Reports

Spatial manipulation of cells and organelles using single electrode dielectrophoresis

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The selection, isolation, and accurate positioning of single cells in three dimensions are increasingly desirable in many areas of cell biology and tissue engineering. We describe the application of a simple and low cost dielectrophoretic device for picking out and relocating single target cells. The device consists of a single metal electrode and an AC signal generator. It does not require microfabrication technologies or sophisticated electronics. The dielectrophoretic manipulator also discriminates between live and dead cells and is capable of redistributing intracellular organelles.

Dielectrophoresis (DEP) is a proven technique for characterizing and manipulating cells by imposing forces through applied AC electrical field gradients. This technique, which can be used on diverse cell types and multicellular organisms, has been largely worked on in physics and engineering laboratories and has received limited attention as a potentially useful tool for biologists (1–3). Most studies utilizing DEP use sophisticated planar DEP microelectrode arrays coupled to microfluidics systems for large-scale separation of thousands of cells (4–6). These devices have shown significant advances with the advent of photolithography-based microfabrication technologies and new polymers, however they are not suitable for small-scale separations of rare cells within a heterogeneous population and are not commercially available. Similarly, cell sorting flow cytometers are optimized for high-throughput applications but are not appropriate for all cell types and often require large quantities of sample material that not all applications have the fortune of. Additionally, these systems are costly and often require a trained technician for proper operation. Other cell manipulation technologies, including optical tweezers, enable single cell manipulation but also require highly specialized and expensive instrumentation.

We have previously reported a DEP electrode designed to pick out and relocate single target cells from a cell culture (7). The electrode took the form of two electrochemically etched gold wires insulated from each other except for a short region near the electrode tips, which formed the working ends of the DEP "tweezer" design single electrode. Consistent fabrication of this design was difficult, and great care was required in preventing both cell damage arising from contact of cells with electrode tips, as well as damage to the tips themselves. To date, single DEP electrodes have been handmade, making them inherently prone to manufacturing inconsistencies. Fabrication of these designs are technically difficult and require instrumentation not standard to most labs, such as capillary pullers, metal etching equipment, and sputter coaters (7–10). Despite these difficulties, single electrode DEP designs offer advantages over planar designs in single cell manipulation and in small-scale separations of primarily rare cell types. However, both configurations utilizing DEP as a technique have found limited practical use in biological research, in part to technical limitations.

Here, we report the characterization and implementation of an improved DEP electrode. Our design is comprised of a single, commercially available microelectrode and requires only a micromanipulator, microscope, and AC signal generator for use. Unlike prior single and planar DEP electrodes, our design is of simpler composition and commercially available, enabling greater consistency and ease of use. Furthermore, our single electrode design provides the ability to select rare cells from a heterogeneous population and applies exacting positive or negative DEP forces to finite regions of a cell. Our design is capacitively coupled to ground, negating the need for a direct ground in the liquid medium and thus aiding in overall experimental setup. In this report, we demonstrate how this electrode is capable of small-scale separations of single cells and demonstrate its ability to assess viability states without the use of chromogens. Lastly, we demonstrate a novel application of single electrode DEP in the spatial manipulation of intracellular organelles.

Materials and methods

Cell culture

Clonal cell lines CHO (gifted by W. Chowanadisai, UC Davis, USA), HeLa (CCL-2; ATCC, Manassas, VA, USA),

and HeLa mCherry-H2B (gifted by Daniel Gerlich, ETH Zurich) were cultured in medium containing Dulbecco's modified Eagle medium (Gibco, Invitrogen, Carlsbad, CA, USA) containing high glucose and sodium bicarbonate, supplemented with 10% FBS (Gibco) and 100 mg/mL penicillin/streptomycin (Invitrogen). All cell lines were grown at 37°C, in 5% CO_2. *Eremosphaera viridis* (no. LB 2600; UTEX, USA) were grown as previously described (11). Lily pollen grains were germinated in a low calcium Dickinson's medium [5 mM MES, 1.27 mM $Ca(NO_3)_2$, 1 mM KNO_3, 0.16 mM H_3BO_3, 5% (w/v) sucrose, pH 5.5] at ambient temperature.

Fluorophore loading

Cell viability labeling was performed by first trypsinizing cells and washing twice in 1× Hank's balanced salt solution (HBSS; no. 14025-092; Gibco) containing calcium and magnesium. Cells were incubated in 2 µM calcein-AM (AnaSpec, Fremont, CA, USA) and 4 µM ethidium homodimer-1 (AnaSpec) for 20 minutes at room temperature. Serial dilutions were performed to acquire a cell density suitable for cell separation. For cell separations of fluorescent and nonfluorescent cells, the above procedure was followed for loading calcein-AM. Both loaded and nonloaded populations were then mixed together.

Imaging

Scanning electron microscopy was performed on noncoated microelectrodes with a Zeiss Supra 40 VP microscope (Carl Zeiss, Munich, Germany) at an accelerating voltage of 2 KV. All light and fluorescent microscopy was performed on a Zeiss Axiovert 40 CFL (Carl Zeiss).

DEP on cells

Prior to use, mammalian cells were detached from the bottom of the culture dish with trypsin treatment and washed twice in 1× HBSS. Cells were then transferred to a low conductivity medium after two rinses with 0.1× HBSS (conductivity of 114 mS/m) supplemented with 2.25 g/50 mL sucrose (CAS: 57-50-1; Fisher Scientific, Pittsburgh, PA, USA) to attain an osmolarity of ~300 mOsm. The conductivity of the 0.1× HBSS solution was 114 mS/m. A 1% agarose-0.1× HBSS cushion was prepared in 35 × 10-mm Petri dishes (Falcon 35 1008; Becton Dickinson, Franklin Lakes, NJ, USA) to prevent cell adhesion to the bottom of the dish and to prevent collision of the electrode tip with the bottom of the dish. Serial dilutions of cells were performed to acquire a cell density suitable for cell separations. After dilutions and washing in 0.1× HBSS-sucrose, cells were transferred to the agarose bottom dish for DEP experimentation. *E. viridis* and lily pollen were directly transferred from growth media and -80°C storage, respectively, to low calcium Dickinson's medium for experimentation. A 1% agarose bottom cushion containing low calcium Dickinson's medium was used for preparations of these cell types. The DEP single electrode (tip resistance of 1 MΩ stainless steel microelectrode, no. SS30031.0A10; MicroProbes for Life Science, Gaithersburg, MD, USA) was positioned by use of a mounted micromanipulator (Narishige, Tokyo, Japan). A signal generator (model 4045; B&K Precision, Yorba Linda, CA, USA) was used for all sinusoidal wave forms. An aluminum foil ground was made by cutting a 10 cm^2 piece of aluminum and placing a small hole (~0.5 cm diameter) in the center (to enable microscopy). A 5 cm piece of silver wire with a 1 mm diameter (7440-22-4; Alfa Aesar, Ward Hill, MA, USA) was secured to the aluminum foil and to a grounded alligator clip. The foil ground was taped in place to the microscope stage.

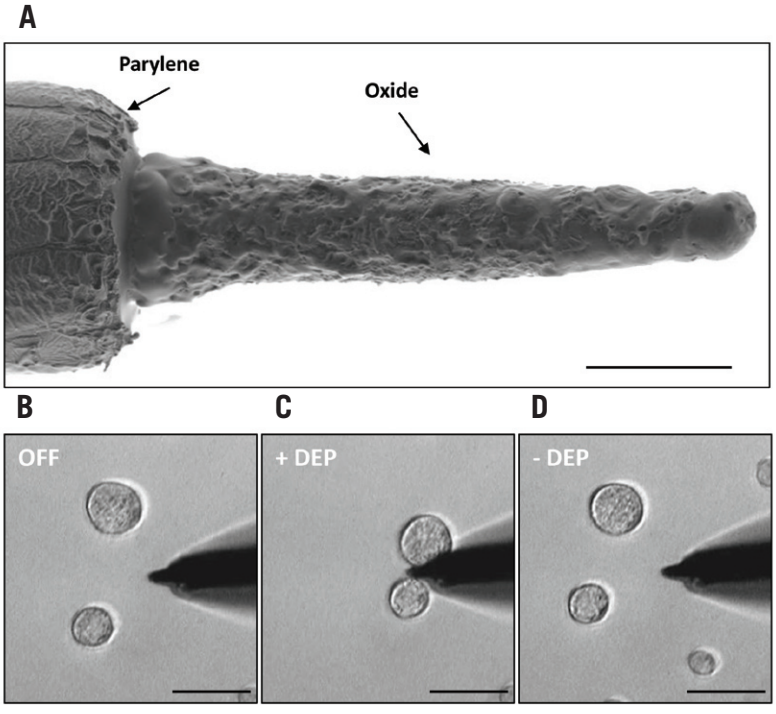

Figure 1. Principles of single electrode DEP. (A) SEM image of a 1 MΩ electrode tip. The etched elgiloy tip has a porous metal-oxide coating. Scale bar is 10 µm. (B) No voltage applied to single electrode. (C) Cells (CHO) attracted to the maximum field gradient at the energized electrode tip. (D) Cells repelled from the energized electrode tip. Scale bars in B–D are 50 µm. Dark region surrounding electrode is diffraction of out-of-focus light off electrode body.

Results and discussion

The DEP cell manipulator described here consists of a single, commercially available metal microelectrode. Although this design generates larger DEP forces at the surface of the electrode than our previous design, cell damage is avoided due to a thin porous metal oxide coating on the microelectrode tip. The oxide coating appears to shield direct exposure of a cell to the energized electrode end, reducing exposure to undesirable AC field exposure effects (12–14). A scanning electron micrograph of a 1 MΩ electrode is shown in Figure 1A. The electrode body is coated with a thin layer of Parylene and the exposed stainless steel tip has a metal-oxide coating.

The DEP force (F_{DEP}) depends on the applied field (E) according to Equation 1:

$$(F_{DEP}) = 2\pi \varepsilon_m R^3 \mathrm{Re}[CM](\nabla E^2_{rms}) \quad (1)$$

where ε_m is the absolute permittivity of the surrounding medium, R is the

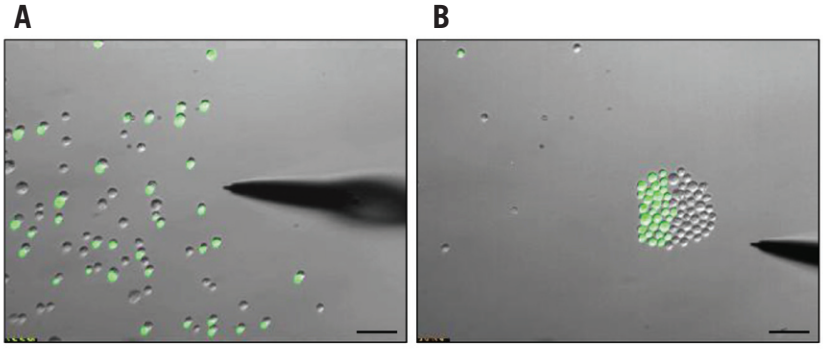

Figure 2. Cell sorting with a single DEP electrode. (A) Overlay of transmitted light and corresponding fluorescence images of a mixed population of calcein-AM loaded and nonloaded CHO cells. (B) Cells were separated into two distinct populations of loaded and nonloaded cells via DEP. Overlay of transmitted light and corresponding fluorescence is shown. Scale bar is 100 µm.

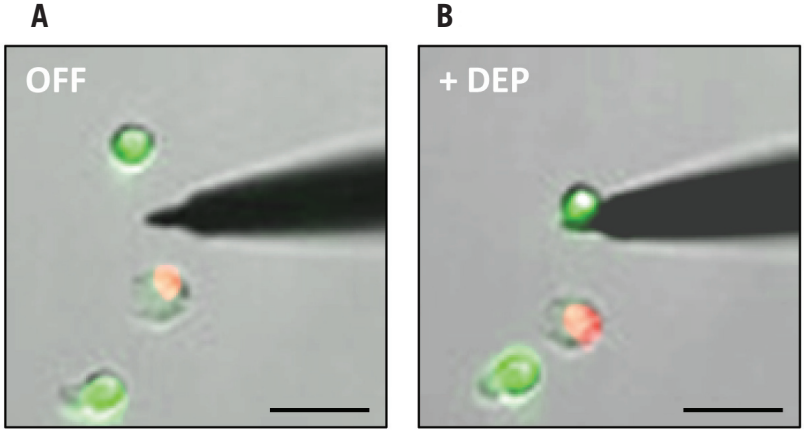

Figure 3. Identification of cell viability with single electrode DEP. (A) CHO cells are labeled with calcein-AM (green) and ethidium homodimer-1 (red), identifying living and dead cells, respectively. No voltage applied. (B) A 10 MHz signal attracts the viable cells and simultaneously repels the dead one. Scale bar is 50 µm.

particle radius, and Re[*CM*] is the real component of the Clausius-Mossotti polarization factor. This relationship assumes that the length scale of the field gradient is large compared to the cell size (2). The polarizability parameter Re[*CM*] can have a value ranging between +1.0 and -0.5, depending on the particle's effective polarizability compared to that of the surrounding medium. Thus, a particle can respond by moving either up (positive DEP) or down (negative DEP) the field gradient generated at the energized electrode tip (Figure 1, B and D).

The experimental arrangement we used for initial testing and use of the DEP microelectrode was comprised of an inverted microscope, an AC signal generator capable of reaching frequencies in the megahertz, an aluminum foil ground, and a micromanipulator positioned with an electrode holder to enable precise three-dimensional control over the DEP microelectrode with reference to a target cell (see the Supplementary material). The AC signal generator enables control of positive (attractive) and negative (repulsive) DEP forces of the electrode through controling amplitude and frequency settings. The counter electrode, acting as the electrical return path to ground potential, can be located either in the cell suspension fluid or as an aluminum foil ground placed outside the bath, between the microscope stage and the bottom of the cell culture dish (see the Supplementary material). For the case of the grounded aluminum foil beneath a 35 × 10 mm Petri dish (Becton Dickinson), the parallel capacitance and resistance of the electrical coupling between the electrode and ground was measured at 9.94 pF and 6.6 kΩ, respectively, with the electrode tip submerged 0.25 cm below the fluid surface. These values changed, as measured with an impedance bridge,

to 10.04 pF and 5.9 kΩ at an electrode tip submersion depth of 1.0 cm. Thus, the effective capacitive reactance of the single electrode is such that capacitive coupling to ground exists for frequencies above ~50 kHz. It was found that single electrode DEP can be operated without a grounded foil or counter electrode altogether. In either case, the electrical return path used is the microscope or nearest ground plane. Without a grounded counter electrode, however, the DEP forces were observed to be slightly weaker.

To demonstrate the overall usefulness of the single DEP electrode in spatial manipulation of living cells, mammalian clonal cell lines Chinese hamster ovary (CHO) and HeLa, were separated from heterogeneous populations (see the Supplementary material for HeLa results). In Figure 2, a mixed population of calcein-AM loaded and nonloaded CHO cells were plated on a cushion of 1% agarose in a Petri dish containing 114 mS/m 10% HBSS supplemented with sucrose as an osmoticum (300 mOsm). No significant cell death was observed within these conditions after 2 hours for both CHO and HeLa cell lines, as measured by cell viability assays (data not shown). Cells labeled with calcein-AM and nonloaded cells were actively separated from the mixed population using single electrode DEP. Voltage amplitude [root mean squared (rms)] and frequency settings of 1 V, 10 MHz and 1 V, 50 kHz were used for positive DEP and negative DEP, respectively. Disruptive effects such as electrolysis and electro-osmotic driven fluid flow were avoided by operating in a low conductive solution (≤250 mS/m), under low voltage (≤4 V rms) and high frequency (≥10 kHz), with no DC voltage bias coupled to the electrode (7,12–14).

The organization of cells shown in Figure 2B was achieved by actively positioning the single electrode within 50 µm of a target cell, applying a 10 MHz signal to attract the cell to the electrode tip, repositioning (by use of micromanipulator or stage), and then repelling the cell from the electrode by changing the frequency to 50 kHz. Using this approach, a single cell or multiple cells (up to 10) can be moved simultaneously. The frequency values of 10 and 50 kHz were determined through preliminary tests to identify optimal frequencies for cell capture and cell repulsion, by positive and negative DEP, respectively. The experimental parameter of relevance is the DEP cut-off frequency (f_{xo}) that defines where a particle makes the transition from a negative to positive DEP response. An estimate of f_{xo} for a viable cell can be derived from Equation 2 (1,2,7):

$$f_{xo} = \sqrt{2\sigma_m/(2\pi R\, C_m)}$$

where σ_m is the conductivity (S/m) of the cell suspending medium, and C_m is the capacitance of the plasma membrane. A cell of radius 10 µm and a typical C_m of 10 mF/m^2, in a 100 mS/m solution, will thus exhibit a DEP crossover at ~225 kHz. A viable cell will exhibit negative DEP (repulsion) at frequencies below ~ $f_{xo}/4$, and positive DEP (attraction) at frequencies above ~$4f_{xo}$. Following the procedure described previously by Menachary et al, the field gradient parameter (∇E^2) of Equation 1 was determined to be ~5 × 10^{11} V^2/m^3 at a radial distance of ~40 µm from the electrode tip with an applied voltage of 1 V (rms) (7). With all other experimental factors remaining constant, this represents an enhanced DEP force compared to the DEP tweezer, without producing any noticeable electrical damage to the cells.

Cell viability is commonly identified through the use of chromogens and fluorophores. However, DEP can also distinguish between live and dead cells (1,2). The plasma membrane of a dying cell loses its high resistance to passive ion leakage. This is manifested as an increase of the DEP crossover frequency defining where the polarizability parameter Re[*CM*] of Equation 1 changes from a negative to positive value as the field frequency is increased (2,7). We used DEP to demonstrate that the single electrode could identify living and dead CHO cells within a population. In Figure 3, cells were first labeled with calcein-AM and ethidium homodimer-1 fluorophores to visually verify living and dead cells, respectively. Living cells displayed cell attraction at 10 MHz and repulsion from the electrode tip at 50 kHz, whereas dead cells where consistently repelled at both 10 and 50 kHz.

Lastly, we explored the use of single electrode DEP on smaller diameter, polarizable biological entities. Freshwater green algae, *Eremosphaera viridis* and growing lily pollen tubes were used for their pellucid unicellular bodies, which contain several chloroplasts and rapidly trafficking organelles, respectively. Both *Eremosphaera* and lily pollen tubes thrive in low conductivity media, making them amenable to DEP studies. In the following experiments, a single DEP electrode was positioned near either a single *Eremosphaera* cell (Figure 4A) or a growing lily pollen tube (Figure 4C). A signal of 100 kHz, 5 V (rms) was applied to both cells (Figure 4, B and D). The *Eremosphaera* were attracted to the electrode by positive DEP, indicating that

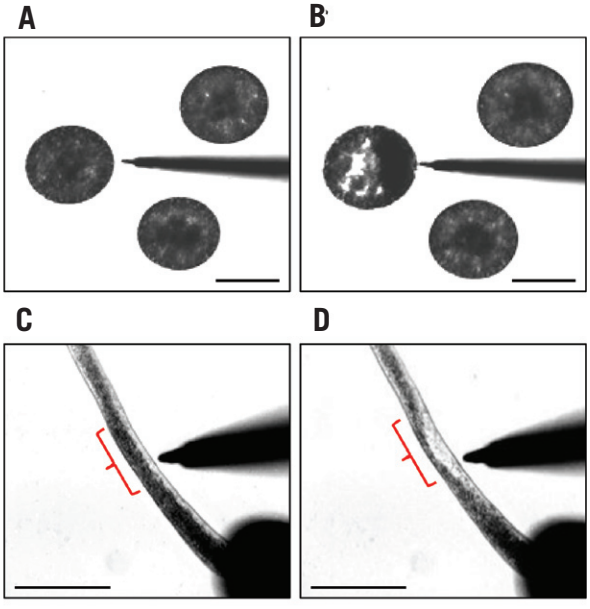

Figure 4. Redistribution of intracellular organelles. (A) Single electrode positioned near an *E. viridis* cell without an applied voltage signal. (B) A 100 kHz, 5 V (rms) signal applied for 10 min. Scale bar is 100 µm. (C) Single electrode positioned near a growing lily pollen tube without an applied voltage signal. (D) A 100 kHz, 5 V (rms) signal applied for 1 min. Brackets emphasize region subject to DEP. Scale bar is 100 µm.

the electric field penetrated the outer membrane and into the cell interior. The intracellular chloroplasts (Figure 4B) were also attracted towards the electrode tip. In the growing pollen tube, the flowing stream of organelles (Figure 4D) were repelled by negative DEP, greatly slowing the rate of cytoplasmic streaming within the growing tube. Both types of intracellular organelles exhibited strong redistributions in response to the DEP force. Upon removal of the voltage signal, the intracellular organelles evenly redistributed themselves in the *Eremosphaera* after approximately 1 hour, and cytoplasmic streaming recommenced within the lily pollen tube after seconds (data not shown).

The single electrode DEP design described provides a simpler and more cost-effective solution to small-scale cell manipulation over costly systems. The uncomplicated nature of its design and commercial availability provide significant advantages over previous single DEP electrodes. We have shown it is suitable for single cell manipulation, determining cell viability states, and redistributing intracellular organelles. These abilities may ultimately aid researchers interested in studying rare cells found within heterogeneous populations or exploring unknown functions of intracellular organelles. Moreover, use of our single electrode DEP design could be automated for higher throughput applications. The ability to spatially manipulate both cells and intracellular organelles opens doors toward studying multiple exciting and intriguing biological phenomena. For example, how are cell division, migration, and/or growth influenced by asymmetric organellular distribution? Its plasticity toward a wide range of cell types and efficiency make it amenable as a biological tool.

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Competing interests

The authors declare no competing interests.

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