Supporting information

Effect of AFM-based electrical stimulation on electrophysiological and mechanical characteristics of cardiomyocytes

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S1. Schematic diagram for testing electrical and mechanical properties

(1) Specific parameters and measurement mode of lab-made AFM

The effects of the electrical stimulation (ES) on the action potential and contractile force of cardiomyocytes were measured by lab-made AFM. The electrical signal detection module mainly consists of the conductive probe, conductive probe holder, four-channel data acquisition card (Input channel: 4; Input voltage range: ±42.4 V; Sampling rate: 204.8 kS/s; Resolution: 24 bits; Signal-to-Noise Ratio (SNR): 118 dB; Software-selectable IEPE signal conditioning and AC/DC coupling; National Instruments NI4462) and electrical signal acquisition software (LabVIEW). Using the conductive probe (SCM-PIC-V2, Burker; Length: 450 μm; Width: 35 μm; Thickness: 1.8 μm; Half-cone angle: 20°; Tip radius: 25 nm; Spring constant: 0.1 N/m; Resonance frequency: 10 kHz) combined with a force feedback system, the contractile force and action potential of cardiomyocytes can be recorded, simultaneously. The system has a measurement range of 100 μm × 100 μm in the X-Y direction and 15 μm in the Z direction, and it is controlled by closed-loop system. The resolution in the X-Y direction was 0.2 nm, the resolution in the Z direction was 0.1 nm, and the mechanical resolution was 30 pN. The acquired data were processed using the equipped data analysis software, including filtering, baseline correction, contact point identification and adjustment and elastic model fitting, and the Hertz-Sheldon model was used to fit the resultant force-distance curves. Therefore, the system has nano-scale spatial resolution and pico-newton force resolution. The developed constant force contact mode was adopted in the contraction-relaxation process of cardiomyocytes, and the force exerted on the cell membrane was kept constant through the force feedback system. In addition, the force applied to the cardiomyocyte membrane during the measurement was 0.58 nN, and both the approach and retraction speeds were set to 10 μm/s. In addition, the non-contact area between the probe and probe holder was designed in an insulation structure, and low-pass filtering and other algorithms were used to reduce noise.

Constant force contact mode: In the process of the contraction-relaxation process of cardiomyocytes, the force exerted on the cardiomyocyte membrane was always kept constant through the force feedback system. In this process, the conductive probe moves up and down in the Z-axis direction with the contraction-relaxation of cardiomyocytes. Conductive probes always keep constant contact force with cardiomyocytes, so that cells and conductive probes always keep a stable sealing relationship. The force applied to the cardiomyocyte membrane was 0.58 nN in the constant force contact mode, and the height (Z Height) change of the piezoelectric actuator in the Z-axis direction was recorded during the contraction-relaxation process of cardiomyocytes.

(2) Specific parameters of the electrochemical comprehensive test system

Electrochemical comprehensive test system: The electrochemical comprehensive test system is a high-performance instrument with a high voltage range of ±48 V and a maximum current output of ±4 A. It supports a variety of experimental applications, including electrochemical research, corrosion and coating testing, sensor development, biomedical research and nanotechnology-related experiments. This equipment has the characteristics of high precision (Resolution is 1.2 fA), high-speed sampling (1 μs) and a wide frequency range (10 μHz~10 MHz), which makes it suitable for various scientific research fields.

(3) Circuit scheme and setup scheme of electrical stimulation of cardiomyocytes

As shown in Fig. S1(a), welding points were fabricated on the conductive glass (ITO) with wires connected to serve as the ground electrode, while a probe was used as the working electrode, enabling the simultaneous measurement of the electrophysiological properties and contractile behavior of cardiomyocytes. In Fig. S1(b), a function generator (AFG3022C, Tektronix) was used to apply a constant voltage to the cardiomyocytes in the group of experimental, in which the electric field direction was parallel to the long axis of the cardiomyocytes. In Fig. S1(c), biphasic rectangular electrical pulses with different frequencies were applied to the cardiomyocytes of the group of experiments by function generator. As shown in Fig. S1(d), the electrochemical comprehensive test system (AMETEK, PARSTAT 4000A) stimulated the cardiomyocytes with the DC currents of 0.04 nA, 0.08 nA, 0.12 nA, 0.16 nA, 0.2 nA, 0.24 nA, 0.28 nA and 0.32 nA, respectively.

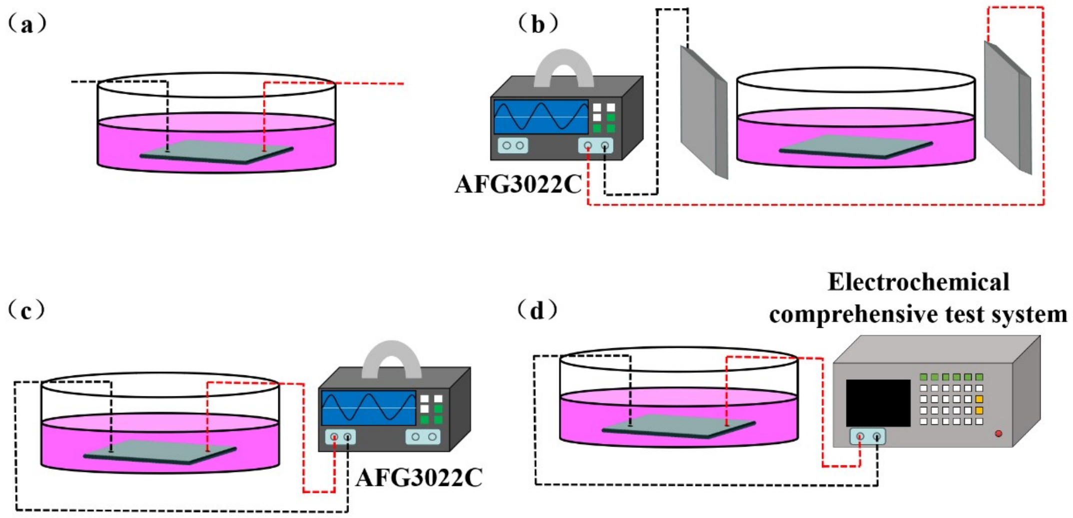

Fig. S1 Schematic diagram of the electrical stimulation of cardiomyocytes. (a) Cardiomyocytes were cultured on ITO. (b) Electric field stimulation. (c) Voltage frequency stimulation. (d) Direct current (DC current) stimulation.