Supplementary Information (SI) for Lab on a Chip.

Stretchable impedance electrode array with high durability for monitoring of cells under mechanical and chemical stimulations

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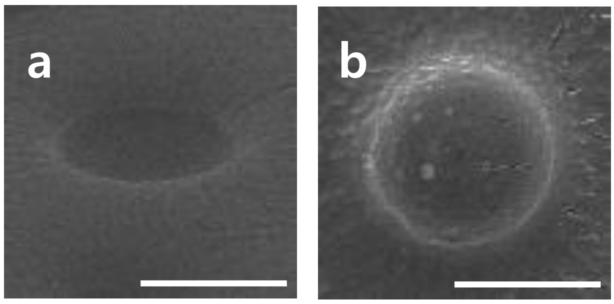
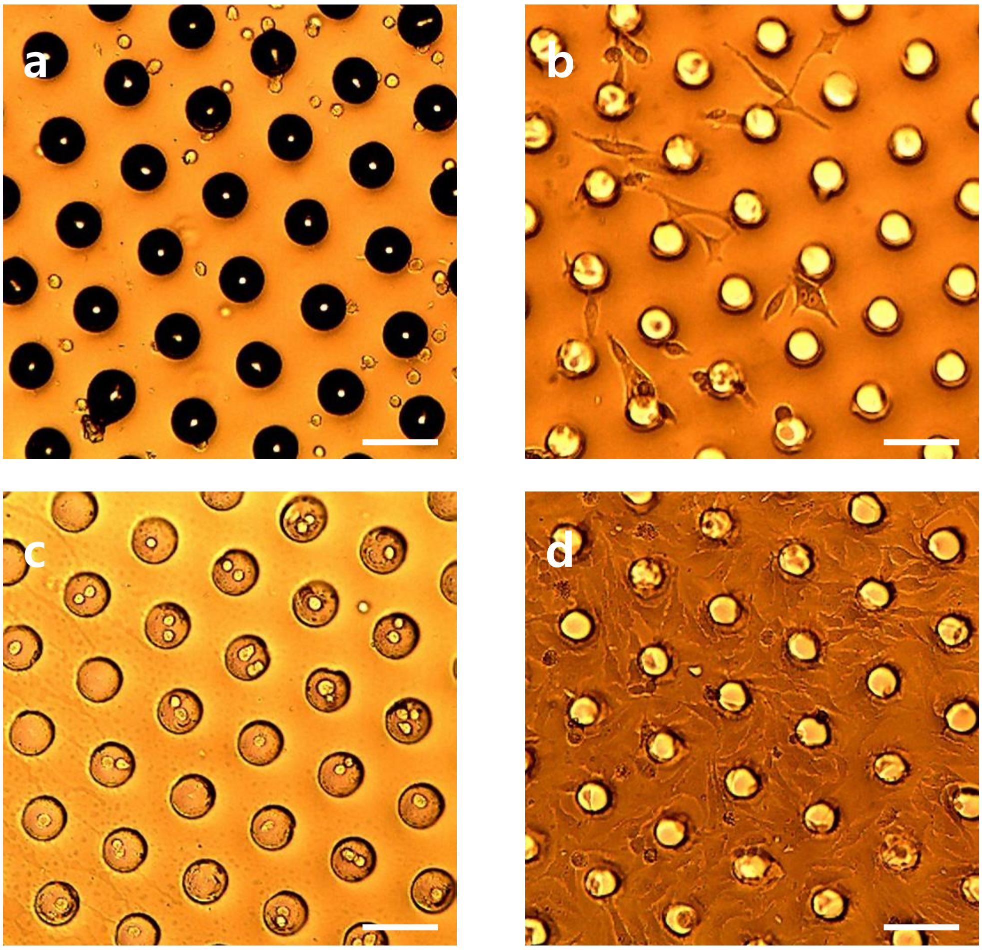
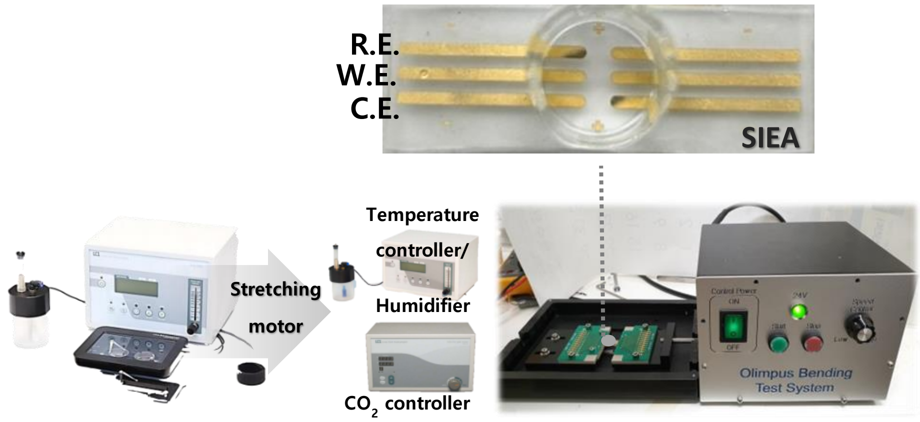


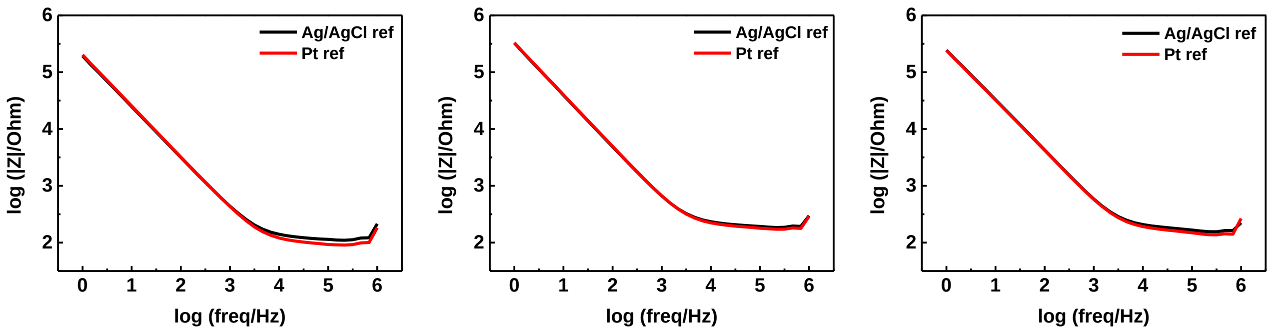
Supplementary Fig. 1. FE-SEM images of 3D micro-patterned substrate. The 3D micro-patterned substrate has both the bumps and valleys with their distance of 60 μm and height of 60 μm from the bottom of the valley to the top of the bump. The scale bar is set at 50 μm.



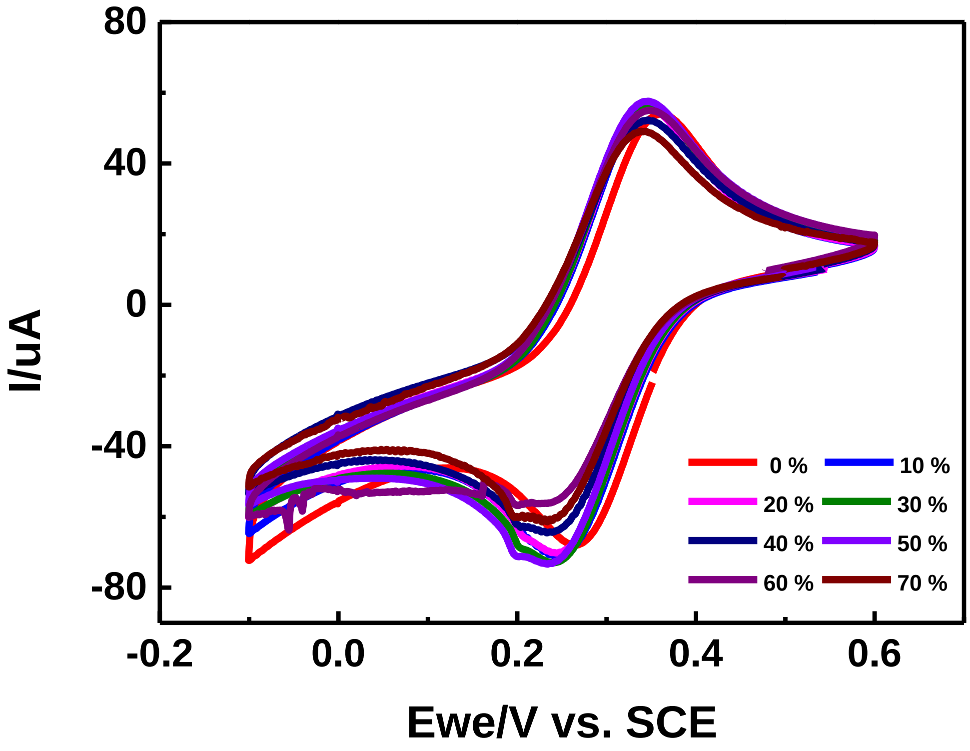
Supplementary Fig. 2. Surface treatment for enhancing cell attachment. a, Optical images after cell seeding on 3D micro-patterned substrate with no surface treatment. b, Optical images 3 days after cell seeding on 3D micro-patterned substrate with no surface treatment. c, Optical image after cell seeding on 3D micro-patterned substrate following O_2 plasma treatment. d, Optical images 3 days after cell seeding on 3D micro-patterned substrate following O_2 plasma treatment. The HeLa cells on the plasma-treated surface adhered well and displayed extensive spreading compared to the non-treated surface. The scale bar is set at 100 μm.



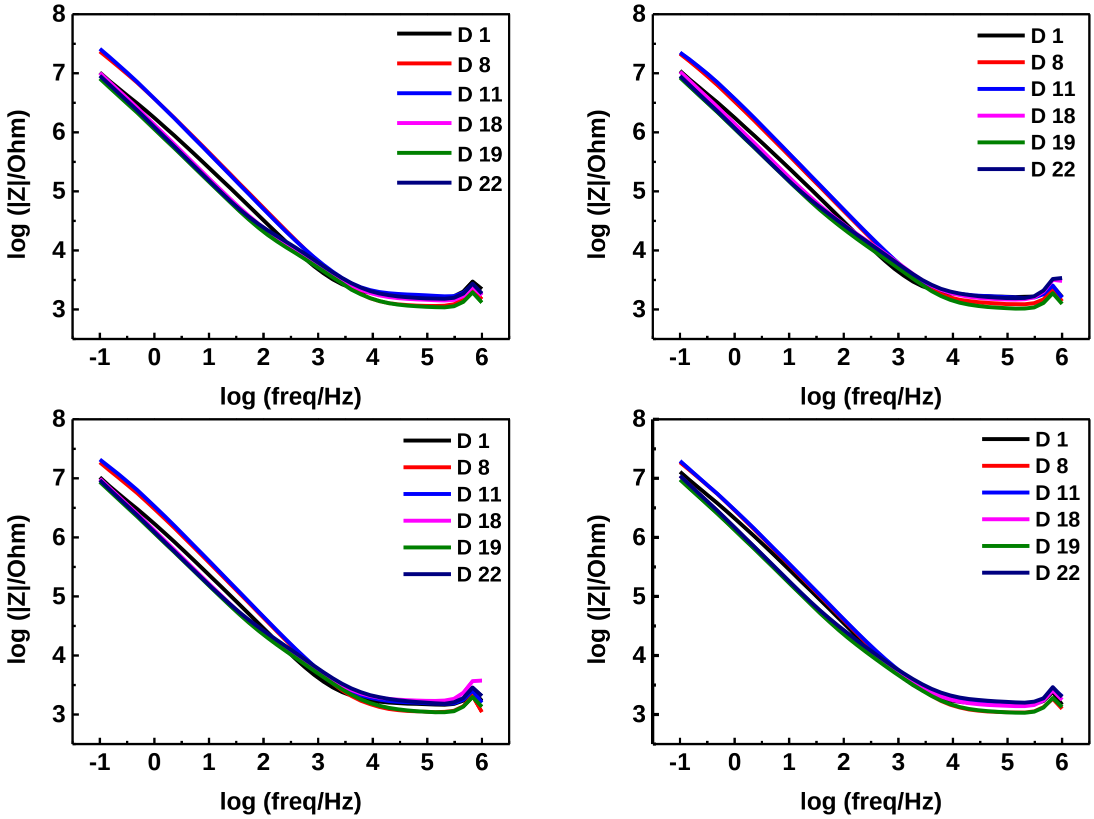
Supplementary Fig 3. Photograph of the actual captured SIEA and stretching system which is modified mini-incubator. The adapted mini-incubator setup accommodates the SIEA apparatus along with a motor controller designed for cyclic stretching and a culture well. This compact incubator system possesses the functionality to regulate CO_2 level, temperature, and humidity. Additionally, it interfaces with an electrochemical potentiostat for impedance measurements.



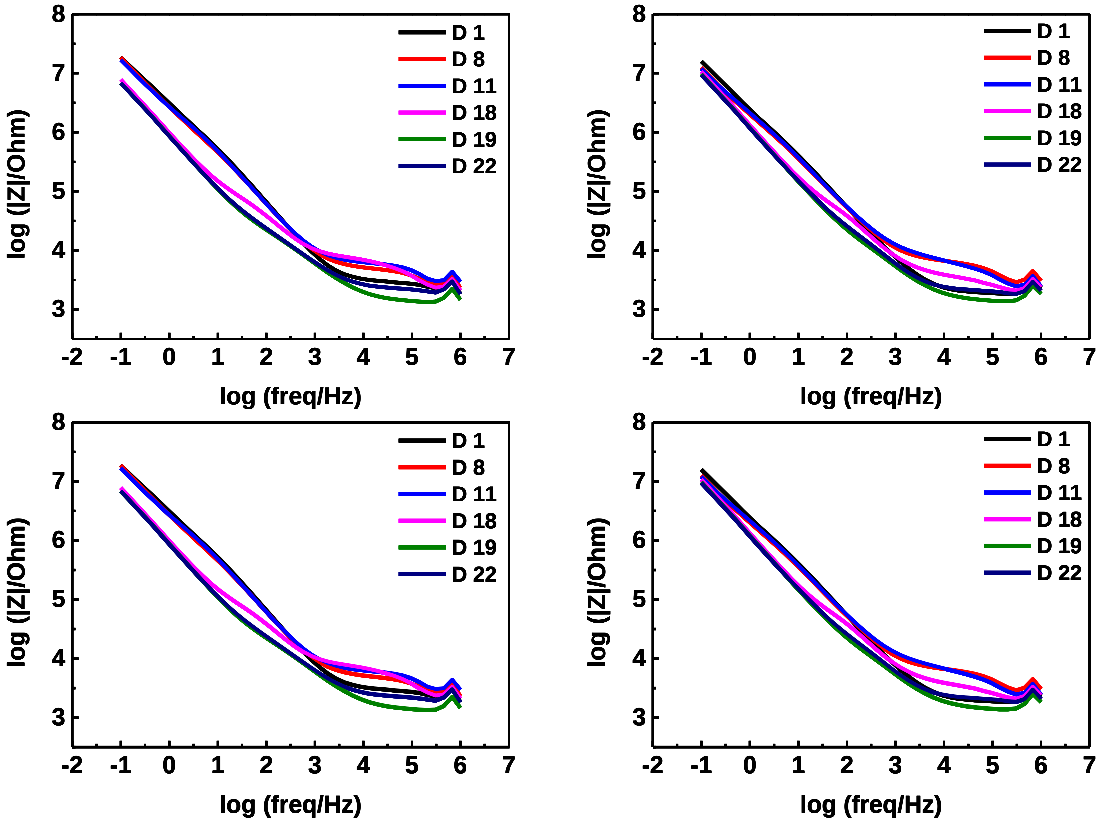
Supplementary Fig. 4. Impedance measurement of a reference electrode with commercial Ag/AgCl electrode and Pt-coated reference electrode. The comparison was made between measurements conducted by inserting a commercial Ag/AgCl reference electrode into the culture chamber integrated with the device having Au working and counter electrodes and *in-situ* impedance measurements of working and counter electrodes and Pt-coated electrode as the reference electrode.



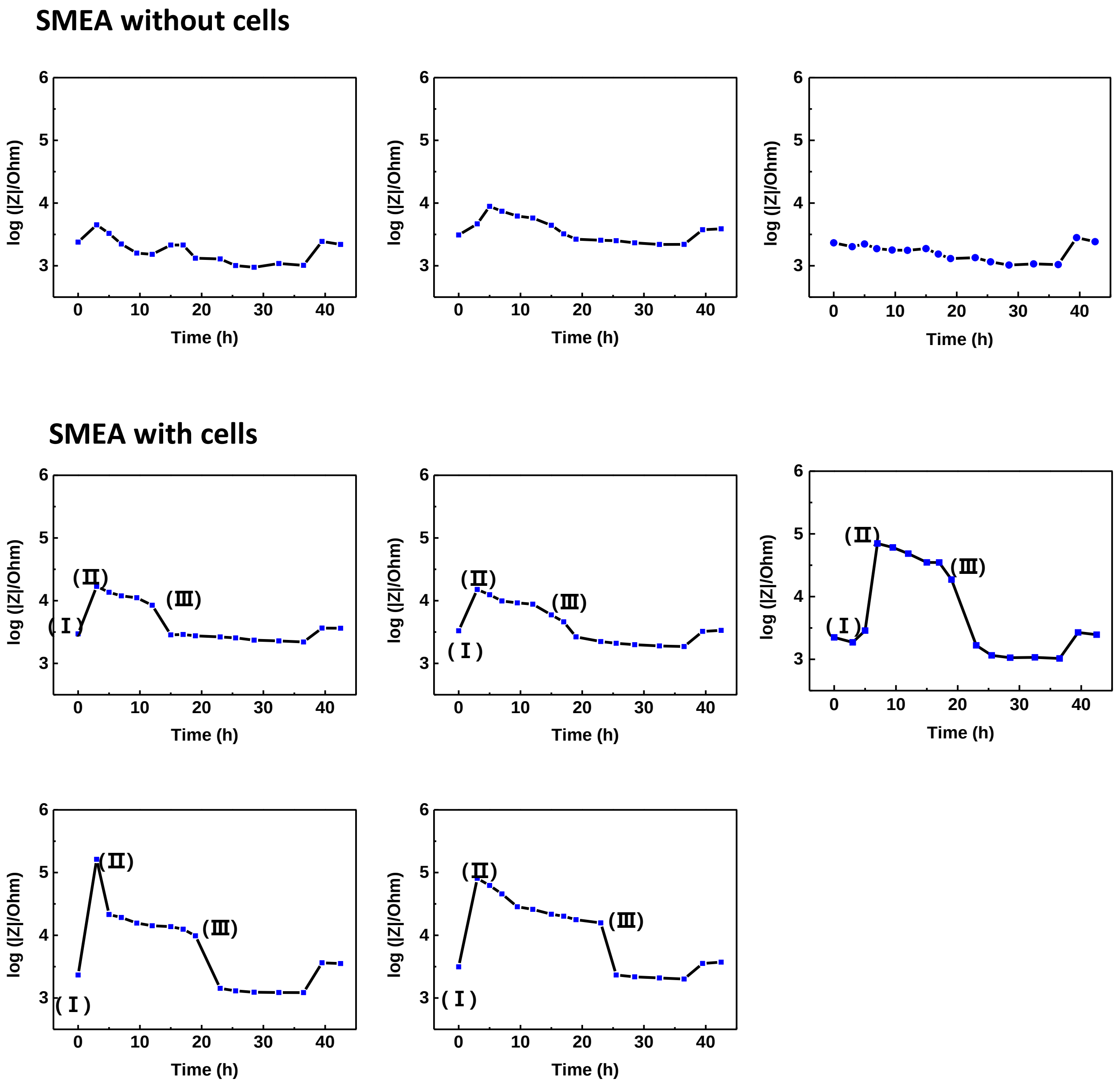
Supplementary Fig. 5. Durability evaluation of Pt-coated reference electrode. After stretching by varying the uniaxial strain at the range of 0 – 70%, we measured cyclic voltammogram (CV) characteristics of the Pt-coated electrode under released state using a commercial Pt counter electrode and a commercial Ag/AgCl reference electrode. The results indicate that the Pt-coated reference electrode is durable and electrochemically stable after large deformation.



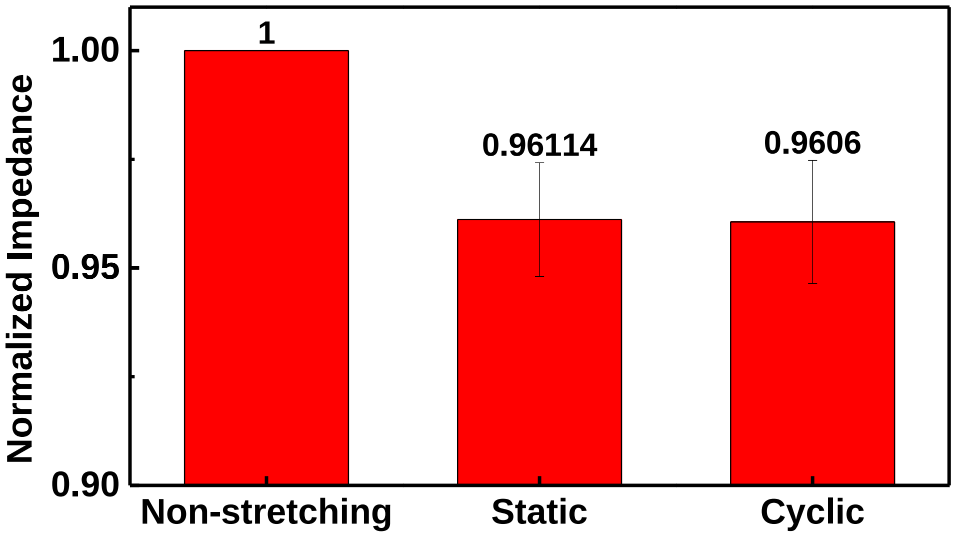
Supplementary Fig. 6. Long-term impedance measurement using SIEA-integrated cell culture device without loading of cells. The impedance was monitored in the SIEA-integrated cell culture chambers without loading of cells. In addition to the device shown in Fig. 2e, four culture devices were additionally used for the measurements. The impedance spectra illustrate good electrochemical stability over 22 days.



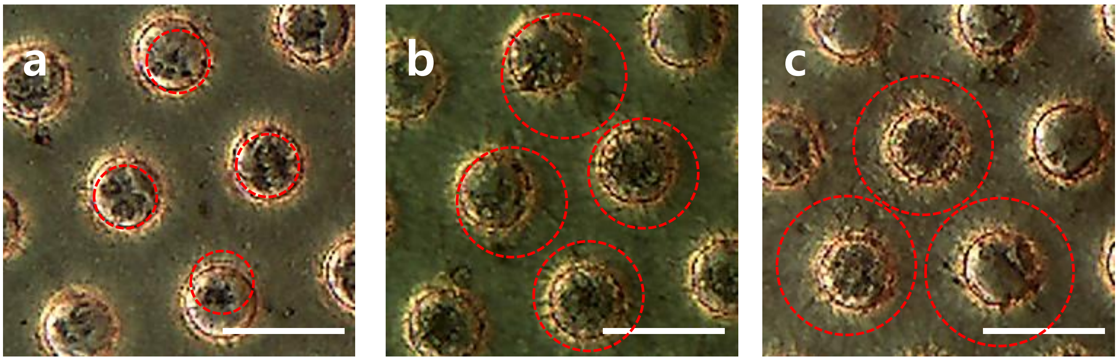
Supplementary Fig. 7. Long-term impedance measurement using SIEA-integrated cell culture device without loading of H9C2 cells. The impedance was monitored for the cells loaded in the SIEA-integrated cell culture chambers after culturing in a cell incubator. In addition to the device shown in Fig. 2f, four culture devices were additionally used for the measurements. The impedance values are different from those of the SIEA without cell loading. The H9C2 cells are attached and detached alternatively on the electrodes, indicating a dynamic and evolving cell condition throughout the observed period.



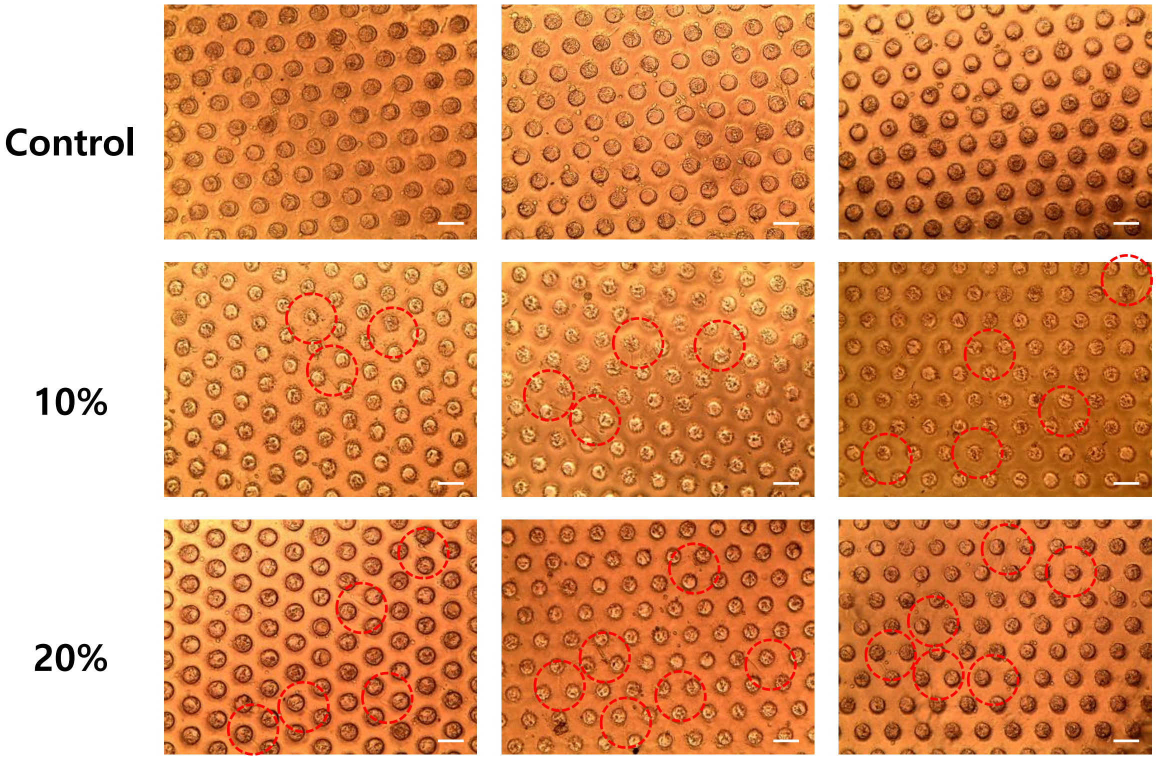
Supplementary Fig. 8. Long-term intermittent impedance monitoring. The impedance values (log|Z|) f the SIEAs without loading the cells and with H9C2 (rat cardiomyocyte) cells loaded were measured at the frequency of 10 kHz and compared. The medium was replaced 14 hours after seeding. And the measurements were carried out every 2 hour for a total of 43 hours. The impedance changes from the device containing cells were notably higher than those from the cell-free device. This observation suggests that the variations in the impedance values are attributed to the processes of cell seeding (I), attachment (II), and detachment (III).



Supplementary Fig. 9. The impact of static and cyclic stretching on the normalized /Z_i values. The graph illustrates a decrease in the normalized impedance (Z/Z_i) following both static (uniaxial extension at 10 % strain for 10 s and releasing) and cyclic stretching (10% for 60 s with the frequency of 1 Hz). There was observed no significant differences in the impedance values after static and cyclic stretching. (All sample number n = 4)



Supplementary Fig. 10. Optical images of the H9C2 cells on the electrode of SIEA for 5 days. The images of the cells were taken during the experiment presented in Fig. 3c. Cyclic stretching at 20% strain was applied for 1 hour at D 2 and D 4. a, optical image at 24 h after H9C2 cell seeding (D 1). b, optical image at D 3. c, optical image at D 5. The scale bar is set at 100 μm.



Supplementary Fig. 11. The optical micrographs of the H9C2 cells after cyclic stretching. The H9C2 cells seeded onto the SIEAs were subjected to cyclic mechanical stimulations of 0%, 10%, and 20% strains with 1 hour per day over a period of 3 days. These optical images were captured at D5, as indicated in the experiment shown in Fig. 3c. A comparative analysis of cell morphology, adhesion, and alignment was carried out. Imaging was obtained from three different locations for each stimulation condition. The scale bar is set at 100 μm.