Supporting Materials

Supporting Figure S1: Displacement of point A (see Fig. 1b) as a function of the displacement of the probe. Five curves represent five cycles of probe motion. The displacement of A is about 1/3 the probe displacement. The linearity of the curves is due to linear elastic material property of the PA gel within the strain it has undergone during the motion. Monotonicity of the curves implies that there is no stick-slip motion of the probe. This 3:1 ratio is used in the computational studies to simulate the probe experiment with the single cell.

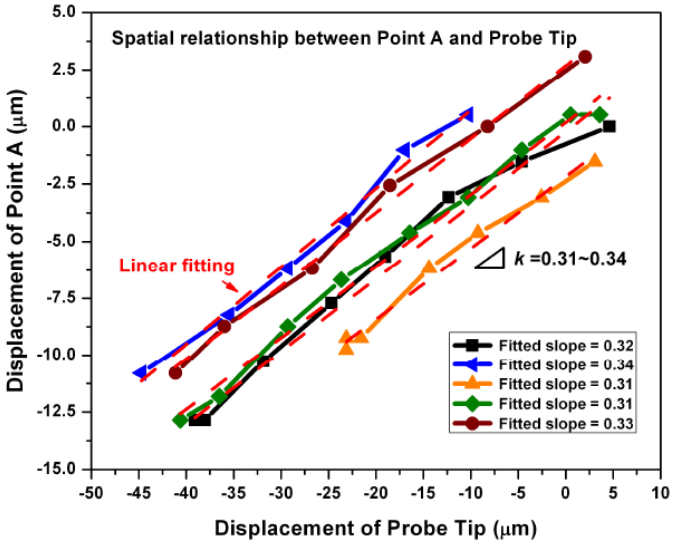
Supporting Figure S2: Histograms of the periods of mechanical stretching of cardiac cells by a mechanical probe and the activated cells. The cells are confirmed as quiescent, i.e. not beating, by 4 minutes of continuous observation prior to stimulation. The quiescent cardiac cells begin to beat after a few cycles of stimulation. The beating frequency of the stimulated cells is high during stimulation. After stimulation is removed, beating frequency drops. The post stimulation periods of beating are shown in the histogram.

Supporting Figure S3: Finite element modeling of a cardiac cell, the soft substrate, and a mechanical probe. The rigid probe and the cell are attached to the soft substrate. The cell is 4 μm thick, and 45 μm in diameter. The probe is 128 μm in diameter. The probe applies a prescribed in-plane displacement to the substrate. The corresponding displacement and the stress fields of the substrate and the cell, as well as the cell-substrate traction are computed by finite element method. The probe actuation is represented by a prescribed displacement field over a circular region of the substrate. Substrate elastic modulus is 1KPa, and the cell modulus is varied until the computed stretch of the cell due to the displacement of the probe matches the experimentally measured stretch (see Fig. 5). Point A in the figure represents a reference marker on the substrate found in the experiment.

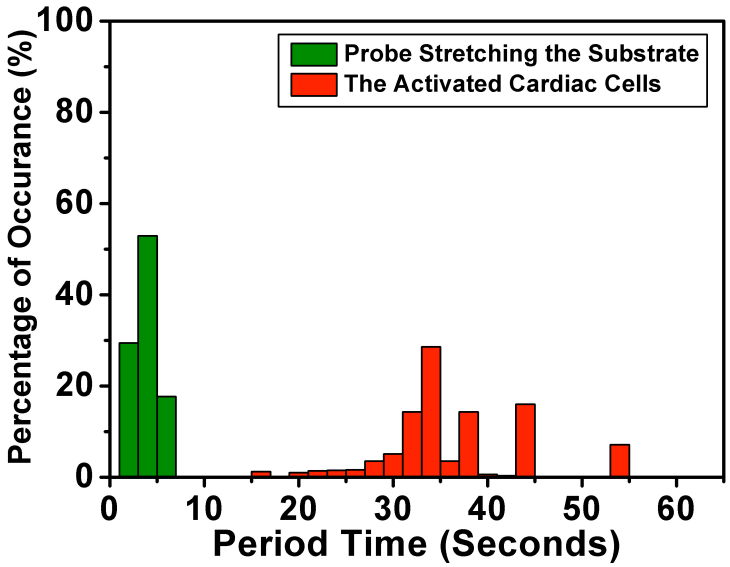
Supporting Figure S4: The mechanical stiffness of soft substrate (polyacrylamide gels) is calibrated by Atomic Force Microscope (with Silicon nitride tip). The thickness of polyacrylamide gels is 70 μm. Indentation depth of Silicon nitride tip on the gels surface is limited to 0.5 - 2 μm to avoid glass substrate effect. The least-square fitting of Hertz theory (see supplementary Materials and Methods) is applied to fit experimental force-indentation data. The stiffness of gels are determined as $E = 1.05 \pm 0.17$ kPa ($n = 45$). The stiffness of 47 kPa gels was confirmed by following the same methods.

Supporting Figure S5: How far the cells can see each other depends on the stiffness of the substrate and the distance between the cells. The figure shows the qualitative relationship (solid line) between the elastic modulus of a substrate and the distance between two cells to achieve a prescribed stretch (or strain ε) in a cell due to the contraction in the other. Here ε_{th} is a threshold strain for which a cell gets stimulated. For $\varepsilon < \varepsilon_{th}$, the cells become mechanically decoupled. And their beating may become incoherent, or they may cease to beat. If the tissue stiffens, the cells need to be closer for mechanical coupling.

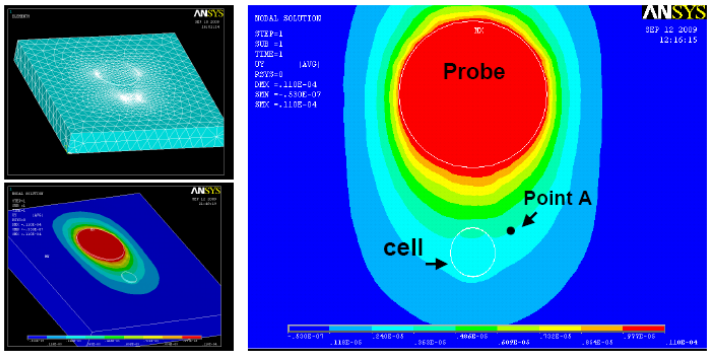
Supporting Figure S1:



Supporting Figure S2:



Supporting Figure S3:



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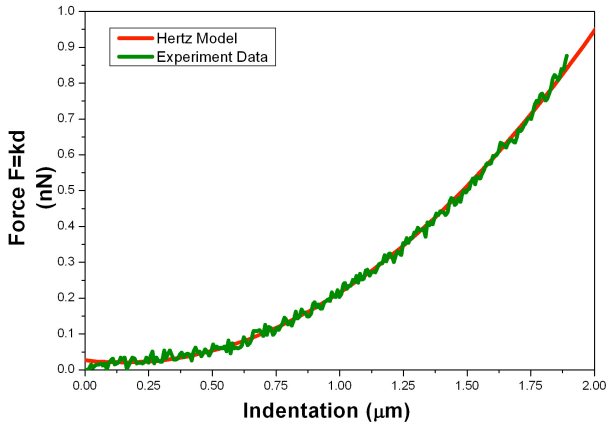
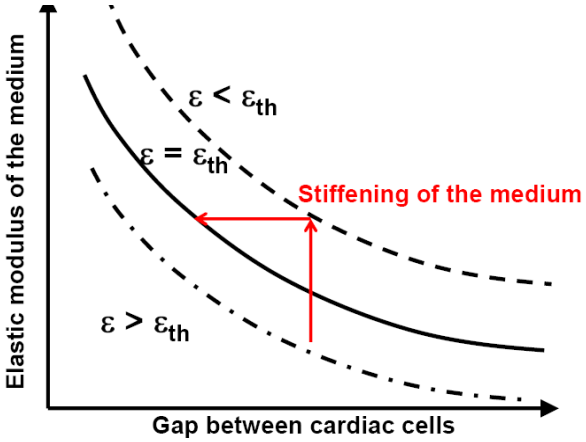


Supporting Figure S5:



Supporting Movies

Supporting Movie S1: A group of beating cardiac cells on soft (1 KPa) substrate. The nearby pair (cell 1 and cell 2), about 42 μm apart, beat with same frequency (64 cycles/min), although their beating is phase lagged by about half a period. The cell 3, which is farther apart, about 65 μm away from cell1 and 89 μm away from cell2, beat with a frequency of 70 cycles/min. Cell 2 stretches cell1 by about 2.5 ± 0.8 %.

Supporting Movie S2: On soft (1 KPa) substrate, two cardiac cells are connected by connecting tissues over long distances. They become mechanically coupled, and they can synchronize their beating (both beating frequency and beating phase).

Supporting Experimental Materials and Methods:
Gel preparation and AFM calibration

Polyacrylamide (PA) gels were prepared following the protocols described by Y. L. Wang *et al* [1]. The soft PA gels contain acrylamide solution (Bio-Rad Laboratories, Hercules, CA) with concentration 8% (mol/l) and N, N'- Methylene bis-acrylamide solutions (Bio-Rad Laboratories, Hercules, CA) with concentration 0.01 % (mol/l) to induce crosslinking levels. The gels are adhered to the activated glass cover-slides (Fisher Scientific, Pittsburgh, PA). All the substrates were covalently coated with 4 μg/mL laminin (BD Company, Franklin Lakes, NJ) to enhance cardiac cell adhesion. Asylum atomic force microscope (AFM) with pre-calibrated silicon nitride tip was used to characterize the stiffness of PA gels in phosphate buffered saline (PBS, Invitrogen, pH=7.4) solution. The spring constant of the AFM cantilever was 148.14 pN/nm. It was used to perform the indentation test with a speed of 0.1 μm/sec. Hertz theory (Eq. 5) was used to extract the elastic modulus of the indented substrates [2-4].

$$z - z_0 = (d - d_0) + \sqrt{\frac{k(d - d_{0)}}{\frac{2}{\pi} E(1 - v^2)\tan(\alpha)}} \qquad \text{(Eq. 5)}$$

In Equation 5, k is the spring constant of cantilever calibrated by resonant frequency counting. Z and d are cantilever's base displacement and tip deflection, respectively. Z_0 is the piezo-controller's vertical position as the AFM tip touches the gel surface, and d_0 is the initial cantilever deflection prior to bending. v is the Possion's ratio of sample (0.5 for hydrated gels in the present study). $\alpha = 35^o$ is the half open-angle of cantilever tip and E is the elastic modulus of sample. A representative example of force vs. indentation of the substrate and the theoretical fitting curve is shown in supplementary materials. Soft PA gels' stiffness is calibrated as 1.05 ± 0.17 kPa (see Supporting Fig.S4).

Cell Extraction and Cell Culture

Cardiac cells were extracted from chicken embryo between the 32-35 Hamburger Hamilton stage, roughly when the chicken embryo was 8 days old [5]. The eggs were removed from the egg incubator after they were 8 days old and candled to check for the growth of the embryo. Only eggs with developed embryos were used. The eggs were sterilized using 70% ethanol and then they were opened. The embryo was removed and placed in a petri dish containing Puck's Saline and its head was decapitated. The thoracic region of the embryo was opened and the heart was isolated. The heart was then rinsed a couple of times in a PBS solution to remove the blood. The heart was put in a solution containing 0.05% trypsin/PBS (Invitrogen) and was left there for about 15 minutes after which the solution was pipetted gently to break the heart. The cells were plated on the laminin coated substrates and cultured in minimum essential medium (MEM) with alpha modification (Sigma), supplemented with 10% fetal bovine serum (FBS) (Sigma) and antibiotics (penicillin, 1000 IU/mL and streptomycin, 1000 μg/mL) (Gibco). The cells were left undisturbed in the incubator overnight and measurements were started only on the next day. The cell culture media was changed every two days. During data acquisition, the gels and the culture dishes were kept on heated microscope stage to maintain a temperature of 37^{o}C and a tube releasing 5% CO_2 was kept over the dishes to maintain a physiologically relevant pH.

Tungsten Probe Stretching System Setup

A rigid Tungsten probe is mounted on the X-Y-Z micro-positioning stage. The X-Y-Z micro-positioning stage is mounted beside the inverted phase-contrast microscope (Olympus IX81). The probe tip is immersed into culture medium in culture dish and lightly indents the soft substrate at the region 40 ~ 70 μm away from the quiescent cardiac cells (Figure 1a). The target quiescent cardiac cells are selected after more than 3-minute observation to ascertain they are not beating with very low frequency. Driving by the X-Y-Z micro-positioning stage, the Tungsten probe cyclically pulls the soft substrate beside the cells in a pulsatile or sinusoidal fashion at the frequency 20 ~ 45 cycles per minute. As the soft and flexible substrate is cyclically pulled, the cells which adhere on substrate and near the probe pulling region (40 ~ 70 μm away) are in turn cyclically stretched. The Tungsten probe is selected because of its high stiffness and effective force application. The Tungsten probe is not chemically functionalized and sterilized by Ultra-violet ray (UV) before experiment. Therefore, no biochemical cues but only mechanical stimulus are introduced during study.

Imaging and data analysis

The videos of the beating cells were taken using a digital Cannon camera and a high speed SPOT camera. The image analysis and processing are done using ImageJ, Matlab 7, and Photoshop CS2.

References in Supporting Materials and Methods

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3. A. J. Engler, F. Rehfeldt, S. Sen and D. E. Discher, *Methods in Cell Biology*, 2007, **83**, 521-545.
4. R. Dong, T. W. Jensen, K. Engberg, R. G. Nuzzo and D. E. Leckband, *Langmuir*, 2007, **23**, 1483-1488.
5. V. Hamburger and H. Hamilton, *Journal of Morphology*, 1951, **88**, 49-92.