Supporting Information

Highly Sensitive Hair Springs to Measure the Contraction Force of Engineered Cardiac Tissues

Qian-Ru Xiao, Si Sun, Nihad Cheraga, Kai-Hong Wu, Yong Jiang, Ning-Ping Huang*

Experimental Section

Fabrication of the Hair Springs: First, newly cut human hairs were rolled around stainless-steel needles with different diameters (1.0 mm, 1.2 mm and 1.6 mm) into springs with different turns (50, 80 and 100) and fastened with cotton threads. Next, the needles with rolled hairs were heated in boiling water for 20 min then dried in room temperature. Hair springs with different diameters and different turns were obtained after being removed from the needles.

Measurement of the Constant Factors of the Hair Springs. To measure the constant factor k, hair springs were hanged vertically by fixing one end. Then different numbers of balls with different weights (1 ball: 0.0043 g, 2 balls: 0.0077 g, 3 balls: 0.0115 g, 4 balls: 0.0163 g and 6 balls: 0.0229 g) were hanged at the other end. Digital vernier caliper was used to measure springs' initial length (x0) and final length (x1) after stretching. The displacement of the springs (Δx) was calculated by subtracting x0 from x1. Then k values of hair springs could be calculated according to the Newton's Second Law of Motion-Force and Acceleration ($F= m \bullet g$) and Hooke's law ($F= k \bullet \Delta x$).

Cyclic Tensile Loading Test: The spring was stretched 1000 times and the length of the spring before and after stretching at 10 different time points (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000) was measured and the corresponding k value at each time point was calculated.

Cardiac Induction of Human Induced Pluripotent Stem Cells (hiPSCs): The human induced pluripotent stem cells, purchased from Beijing Cellapy Biotechnology (China), were generated from urine epithelial cells. These hiPSCs were cultured on Matrigel-coated (BD

Biosciences, Canada) culture flasks with PSCeasy medium (Beijing Cellapy Biotechnology, China). Matrigel was diluted at a ratio of 1:100 with cold sterile Dulbecco's modified eagle medium (DMEM; Hyclone, USA). When hiPSCs maintained on Matrigel achieved 80%~90% confluence on day 0, cells were treated with the Wnt agonist CHIR 99021 (6 μM, Selleck) in RPMI1640 (Gibco) with B27 minus insulin (Thermo Scientific) from day 0 to day 1. After 24 h, the medium was changed to RPMI1640 with B27 minus insulin (RPMI/B27-insulin). From day 3 to day 6, cells were treated with the Wnt inhibitor IWR-1 (5 μM, Selleck) in RPMI/B27-insulin. Then the medium was changed to RPMI/B27-insulin. From day 7 onwards, cells were maintained in the CardioEasy medium (Beijing Cellapy Biotechnology, China) and the medium was changed every 3 days. All cells were cultured under standard cell culture conditions in a humidified incubator (37 ^{o}C, 5% CO_2).

Establishment of the Hair Spring Dynamometer: Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) mixture (cross linker to base in a ratio of 1:10) was poured into a culture dish (φ 60 mm) and baked at 65 ^{o}C overnight. Then the solid PDMS was cut into a rectangular well (40 mm × 5 mm × 5 mm) and two short straight hairs paralleled with each other were fixed at one side of the well. Then a hair spring was set in the well with one end fixed at the other side of the well and the other end of the spring attached to one of the straight hairs. Before cell seeding, the spring dynamometer was soaked in 75% ethanol overnight then transferred into the clean bench, sterilized with UV light for 1 h and washed three times with sterile phosphate buffer solution (PBS, homemade).

Hydrogel preparation: Collagen hydrogel solution (200 μL) was prepared on ice by mixing high concentration rat tail collagen (164.8 μL, at 3.64 mg/mL, Corning) with 10 X Hank's balanced salt solution (HBSS, 20 μL, homemade), deionized sterile H_2O (11.4 μL) and NaOH (1 mM, 3.8 μL, homemade) to obtain a final collagen concentration of 3.0 mg/mL.

Generation of Engineered Cardiac Tissues: Cardiomyocytes derived from hiPSCs were dissociated and resuspended in the CardioEasy medium. About 200 μL of cell solution was

mixed with the collagen hydrogel solution on ice and seeded into the area of the two paralleled straight hairs. The whole device was subsequently incubated at 37 ^{o}C for 30 min for polymerization. Then, 5 mL CardioEasy medium was added into the dish and cardiac tissues were further cultured in the incubator.

Measurement of the Contraction Force Generated by the Engineered Cardiac Tissues: After the cell-hydrogel mixture compacted and turned into a beating 3D construct, the contraction of cardiac tissues has pulled the spring causing its displacement. Bright-field videos of the movement of both the 3D cardiac tissues and the spring were taken at 15 frames per second (fps) over 15-20 seconds and the spring's displacement could be measured by analyzing videos using the motion tracking software. Then the contraction force was calculated according to Hooke's law.

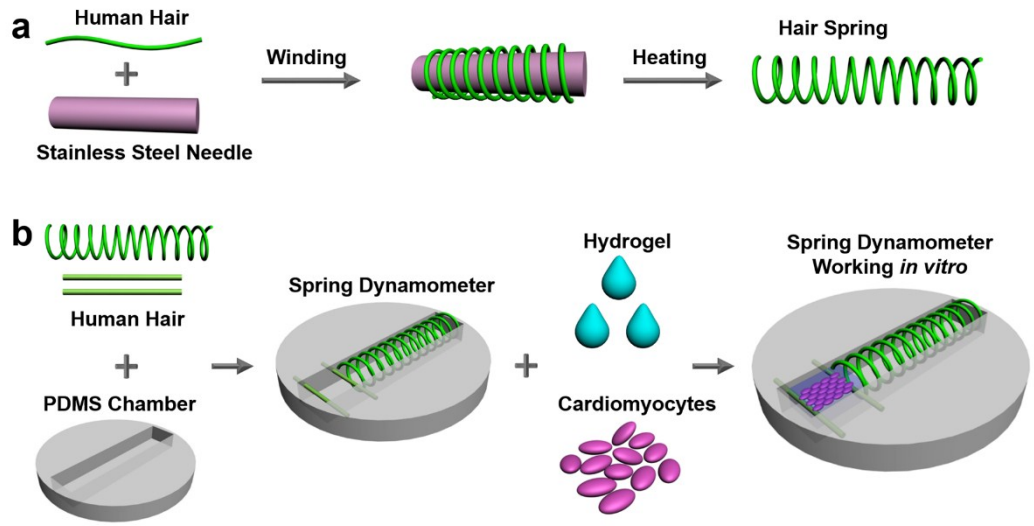


Fig. S1 The fabrication process of the hair spring and the spring dynamometer. a, Illustration of the fabrication process of the human hair springs. Step 1, winding human hair around the stainless steel needle to get a spring shaped construct; step 2, heating the spring shaped construct in boiling water to get a steady helical hair spring. b, Illustration of the assembling of the hair spring dynamometer and its working process. The spring dynamometer consisting a hair spring and two straight hairs and measuring the contraction force generated by the hydrogel-cardiomyocyte mixture cultured in the PDMS chamber.

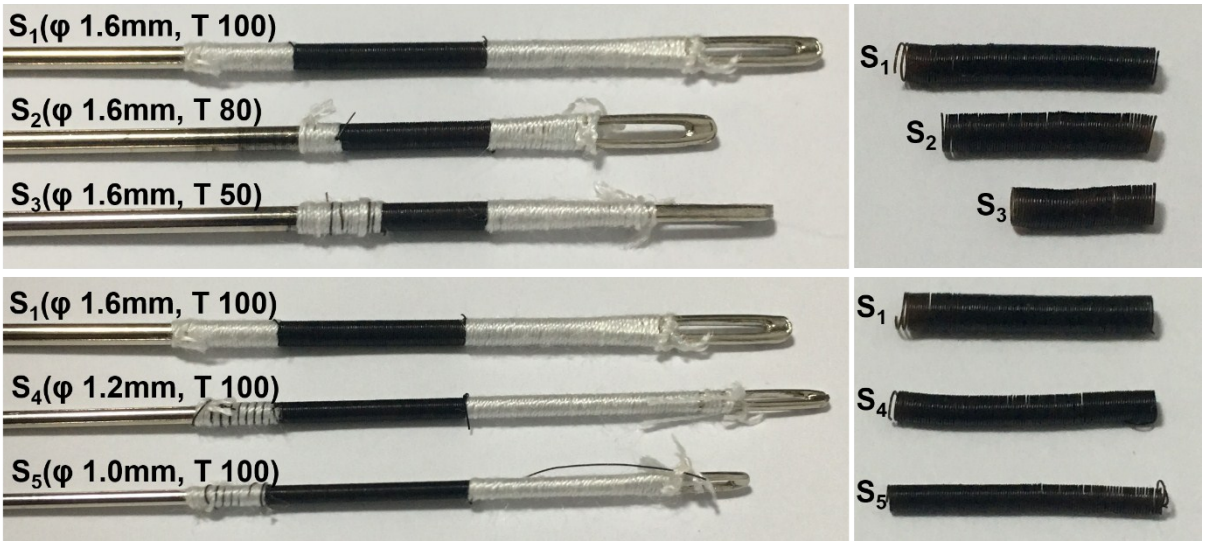
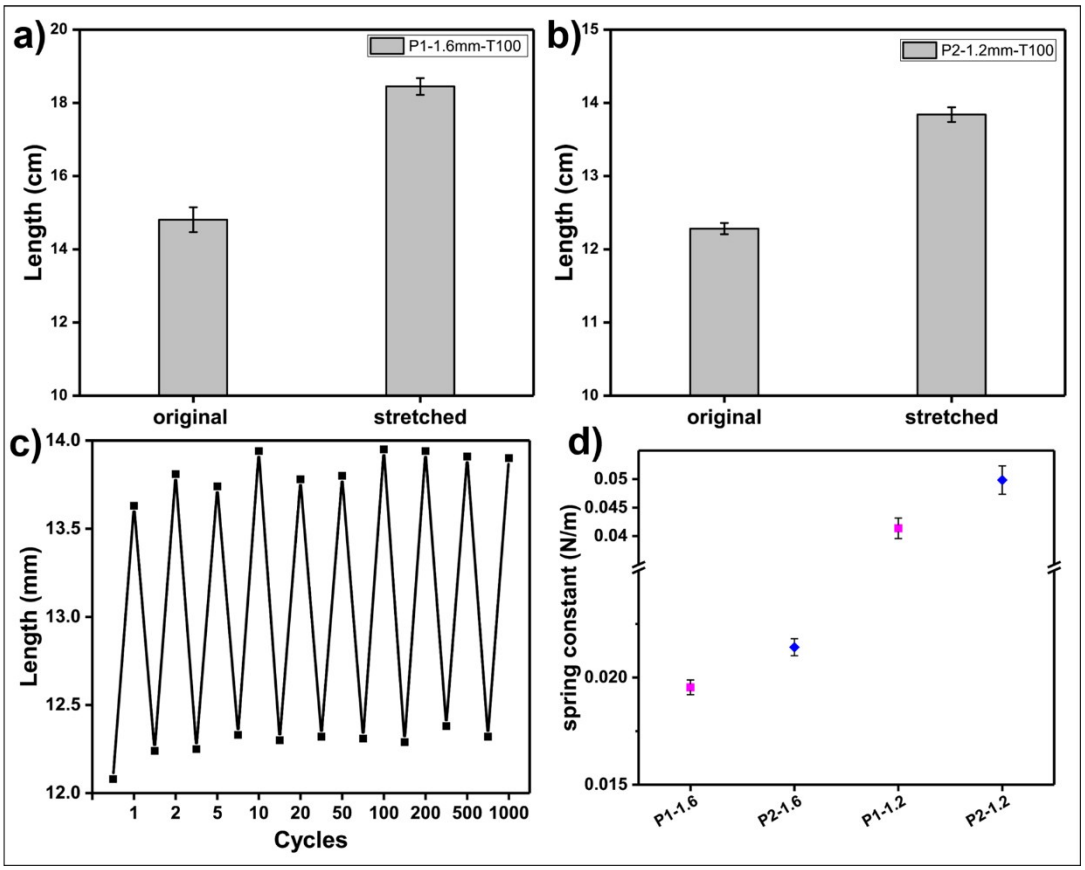


Fig. S2 Photos of hair springs. Hair springs with different turns and different diameters: S1 (φ1.6 mm, T100), S2 (φ1.6 mm, T80), S3 (φ1.6 mm, T50), S4 (φ1.2 mm, T100) and S5 (φ1.0

mm, T100) fixed around stainless-steel needles with cotton threads (white) and untied from



the needles.

Fig S3 (a, b) The average length of the spring (a, from Person 1, φ 1.6 mm, T 100; and b, from Person 2, φ 1.2 mm, T 100) before and after being stretched. The p value for the original length is 0.149 (a) and 0.104 (b) respectively. (c) The length of the spring (from Person 2, φ 1.2 mm, T 100) before and after being stretched at 10 different time points (1, 2, 5, 10, 20, 50, 100, 200, 500, 1000) during the cyclic tensile loading test. (d) The spring constant of hair springs (pink: from Person 1, T 100, φ 1.6 mm and 1.2 mm; blue: from Person 2, T 100, φ 1.6 mm and 1.2 mm).

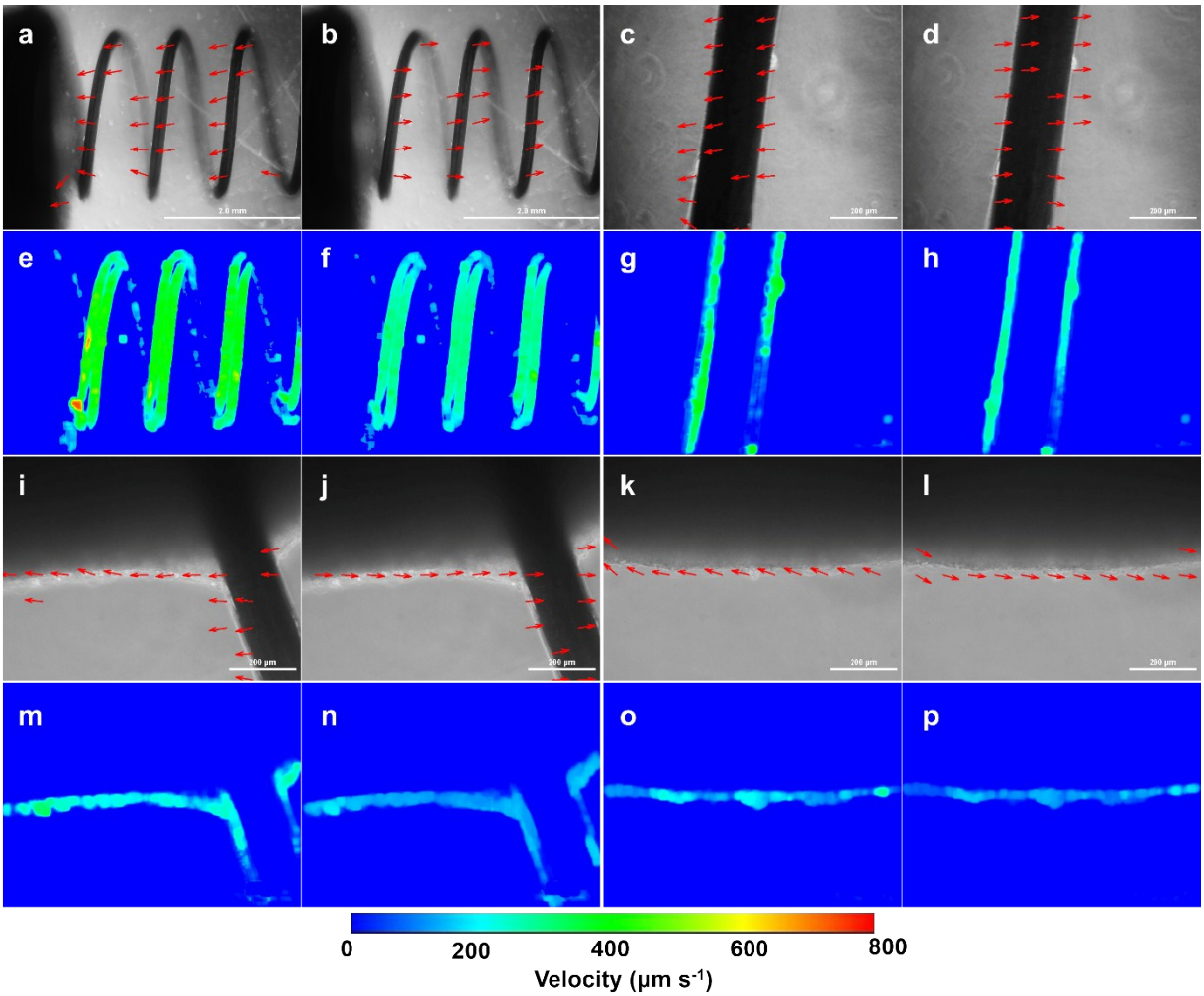


Fig S4 Representative images of the working hair spring dynamometer. (a, b) Bright-field images of the hair spring dynamometer overlaid with motion vectors showing the direction of stretching and retraction. (c, d) Magnified bright-field images of the hair spring overplayed with motion vectors showing the direction of stretching and retraction. (i, j) Bright-field images of the cardiac tissue and the straight hair overlaid with motion vectors showing the direction of contraction and relaxation. (k, l) Bright-field images of the cardiac tissue between the two straight hairs overlaid with motion vectors showing the direction of contraction and relaxation. e, f, g, h, m, n, o, p, Corresponding heat maps of images a, b, c, d, i, j, k and l.

Video S1 Beating cardiomyocytes differentiated from human induced pluripotent stem cells.

Video S2 The motion of the hair spring dynamometer on Day 29.

Video S3 The motion of the free end of the hair spring on Day 29.

Video S4 The motion of the cardiac tissue and the straight hair on Day 29.

Video S5 The motion of the cardiac tissue between the two straight hairs on Day 29.

Consent Statement

Title: Highly Sensitive Hair Springs to Measure the Contraction Force of Engineered Cardiac Tissues

Name of Researcher(s): *Qian-Ru Xiao, Si Sun, Nihad Cheraga, Kai-Hong Wu, Yong Jiang and Ning-Ping Huang*

1. I confirm that I have read and understand the information about this project. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and I agree to participate in the above research.

3. I agree to allow the researcher(s) to take my hair and grant permission for the hair to be used by the researcher(s) and their project partners in publications, press articles and websites.

Hair Providers: *Qian-Ru Xiao, Zu-Xiang Hu*

Date: 22/01/2020