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Dielectrophoresis tweezers for single cell manipulation

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Abstract Positioning single cells is of utmost importance in areas of biomedical research as diverse as in vitro fertilization, cell-cell interaction, cell adhesion, embryology, microbiology, stem cell research, and single cell transfection. Here we describe dielectrophoretic tweezers, a sharp glass tip with electrodes on either side, capable of trapping single cells with electric fields. Mounted on a micromanipulator, dielectrophoresis tweezers can position a single cell in three dimensions, holding the cell against fluid flow of hundreds of microns per second with more than 10 pN of force. We model the electric field produced by the tweezers and the field produced by coaxial microelectrodes. We show that cells are trapped without harm while they divide in the trap. In addition, dielectrophoretic tweezers offer the possibility for trapping, electroporating, and microinjecting a single cell with one probe.

Keywords Dielectrophoresis · Single cell manipulation · Tweezers · Single cell electroporation.

As biologists struggle to understand and manipulate living systems, they increasingly turn to single cell analysis (Brehm-Stecher and Johnson, 2004). With the advance of miniaturization, it is now possible to perform mRNA analysis on a single cell (Eberwine, 2001), transfect a single cell to change its gene expression, observe how one cell differentiates, or study how two individual cells interact. The quantum of biology is the cell, and biologists strive to reach into that world. For over one hundred years, the standard technique for single cell manipulation has been to grasp a cell with suction through a hollow glass micropipette tip (Barber, 1904). This manipulation process requires a very skilled operator and can easily damage the cell membrane or cytoskeleton (Fleming and King, 2004). Optical tweezers are an alternative (Ashkin et al., 1987), requiring intricate optics and producing limited manipulation force. Microactuators for physically holding cells (Chronis and Lee, 2004) are possible to build but have not been widely adopted. Local magnetic fields can be used to move cells (Gosse and Croquette, 2002; Lee et al., 2004) if the cells are tagged with magnetic beads. Cells can be trapped with arrays of microfluidic chambers and valves (Yun and Yoon, 2005) or electrodes (Manaresi et al., 2003), which are well suited for parallel, high throughput experiments. However, a micromanipulator based trap has the advantage of being able to arbitrarily position single cells in three dimensions. Here we present dielectrophoretic (DEP) tweezers shown in Fig. 1 DEP tweezers use electric forces to hold single cells at the end of a micromanipulator. This technique is simple, robust, label free, and does not damage cells. DEP tweezers with electrodes tens of nanometers apart could be useful tools for manipulating nanoparticles or macromolecules.

Figure 1(a) is an illustration of how DEP tweezers work. Two electrodes a few μm apart provide a non-uniform electric field which polarizes a nearby cell. If the cell is more polarizable than the solution, it will be attracted to the field maximum at the tip of the electrodes. The movement of the cell due to the force on the induced dipole is called DEP. In more detail, the DEP force on a spherical particle is given by $F_{\mathrm{DEP}} = 2\pi\varepsilon_f r^3 \mathrm{Re}[\mathrm{CM}(\omega)]\nabla E^2_{\mathrm{rms}}$, where r is the radius

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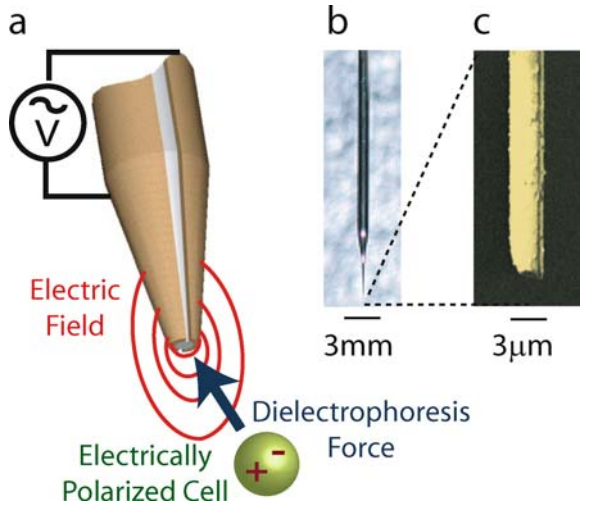

Fig. 1 DEP tweezers for cell manipulation. (a) Schematic of DEP tweezers in operation. A voltage across two electrodes on either side of a sharp glass tip creates an electric field which polarizes a cell and pulls the cell into the field maximum at the end of the tip. (b) Photograph of DEP tweezers. (c) SEM image of the tweezer tip. Electrodes appear light while the insulating gap between electrodes is dark

of the particle, E_{rms} is the root mean squared electric field, ε_f is the fluid permittivity, ω is the frequency of the electric field, and CM(ω) is the Clausius-Mossotti factor which depends on the permittivity and conductivity of the cell and the fluid. (Pohl, 1978) For a given cell, a positive CM(ω) can be achieved by adjusting the frequency of the electric field and the conductivity of the fluid, which will trap the cell at the electric field maximum at the tip of the DEP tweezers. The tweezers are also capable of being used when the CM factor is negative, in which case, cells will be pushed away from the tip. Using MHz AC voltages for DEP minimizes the induced potential across the trapped cell membrane and the electro-osmotic flow of the charged double layer (Jones, 1995).

A photograph of the DEP tweezers is shown in Fig. 1(b). The first step to fabricate DEP tweezers is a standard method for fabricating micropipettes (Fleming and King, 2003). A 1 mm diameter glass rod is pulled to a sharp tip in a pipette puller that controllably heats the center section of the rod and pulls on either end until the rod tapers and breaks. To produce uniform, repeatable tips with a desired radius, the tapered tip was examined under a microscope and fractured at the correct radius.

To deposit electrodes, the sharpened glass rod was placed in a high-vacuum thermal evaporator. 7 nm Ti and 20 nm Au were evaporated on one side of the rod, the rod was flipped, and Ti-Au deposited on the opposite side. Most electrode pairs produced with this method were electrically isolated. If a small metal bridge connected the two electrodes, applying a few volts between the electrodes burned out the thin film of metal that caused the short.

To use the DEP tweezers, a micromanipulator was mounted on top of an inverted microscope. Spring steel clips coated with soft indium held the DEP tweezers on the micromanipulator and made electrical contact to the electrodes on either side of the tweezers. A function generator provided voltage for the electrodes with adjustable frequency and amplitude.

Figure 1(c) is an electron micrograph of the tweezer tip. We manufactured tweezer tips 3 μm in diameter to manipulate yeast cells approximately 6 μm in diameter. Sharper tips produce higher field gradients than broad tips for a given electric field. However, if a tip is much sharper than the size of the cell being trapped, the electric field gradient produced by the tip will die off significantly across the diameter of the cell, resulting in reduced trapping force. Our tip diameter was chosen to maximize the trapping force by producing a high electric field gradient across the entire diameter of the cell.

Figure 2(a) shows a finite element simulation of the electric field at the tip of the tweezers with an applied voltage of 10 V peak to peak. The simulation software (Maxwell 3D, Ansoft) solves Poisson's equation on a mesh of tetrahedrons optimized to fit the actual tip geometry. The simulation included a glass cylinder 3 μm in diameter with dielectric constant 5.5 ε_0 immersed in water with dielectric constant 81ε_0. Two metal electrodes wrap 170 degrees around the cylinder with 10 degree gaps between the electrodes. The electric field maximum is approximately 2×10^6 V/m and dies away to 10^4 V/m within a distance to the tweezer tip, $r < 20$ μm. The electrodes do not form a perfect point dipole so the field dies off slower than $1/r^3$. It is possible to produce a more concentrated electric field distribution with coaxial electrodes, simulated in Fig. 2(b). A 1 μm diameter inner conductor is surrounded by an insulating glass layer and a 3 μm diameter,

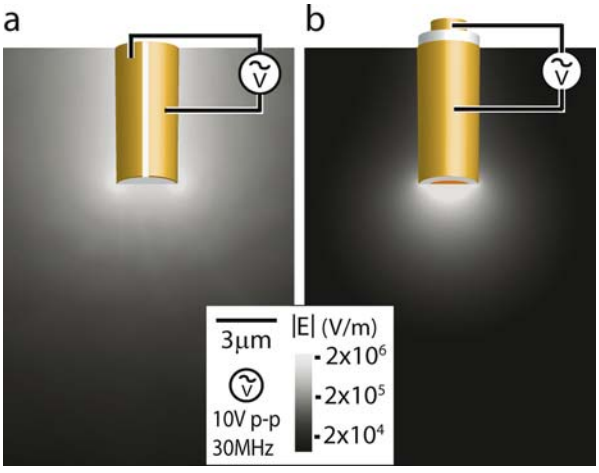

Fig. 2 Electric field simulations near the DEP tweezer tip. Finite element model shows the field magnitude produced by DEP tweezers in a plane through the axis of the tweezers. (a) DEP tweezers as fabricated. (b) Field produced by a coaxial tweezer

100 nm thick outer conductor. The coaxial geometry produces a very high field gradient that dies off with $1/r^3$ and provides strong trapping forces close to the tip. The outer conductor also serves to shield the surrounding liquid from electric field everywhere except at very end of the tip, which reduces joule heating and the resulting convection of liquid. However, the sharper field distribution of coaxial electrodes results in a reduced trap radius compared to the fabricated DEP tweezers (Fig. 2(a)).

Modeling a yeast cell (Kotnik and Miklavcic, 2000) trapped at the tip of the tweezers in Fig. 2(a), with 10 V at 30 MHz applied to the electrodes, the induced voltage across the cell membrane is less than 40 mV. To further decrease the voltage across the cell membrane when the cell is trapped at the tweezer tip, the voltage on the tweezers can be reduced so that the electric field provides only the necessary trapping force for a particular manipulation.

Trapping a single yeast cell at the end of the tweezers is shown in the micrograph sequence in Fig. 3(a). Baker's yeast, (*Saccharomyces cerevisiae*) were suspended in standard yeast growth medium (YPD broth, BD) with electrical conductivity 29 mSiemen/m. The tweezers were energized with 10 V peak to peak at 30 MHz and a nearby yeast cell was pulled into the maximum of the field at the tip of the tweezers. By moving the micromanipulator, the trapped cell could be translated through the fluid at hundreds of microns per second without escaping from the tweezers. Stokes drag yields an estimate of the DEP force pulling a yeast cell toward the tip of the tweezers. For a spherical cell in laminar flow, $F_{\text{drag}} = 6\pi\eta a v$, where η is the viscosity of water, a is the radius of the cell ($\sim 3\ \mu$m), and v is either the maximum velocity of a cell as it is pulled onto the tweezer tip ($\sim$0.2 mm/s) or the maximum velocity that the tweezers can be translated through fluid without releasing the cell ($\sim$0.5 mm/s). A single yeast cell is trapped by DEP tweezers with 10–50 pN force.

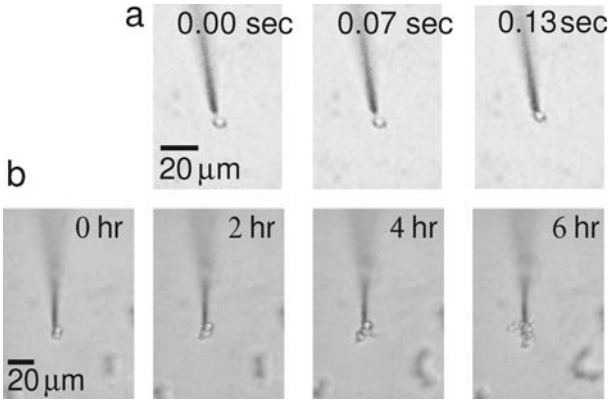

Fig. 3 Cells held by DEP tweezers. (a) DEP tweezers capturing a yeast cell. The electrodes were energized at 0.0 s and the yeast was rapidly pulled into the field maximum at the tip of the tweezers. (b) Yeast dividing while trapped at the tip of the tweezers

To demonstrate that the strong electric field at the tip of the tweezers did not harm yeast cells, we trapped cells for many hours and observed them dividing in the trap. Figure 3(b) shows two yeast cells trapped by the tweezer. Both cells budded and formed daughter cells in 2 h, producing a cell mass with many cells within 6 h, still trapped by the field of the DEP tweezers. The voltage induced by the DEP tweezers across the cell membrane of yeast does not interfere with essential cellular mechanisms necessary for growth and reproduction. To manipulate cells that are more sensitive to transmembrane potentials, it is possible to reduce the voltage on the DEP tweezers at the expense of trapping force.

DEP tweezers are a powerful tool for manipulating individual cells in physiological conditions which is an increasingly important technique in biomedical research. It is straightforward to add electrical contacts to a suction-based micropipette micromanipulation system, allowing the use of DEP tweezers instead of suction for cell manipulation. Furthermore, by applying a high voltage pulse to the DEP tweezer electrodes, it should be possible to selectively and controllably electroporate a single cell. If the DEP tweezers are fabricated with a hollow pipette tip, it may be possible to hold a cell with electric field, electroporate the cell with a voltage pulse, and perform a microinjection with a single probe. This simple technique would greatly facilitate in vitro fertilization and single cell transfection studies. Another promising application for DEP tweezers is to trap and position nanoparticles. It is possible to make electrodes on an AFM tip (van der Weide and Neuzil, 1996) or a very sharp glass tip. The electric field produced by such a sharp tip could be increased until the field reached the dielectric breakdown strength of water. An electric potential of 1 V across a 100 nm gap would allow stable trapping of objects with 5 nm diameters.

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