Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

In vitro Cardiomyocytes-Driven Biogenerator Based on Aligned Piezoelectric Nanofibers

Xia Liu [a,b], *Hui Zhao* [c], *Yingxian Lu* [a,b], *Song Li* [d], *Liwei Lin* [e], *Yanan Du* [c], *Xiaohong Wang* [a,b*]

a. *Institute of Microelectronics, Tsinghua University, Beijing 100084, PR China.*

b. *Tsinghua National Laboratory for Information Science and Technology, Tsinghua University, Beijing 100084, PR China*

c. *Department of Biomedical Engineering, School of Medicine, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, Tsinghua University, Beijing 100084, PR China*

d. *Department of Bioengineering, University of California, Berkeley 94720, USA*

e. *Department of Mechanical Engineering, University of California, Berkeley 94720, USA*

*Address correspondence to: wxh-ime@tsinghua.edu.cn

EXPERIMENTAL METHODS

Controlling viscosity of PDMS thin film

Once mixed, the PDMS prepolymer slowly increases in viscosity. We made use of the varied viscosity of PDMS under heat curing to guarantee the robustness of the biogenerator devices. The curve of viscosity to time is dependent on the temperature (Figure S1). For example of a 4-μm-thick PDMS film, the viscosity maintains constant at room temperature within 100 minutes, while the PDMS layer was solidified at 100 °C for 60 minutes. In both cases, the viscosity retention is difficult to control. Then at 60 °C the viscosity increases linearly within 30 minutes and then parabolically.

Deflection response of the biogenerator

After about five days of cell culture, samples were placed at room temperature, so that as the PNIPAM layer was cooled below 35 °C, it transitioned from a hydrophobic state to a hydrophilic state and began to dissolve. Once the PNIPAM layer dissolved, the samples were cut into rectangular shapes (6 mm x 9 mm) along the gold wires and then the contractions of the cardiomyocytes pulled the CCDPN biogenerators to remove from the cover slip. The wires were fixed at the edge of the Petri dish. The length of the wires is 3 cm enough to enable the biogenerator to swim freely.

Electrical output measurement

The CCDPN biogenerator was tested full-course in an anechoic chamber that makes a quite difference in collecting electrical output. The biogenerator performed in the culture medium at 37 °C. The open-circuit voltages and short-circuit currents were measured using a nanovoltmeter (2282A, Keithley) and a picoammeter (6485, Keithley) with Labview software, respectively.

SUPPORTING TEXTS

Additional information of the influence of the experimental conditions on the nanofiber piezoelectricity

The ratio of DMAC/acetone is kept in 4:6. The experiments of electrospinning were carried out at room temperature and 60% humidity.[1] The electrospinning setup worked in a windtight chamber to avoid the airflow to influence the nanofiber alignment.[2] High voltage of 30 KV is applied to increase in the piezoelectric β phase fraction.[3]

Assembling the piezoelectric nanofibers and the generator electrodes

The aligned PVDF nanofiber mat we fabricated consists of two to four layers of nanofibers. In order to directly connect the piezoelectric nanofibers with the gold wires, we conceived a semi-curing method that is the PDMS substrate is semi-cured with certain viscosity maintained. The semi-cured PDMS substrate is used to support the gold wires and then collect the as-electrospun nanofibers across the gold wires, as shown in Figure S3. In this case, fused junctions are formed between the nanofibers and the gold wires. The contact resistance is quite lower than using conductive silver adhesive to connect the nanofibers with the generator electrodes.[4] The optimized voltage output is close to the potential of the two ends of the nanofiber mat.

Supplementary References

[1] D. Dhakras, V. Borkar, S. Ogale, J. Jog, *Nanoscale* **2012**, *4,* 752.

[2] E. Cozza, O. Monticelli, E. Marsano, P. Cebe, *Polym Int* **2013**, *62,* 41.

[3] J. Fang, H. Niu, H. Wang, X. Wang, T. Lin, *Energy Environ. Sci.* **2013**, *6*, 2196.

[4] B. Hansen, Y. Liu, R. Yang, Z. Wang, *ACS Nano* **2010**, *4*, 3647.

SUPPORTING FIGURES

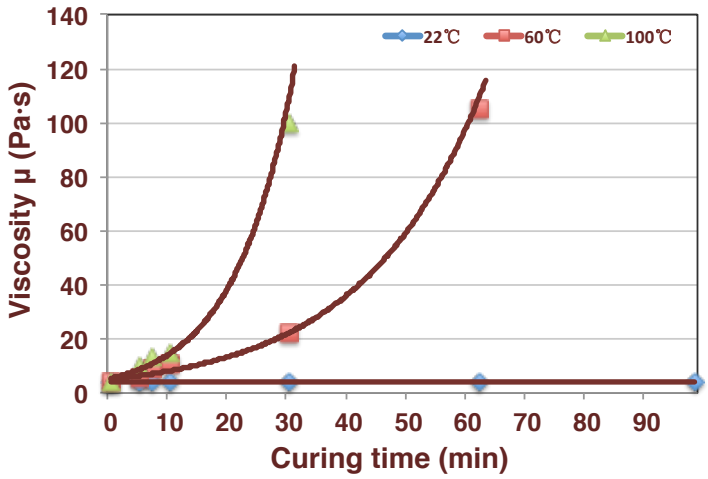

Figure S1. Dependence of PDMS viscosity on curing time. The viscosity (μ) increases with the curing temperature increasing exponentially at 60 ℃ and 100 ℃, while the viscosity almost maintains constant at room temperature (22 ℃) within about 100 minutes. (The data was collected based on the condition that the thickness of the PDMS film is 4 μm.)

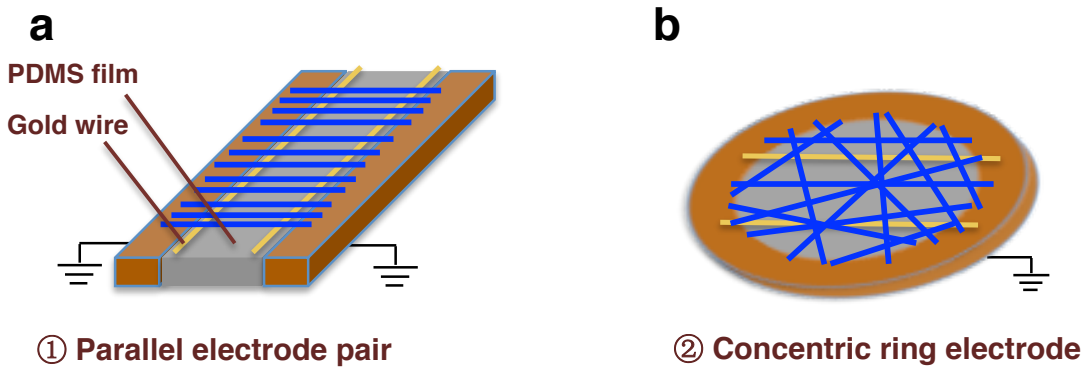

Figure S2. Schematic drawing of the electrodes for guiding and collecting the as-electrospun nanofibers on the PDMS film. (a) The parallel electrode pair was designed to collect uniaxially aligned PVDF nanofiber mat. The as-electrospun nanofibers crossed the gold wires and were settled down on the PDMS film. (b) The concentric ring electrode grounded was expected to pattern monolayer of isotropic nanofibers.

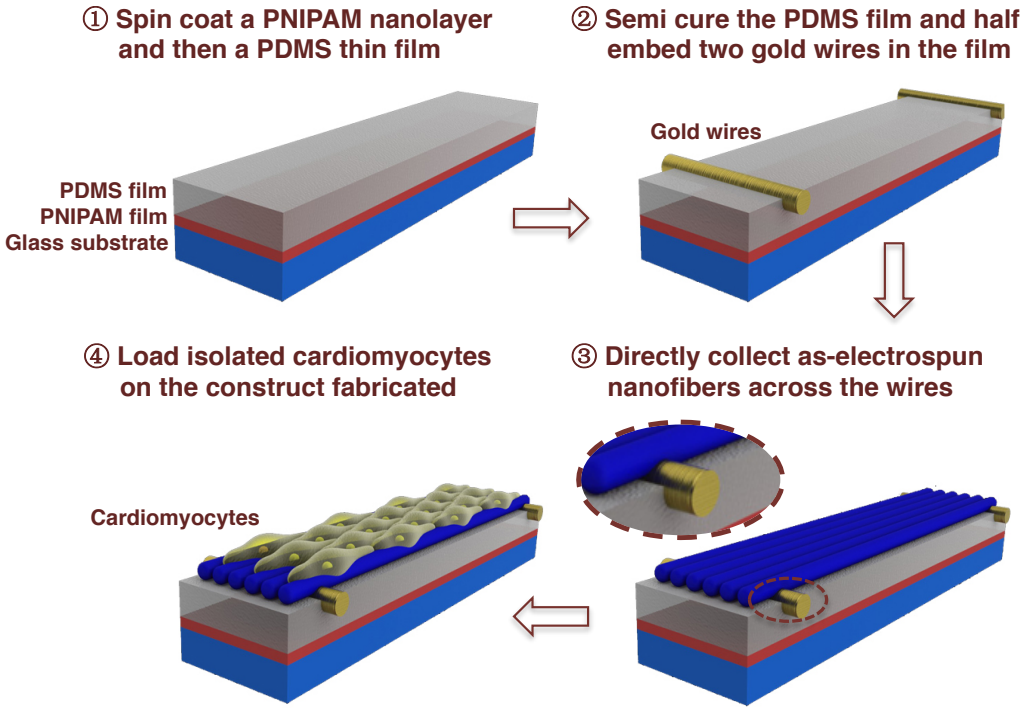

Figure S3. Fabrication schematic of the CCDPN biogenerator device. (1) The substrate was fabricated on a glass slide with PNIPAM nanolayer that acted as a satisficing film to release the top layer, the PDMS microfilm. PNIPAM, a thermally responsive polymer, has a sharp lower critical solution temperature of ~32 °C, a hydrophobic state at temperatures greater than this temperature. (2) The PDMS was semi cured with certain viscosity maintained. Then two gold wires (their diameter is 1.5 μm) as the biogenerator electrodes were partially embedded in the PDMS film. (3) The substrates were used to directly collect PVDF nanofibers crossing the parallel wires through electrospinning technique. (4) Isolated cardiomyocytes were placed on the construct fabricated and then self assembled on the nanofiber mat. The fabrication of CCDPN biogenerator based random pattern is similar as the depicted, except for the nanofiber pattern.

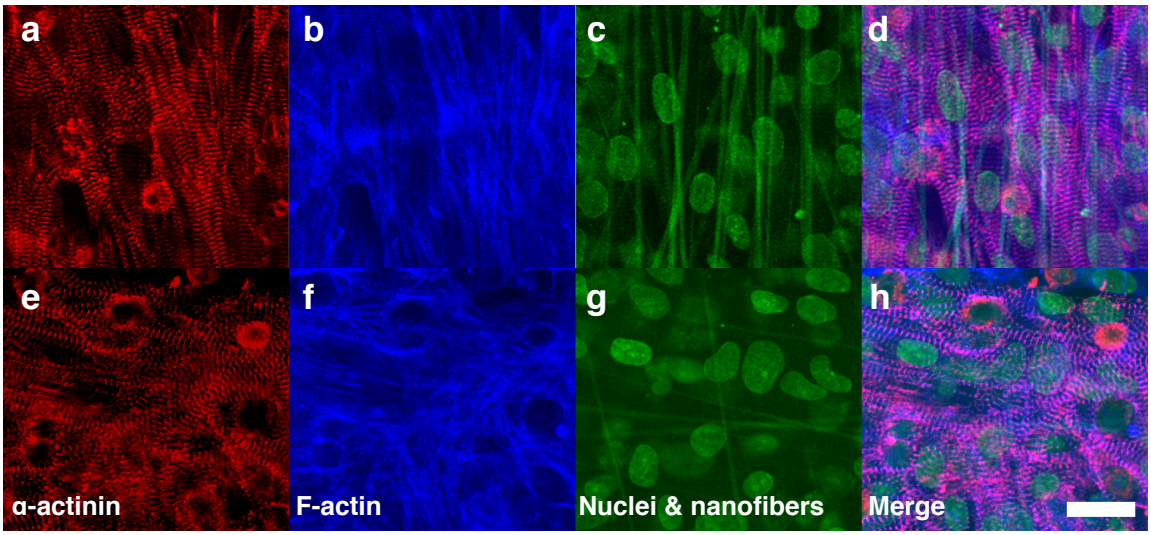

Figure S4. Confocal immunofluorescence of sarcomeric α-actinin (red) F-actin (blue), and nuclei and nanofibers (green) shows cell alignments along the fiber axises. (a-d) Anisotropically patterned cardiomyocytes on uniaxially aligned nanofiber mat. (e-h) Isotropically patterned cardiomyocytes on randomly distributed naonfiber mat. After five days of cell culture, cardiomyocytes spread out and grow along nanofibers (c,g) showing the resultant same similar pattern with the nanofiber pattern (a,b,e,f). (The scale bar is 20 μm.)

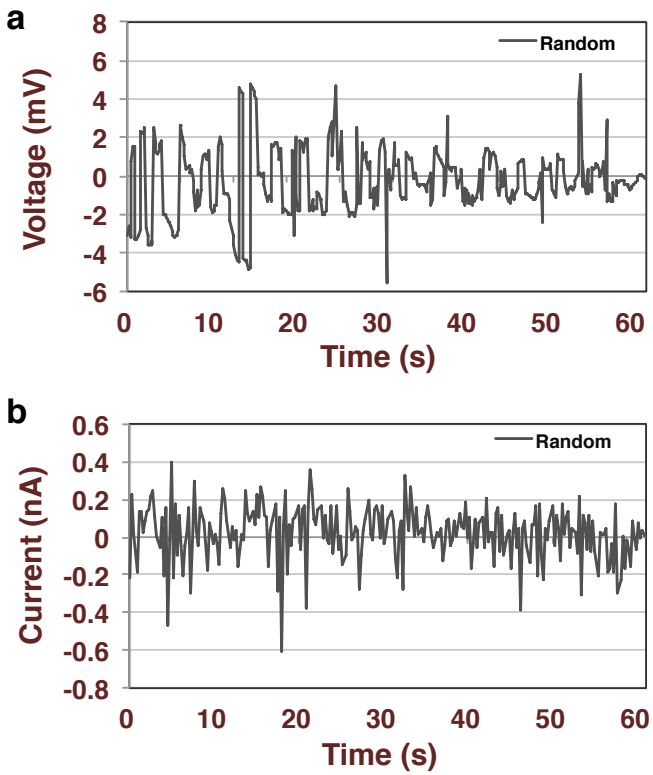

Figure S5. Electrical output of the CCDPN biogenerator based on random pattern. (a) Open-circuit voltage signal from the biogenerator. (b) Short-circuit current signal from the biogenerator.

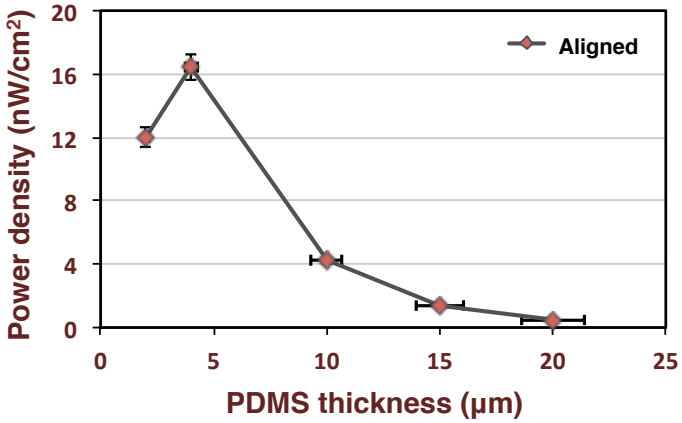

Figure S6. Dependence of power density on PDMS thickness. The power density decreases with the PDMS film thickness increasing, except when the thickness of the nanofiber mat is less than that of the PDMS film. Since the pizoelectric nanofiber mat has maximal electrical output when it works at either a compression state or a tension state. (The concentration of cardiomyocytes on uniaxially aligned nanofiber mat is 1.0 million/ml.)

SUPPORTING MOVIE CAPTIONS

Movie S1. Anisotropic contraction while measuring by AFM

The myocardial cell sheet from Movie S1 is under the cantilever of atomic force microscopy. When a constant force is applied on the cantilever, the contraction characteristics (like vertical deflection, height) are recorded real-time.

Movie S2. Isotropic contraction while measuring by AFM

Under the same condition as Movie S1, the contraction characteristics of the random myocardial cell sheet are recorded in real time.

Movie S3. Buckling of the aligned bio-bot film after releasing

The bio-bot film consists of anisotropic myocardial cell sheet along the long axis of nanofibers. The film exhibits self-exciting behavior powered by successive contraction.