectric fiber to bend periodically, as shown in the meshed of Figure 1A. A bio-hybrid energy harvester combined living cardiomyocytes, PDMS polymer substrate and piezoelectric PVDF film with the electrical output of peak current 87.5nA and peak voltage 92.3mV, as shown in Figure 1B. However, the bending angle of the bio-hybrid film just attains less than 20°, which is much lower than expected. There are several challenges existing in the design and fabrication of the device. The thickness of the cardiomyocyte cultured on a two-dimensional substrate is much less than that of the piezoelectric film. The Micro Contact Printing method used in cell pattern on the PDMS thin film has tough requirement for the film surface [7]. As such, we fabricated nanofiber-constructed PDMS thin film to realize cell pattern, due to PVDF nanofibers with better piezoelectricity and microstructures of nanofiber mats guiding cell distribution. The conceptual drawing of the cell-based energy harvester in Figure 1C consists of myocardial cell sheet and a PDMS substrate with a PVDF nanofiber mat on. The PVDF nanofibers were electrospun with specific collectors used.

2. Experimental
To construct the structure of the piezoelectric bio-bot, we applied far-field electrospinning technology to produce and used specific grounded gap collectors collecting electrodes to settle monolayer of PVDF piezoelectric nanofibers on a semi-cured PDMS thin film with two gold microwires partially imbedded in. The schematic of the device design is outlined in Figure 1C, and shows several key features. First, the piezoelectric nanofiber arrays are partially imbedded into the underlying PDMS substrate. Second, two gold microwires, as the device electrodes, are sandwiched between the nanofiber arrays and the PDMS film. We observed both nanofiber patterns for their ability to support

![Figure 1.](image-url)
cardiomyocytes and provide a compliant settlement for myocardial cell sheet contraction. In Figure 2A, a pair of parallel electrodes designed as the gap collector was grounded to collect uniaxially aligned nanofibers. When one end of the electrospun nanofiber lies across one of the two electrodes, the other end of the fiber is enforced to settle down across the other electrode under the electrostatic force between the electrospun nanofiber and the grounded electrodes. So the monolayer of PVDF nanofibers was formed and self-ordered on the PDMS film substrate with their ends across the collecting electrodes (Figure 2B). Scanning electron micrographs yield quantitative information on the degree of alignment, as shown in Fig. 2C. The nanofiber alignment is with up to 75% perpendicular between nanofibers and the collecting electrode. In contrast, randomly distributed straight nanofibers were realized by concentric ring electrode (Figure 2D) and the statistical result of the angle is uniformly distributed (Figure 2E&2F).

To extract quantitative information about cardiomyocyte contractions, the piezoelectric response of PVDF nanofibers must be fully characterized. We analyzed crystalline structure and dipole orientation of the nanomaterial. X-ray diffraction (XRD) patterns provide information on the long-range order and the crystalline structure of electrospun PVDF nanofibers, shown in Figure 3. The results indicate that the overall crystallinity of the material in the aligned arrays is as the same as that of in the random networks. The nanofiber exhibits a prominent peak at $2\theta=20.6^\circ$, corresponding to the (110) reflection of the $\beta$ phase formation. A distinguishing feature of the electrospun nanofibers is that the 30 kV high voltage significantly enhances the fraction of the polar $\beta$-phase.
Results and discussion

We engineered two kinds of 2D myocardial tissue on fibronectin-coated monolayer PVDF nanofibers: anisotropic (Figure 4A) or isotropic (Figure 4B) cell sheets by passive seeding of dissociated ventricular cardiomyocytes. The loaded cells exerted traction forces on the nanofibers via integrin attachments to compact the cells into a 2D muscle strip over time. For both kinds of nanofiber pattern, the nanofibers served as physical cues for the inter- and intracellular organization of cardiomyocytes into a tissue and the uniaxial coupling of sarcomere ensembles over length scales from micrometers to centimeters. Synchronized actuation of the array of contractile cardiomyocytes is critical for optimal functionality and can be achieved by their electrical coupling through gap junctions. From the 1st day to the 5th day of cell culture, the cardiomyocytes loaded on the aligned nanofibers are adhering to the nanofibers gradually and forms the uniaxial cell pattern eventually. Compared with Figure 4B, the 5th-day cell image in Figure 4A shows uniaxially aligned living cardiomyocytes on parallel nanofibers. The muscle strips therefore displayed functional behavior characteristic to physiological skeletal muscle. Anisotropic 2D myocardium had uniaxial alignment of cell bodies (Figure 4A). The 3D deflection of the bio-bot film depended on the direction of cell alignment relative to the nanofiber pattern. Instead of relying on external signal simulation, the contractions of the myocardial cell sheets can be controlled and paced via spontaneous muscle contraction. This bio-inspired design mimics the in vivo musculoskeletal arrangement in which force transmission occurs from a contracting muscle to bone through a connecting tendon.

Results presented here indicate that aligned PVDF nanofibers can be formed into flexible, settled monolayer, by use of electrospinning onto a specifically designed gap collector. The process yields alignment at both the level of the fibers and the cardiomyocytes, thereby enabling high piezactive β-fraction without further processing and excellent contractile response, respectively. At one hand, soft robotic film can form a biocompatible interface with cells to act as sensitive extracellular probes to detect minute cellular deformations. At other hand, this can generate electricity to supply implanted devices, acting as biogenerator. As expected, the stiffer PDMS structures offered a greater resistance to bend; thus, films with higher elastic moduli exhibited a lower deflection in response to passive tension forces exerted by the muscle strips.
4. Conclusion
To summarize, we demonstrated a cell pattern method on piezoelectric nanofiber mats with applications in energy harvesting. From sarcomeregenesis to the integration of the biochemical and electrical networks, engineered muscle remains an attractive method for building actuators and powering devices from the micro to macro scales. Our research shows a feasible approach to scavenge the biomechanical energy inside the body, such as heartbeat, blood flow, muscle stretching, or even irregular vibration. The new epidermal bioenergy paradigm thus holds considerable promise as a viable autonomous power source for implantable self-powered electronics.

5. Acknowledgements
This work is supported by grants from Tsinghua National Laboratory for Information Science and Technology (No. 042003130).

6. References
[1] C. L. Schmidt and P. M. Skarstad 2001 *Journal of Power Sources* 97–98 742
[2] L. R. Hochberg, M. D. Serruya, G. M. Friehs 2006 *Nature* 442 164
[3] R. A. Roeder, G. C. Lantz, L. A. Geddes 2001 *Biomedical Instrumentation and Technology* 35 110
[4] S. M. Kurtz et al. 2010 *Pacing and Clinical Electrophysiology* 33 705
[5] Vunjak-Novakovic G, et al. 2010 *Tissue Eng Part B Rev* 16 169
[6] Xi J, Schmidt J J, Montemagno C D 2005 *Nat Mater* 4(2): 180
[7] Feinberg A W, et al. 2007 *Science* 317 1366
[8] Kim J, et al. 2007 *Lab Chip* 7 1504
[9] X. Liu et al., *Proc. MEMS 2014*, pp. 159-162.

**Figure 4.** Microstructures of 2D cardiomyocyte sheets with piezoelectric PVDF nanofiber mats: with uniaxial alignment (A) and random pattern (B), respectively. (A) From the 1st day to the 5th day of cell culture, the cardiomyocytes loaded on the aligned nanofibers are adhering to the nanofibers gradually and forms the uniaxial cell pattern eventually. (B) The growing process of the cardiomyocytes is as the same way as (A), but causes the random cell pattern. (Scale bar: 50um)