Supplementary Material (ESI) for Lab on a Chip

Enhancement of an Electroporation System for Gene Delivery using Electrophoresis with Planar Electrode

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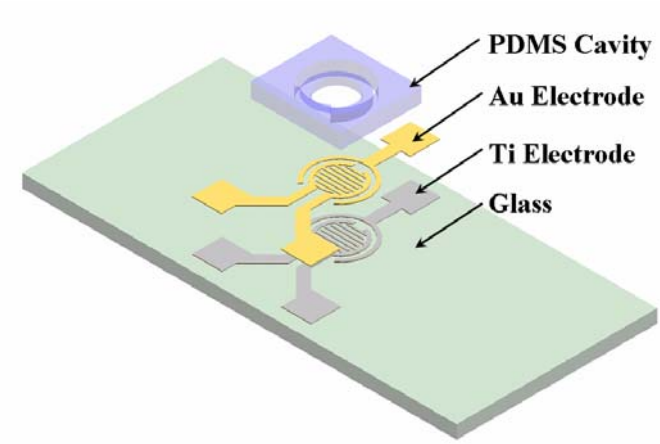
Supplementary Data

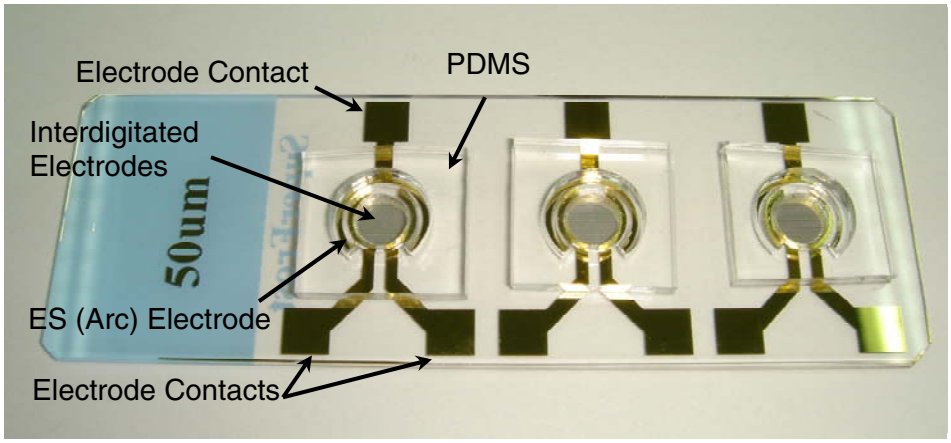
1. Fabrication of an ES-EP Microchip

The developed ES-EP microchip, as shown in Figs. S1a and S1b, consist of two components: a cell-accommodation cavity and the thin-film electrodes. The ES-EP microchip was fabricated on glass slides (Menzel-Glaser, Germany). Microfabrication processes, such as thermal evaporation, photolithography and wet etching, were used to fabricate the thin-film electrodes [1-6]. Gold and titanium thin films were thermally evaporated onto microscopic glass slides. Photolithography was used to transfer the electrode pattern. After chemical wet etching, the ES and EP electrodes were formed. A thick layer of poly-dimethyl siloxane (PDMS), as shown in Fig. S1c, was bonded to the glass surface with patterned electrodes to form the fixed-volume (approximately 100 μL) cell-accommodation cavity. In addition, EP (interdigitated) electrodes are in the cell-accommodation cavity. The ES (arc) electrode is below the hollow horseshoe form of PDMS and not in the cell-accommodation cavity.

The thin-film electrodes, as shown in Fig. 1d, were made of 10 nm titanium and 200 nm gold, the electrode width was 50 μm and the spacing of the electrodes was 50 μm. The interdigitated electrodes are designed for providing adequate electric fields to perform EP in a large area, and the arc electrode is designed for providing a DNA-attracting electric field to perform ES. The interdigitated-electrode design with small spacing (50 μm) can increase the effective electric field area while maintaining low applied voltages.



(a)



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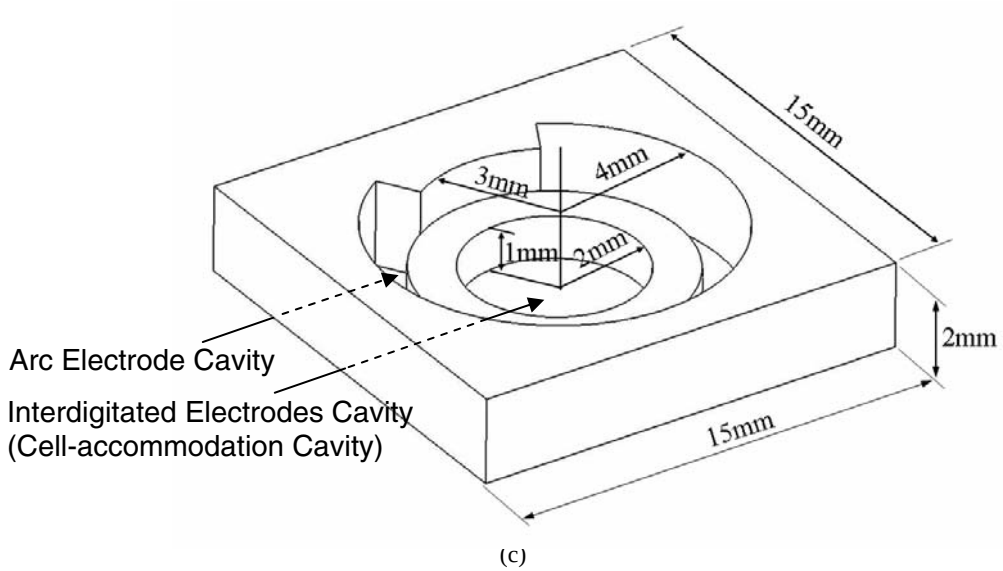
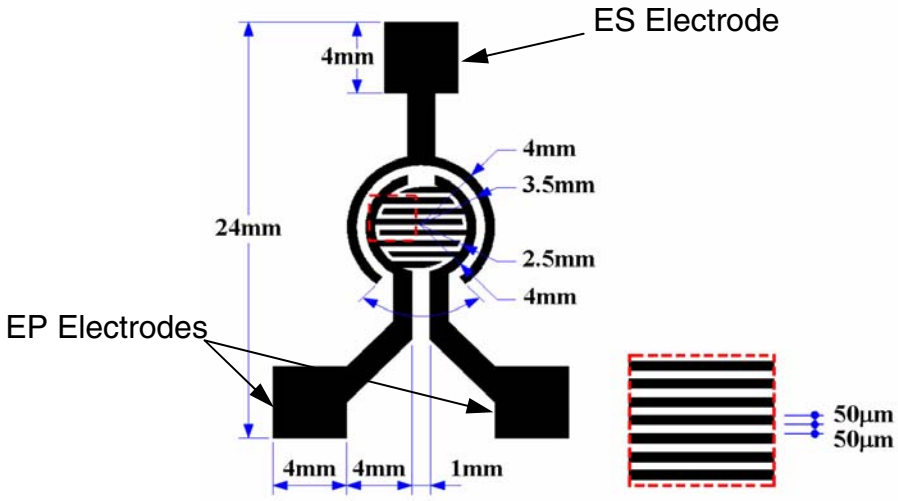


(c)



(d)

Figure S1. Schmatic drawings and photo of the ES-EP microchip: (a) expanded view of the ES-EP microchip, (b) actual photo of the ES-EP microchip, (c) specification of the PDMS cell-accommodation cavity, and (d) close-up image of the interdigitated electrodes.

2. Design of a Logic Circuit for the ES-EP System

There is no ES function provided in commercial EP circuits. The circuit diagram is shown in Fig. S2a. We used a programmable IC to control the output voltage signal for the generation of ES and EP multi-waves. The hardware of this logic circuit was composed of two micro central processing units (CPUs), D/A IC for the applied voltage signals, a digital user interface, and an IC for data-saving. For the higher programmable flash and the faster processing speed in the micro CPUs, we used two AT89C55 ICs (Atmel, USA), between which data transfer is performed by series connection. The applied signal voltage was precision controlled using a DAC08 reference amplifier (Analog Devices, USA). For the digital user interface section, a 16×2 character digital liquid crystal display (LCD) and a 4×4 keyboard were adopted. The memory IC, M93C66 (STMicroelectronics, USA), was selected for saving data. The electrical pulses were delivered to the electroporation microchip via the electrical wires connected from the pulse generator to the contact pads of the ES-EP microchip (Fig. S2b).

The operating procedures of this circuit can be divided into three parts. First, input wave form, voltage magnitude, and delay time are determined. Second, one of two AT89C55 computers is responsible for operating data settings and saving those data to the memory IC. Third, when the “working” command is inputted, the other AT89C55 will retrieve the information needed for output in the memory IC and will let the DAC08 work to obtain the correct output voltage signal. The functions and tuning range of this logic circuit system include: (i) input AC 110 V universal, (ii) output voltage range from -10 V to +10 V, (iii) the minimal time of pulse length and interval is 100 μs and (iv) the wave forms can be changed according to different demands. Figure S2c shows an example of the electric waveform under the ES and EP processes by the logic circuit.

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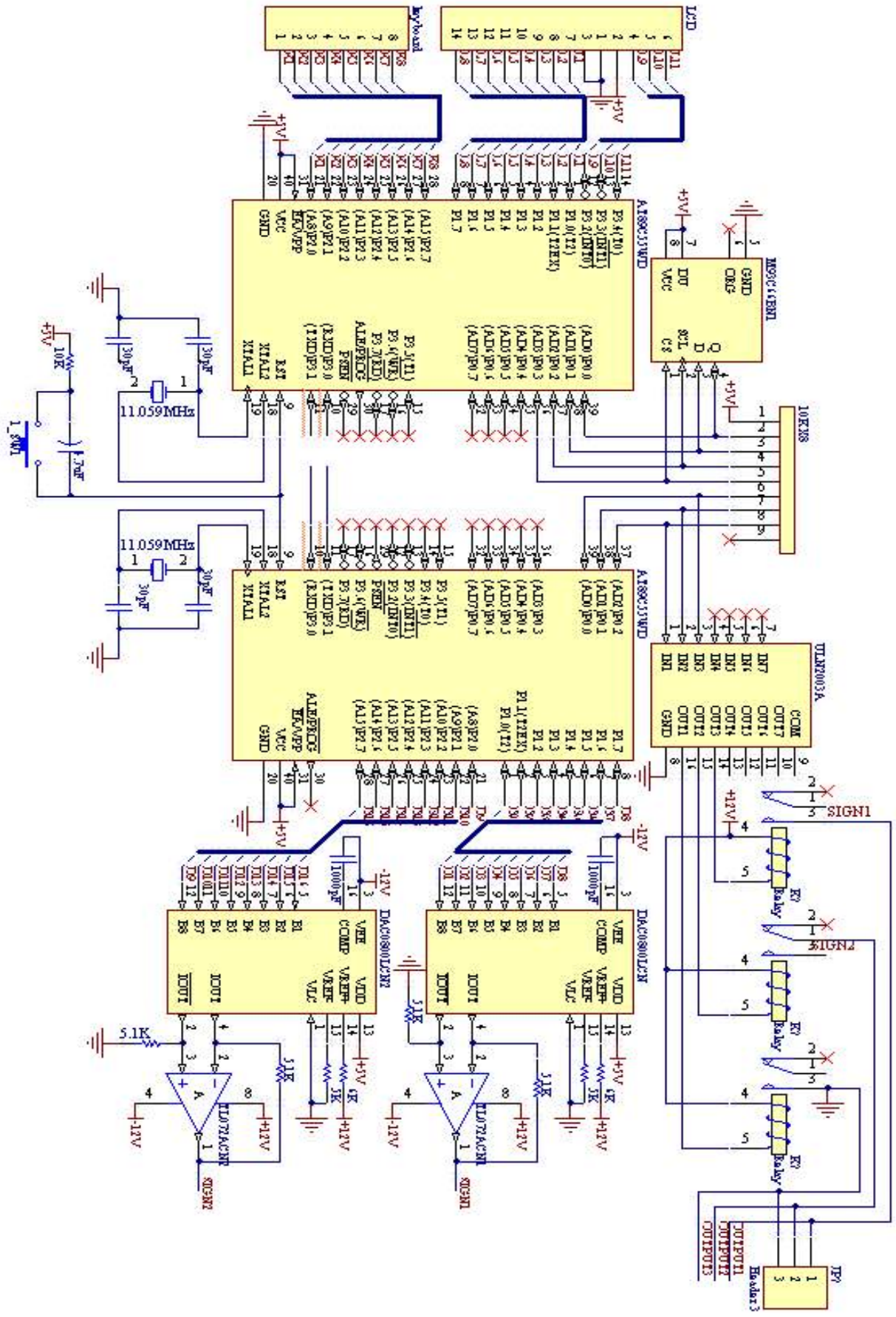
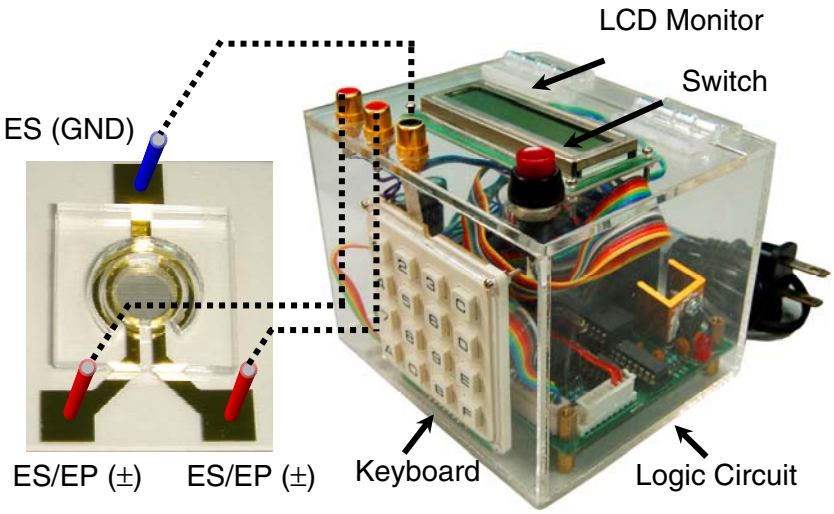
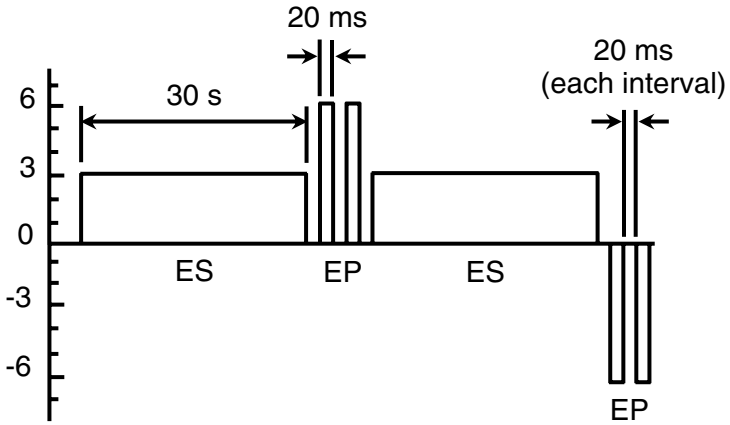


(a)



(b)



(c)

Figure S2. The photos of (a) the circuit diagram, (b) the actual logic circuit, and (c) the sample of the electric waveform with ES and EP (not in scale).

3. Plasmids Preparation and Cell Culture Maintenance

The pEGFP-N1 plasmid (Clontech, USA) coding for green fluorescent protein (GFP) gene was extracted and purified from E.coli using a Plasmid Isolation Kit (Qiagen, Germany). The plasmid was then dissolved in a Tris-EDTA buffer and stored at -20°C until use. A basal cell carcinoma (BCC) cell line was maintained at 37°C, under 5% CO_2 in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma), penicillin (100 units/mL), streptomycin (100 µg/mL), and L-glutamine (4 mM).

4. Detection System

The detection system consists of a microscope and a digital camera. The excitation light is generated using a mercury lamp, filtered by a 420-480 nm band-pass filter and focused on the cell surface through an optical microscope (BX60, Olympus, Japan). The induced fluorescence from the cells is filtered using a 515 nm high-pass filter and is then captured through an optical microscope using a digital camera (DP70, Olympus, Japan).

References

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