Supporting Information for

Multimodal Characterization of Cardiac Organoids Using Integrations of Pressure-Sensitive Transistor Arrays with Three-Dimensional Liquid Metal Electrodes

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This PDF file includes:

Supplementary Materials and Methods

Materials

Single-crystalline silicon channels for pressure-sensitive transistors were patterned by isolating them from silicon-on-insulator wafers (thickness of top Si layer: 160 nm, buried oxide: 400 nm, Soitec, France). As a liquid metal ink, EGaIn (75.5% gallium and 24.5% indium alloy by weight, Changsha Santech Materials Co. Ltd.) was used for the printing of the 3D electrode. All materials used in the fabrication of the hiPSC-derived cardiac organoid are listed in the Tables of Supplementary Materials.

Fabrication of cardiac organoid

The cardiomyocytes were generated from the hiPSC line (KYOU-DXR0109B, American Type Culture Collection (ATCC), Manassas, VA, USA). The study with hiPSCs was approved by the Institutional Review Board (IRB) of Yonsei University (Permit Number: 7001988-202004-BR-844-01E). The differentiation of hiPSCs to cardiomyocytes was conducted following the protocol previously reported (Figure 3a).[39] When the cardiomyocytes started beating from day 17 to day 21, they were detached by treatment of 290 U/ml of collagenase type II at 37 ℃ for 1 hour. The detached cardiomyocytes were seeded into fabricated PDMS microwell at a concentration of 10^5 cells/μL using a pipette. Before seeding the cardiomyocytes, PDMS microwells were sterilized with 70% ethanol and ultraviolet irradiation for 30 minutes, and then washed three times with phosphate buffered saline (PBS, Sigma, St. Louis, MO, USA). The cell concentration and the dimension of the microwell were optimized to obtain cardiac organoids with the most geometrical shape suitable for the fabricated multimodal sensory device. The surrounding cells that did not enter the PDMS microwell were washed away and then PDMS microwells containing cardiomyocytes were incubated at 37 °C with 5% CO_2. In

order to avoid the destruction of liquid-metal 3D electrodes, instead of inserting our 3D electrodes directly into a cardiac organoid, we cultured and formed the organoid in our culture device where the liquid metal 3D electrodes were placed inside a polydimethylsiloxane (PDMS) microwell (the bottom right image of Figure 1e). When the human iPSC-derived cardiomyocytes were seeded into this PDMS microwell, they were spontaneously aggregated into a 3D cardiac organoid where the 3D electrodes were naturally inserted inside (Figure 3d). This method did not destroy the structure of 3D electrodes even after organoid formation, as shown in Figure 5b and Figure S13.

Preparation of the pressure sensor

An array of silicon channels was patterned photolithographically using a positive photoresist (S1818, MicroChem) on the SOI wafer. For the channel isolation, the Si channels were etched using a reactive ion etching (RIE) system with sulfur hexafluoride plasma (SF_6 25 sccm, Ar 55 sccm, 300 W, 40 sec). The remaining photoresist residue was removed by exposure to Piranha solution for 10 minutes. For the formation of interconnects, metal layers (Cr: 5 nm, Au: 100 nm) were deposited onto the Si channels by e-beam evaporation. Then, the source/drain/interconnect electrodes were patterned photolithographically with an S1818 photoresist and then etched using gold and chrome etchants. The remaining photoresist was removed and rinsed with acetone, IPA and distilled water. At each interval, the sample was dried with N_2 gas. A negative photoresist (thickness: 1 μm, SU-8 2020, MicroChem) was patterned photolithographically to define the air-dielectric area and to passivate the metal interconnects. For the preparation of a pressure-sensitive elastomeric layer, a 30 μm-thick flat PDMS layer in which microdroplets of glycerol (diameter: 1 – 2 μm) were dispersed uniformly in a weight ratio of 1:10 (glycerol: PDMS) was prepared (denoted as G-PDMS). Subsequently, the G-PDMS with laser-patterned vacant air-dielectric holes was subsequently transferred. For

the gate panel, metal layers (Cr: 5 nm, Au: 70 nm) were deposited and patterned on a thin polyimide (PI) film (thickness: 3 μm) which was thermally pre-imidized on a bare Si wafer. After delaminating this PI sample from the Si wafer, metal layers (Cr: 5 nm, Pt: 30 nm) for the interconnect pads of the 3D electrodes were deposited on the opposite side of the PI film using a shadow mask. A thin encapsulation layer of parylene C (thickness: 1 μm) was deposited and then patterned to open only the printing site of the 3D electrodes. Using a high precision aligner, the gate film was assembled with the transistor channels, facing the gate electrodes. After direct printing of the 3D EGaIn electrodes on the open area of the Pt pads, a PDMS microwell (thickness: 700 μm) for the development of the organoid was attached on the top surface of this gate film. To remove any residual water vapor before each assembling process, this sample was dried on a hotplate at 100 ℃ for 30 minutes.

Direct printing of 3D electrodes

A nozzle was prepared from a glass pipette (outer diameter: 1.0 mm, inner diameter: 0.5 mm, World Precision Instruments) that was pulled by an automatic pipette puller (Sutter P-1000), resulting in the formation of a nozzle with its inner diameters from 10 to 30 μm. The height between the tip of the nozzle and a substrate was set to about 5 μm before starting the printing of EGaIn. Using a pneumatic regulator, a pressure of 30 psi was applied to the ink storage (i.e., syringe) to deliver the ink from the syringe to the tip of the nozzle. Next, the distance between the tip of the nozzle and the substrate was kept around 5 μm with the ink in contact with the substrate surface. By toggling the pneumatic pressure, we coordinate the on and off sequences of the direct printing process. When the ink was in contact with a substrate, the printing stage was moved vertically downward at a velocity of 25 μm/s for the 3D printing of EGaIn.

Calculation of pressure of air-dielectric Si pressure sensor

The drain current, I_D, in the saturation regime can be written as the equation regarding the metal-oxide-semiconductor FET model

$$I_{D,sat} = \frac{C_i \mu_{sat} W}{2L} \times (V_G - V_T)^2$$

, where C_i, W, V_T, and L are defined as the capacitance, width of channel, threshold voltage, and length, respectively. The key mechanism of the pressure sensor is utilizing the change in the I_D with respect to the modulation of gate dielectric layer thickness in the FET. The changes in I_D correspond to the changes in channel dimension but the relative change in I_D ($\Delta I_D/I_0$) is independent from channel width variations. $\Delta I_D/I_0$ is only derived from the changes in the thickness of the dielectric layer (d), according to the following equations:

$$I_D = \frac{W}{L} \mu C (V_{GS} - V_T)^2$$

$$C = \frac{\varepsilon_0 \varepsilon}{d}$$

, where L is length of channel, μ is electron mobility, W is width of channel, C is capacitance of dielectric layer, V_{GS} is gate-source voltage, V_T is threshold voltage, ε_0 is vacuum permittivity, ε is permittivity of dielectric material, and d is dielectric thickness.

Conditioning of organoid for fluorescent immunostaining

Organoid fixation (4% (w/v) paraformaldehyde, Sigma) is performed for 1 hour at room temperature. Subsequently undergoes permeabilization (0.1% Triton X-100, Sigma) for 30 min at room temperature. Then, blocking (5% Bovine Serum Albumin, Fraction V, MP biomedicals, Asse-Relegem, Belgium) is performed for 2 hours at room temperature. It is incubated using primary antibody overnight at 4 ℃. It is washed with PBS three times. Incubation using secondary antibody is performed overnight at 4 ℃. Again, it is washed with PBS three times. Nuclei were then stained with 2-(4-amidinophenyl)indole-6-carboxamidine dihydrochloride (DAPI, TCI, Tokyo, Japan) for 30 min at room temperature.

Organoid dehydration process for scanning electron microscopy (SEM)

Organoid is fixed (4% (w/v) paraformaldehyde, Sigma) for 1 hour at room temperature. For dehydration procedure, organoid is bathed in 50% ethanol (1 hour), 75% ethanol (1 hour), 90% ethanol (1 hour), and 100% ethanol (1 hour) sequentially all at room temperature. The organoid is then vacuum dried overnight before SEM imaging.

Organoid preparation for cryosectioning

Cell seeding after sterilization of substrate. Organoid is fixed (4% (w/v) paraformaldehyde, Sigma) for 1 hour at room temperature. Organoid is then immersed in 30% sucrose solution and left overnight at 4 ℃. Immerse the sample in liquid oct compound (CellPath, United Kingdom) and freeze the sample at -80 °C. After taking out the sample and going through a suitable trimming process, place the sample in the direction of the desired section and use the oct compound again to make an oct block. The block is then sectioning at the thickness of 10 μm using a microtome. The organoid is conditioned for fluorescent immunostaining using the previously reported steps.

Assessment of biocompatibility

To confirm the biocompatibility of 3D electrode, Live/Dead assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was applied to cardiac organoids formed with or without 3D electrodes. The staining procedure was conducted according to the manufacturer's protocol. Live cells stained with 2 μM calcein-AM showed green fluorescence and dead cells stained with 4 μM ethidium homodimer-1 showed red fluorescence. Fluorescent images of cardiac organoids were obtained using confocal microscopy (LSM 880, Carl Zeiss, Jena, Germany). Cell viability was quantified based on Live/Dead stained images using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

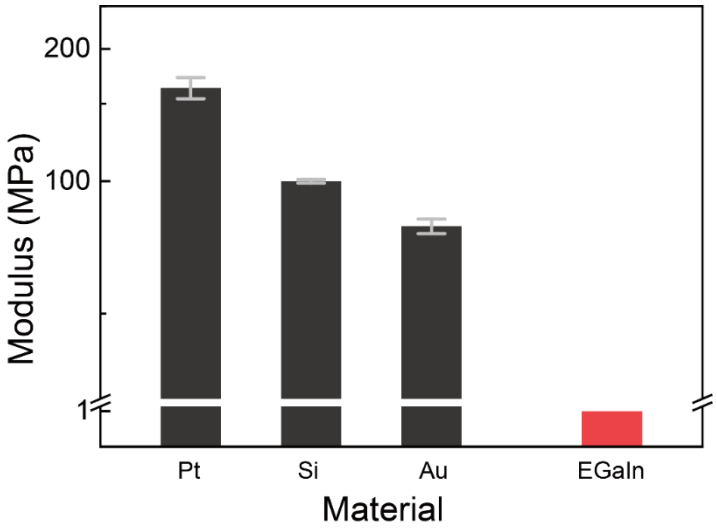

Figure S1. Modulus values of conventional materials used for bioelectronic electrodes. Red bar represents the modulus of 3D EGaIn electrode (204 kPa).

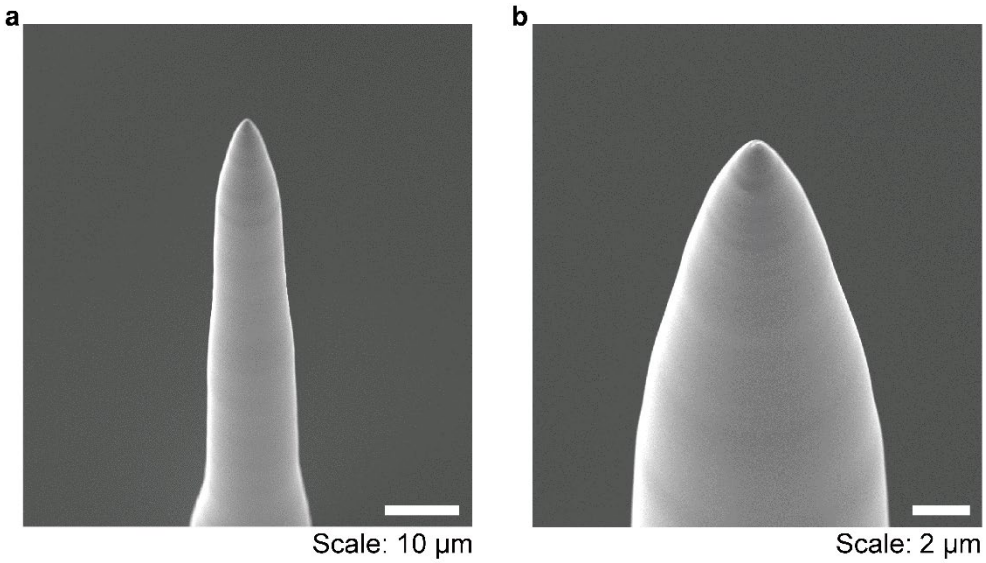

Figure S2. a) SEM image of the 3D electrode printed with a 10 µm nozzle. b) Close-up SEM image of the 3D electrode tip.

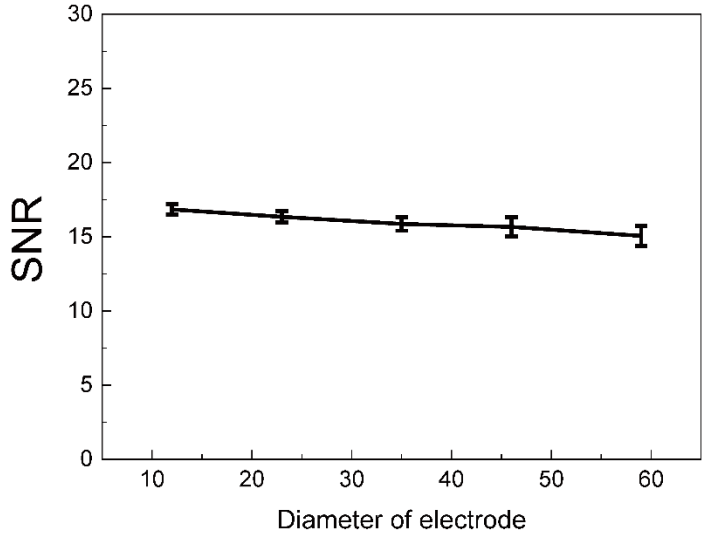

Figure S3. Plot of the ECG signal SNR versus the diameter of the 3D electrodes.

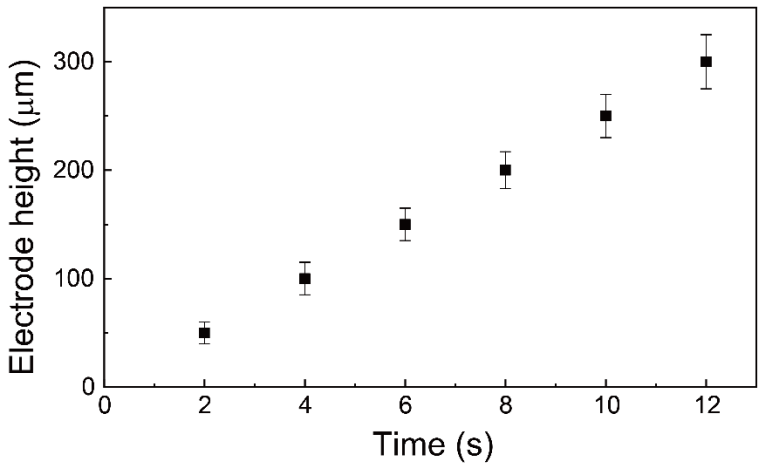

Figure S4. The plot of the electrode height versus printing duration at vertical printing velocity of 25 μm/s.

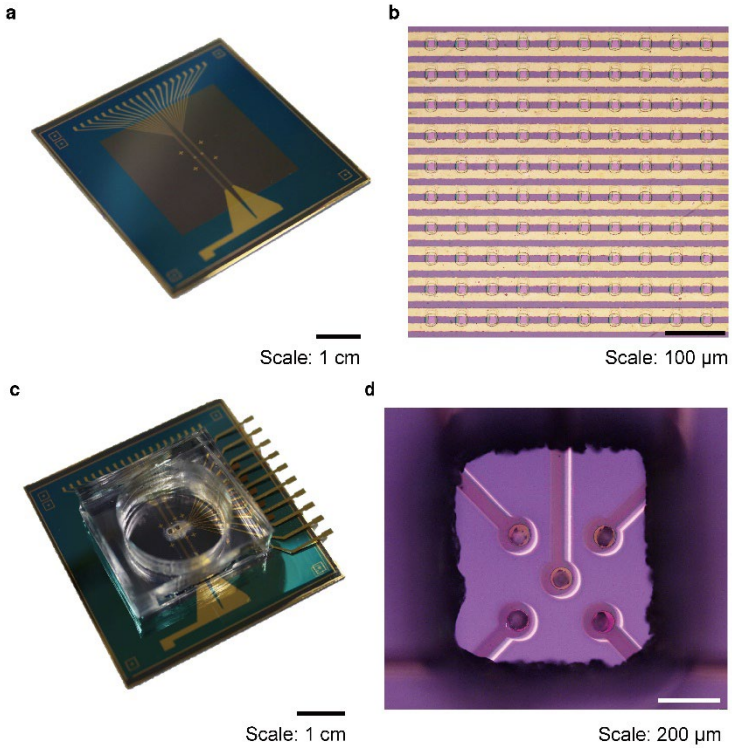

Figure S5. a) Photograph of the bottom FET panel before its assembly with the gate electrode panel. b) Optical micrograph of this bottom panel to show source/drain, channel, and the SU8 layer. c) Photograph of the completed multimodal device platform after the assembly of all components, including the 3D liquid metal electrodes. d) Optical micrograph of the liquid metal 3D electrodes inside a PDMS microwell (top-view).

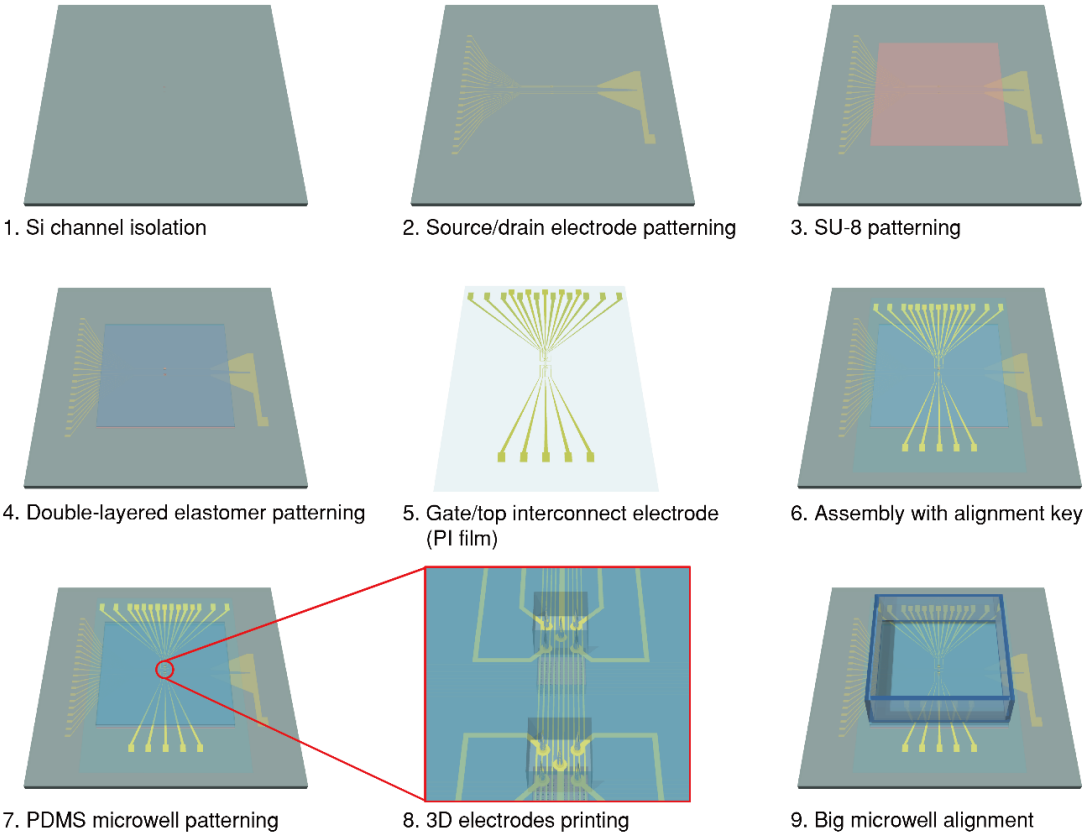

Figure S6. Schematic illustrations for fabrication method of multimodal organoid sensor.

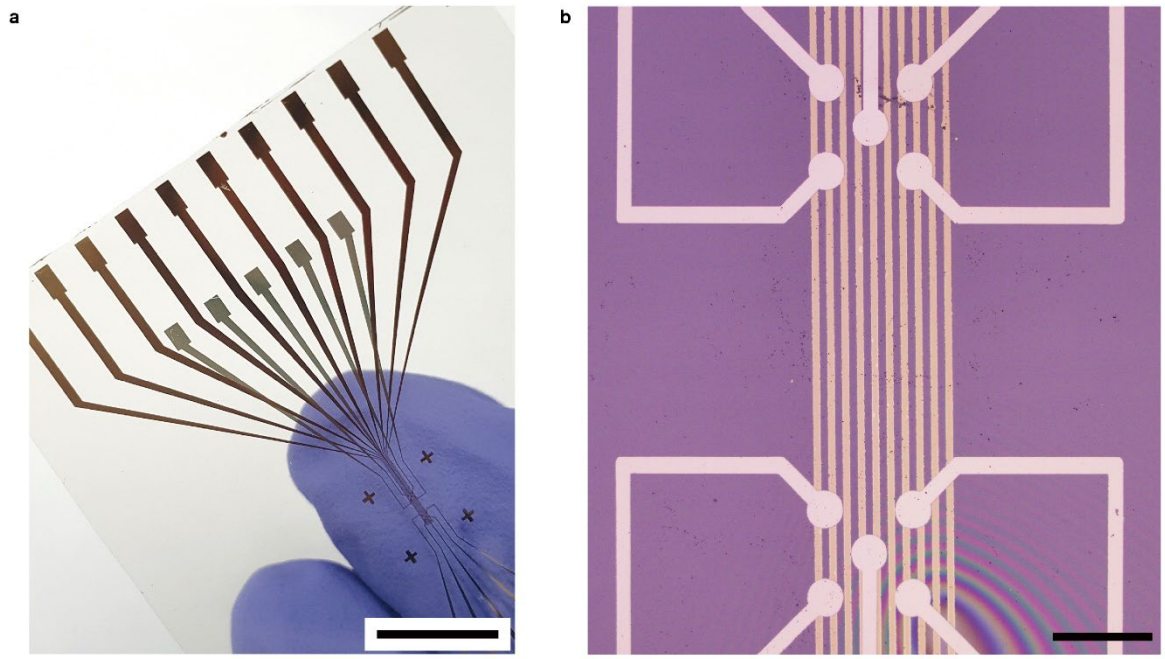

Figure S7. a) Photograph of gate electrode and interconnect contact pad on polyimide film. Scale bar, 1 cm. b) Optical micrograph of the PI film at top view. Scale bar, 300 μm.

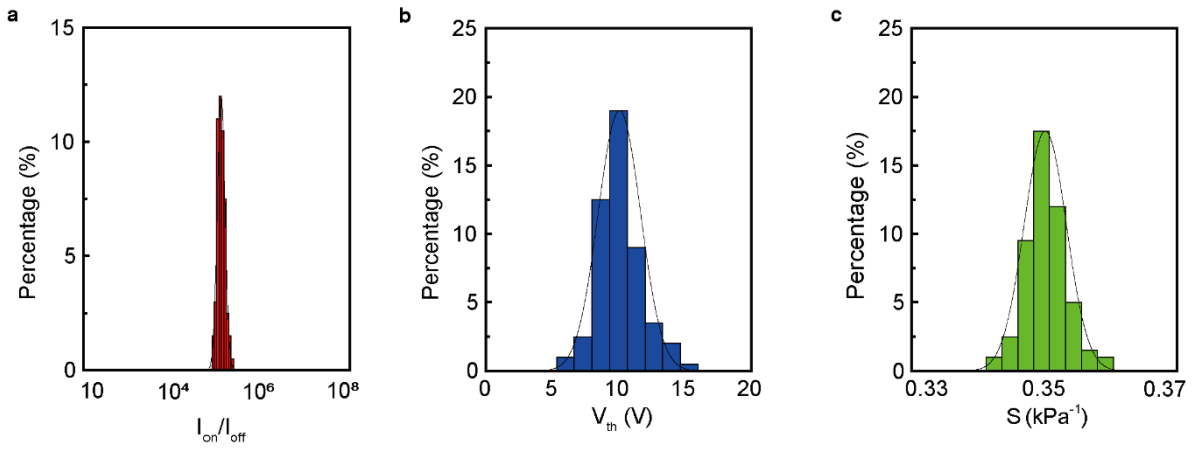

Figure S8. a) On/off ratio, b) threshold voltage, and c) pressure sensitivity of 10 × 10 Si FET arrays.

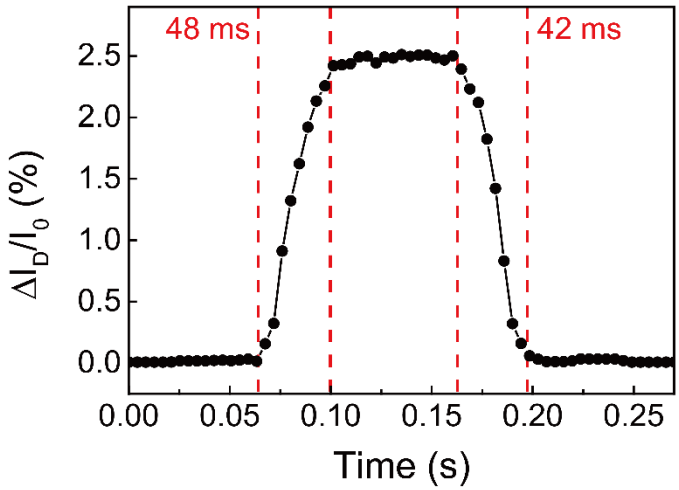

Figure S9. Response time and recovery time of pressure sensor during pressure loading and unloading.

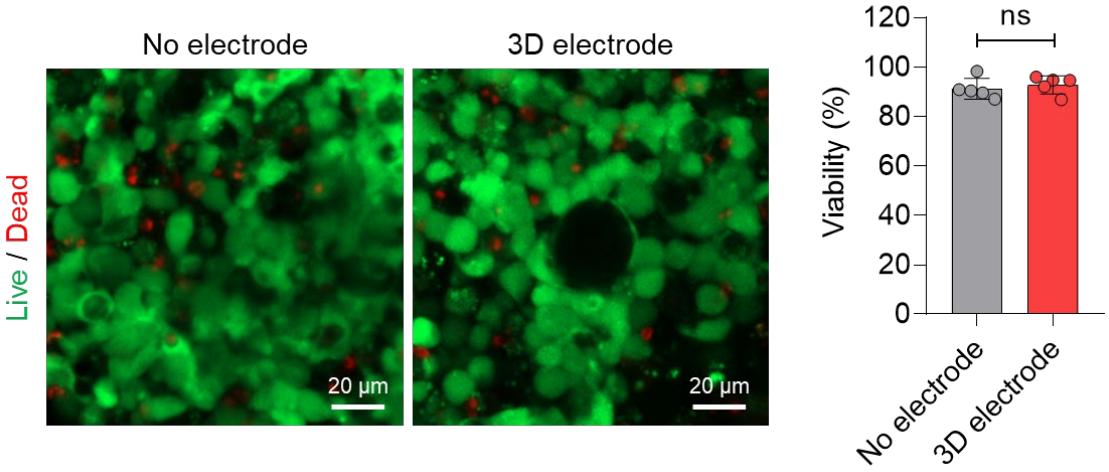

Figure S10. a) Live/Dead staining images and b) viability of cardiac organoids formed with or without the 3D electrodes for 3 days after the organoid generation (Scale bars = 20 μm, N = 5). Non-significant statistical difference was marked as ns.

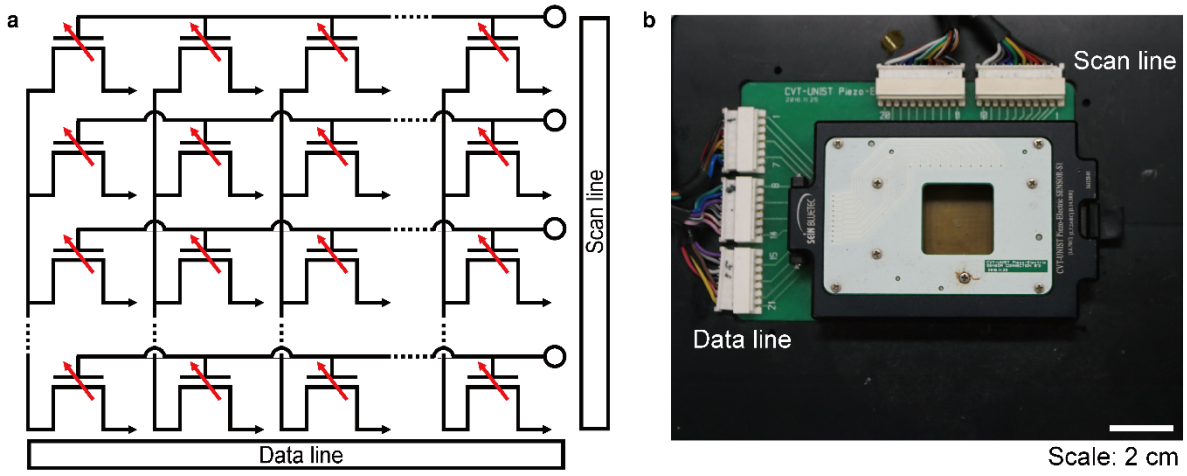

Figure S11. a) The circuit diagram of the 10 × 10 pressure-sensitive transistor array. b) Photograph of the custom-made jig of the acquisition system.

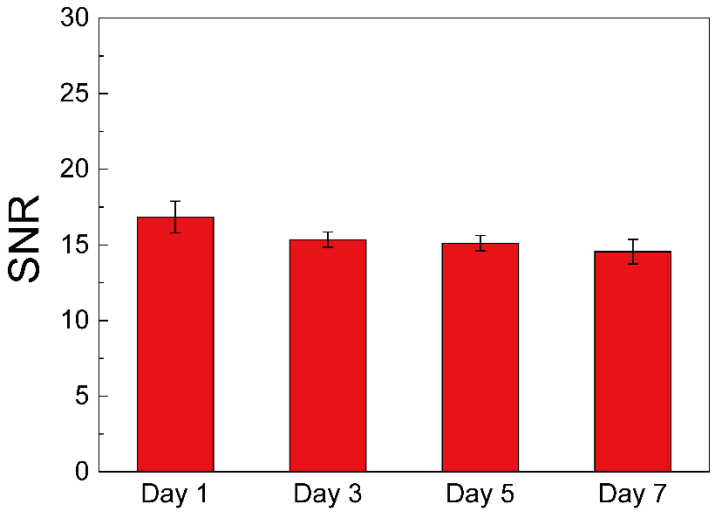

Figure S12. Recording of the ECG signal of a cardiac organoid using the 3D electrodes.

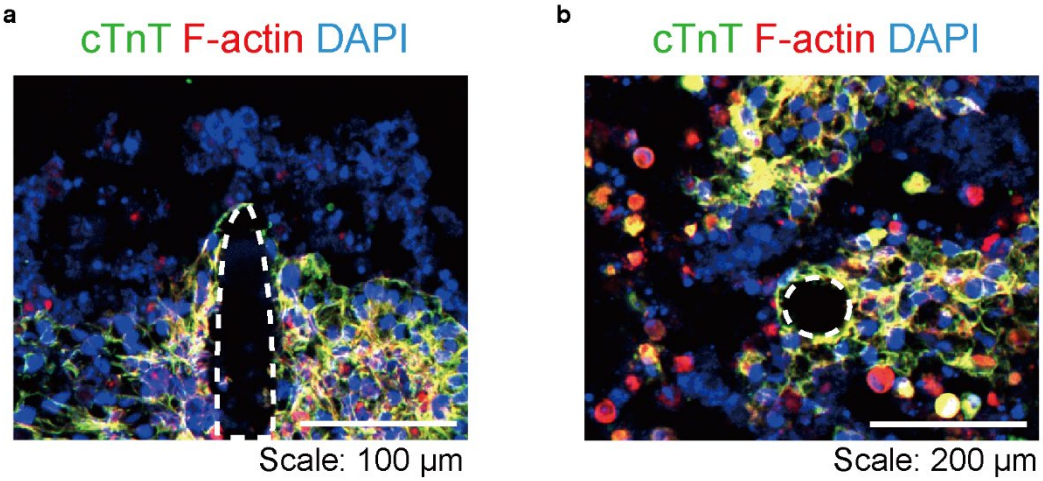

Figure S13. a) Cross-sectional view and b) top-view of immunofluorescent images of α-actinin, and F-actin in hiPSC-derived cardiac organoid with the imbedded 3D electrodes after cryosectioning.

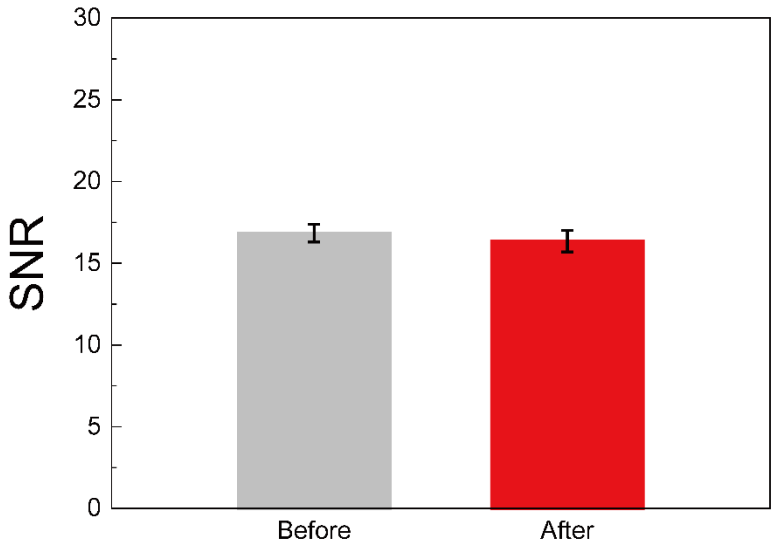

Figure S14. SNR of the ECG signals recorded before and after the application of electrical stimulation.

Sensing Type	Advantages	Disadvantages	Sensitivity	Detection Range	Ref.
Piezoresistive	Simple structure Fast response time	Low pixel density High crosstalk effect	0.48 kPa^{-1}	1.5 kPa ~ 6.5 kPa	1
			41.0 MPa^{-1}	10 kPa ~ 500 kPa	2
			0.76 kPa^{-1}	1.7 Pa ~ 6 kPa	3
Piezocapacitive	Simple structure Simple principle	Neighboring Interference High crosstalk effect	0.137 kPa^{-1}	50 Pa ~ 6 kPa	4
			7.70 kPa^{-1}	13 Pa ~ 7.4 kPa	5
			0.7 kPa^{-1}	12 Pa ~ 430 kPa	6
Piezoelectric	High sensitivity Fast response time	Pyroelectric High crosstalk effect	32.6 $nA{\cdot}kPa^{-1}$	0.125 kPa ~ 22.5 kPa	7
			0.018 kPa^{-1}	1 kPa ~ 30 kPa	8
			7.1 $mV{\cdot}kPa^{-1}$	1 kPa ~ 10 kPa	9
Pressure-sensitive transistor (In this work)	Active matrix Fast response time High spatial contrast High resolution Low crosstalk effect	Complex structure	0.35 kPa^{-1}	37 Pa ~ 5 kPa	

Table S1. Comparison in characteristics and performances of pressure sensors for biomedical applications.

Agent	Manufacturer
Essential 8 (E8) medium	Thermo Fisher Scientific, Waltham, MA, USA
Matrigel hESC-qualified matrix	Corning, Corning, NY, USA
RPMI 1640 medium	Thermo Fisher Scientific
B27 Supplement minus insulin	Thermo Fisher Scientific
B27 Supplement	Thermo Fisher Scientific
CHIR99021	LC laboratory, Woburn, MA, USA
IWP4 Wnt inhibitor	Stemgent, Cambridge, MA, USA
RPMI 1640 Medium, no Glucose	Thermo Fisher Scientific
Sodium DL-Lactate solution	Sigma, St. Louis, MO, USA
L-Ascorbic Acid	Sigma
Albumin human	Sigma

Table S2. Information regarding biochemical agents and their respective manufacturers used in the culturing of organoids.

Agent	Manufacturer
Anti-α-Actinin antibody	A7811, Sigma
Anti-Cardiac Troponin I antibody	ab47003, Abcam, Cambridge, United Kingdom
TRITC-conjugated phalloidin in Focal Adhesion Staining Kit	FAK100, Sigma
anti-mouse Alexa Fluor 488 antibody	Thermo Fisher Scientific
anti-rabbit Alexa Fluor 488 antibody	Thermo Fisher Scientific

Table S3. Information regarding biochemical fluorescence agents and their respective manufacturers used in the immunostaining procedure.

Video S1. Fabrication of soft 3D EGaIn electrode using direct printing method.

Video S2. Beating of hiPSC cardiac organoid after cultivation in PDMS stencil.

Video S3. Time-lapsed 2D pressure distribution of the cardiac organoid with calcium flux imaging.

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