Electrical Forces For Microscale Cell Manipulation

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electrophoresis, dielectrophoresis, lab-on-a-chip, cell separation, cell patterning

Abstract

Electrical forces for manipulating cells at the microscale include electrophoresis and dielectrophoresis. Electrophoretic forces arise from the interaction of a cell's charge and an electric field, whereas dielectrophoresis arises from a cell's polarizability. Both forces can be used to create microsystems that separate cell mixtures into its component cell types or act as electrical "handles" to transport cells or place them in specific locations. This review explores the use of these two forces for microscale cell manipulation. We first examine the forces and electrodes used to create them, then address potential impacts on cell health, followed by examples of devices for both separating cells and handling them.

INTRODUCTION

Electrophoresis (EP): the force on a charged particle in an electric field

Dielectrophoresis (DEP): the force on a polarizable particle—such as a cell—in a spatially nonuniform electric field

Manipulating cells is fundamental to much of biology and biotechnology. From cell-based screens for basic science (1) to surface immunophenotyping for diagnosis (2), from studying how cell shape affects differentiation (3) to detecting pathogenic bacteria in food supplies (4), cell-based assays are crucial to bioscience. Integral to these assays is the need to manipulate the physical location of cells, either to separate them from phenotypically different cells or to organize them in vitro. Owing to the small size and typically large numbers of cells, we need surrogate "hands" to provide efficient physical access to cells that our fingers cannot grasp.

Many techniques exist to physically manipulate cells, including optical tweezers (5), acoustic forces (6), and surface modification (7). Electrical forces are an increasingly common approach for enacting these manipulations. Electrical forces can be classified into electrophoresis (EP) or dielectrophoresis (DEP) depending on whether they act on a particle's fixed or induced charge, respectively. From these, a whole host of different forces can be created to translate, rotate, stretch, or otherwise manipulate particles. As discussed below, these forces tend to scale favorably for manipulating cells in microfluidic devices, explaining their popularity.

In this review, I focus on the use of EP and DEP to manipulate cells. I distinguish separation, where one wishes to move one type of cell relative to another, from handling, where one wishes to move one type of cell relative to the device within which it resides. As defined, separation is a subset of handling. Given the predominance of DEP over EP for such manipulations, this review gives more attention to DEP. Additionally, I focus on research that relates to cells rather than colloids to highlight the specific challenges one faces when working with living things. In this context, the term particles includes cells from hundreds of nanometers to tens of micrometers.

Owing to space limitations, I do not discuss electrical methods to manipulate proteins, nucleic acids, or other subcellular entities, nor do I discuss indirect electrical forces, such as electrohydrodynamics, where electric fields move liquids which in turn move cells (reviewed in 8).

THEORY

Cells can be manipulated in electric, magnetic, and electromagnetic fields. Electromagnetic fields are the most general case, and are commonly used to manipulate particles when used as optical tweezers (5). At low frequencies or in systems that are small compared with the wavelength of the field, the electric and magnetic components of the electromagnetic field decouple and one will dominate (9). In microsystems, where the characteristic length is <1 mm, fields at frequencies $\ll 30$ GHz will decouple. This decoupling leads to the quasistatic approximation (9), and the resulting fields are known as electroquasistatic and magnetoquasistatic fields. These are commonly referred to as the electric and magnetic fields, respectively, although that is only strictly true at DC. In this review, I use the terms electric and electroquasistatic interchangeably.

Magnetoquasistatic fields are used to manipulate cells either due to an intrinsic magnetizability, such as the iron in red blood cells (10), or by attaching magnetic materials to the cells for magnetically activated cell sorting (11). The resulting force is due to the interaction of a magnetic dipole with a nonuniform magnetic field and is known as magnetophoresis. Magnetophoresis has the important attribute of being highly specific because the vast majority of material does not respond strongly to magnetic fields. Thus, targeting a magnetic material to a cell makes it very easy to then separate that cell from the milieu. The drawback of being highly specific is that the technique requires a label (except for red blood cells), whereas techniques that rely on electrical properties can be label-free and are thus intrinsically more universal.

Forces of Electrical Origin

Electroquasistatic forces result from the interaction of electric charges and the fields that they generate. These electrical forces can be subdivided into two categories: Coulomb forces and dielectrophoretic forces.

Electrophoresis. The Coulomb force is given by $\mathbf{F} = q\mathbf{E}$, where q is the net charge on the object and $\mathbf{E}$ is the applied electric field. Most cells [except for some bacteria (12)] are covered with negatively charged functional groups at neutral pH (13). Because the cells are charged, they can be acted upon by electric fields. In water, the cells will move at a velocity given by the balance of the Coulomb and viscous drag forces, a process known as EP (**Figure 1*a***, *left*). The electrophoretic mobility (μ) relating electric-field intensity to velocity is, to first order, given by $\mu = \varepsilon_m \xi/\eta$, where ε_m is the permittivity of the liquid, η is the liquid viscosity, and ξ is the zeta potential, which is primarily related to the particle's charge density and the ionic strength of the liquid (14). For most biological cells, the EP mobility is $\sim 10^{-4}$ cm^2/V-s, or 1 μm/s in a field of 1 V/cm (13). Any use of EP, therefore, to separate different cell types is dependent on the two cells having different zeta potentials.

Dielectrophoresis. Whereas EP arises from the interaction of a particle's charge and an electric field, DEP (in its simplest form) is due to the interaction of a particle's dipole and the spatial gradient of the electric field (see texts 15–18 and reviews 19–21). One general form of the expression is

$$\mathbf{F}_{dep} = \mathbf{p} \cdot \nabla \mathbf{E}, \tag{1}$$

where $\mathbf{p}$ is the particle's dipole moment. One sees that the gradient of the electric field ($\nabla \mathbf{E}$) must be nonzero for the force to be nonzero, which can be explained with reference to **Figure 1**. Here we see that if each half of a dipole sits in the same electric field (**Figure 1*a***, *right*), then the cell will experience equal ($\mathbf{F}_- = \mathbf{F}_+$) opposing forces and no net force. If, however, each half of the dipole is in a field of different magnitude ($\mathbf{F}_- > \mathbf{F}_+$, **Figure 1*b***), then the net force will be nonzero, driving the cell up the field gradient. We also note that if the dipole is not oriented along the field, then a nonzero torque will be created, forming the basis of electrorotation (22). In both uniform and nonuniform fields, the cell will also experience an electrodeformation

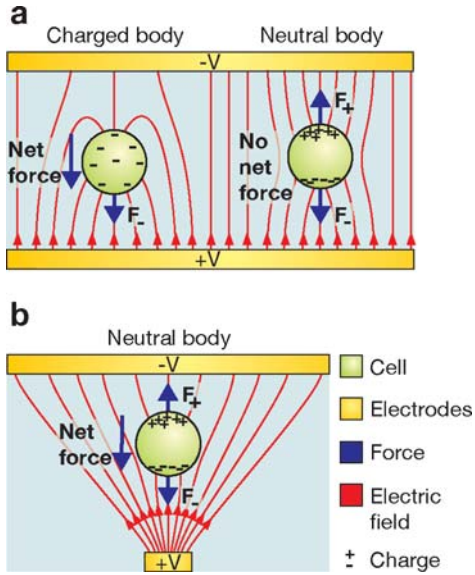

Figure 1

EP and DEP. (*a*) Charged and neutral particle in a uniform electric field. The charged particle (*left*) feels an EP force, whereas the dipole induced in the uncharged particle (*right*) will not result in a net force ($F_- = F_+$). (*b*) A neutral particle in a nonuniform electric field. The particle will experience a net force toward the electric-field maximum because the field magnitude is different at each end of the particle ($F_- > F_+$).

force proportional to $|\mathbf{E}|^2$ (23, 24). This force is usually negligible, but can be used intentionally to enhance cell electroporation and electrofusion (24).

To obtain a practical force expression, we need to determine the dipole moment $\mathbf{p}$. For cells, the dipole moment is induced by the applied electric field. When cells (and other polarizable particles) are placed in an electric field, a dipole is induced to satisfy the boundary conditions on the electric field. This induced dipole can be created by free charge, by polarization charge (e.g., water), or in general by a combination of the two. The exact constitution of the dipole will be related to the frequency of the applied field; at low frequencies (down to DC) free-charge dipoles dominate, whereas polarization-charge dipoles dominate at high frequencies. One typically uses AC fields (rather than DC) for DEP because that will damp out EP-induced motion while minimizing physiological impact on the cells and any electrochemical reactions at the electrodes (both described below).

For a uniform sphere placed into a sinusoidal electric field given by $\mathbf{E}(\mathbf{r},t) = \mathrm{Re}[\underline{\mathbf{E}}(\mathbf{r})e^{j\omega t}]$, where $\underline{\mathbf{E}}(\mathbf{r})$ is the complex electric-field phasor that contains spatial information on the field intensity and polarization, the overall induced dipole is given by

$$\underline{\mathbf{p}}(\mathbf{r}) = 4\pi\varepsilon_m R^3 \left(\frac{\underline{\varepsilon}_p - \underline{\varepsilon}_m}{\underline{\varepsilon}_p + 2\underline{\varepsilon}_m}\right) \underline{\mathbf{E}}(\mathbf{r}) = 4\pi\varepsilon_m R^3 \cdot \underline{K}(\omega) \cdot \underline{\mathbf{E}}(\mathbf{r}), \qquad (2)$$

where R is the radius of the particle; $\underline{\varepsilon}_m$ and $\underline{\varepsilon}_p$ are the complex permittivities of the medium and the particle, respectively, and are each given by $\underline{\varepsilon} = \varepsilon + \sigma/(j\omega)$, where ε is the permittivity of the medium or particle, σ is the conductivity of the medium or particle, and j is $\sqrt{-1}$. $\underline{K}$ is known as the Clausius-Mossotti (CM) factor.

In a linearly polarized sinusoidal field [e.g., $\underline{\mathbf{E}}(\mathbf{r}) = \mathbf{E}(\mathbf{r})$], we can combine Equations 1 and 2 to arrive at an expression for the time-average DEP force, given by

$$\left\langle \mathbf{F}_{dep}(\mathbf{r}) \right\rangle = \pi \varepsilon_m R^3 \mathrm{Re}\left[\underline{K}(\omega)\right] \cdot \nabla \left|\mathbf{E}(\mathbf{r})\right|^2, \tag{3}$$

where $\langle\,\rangle$ denotes the time average. If the relative polarizability of the cell is greater than that of the medium, then $\mathrm{Re}[\underline{K}(\omega)]$ will be positive (known as positive DEP, or pDEP), and the force will be directed up the field gradient. If the cell is less polarizable than the medium, then $\mathrm{Re}[\underline{K}(\omega)]$ is negative, and the force will be directed down the field gradient (negative DEP, or nDEP). Examining the expression for $\underline{K}(\omega)$ in Equation 2, one sees that $\mathrm{Re}[\underline{K}(\omega)]$ can only vary between $+1$ and -0.5.

Of course, cells are neither uniform (e.g., they are multilayered particles with a membrane, cytoplasm, etc.) nor necessarily spherical (e.g., red blood cells, some bacteria). These complications do not alter the fundamental physics, but rather result in more complicated expressions for the induced dipole (and the resulting DEP force) (15, 16, 25). The complicated internal cellular structure primarily manifests itself in the Clausius-Mossotti factor ($\underline{K}$).

In **Figure 2** we show the calculated CM factor for a typical mammalian white blood cell. Several features are apparent. First, the CM factor is a function of frequency. This is due to the fact that the polarizability of the cell has contributions from the different internal compartments, each having unique kinetics. For instance, at low frequencies ($\lesssim$100 kHz), the cell appears insulating and therefore less polarizable than typical ionic media, and will thus experience nDEP. At higher frequencies ($\sim$1–100 MHz), the field bridges the membrane and the CM factor will compare the cytoplasm and the media conductivities, resulting in pDEP at low solution conductivities and nDEP at high solution conductivities. Finally, at high frequencies (>1 GHz) the CM factor compares the cytoplasmic and media permittivities. For these cells this results

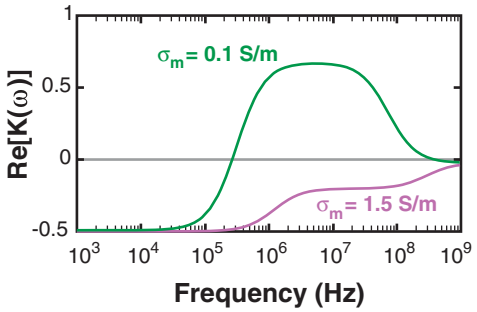

Figure 2

Real part of the CM factor of mammalian cells at low (0.1 S/m) and high (1.5 S/m) solution conductivities. Cell electrical properties, which are representative of white blood cells, were obtained from Reference 66.

in nDEP, likely due to cytoplasmic proteins that impart a net permittivity lower than water.

Traveling-wave DEP (twDEP): the DEP force on a particle in a field with spatially varying phase

An important consequence of the preceding discussion is that one can probe different compartments of the cell by varying the frequency. In fact, this is the basis for devices aimed at finding electrical manifestations of phenotypic differences to enable electrical separation of different cell types. A second important consequence is that cells will experience nDEP or pDEP depending on the applied frequency, the cells' electrical properties, and the conductivity (or permittivity) of the solution. Conductivity is easiest to adjust and so is the most common variable, although one can vary permittivity by using buffers that include molecules with large dipole moments (26, 27).

One can impose a translational force on cells by placing them in a field with spatially varying phase [e.g., when $\underline{\mathbf{E}}(\mathbf{r})$ is complex] (28). One then gets an additional term in the force expression of Equation 3, which now includes a force due to traveling-wave DEP (29):

$$\left\langle \mathbf{F}_{tw\,dep}(\mathrm{r}) \right\rangle = 2\pi\varepsilon_m R^3 \mathrm{Im}\left[\underline{K}(\omega)\right] \cdot \nabla \times \left(\mathrm{Im}\left[\underline{\mathbf{E}}(\mathbf{r})\right] \times \mathrm{Re}\left[\underline{\mathbf{E}}(\mathbf{r})\right]\right). \quad (4)$$

In general, not only dipoles but also quadrupoles and higher-order multipoles are induced in the cell, and these must sometimes be taken into account to describe particle behavior (30–33). Most generally, the electrical force on particles in arbitrarily polarized sinusoidal electric fields can be expressed in a compact tensor expression, first introduced by Jones & Washizu in 1996 (29), and given by

$$\left\langle \mathbf{F}^{(n)}_{\mathbf{dep}}(\mathbf{r}) \right\rangle = \frac{1}{2}\mathrm{Re}\left[\frac{\underline{\underline{\underline{p}}}^{(n)} [\cdot]^n (\nabla)^n \underline{\mathbf{E}}^*}{n!} \right], \quad (5)$$

where n refers to the force order ($n = 1$ is the dipole, $n = 2$ is the quadrupole, etc.), $\underline{\underline{\underline{p}}}^{(n)}$ is the complex multipolar induced-moment tensor, and $[\cdot]^n$ and $(\nabla)^n$ represent n dot products and gradient operations. Thus one sees that the n-th force order is given by the interaction of the n-th-order multipolar moment with the n-th gradient of the electric field. For $n = 1$, the result reverts to the force given by Equation 1, whereas for $n = 0$ (zeroth-order dipole or monopole), one obtains the Coulomb force.

Finally, when cells in electric fields approach each other, the induced dipoles will attract each other, causing the cells to form "pearl chains" (15). This "chaining" can be both beneficial (e.g., to enhance cell fusion) or deleterious (e.g., if one is trying to keep cells separate).

SCALING

To understand the popularity of applying electrical forces at the microscale, it helps to examine how the forces scale with size. If the length that characterizes electric-field variations is L then the EP force will scale as $\mathbf{F}_{ep} \sim V/L$, where V is the applied voltage. This means that when going from a macro- to a microsystem, one can either reduce V along with L to keep the field constant, or, by keeping V constant as L decreases, get higher fields for the same voltage.

The DEP force scales more strongly with L, as $\mathbf{F}_{dep} \sim V^2/L^3$. This means that in scaling from a macroscopic system with $L = 1$ cm to one where $L = 100$ μm, one can reduce the voltage by ∼1000× and get the same force. Thus, one would need to apply 1000 V in the macroscale system to get the same force achievable with 1 V in a microscale system. Or, keeping the voltage constant, one gets a stronger force by scaling down the characteristic length of the system. More practically, the AC fields needed for operation with cells are easier to generate for small voltages due to amplifier slew-rate limitations. Essentially, it is much easier at 1 MHz (and any other frequency) to generate a 1 V sine wave than a 1 kV sine wave. The former can be created with an inexpensive op-amp, the latter requires expensive instrumentation.

Miniaturization also has important implications for heating. The temperature rise due to Joule heating will scale as $\Delta T \sim L^2|E|^2$ (34), so decreasing L decreases ΔT, enabling the use of highly conductive liquids such a cell-culture media.

ELECTRODES

To manipulate cells, one must create an electric field of a certain shape and strength. The two approaches to doing this are to either integrate electrodes within the microsystem or have them be external.

Internal Electrodes

Internal electrodes, uncommon in EP systems, are the predominant approach to generating electric fields for DEP, mainly because of the superior control they offer for locally shaping the field. Electrodes act as equipotential surfaces that shape the field by controlling their location and voltage. Above, we saw that reducing L favorably affected manipulation forces, and the simplest way to do this is to place electrodes close together. This necessitates electrodes that are internal to the system.

The primary consideration that arises when using internal electrodes is that the electrodes can adversely interact with the electrolyte via either gas generation or corrosion. Because current is carried in metals by electrons and in electrolytes by ions, electrochemistry must occur at the electrode-electrolyte interface to transform the electron current into an ion current. The net result can be the production of gas (H_2, O_2, etc.) or dissolution of the electrode, both of which can disrupt operation of the device. This is a serious concern at DC, explaining the relative absence of EP systems that use internal electrodes. For DEP systems, electrochemical effects are typically avoided by operating at >10's kHz in saline (lower frequencies can be tolerated in liquids of lower ionic strength). Gas generation is also voltage dependent, so higher voltages are more likely to lead to deleterious effects. Given that one usually wishes to operate at high frequency (>100's kHz) when using DEP with cells, electrochemistry does not pose any practical limitations.

External Electrodes

The alternate approach is to use external electrodes. Very common in EP systems, external electrodes are rare in DEP systems. One advantage of using external

electrodes is that they do not have to be fabricated with the device, resulting in a simpler fabrication process. The electrodes themselves often consist of platinum wires that are inserted into the port holes of the device. The electric field in systems that use external electrodes is shaped by using a straight channel (for EP) or inserting low-polarizability obstructions [such as glass or polymer posts (35, 36)] in the channel (for DEP).

There is no theoretical limit to the electric fields and forces that can be generated using external electrodes, although there are practical limits. Higher overall fields (such as for EP) are created by increasing the voltage or decreasing the spacing between the obstructions, limited by the fact that openings cannot be made any smaller than the cells. Additionally, the fields exist throughout the volume of the electrolyte, which can lead to significant heating. Thus, published DEP devices for use with cells have typically used very-low conductivity water to minimize heating (36, 37). Finally, fairly large voltages (~0.1–1 kV) are needed to generate the required fields, and thus these devices are limited to DC or low-frequency operation. This is fine for EP, which operates at DC, but restricts DEP manipulations to only the low-frequency region of the CM factor. The need to minimize solution conductivity, operate at or near DC, and minimize chamber volume make external electrodes best suited for use with robust cells—such as bacteria—that can tolerate being placed in low-conductivity buffers or as endpoint analysis on cells that are not needed for downstream use.

EFFECTS OF ELECTRIC FIELDS ON CELLS

Because electrical cell manipulation exposes cells to strong electric fields, one needs to know how these electric fields might affect cell physiology. Ideally, one would like to determine operating conditions that will not affect the cells and use those conditions to constrain the design. Of course, cells are poorly understood complex systems and thus it is impossible to know for certain that one is not perturbing the cell. However, all biological manipulations—cell culture, microscopy, flow cytometry, etc.—alter cell physiology. What is most important is to minimize known influences on cell phenotype and then use controls to account for unknown influences.

The known influences of electric fields on cells can be split into the effects due to current flow, which causes heating, and direct interactions of the fields with the cell. We consider each of these in turn.

Current-Induced Heating

Electric fields in a conductive medium will cause power dissipation in the form of Joule heating. The induced temperature changes can have many effects on phenotype because temperature is a potent affecter of cell physiology (38). Very-high temperatures (>4°C above physiological) are known to lead to rapid mammalian cell death. Less-extreme temperature excursions also have physiological effects, possibly owing to the exponential temperature dependence of kinetic processes in the cell (39). One well-studied response is the induction of the heat-shock proteins (38, 40). These

proteins are molecular chaperones, one of their roles being to prevent other proteins from denaturing when under environmental stress.

Although it is still unclear as to the minimum temperature excursion needed to induce responses in the cell, one must try to minimize any such excursions. A common rule of thumb for mammalian cells is to keep variations to $<1^{\circ}$C above physiological temperatures (37°C), which is the approximate daily variation in body temperature (39). In this regard, internal electrodes that minimize temperature excursions are superior to external electrodes. Because temperature rise is independent of frequency, it affects DEP and EP equally.

Direct Electric-Field Interactions

Electric fields can also directly affect cells. A simple membrane-covered sphere model for mammalian cells can be used to determine where the applied electric fields are likely to reside in the cell (41), and therefore which pathways are likely to be affected by the fields (41, 42). Using this model, one finds that the imposed fields can exist within the cell membrane or the cytoplasm.

At the frequencies used for electrical manipulation—DC to tens of MHz—the most probable route of interaction between the electric fields and the cell is at the membrane (43). This is because electric fields already exist at the cell membrane, generating endogenous transmembrane voltages in the tens of millivolts, and these voltages can affect voltage-sensitive proteins [e.g., voltage-gated ion channels (44)]. The imposed transmembrane voltage, which is added onto the endogenous transmembrane voltage, can be approximated as (45)

$$|V_{tm}| = \frac{1.5\,|\mathbf{E}|\,R}{\sqrt{1+(\omega\tau)^2}}, \tag{6}$$

where ω is the radian frequency of the applied field and τ is the time constant given by

$$\tau = \frac{Rc_m\left(\rho_{cyto}+1/2\rho_{med}\right)}{1+Rg_m\left(\rho_{cyto}+1/2\rho_{med}\right)}, \tag{7}$$

where ρ_{cyto} and ρ_{med} are the cytoplasmic and medium resistivities (Ω-m) and c_m and g_m are the cell membrane specific capacitance (F/m^2) and conductance (S/m^2), respectively.

At DC and low frequencies we see that $|V_{tm}|$ is constant at $1.5|\mathbf{E}|R$ and decreases rapidly ($\sim\omega^{-1}$) above a characteristic frequency [~1 MHz for mammalian cells in saline and decreasing approximately linearly with media conductivity, but depends on cells and solution properties (45)]. Therefore, at DC a 10-μm cell in a 10 kV/m field will incur a 75 mV imposed potential, approximately equal to the endogenous potential. Thus, DC fields—such as used in EP—will impose the greatest stress on the cell membrane, whereas use of DEP above the characteristic frequency is more benign.

Many studies have investigated possible direct links between electric fields and cells, although most are focused on extremely low- (46) or high-frequency (47)

electromagnetic fields. DC electric fields have been investigated, and have been shown to affect cell growth (48) as well as reorganization of membrane components (49).

Electrical phenotype: the aggregate electrical properties of a cell, including net charge and polarizability

In the frequency ranges involved in DEP, Tsong has provided evidence that some membrane-bound ATPases respond to fields in the kHz-MHz range, providing at least one avenue for interaction (43). Electroporation and electrofusion are other, more violent, electric field–membrane coupling mechanisms (50). In the context of DEP, most studies have examined viability, growth, and other endpoints to integrate responses from a number of molecular pathways. Some studies have tried to measure or estimate the induced transmembrane potentials at the cells (51), but these have been difficult to measure, not least because the intrinsic nonuniformity of the electric field in DEP makes it impossible to assign it a unique value. In most studies, however, researchers have found no measured effects owing to field exposure (51–53). One study that did find an effect at low frequencies (1 kHz) found that it was due to hydrogen peroxide generation at the electrodes (54). In general, though, studies specifically interested in the effects of kHz-MHz electroquasistatic fields on cells thus far demonstrate that choosing conditions under which the transmembrane loads and cell heating are small—e.g., >MHz frequencies, and fields in approximately tens of kV/m range—can obviate any gross effects. Subtler effects, such as upregulation of certain genetic pathways or activation of membrane-bound components could still occur, and thus DEP, as with any other biological technique, must be used with care.

CHARACTERIZATION AND SEPARATION

One use of electrical forces at the microscale is to exact forces on the cells that are dependent on the electrical phenotype of the cell, either to characterize those properties or to separate different cells from each other. The electrical phenotype of a cell is the collection of its electrical properties, most notably its net charge and polarizability. Electrical phenotypes become useful when they are specific to biologically relevant differences in cells (e.g., biological phenotypes). If this occurs, then two biologically different cells can be distinguished using either EP (for charge) or DEP (for polarizability).

EP Characterization and Separation

The net charge of the surface of the cell is one part of the electrical phenotype. Most studies have focused on bacteria, with much less effort on eukaryotic cells (see reviews in 14, 55). Using conventional EP systems, researchers can readily distinguish different bacterial cell types (56). There are reports that one can also distinguish subtler phenotypic differences in mammalian cells, such as apoptosis (57) and metastatic potential (58).

At the microscale, there have been few reports of using EP to separate or characterize cells. One possible reason is that the advantage conferred by miniaturization onto chip electrophoresis (over capillary electrophoresis, CE) of molecules is primarily in the ability to generate narrow sample plugs, not in the separation mechanism itself. Because cells are much larger than molecules and have correspondingly smaller

diffusivities, it is much easier to create narrow plugs using conventional CE systems. Another possibility concerns specificity, discussed below.

DEP Characterization and Separation

DEP can be used to characterize the polarizability aspect of electrical phenotype. The polarizability of cells is primarily due to the cell wall (if present), membrane, and/or cytoplasmic electrical properties, depending on the applied field frequency. The effects of polarizability manifest themselves in the CM factor. However, given the strong dependence of the DEP force on size (Equation 3), one must be careful when trying to distinguish electrical properties to ensure that one is not just trivially distinguishing cell size.

Sign of Re[K(ω)]. For cells with very different electrical phenotypes, one can find a frequency and solution conductivity where one population of cells experiences pDEP (Re[K] > 0) and another nDEP (Re[K] < 0). This allows for an easy separation, where one cell type will be attracted to the electrodes and the other repelled (**Figure 3*a***). This approach has been used to separate or characterize live versus dead cells (59–61), different species from each other (62), and cancer cell lines from dilute whole blood (63, 64). One of the most complex separations to date has been to use DEP to enrich hematopoietic stem cells from whole blood (65).

If the electrical phenotypes are similar, then the CM factors for two cell populations will be similar, and at frequencies where the CM factor of one population is positive and the other negative, the magnitudes of both CM factors will be quite small. This makes separation difficult because the DEP forces for both cell types will also be small.

Magnitude of Re[K(ω)]. To overcome the challenge of separating subtly different cells, researchers have developed separation approaches that rely on differences in the magnitude of the CM factor. The primary technique, introduced in the late 1990s (66, 67), is a type of field-flow fractionation, where a perpendicular force (in this case, the DEP force) moves cells to different heights in a parabolic flow chamber, where they then experience different drag forces and separate into bands (**Figure 3*b***). The most common implementation, hyperlayer DEP-FFF, levitates cells above an interdigitated electrode array via nDEP. The DEP forces are balanced by the weight of the cell, resulting in a uniquely defined height.

There are several appealing qualities to this technique. First, one can separate closely related cell types without having to operate near cross-over frequencies. Second, it is possible to separate more than two cell types with this approach, enabling complex separations. Third, DEP-FFF is a one-step separation process, as opposed to approaches that use pDEP and nDEP, where one must first trap and then separate. Forth, and most importantly, DEP-FFF removes any first-order size dependence from the separation process. This is because the levitation height is determined by a balance of the DEP force and weight of the cells, both of which scale with R^3, canceling any R dependence and increasing specificity.

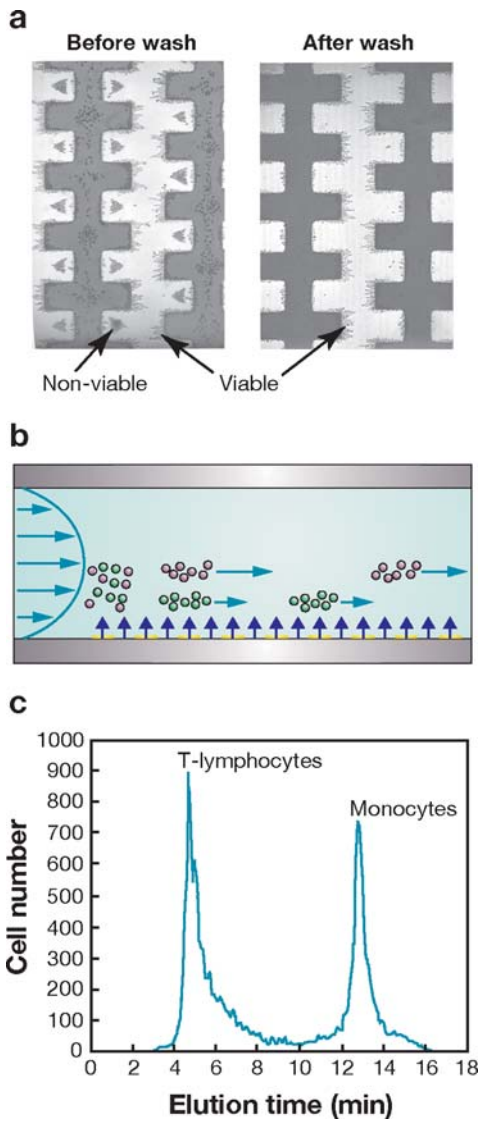

Figure 3

(*a*) Separation of viable and nonviable yeast. The left panel shows viable (experiencing pDEP) cells collecting on the electrodes and nonviable yeast (experiencing nDEP) collecting in between the electrodes. The nonviable cells can be removed by applying a fluid flow (*right*). Reprinted from Reference 59 with permission. (*b*) Schematic of DEP-FFF (*side view*), showing that two cell populations, levitated to different heights by a DEP force, separate along the direction of flow in parabolic flow. (*c*) Separation of a mixture of human T-lymphocytes from monocytes using DEP-FFF. The monocytes are levitated to a lower average height and thus elute later than the T-lymphocytes. Adapted from Reference 68 with permission from the Biophysical Society.

As a result, DEP-FFF has been used for fairly subtle separations. For example, researchers have separated premixed samples of human leukocyte populations (e.g., T lymphocytes from monocytes) (68) (**Figure 3*c***) and enriched leukocytes with respect to erythrocytes in diluted whole blood (69).

There are some limitations to hyperlayer DEP-FFF. The first relates to separating small cells. Small cells such as bacteria (~1 μm) undergo significant Brownian motion that causes a time-dependent variation in their levitation height. Because the separation critically depends on this levitation height, the specificity of the separation will decrease for these small cells. Second, DEP-FFF is a batch separation technique, where sample plugs are repeatedly injected and separated. This can limit throughput and make operation more difficult than a continuous separation technique. Another implication of separating plugs is that the separation time or distance depends on the width of the initial plug of cells, and thus creating a tight plug leads to more efficient separations. Because these systems use pressure-driven flow, narrow sample plugs can be difficult to create owing to Taylor dispersion.

Specificity of Electrical Phenotypes

The separations described above provide strong evidence that electrical phenotypes are specific to cell type (e.g., yeast versus bacteria, *Escherichia coli* versus *Bacillus subtilis*, erythrocytes versus leukocytes) and viability. This is entirely reasonable because different cell types could be expected to have widely different expression patterns (and thus have different charge and polarizability) and nonviable cells with compromised membranes would have different polarizability than viable cells with intact membranes.

The question of the ultimate specificity of electrical phenotypes, however, is unresolved. Impressive examples do exist of electrically distinguishing subtly different biological phenotypes (57, 58, 70–73). However, it is unclear how robust these electrical phenotypes are to variations in preparation conditions, different individuals, cell cycle, time, etc. Indeed, there are few reports where a specific operating condition has been used to predictively separate out cells with useful phenotypes from unknown (rather than premixed) samples [Stephens et al. is a notable exception (65)].

Thus, it seems that electrical techniques are currently competent for separating unknown mixtures of low complexity when one has appropriate positive and negative controls to establish the operating conditions. This can be quite useful because low complexity does not imply low utility. Electrical phenotypes also have the distinct benefit that they can be probed without resorting to labels, as opposed to affinity techniques. When no affinity labels exist or overly complicate the assay, electrical techniques may be the method of choice.

The lower specificity of electrical phenotypes (as compared to using affinity tags) is not difficult to understand. Cell proteomes consist of thousands of distinct proteins, each at different copy numbers. That collection of proteins in turn determines the charge state and polarizability. Because it is possible for many different proteomes to result in the same electrical phenotype, this intrinsically limits specificity. These facts are widely appreciated at the molecular scale. For instance, capillary zone

electrophoresis, which separates proteins based on charge/mass ratio, is not useful for complex samples because the charge/mass ratio is not specific enough to separate many proteins from each other. Analytical chemists typically use electrophoresis in conjunction with gels or micelles to increase specificity. At the current time, no such options exist for electrical phenotypes. As a final note, the DEP component of the electrical phenotype, because it integrates more information about the cell structure, will be more specific than the EP component, which is based only on net charge. This helps to explain increased attention to DEP rather than EP cell separations.

HANDLING

A different class of devices uses electrical forces as "handles" to physically position cells. Here the goal is not to characterize or separate subclasses of cells based on their electrical phenotype but rather to move cells to known locations within the device. Most of these systems move cells by creating and/or moving electrical potential energy wells, or traps.

Theoretical Existence of EP and DEP Traps

By considering the conditions under which traps can be created, we can understand when EP or DEP might be applicable for handling cells. The fundamental requirement for any deterministic particle trap is that it creates a region where the net force on the particle is zero. Additionally, the particle must be at a stable zero, in that the particle must do work on the force field to move from that zero (74). The important forces in these microsystems are EP, DEP, fluid drag, fluid lift, gravity, and restoring forces due to solid surfaces.

Earnshaw's theorem states that a charged particle cannot be stably held in an electrostatic field owing to the saddle shape of the electric fields that result from Laplace's equation. Although this would seem to preclude EP traps, one can get around this problem by taking advantage of other forces. For instance, one can use the substrate to add a restoring force that creates a stable holding point.

Related to this, one can also show that a dipole oriented parallel to the field also cannot be stably held. This is the situation encountered in pDEP, where the induced dipole is always oriented with the field. Thus, similar to EP, one needs an additional force to stably trap a cell using pDEP, such as a rigid surface. The third situation, that of nDEP, is that of a dipole oriented antiparallel to the field. In this case one can stably hold the dipole at field minima, as shown by Jones & Bliss in 1977 (75). Taken together, we would expect to trap cells at rigid surfaces (e.g., electrodes, substrates, etc.) using EP or pDEP and away from surfaces when using nDEP.

EP Cell Handling

Negatively charged cells move toward the positive electrode in an EP system. In this fashion, cells can be transported along channels. Li & Harrison transported yeast, erythrocytes, and *E. coli* in conventional electrokinetic microchannels using a

combination of EP and electroosomosis (76). They were able to attain velocities of ~−0.1–0.2 mm/s in a 100 V/cm field, in line with the expected EP mobility of cells. This points to one of the advantages of EP cell handling, which is that one can create electric fields—and thus transport cells—over large distances (up to centimeters). It is more difficult to transport cells using DEP [although twDEP can be used (77)].

Cabrera & Yager developed a bacterial concentrator that used EP and isoelectric focusing (IEF) in a multilayer polymeric device (78). They were able to pull the bacteria to a specific zone (either the positive electrode for EP or the isoelectric point for IEF) while applying a perpendicular pressure-driven flow, resulting in a concentrated bacterial sample.

One can also make an EP trap by attracting cells to positively charged internal electrodes. Ozkan et al. (79) developed a simple platform for EP patterning of cells. The device used a bottom substrate with round electrodes (defined as openings in an agarose film) and a top slide that served as the counter-electrode. By applying modest electric fields (~40 V/cm), they were able to position a variety of cells (3T3 fibroblasts, neural stem cells) on the electrodes. They showed that cells exposed to these voltages for 1 min did not exhibit any morphological changes upon replating. They observed cell velocities of <1 μm/s, lower than would be expected at these fields, suggesting a significant voltage drop at the electrode-electrolyte interface.

Given the relative simplicity of these systems, it is interesting that few reports exist for microscale EP cell handling. This is perhaps due to the fact that DC fields are most harmful to cells, limiting the fields that one can use. Additionally, because EP cell trapping uses internal electrodes, there is a difficulty in operating internal electrodes at DC without generating bubbles or harmful electrochemical species.

DEP Cell Handling

DEP has been used extensively to handle cells, both for positioning and for transporting cells. Cell positioning typically uses stationary traps that may be turned ON or OFF in time, whereas cell transportation uses potential energy wells that can be moved in space.

Positioning cells. Cell positioning is used either to create long-term (more than several hours) patterns of cells on a substrate or for short-term (minutes to hours) observation of cells in specific locations. Both pDEP and nDEP and many different electrode geometries can be used for these purposes.

Interdigitated electrodes. Interdigitated electrodes are one of the simplest geometries for patterning cells. Its primary advantages are that they are easy to model (see, for example, 80–82) and easy to create large arrays. Interdigitated electrodes are useful for patterning many cells where subsequent washes are not necessary. The long extent of the electrodes in one direction creates an essentially 2-D field geometry and thus no trapping is possible along the length of the electrodes. This means that it is not possible to create an electric-field minimum sized to one cell. Additionally, in the most common configuration, where the electrodes are on the bottom substrate,

trapping in the z-direction is due to the balance of upward-pushing nDEP forces against gravity (as in DEP-FFF). Thus, to pattern cells on a substrate, one needs to operate at small voltages that do not levitate the cells. This means that the sideways confining forces will be small, and thus the DEP forces will not be able to resist strong drag forces. Thus, any washes will take a long time. In fact, the interdigitated electrode trap is strongest at the voltage just before the cells are levitated (discussed below), and thus one cannot simply increase voltage to increase strength. A different approach to patterning via interdigitated electrodes was introduced by Matsue et al. and extensively characterized by Albrecht et al., where they put the electrodes at the top of the chamber and pushed the particles down, in the same direction as gravity (83, 84). This has the advantage that the particles are patterned onto a blank substrate, with the concomitant disadvantage that the patterning DEP forces are smallest at the end of patterning, increasing patterning times.

Insulating-post geometry. One notable example of an external-electrode device is an nDEP cell concentrator developed by researchers at Sandia National Laboratories (36). They use insulating cylindrical posts etched into a channel to create the field obstructions necessary for DEP (**Figure 4*a***). By applying a DC voltage to the device, they obtain EP, DEP, and electroosmotic flows. Because DEP is quadratic with electric field, whereas electrokinetic phenomena are linear with electric field, they apply a large electric field so that DEP dominates and traps particles. To date, they have shown concentration and live/dead discrimination of bacteria (**Figure 4*b***) (36). A distinct advantage of this approach is that the channel is completely passive and can be made out of plastic (85).

Quadrupole electrodes. Quadrupole electrodes are four electrodes with alternating voltage polarities applied to every other electrode (**Figure 5*a***). Some of the first microscale nDEP cell trapping was performed using planar quadrupole electrodes by Fuhr et al. (27, 52) (**Figure 5*c***). Importantly, it is possible to create single-cell traps by spacing the electrodes such that only one cell fits within the electric-field minimum, or, by increasing the electrode spacing, trap many cells. Additionally, one can make arrays of quadrupoles, although the spacing between any two sites in the array will be much larger than the cell diameter, limiting packing density.

Despite all these favorable qualities, planar quadrupoles are not commonly used for handling cells because, like the interdigitated electrodes, they are fairly weak traps. They provide confinement in the in-plane directions, but trap out of plane by balancing the nDEP force against gravity. As with interdigitated electrodes, then, these traps suffer from the drawback that increasing the field only pushes the particle farther out of the trap and does not necessarily increase confinement. We showed this with measurements of the strength of these traps (32). The traps are strongest at an intermediate voltage just before the particle is about to be levitated, and in practice are limited to <1 pN (**Figure 5*b***).

One way to increase the strength is to extend the electrodes into the third dimension, creating extruded quadrupole traps (86, 87). These traps, although much more difficult to fabricate than planar quadrupoles, are orders of magnitude stronger

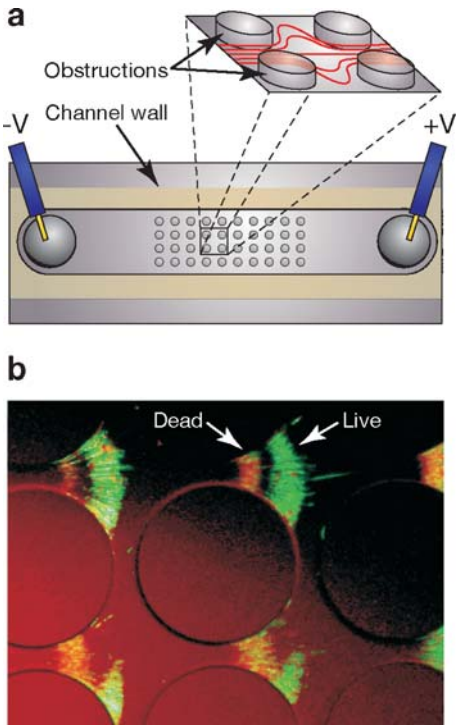

Figure 4

(*a*) Schematic of insulating-post geometry, showing how insulating obstructions create field nonuniformities. (*b*) Image showing spatially separated concentration of live (*green*) and dead (*red*) *E. coli* in a 60 V/mm electric field. Reprinted in part with permission from Reference 36. Copyright 2004 American Chemical Society.

and can successfully hold single cells against significant liquid flows. We have created small arrays of these traps that can be turned ON and OFF by switching the potential at only one electrode (rather than having to turn all four electrodes ON and OFF), and have been able to load, image, and sort cells from these arrays (86). Because the extruded quadrupole positions cells above the substrate, it is most appropriate for observing rather than patterning cells.

Octopole electrodes. Another way to increase the strength of quadrupole electrode traps is to put another quadrupole on the chamber ceiling to provide further particle confinement (**Figure 6*a***). These opposed octopole traps are significantly stronger than planar quadrupoles, and are routinely used for single-cell trapping (88, 89) (**Figure 6*b***). The German team that developed these traps has also combined them with electrorotation (90) to study cell properties.

Octopole electrodes are much simpler to fabricate than the extruded quadrupoles and can be arrayed just like planar quadrupoles. As with the extruded quadrupole,

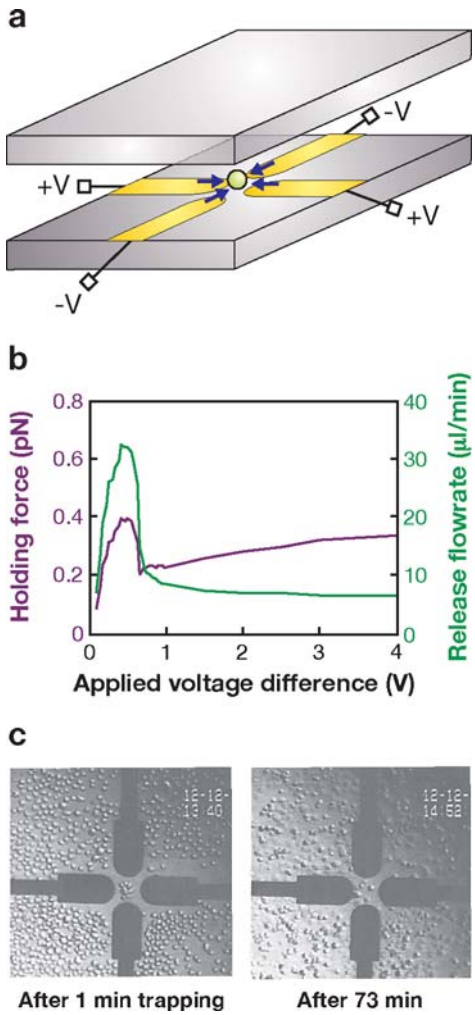

Figure 5

(*a*) Schematic of planar quadrupole, showing voltage polarity and trapped cell. (*b*) Holding strength (force in *purple* and flow rate in *green*) of planar quadrupole with 10-µm bead, adapted from Reference 32 with permission from the Biophysical Society. (*c*) Mouse fibroblast trapping (*left*) and initial attachment (*right*) using planar quadrupole. Reprinted from Reference 52 with permission.

these traps position cells at the midpoint of the flow chamber, although it is possible to adjust the electrode voltages to drive particles away from the midpoint to the substrate. Their primary challenge is that they require precise alignment of the two opposed quadrupoles, and that they limit the overall chamber height because the chamber height must be about the same as the electrode spacing to ensure that the two quadrupoles act in concert; if the chamber height is increased significantly,

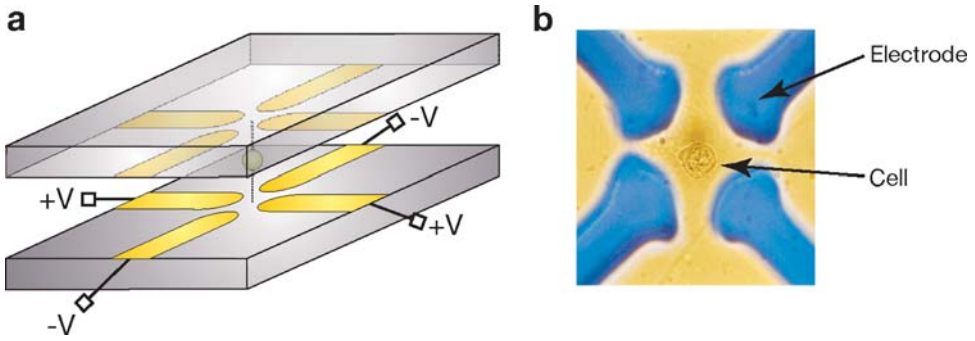

Figure 6

(*a*) Schematic of the opposed octopole, showing the two quadrupoles from which it is constructed. The top quadrupole is excited with opposite polarity from the bottom quadrupole. (*b*) Image of positioned human T-lymphoma cell. Reprinted from Reference 90 with permission.

the fields from the two quadrupoles will not interact and the strength benefits of the octopole will go away.

nDEP microwell. As noted above, the majority of nDEP-based traps are not suitable for cell patterning because they do not position cells against the substrate. This is fundamentally due to the fact that nDEP traps push cells away from electrodes, so if electrodes are on the substrate, they will tend to push the cells off the substrate. To circumvent this, we developed an nDEP trap specifically for patterning single cells (**Figure 7*a***). Our goal was to generate a planar geometry that would push cells toward the substrate, and we did this by confining cells within the fringing fields of two electrodes. As shown in **Figure 7*b***, the strength of this trap increases with higher voltages (as opposed to **Figure 5*c***), and importantly is strong enough to withstand reasonable washes. Additionally, this trap uses electrode patterns that can be created using inexpensive printing technology. We have used these traps to position various types of cells, including fibroblasts, HeLa cells, and mouse embryonic stem cells (**Figure 7*c***).

Points-and-lid geometry. One useful geometry for patterning cells that can tolerate exposure to low-conductivity liquids is the points-and-lid geometry, which traps cells using pDEP. Although several variations exist, all use a uniform top "lid" conductor [typically thin transparent gold or indium tin oxide (ITO)] and a bottom conductor patterned into "points" using some type of insulator (**Figure 8*a***, *left*) (91, 92). We used this geometry to pattern cells to study cell-cell interactions (92) (**Figure 8*a***, *right*). Importantly, experiments showed that the low-conductivity buffer did not affect the gross physiology of the cells at reasonable voltages and short times. Albrecht et al. (91) used this geometry to position mouse fibroblasts and then encapsulate the cells in photopolymerized hydrogel, and found that this did not affect acute cell viability. Importantly, this is one of the few DEP geometries where researchers have positioned

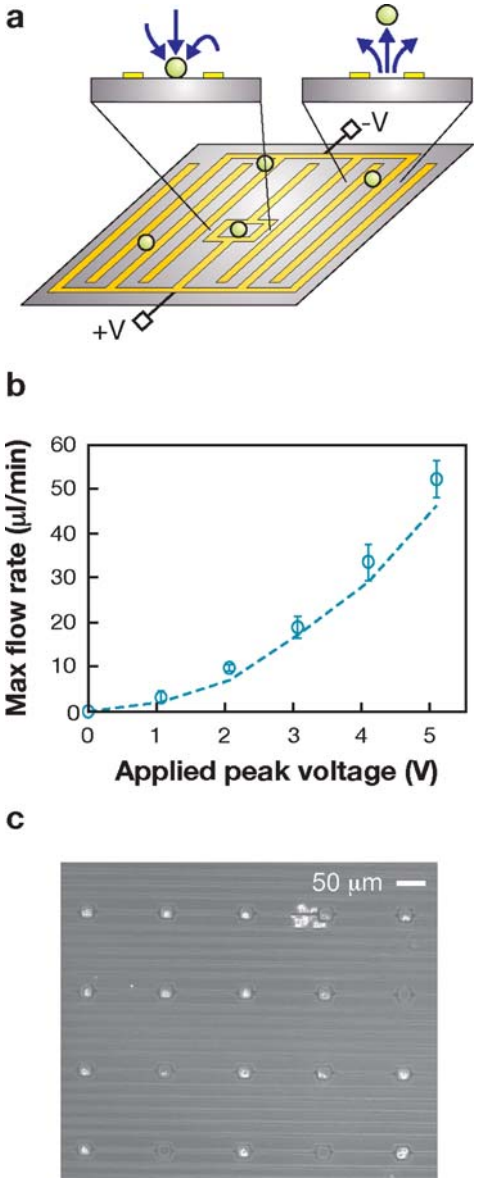

Figure 7

(*a*) Schematic of DEP microwell, showing how cells are repelled from substrate except inside the trap, where the DEP force points downward. (*b*) Holding versus voltage for 9.7-µm beads in a similar trap, adapted from Reference 31 with permission from the Biophysical Society. (*c*) Image of patterned mouse embryonic stem cells.

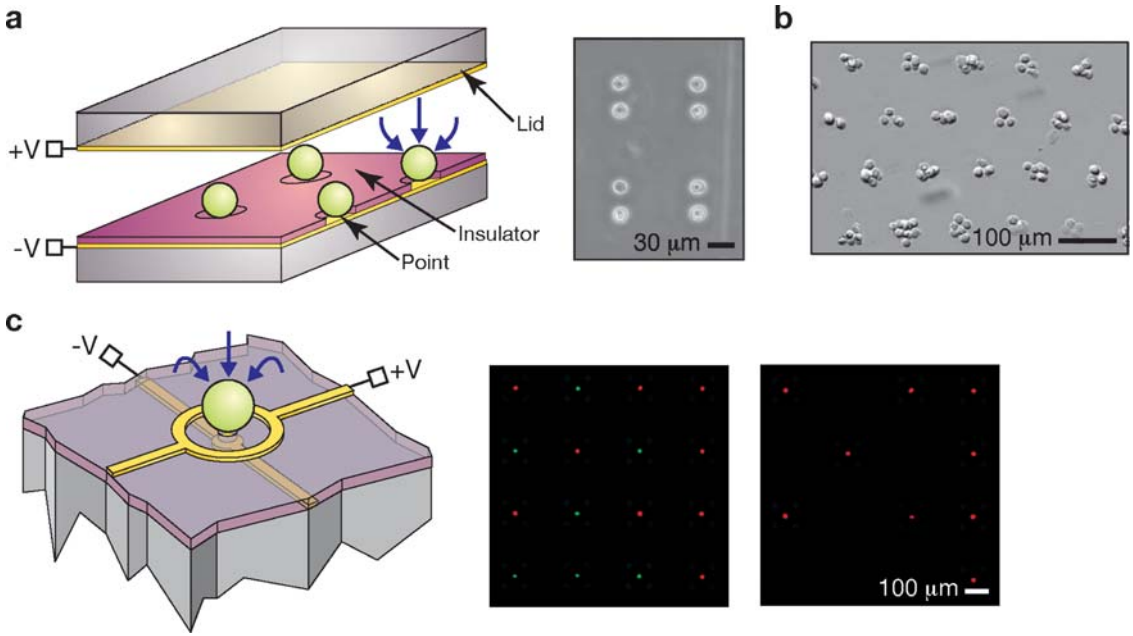

Figure 8

(*a*) Schematic (*left*) of point-lid geometry along with an image (*right*) of endothelial cells patterned using one version of this geometry. Image from Reference 92 reprinted with permission. (*b*) Image of fibroblasts patterned using a different version of the point-lid geometry and embedded in a hydrogel matrix. From Reference 91 reproduced with permission from The Royal Society of Chemistry. (*c*) Schematic (*left*) of ring-dot geometry, along with two images (*right*) showing addressable removal of green-labeled human HL-60 cells from a 4 × 4 trap array.

cells and then had them attach; most other geometries have thus far only been used for positioning.

Ring-dot geometry. The ring-dot geometry consists of an outer ring electrode and an inner round "dot" electrode on a separate metal layer. Cells are attracted via pDEP to the field maximum at the dot. We have used this geometry to develop a scalable addressable trapping array for observing many single cells and then sorting out desired cells (93) (**Figure 8*c***). The distinct advantages of this geometry are that it is planar yet strong and that the number of control electrodes scales as $2\sqrt{n}$, where n is the number of traps. This scaling is improved as compared with standard passive architectures where the number of connections scales linearly with the number of trapping sites (86).

Transporting cells. Microsystems for transporting cells use DEP (sometimes in conjunction with fluid flow) to move cells from one location to another. These are

often used in conjunction with electrical positioning structures to create complete labs-on-a-chip.

Interdigitated electrodes. Interdigitated electrodes can be used in conjunction with nDEP and twDEP to transport cells, as mentioned previously (28, 77). nDEP is used to levitate cells above the electrodes, whereas twDEP applies a translational force to the cells, transporting them. Researchers have created fairly large electrode arrays suitable for transporting cells across >10-mm distances (77). Use of these systems removes the need for external pumps, and can result in cell velocities of 1–100 μm/s (61, 65).

Grid-electrode geometry. A pDEP-based cell transportation device was described by Suehiro & Pethig (94). They used a set of parallel individually addressable ITO electrodes on the top substrate and another set of ITO electrodes on the bottom substrate that were rotated 90° (**Figure 9*b***, *left*). By actuating one electrode on top and bottom, they could create a localized field maximum that could trap a cell, and through careful manipulation of the voltages could release the cell from the trap in a specific direction and then trap it with the neighboring electrodes, enabling deterministic cell movement (**Figure 9*a***, *right*).

Paired-electrode system. The German group that developed the octopole electrode trap extended their approach by adding paired electrodes, which are simply two electrodes opposed from one another, with one on the substrate and one on the chamber ceiling (**Figure 9*b***, *left*). Introduced by Fiedler et al. in 1998 (95), these have been used to create nDEP "barriers" to herd and otherwise transport particles (**Figure 9*b***, *right*), and have now been commercialized to create single-cell handling systems that combine DEP with microfluidics. These structures have even been used for magnitude-based cell separation (96) by controlling the nDEP barrier such that it deflects particles with large $|\mathrm{Re}[K]|$ but passes those with small $|\mathrm{Re}[K]|$.

Transistor-based structures. Recently, a European team has developed an active nDEP-based trapping array (97), consisting of a two-dimensional array of square electrodes and a conductive lid. The key is that incorporating CMOS logic (analog switches and memory) allows each square electrode to be connected to an in-phase or out-of-phase AC voltage in a programmable fashion. By putting a center square at $+V$ and the surrounding squares at $-V$, they can create an in-plane trap. Furthermore, setting the chamber top to $+V$ closes the cage, giving 3-D confinement. The incorporation of CMOS means that very few electrical leads are required to control an indefinite number of sites, creating a readily scalable technology. Using this trap geometry, they have successfully transported both beads and cells, with trap-to-trap movements with approximately second timescales.

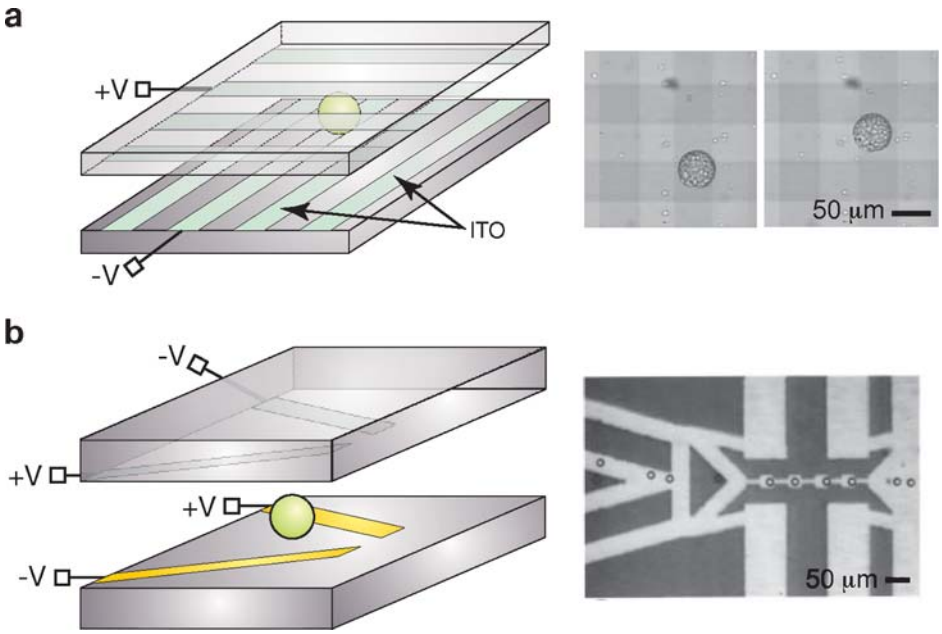

Figure 9

(*a*) Schematic (*left*) of the grid-electrode geometry showing how applied voltages create a pDEP trap, along with two images (*right*) of a protoplast being transported by time-sequencing voltages to the electrodes. Reprinted from Reference 94 with permission. (*b*) Schematic (*left*) of paired-electrode microsystem, showing top ITO and bottom gold electrodes, along with an image of 15-μm beads being funneled and then trapped using this microsystem. Reprinted in part with permission from Reference 95. Copyright 1998 American Chemical Society.

Comparison of nDEP and pDEP for Cell Handling

The preceding discussion raises some important points for DEP cell handling. First, the choice of whether to use pDEP or nDEP is a system-level partitioning problem. For instance, if one absolutely requires (for biological or other reasons) the use of saline or cell-culture medium, then nDEP must be used. If, however, minimizing temperature rises is most important, then pDEP may be better, as the low-conductivity media will reduce temperature rises. This decision may also be affected by fabrication facilities, etc.

In general, pDEP traps are easier to create than nDEP traps because it is easier to hold onto a particle by attracting it than repelling it. Creating effective nDEP traps is more difficult, and requires some sort of 3-D confinement. As noted above, this is difficult (although not impossible) to do with planar electrode structures.

OUTLOOK AND CONCLUSIONS

Electrical approaches to manipulating cells at the microscale have already shown great promise. This is primarily due to the favorable scaling of electrical forces with

system size and the ease of fabricating microscale electrodes. For both separation and handling, DEP has advantages over EP in terms of specificity and cell damage, though some applications, such as long-range cell transport, may favor EP. Looking ahead, an upcoming goal for DEP-based separations will be the demonstration of systems with specificity sufficient to enable separation of real-world samples. For cell handling, we must continue efforts to engineer DEP-based systems that are easy to use and demonstrably innocuous to cell health. Luckily, engineers continue to innovate in both DEP separation (98) and handling (99).

SUMMARY POINTS

1. Electrical manipulation at the microscale primarily uses EP or DEP. EP acts on a particle's charge, while DEP acts on its induced charge.
2. Electrical forces scale favorably as one reduces the size of the system, explaining their prevalence.
3. Electrodes are used to generate and shape the electric fields, and can either be placed interior to the device or remain external. The best approach depends on the application.
4. Studies suggest that cells can be safely manipulated in DC-MHz electric fields as long as heating and imposed transmembrane voltages are limited. Heating is the same for EP and DEP, while imposed transmembrane voltages are typically larger for EP than for high-frequency DEP.
5. Electrical phenotypes are specific enough to distinguish viability and cell type, and polarization is more specific than net charge as an indicator of phenotype.
6. Microsystems have been created to characterize or separate cells based upon both the magnitude and sign of the charge or induced polarization. Magnitude-based DEP separations enable subtler phenotypic distinction.
7. DEP is the most prevalent approach for electrical cell handling. A number of different approaches using both nDEP and pDEP have been used to transport, position, or pattern cells.

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LITERATURE CITED

1. Grimm S. 2004. The art and design of genetic screens: mammalian culture cells. *Nat. Rev. Genet.* 5:179–89

2. Rothe G, Schmitz G, Adorf D, Barlage S, Gramatzki M, et al. 1996. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia* 10:877–95
3. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. 2004. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6:483–95
4. Swaminathan B, Feng P. 1994. Rapid detection of food-borne pathogenic bacteria. *Annu. Rev. Microbiol.* 48:401–26
5. Svoboda K, Block SM. 1994. Biological applications of optical forces. *Annu. Rev. Biophys. Biomol. Struct.* 23:247–85
6. Wu J. 1991. Acoustical tweezers. *J. Acoust. Soc. Am.* 89:2140–43
7. Jung DR, Kapur R, Adams T, Giuliano KA, Mrksich M, et al. 2001. Topographical and physicochemical modification of material surface to enable patterning of living cells. *Crit. Rev. Biotechnol.* 21:111–54
8. Ramos A, Morgan H, Green NG, Castellanos A. 1998. AC electrokinetics: a review of forces in microelectrode structures. *J. Phys. D Appl. Phys.* 31:2338–53
9. Haus HA, Melcher JR. 1989. *Electromagnetic Fields and Energy*. Englewood Cliffs, NJ: Prentice Hall. 742 pp.
10. Melville D, Paul F, Roath S. 1975. High gradient magnetic separation of red cells from whole blood. *IEEE Trans. Magn.* 11:1701–4
11. Radbruch A, Mechtold B, Thiel A, Miltenyi S, Pfluger E. 1994. High-gradient magnetic cell sorting. *Methods Cell Biol. 42*:387–403
12. Jucker BA, Harms H, Zehnder AJB. 1996. Adhesion of the positively charged bacterium *Stenotrophomonas* (Xanthomonas) *maltophilia* 70401 to glass and teflon. *J. Bacteriol.* 178:5472–79
13. Mehrishi JN, Bauer J. 2002. Electrophoresis of cells and the biological relevance of surface charge. *Electrophoresis* 23:1984–94
14. Desai MJ, Armstrong DW. 2003. Separation, identification, and characterization of microorganisms by capillary electrophoresis. *Microbiol. Mol. Biol. Rev.* 67:38–51
15. Jones TB. 1995. *Electromechanics of Particles*. Cambridge, UK: Cambridge Univ. Press. 265 pp.
16. Hughes MP. 2003. *Nanoelectromechanics in Engineering and Biology*. Boca Raton, FL: CRC Press. 322 pp.
17. Morgan H, Green NG. 2003. *AC Electrokinetics: Colloids and Nanoparticles*. Baldock, Hertfordshire, UK: Res. Stud. Press
18. Pohl HA. 1978. *Dielectrophoresis: The Behavior of Neutral Matter in Nonuniform Electric Fields*. Cambridge, UK: Cambridge Univ. Press
19. Fuhr G, Shirley SG. 1998. Biological application of microstructures. In *Microsystem Technology in Chemistry and Life Science*, pp. 83–116. Berlin: Springer-Verlag
20. Pethig R. 1996. Dielectrophoresis: using inhomogeneous AC electrical fields to separate and manipulate cells. *Crit. Rev. Biotechnol.* 16:331–48
21. Gascoyne PRC, Vykoukal J. 2004. Dielectrophoresis-based sample handling in general-purpose programmable diagnostic instruments. *Proc. IEEE* 92:22–42
22. Foster KR, Sauer FA, Schwan HP. 1992. Electrorotation and levitation of cells and colloidal particles. *Biophys. J.* 63:180–90

23. Sukhorukov VL, Mussauer H, Zimmermann U. 1998. The effect of electrical deformation forces on the electropermeabilization of erythrocyte membranes in low- and high-conductivity media. *J. Membr. Biol.* 163:235–45
24. Zimmermann U, Friedrich U, Mussauer H, Gessner P, Hamel K, Sukhoruhov V. 2000. Electromanipulation of mammalian cells: fundamentals and application. *IEEE Trans. Plasma Sci.* 28:72–82
25. Irimajiri A, Hanai T, Inouye A. 1979. A dielectric theory of "multi-stratified shell" model with its application to a lymphoma cell. *J. Theor. Biol.* 78:251–69
26. Arnold WM. 1999. Positioning and levitation media for the separation of biological cells. *Proc. Annu. Meet. IEEE Industry Appl. Soc., 34th, Phoenix*, pp. 2470–77. Piscataway, NJ: IEEE
27. Fuhr G, Arnold WM, Hagedorn R, Muller T, Benecke W, et al. 1992. Levitation, holding, and rotation of cells within traps made by high-frequency fields. *Biochim. Biophys. Acta* 1108:215–23
28. Fuhr G, Hagedorn R, Muller T, Benecke W, Wagner B, Gimsa J. 1991. Asynchronous traveling-wave induced linear motion of living cells. *Stud. Biophys.* 140:79–102
29. Jones TB, Washizu M. 1996. Multipolar dielectrophoretic and electrorotation theory. *J. Electrostat.* 37:121–34
30. Schnelle T, Muller T, Fuhr G. 1999. The influence of higher moments on particle behaviour in dielectrophoretic field cages. *J. Electrostat.* 46:13
31. **Rosenthal A, Voldman J. 2005. Dielectrophoretic traps for single-particle patterning. *Biophys. J.* 88:2193–205**
32. Voldman J, Braff RA, Toner M, Gray ML, Schmidt MA. 2001. Holding forces of single-particle dielectrophoretic traps. *Biophys. J.* 80:531–41
33. **Jones TB, Washizu M. 1994. Equilibria and dynamics of DEP-levitated particles: multipolar theory. *J. Electrostat.* 33:199–212**
34. Castellanos A, Ramos A, Gonzalez A, Green NG, Morgan H. 2003. Electrohydrodynamics and dielectrophoresis in microsystems: scaling laws. *J. Phys. D Appl. Phys.* 36:2584–97
35. Chou CF, Tegenfeldt JO, Bakajin O, Chan SS, Cox EC, et al. 2002. Electrodeless dielectrophoresis of single- and double-stranded DNA. *Biophys. J.* 83:2170–79
36. Lapizco-Encinas BH, Simmons BA, Cummings EB, Fintschenko Y. 2004. Dielectrophoretic concentration and separation of live and dead bacteria in an array of insulators. *Anal. Chem.* 76:1571–79
37. Suehiro J, Zhou GB, Imamura M, Hara M. 2003. Dielectrophoretic filter for separation and recovery of biological cells in water. *IEEE Trans. Indus. Appl.* 39:1514–21
38. Lindquist S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55:1151–91
39. Weaver JC, Vaughan TE, Martin GT. 1999. Biological effects due to weak electric and magnetic fields: the temperature variation threshold. *Biophys. J.* 76:3026–30
40. Carper SW, Duffy JJ, Gerner EW. 1987. Heat-shock proteins in thermotolerance and other cellular processes. *Cancer Res.* 47:5249–55
41. Grosse C, Schwan HP. 1992. Cellular membrane potentials induced by alternating fields. *Biophys. J.* 63:1632–42

31. This paper introduces a planar nDEP trap that effectively confines particles.

33. This is the first in a series of papers by Jones & Washizu developing the multipolar DEP theory.

42. Schwan HP. 1989. Dielectrophoresis and rotation of cells. In *Electroporation and Electrofusion in Cell Biology*, ed. CA Jordan, pp. 3–21. New York: Plenum
43. Tsong TY. 1992. Molecular recognition and processing of periodic signals in cells: study of activation of membrane ATPases by alternating electric fields. *Biochim. Biophys. Acta* 1113:53–70
44. Catterall WA. 1995. Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.* 64:493–531
45. Foster KR, Schwan HP. 1989. Dielectric properties of tissues and biological materials: a critical review. *CRC Crit. Rev. Biomed. Eng.* 17:25–104
46. Lacy-Hulbert A, Metcalfe JC, Hesketh R. 1998. Biological responses to electromagnetic fields? *FASEB J.* 12:395–420
47. Polk C, Postow E. 1996. *Handbook of Biological Effects of Electromagnetic Fields*. Boca Raton, FL: CRC Press. 618 pp.
48. Jaffe LF, Poo MM. 1979. Neurites grow faster towards the cathode than the anode in a steady field. *J. Exp. Zool.* 209:115–28
49. Ryan TA, Myers J, Holowka D, Baird B, Webb WW. 1988. Molecular crowding on the cell surface. *Science* 239:61–64
50. Zimmermann U. 1986. Electrical breakdown, electropermeabilization and electrofusion. *Rev. Physiol. Biochem. Pharmacol.* 105:175–256
51. Glasser H, Fuhr G. 1998. Cultivation of cells under strong ac-electric field—differentiation between heating and trans-membrane potential effects. *Bioelectrochem. Bioenerg.* 47:301–10
52. Fuhr G, Glasser H, Muller T, Schnelle T. 1994. Cell manipulation and cultivation under AC electric-field influence in highly conductive culture media. *Biochim. Biophys. Acta Gen. Subj.* 1201:353–60
53. Docoslis A, Kalogerakis N, Behie LA. 1999. Dielectrophoretic forces can be safely used to retain viable cells in perfusion cultures of animal cells. *Cytotechnology* 30:133
54. Wang XJ, Yang J, Gascoyne PRC. 1999. Role of peroxide in AC electrical field exposure effects on Friend murine erythroleukemia cells during dielectrophoretic manipulations. *Biochim. Biophys. Acta Gen. Subj.* 1426:53–68
55. Kremser L, Blaas D, Kenndler E. 2004. Capillary electrophoresis of biological particles: viruses, bacteria, and eukaryotic cells. *Electrophoresis* 25:2282–91
56. Armstrong DW, Schulte G, Schneiderheinze JM, Westenberg DJ. 1999. Separating microbes in the manner of molecules. 1. Capillary electrokinetic approaches. *Anal. Chem.* 71:5465–69
57. Guo R, Pu XP, Jin OY, Li XR, Luo P, Yang YP. 2002. Capillary electrophoretic determination of apoptosis of rat cerebellar granule cells induced by 1-methyl-4-phenyl pyridium ion. *Electrophoresis* 23:1110–15
58. Price JAR, Pethig R, Lai CN, Becker FF, Gascoyne PRC, Szentgyorgyi A. 1987. Changes in cell-surface charge and transmembrane potential accompanying neoplastic transformation of rat-kidney cells. *Biochim. Biophys. Acta* 898:129–36
59. Markx GH, Talary MS, Pethig R. 1994. Separation of viable and non-viable yeast using dielectrophoresis. *J. Biotechnol.* 32:29–37

60. Li HB, Bashir R. 2002. Dielectrophoretic separation and manipulation of live and heat-treated cells of Listeria on microfabricated devices with interdigitated electrodes. *Sens. Actuators B Chem.* 86:215–21
61. Cen EG, Dalton C, Li YL, Adamia S, Pilarski LM, Kaler K. 2004. A combined dielectrophoresis, traveling wave dielectrophoresis and electrorotation microchip for the manipulation and characterization of human malignant cells. *J. Microbiol. Methods* 58:387–401
62. Markx GH, Huang Y, Zhou XF, Pethig R. 1994. Dielectrophoretic characterization and separation of microorganisms. *Microbiology* 140:585–91
63. Becker FF, Wang XB, Huang Y, Pethig R, Vykoukal J, Gascoyne PRC. 1995. Separation of human breast-cancer cells from blood by differential dielectric affinity. *Proc. Natl. Acad. Sci. USA* 92:860–64
64. Huang Y, Joo S, Duhon M, Heller M, Wallace B, Xu X. 2002. Dielectrophoretic cell separation and gene expression profiling on microelectronic chip arrays. *Anal. Chem.* 74:3362–71
65. Stephens M, Talary MS, Pethig R, Burnett AK, Mills KI. 1996. The dielectrophoresis enrichment of CD34(+) cells from peripheral blood stem cell harvests. *Bone Marrow Transplant.* 18:777–82
66. Huang Y, Wang X-B, Becker FF, Gascoyne PRC. 1997. Introducing dielectrophoresis as a new force field for field-flow fractionation. *Biophys. J.* 73:1118–29
67. Markx GH, Rousselet J, Pethig R. 1997. DEP-FFF: field-flow fractionation using non-uniform electric fields. *J. Liquid Chromatogr. Relat. Technol.* 20:2857–72
68. Yang J, Huang Y, Wang XB, Becker FF, Gascoyne PRC. 2000. Differential analysis of human leukocytes by dielectrophoretic field-flow-fractionation. *Biophys. J.* 78:2680–89
69. Wang XB, Yang J, Huang Y, Vykoukal J, Becker FF, Gascoyne PRC. 2000. Cell separation by dielectrophoretic field-flow-fractionation. *Anal. Chem.* 72:832–39
70. Archer S, Morgan H, Rixon FJ. 1999. Electrorotation studies of baby hamster kidney fibroblasts infected with herpes simplex virus type 1. *Biophys. J.* 76:2833–42
71. Wang XJ, Becker FF, Gascoyne PRC. 2002. Membrane dielectric changes indicate induced apoptosis in HL-60 cells more sensitively than surface phosphatidylserine expression or DNA fragmentation. *Biochim. Biophys. Acta Biomembr.* 1564:412–20
72. Hu X, Arnold WM, Zimmermann U. 1990. Alterations in the electrical-properties of lymphocyte-T and lymphocyte-B membranes induced by mitogenic stimulation—activation monitored by electro-rotation of single cells. *Biochim. Biophys. Acta* 1021:191–200
73. Gascoyne PRC, Pethig R, Burt JPH, Becker FF. 1993. Membrane-changes accompanying the induced-differentiation of Friend murine erythroleukemia-cells studied by dielectrophoresis. *Biochim. Biophys. Acta* 1149:119–26
74. Blake A. 1985. *Handbook of Mechanics, Materials, and Structures*. New York: Wiley. 710 pp.

66–67. These two papers introduce DEP-FFF.

75. Jones TB, Bliss GW. 1977. Bubble dielectrophoresis. *J. Appl. Phys.* 48:1412–17
76. Li PCH, Harrison DJ. 1997. Transport, manipulation, and reaction of biological cells on-chip using electrokinetic effects. *Anal. Chem.* 69:1564–68
77. Morgan H, Green NG, Hughes MP, Monaghan W, Tan TC. 1997. Large-area travelling-wave dielectrophoresis particle separator. *J. Micromechan. Microeng.* 7:65–70
78. Cabrera CR, Yager P. 2001. Continuous concentration of bacteria in a microfluidic flow cell using electrokinetic techniques. *Electrophoresis* 22:355–62
79. Ozkan M, Pisanic T, Scheel J, Barlow C, Esener S, Bhatia SN. 2003. Electro-optical platform for the manipulation of live cells. *Langmuir* 19:1532–38
80. Van Gerwen P, Laureyn W, Laureys W, Huyberechts G, De Beeck MO, et al. 1998. Nanoscaled interdigitated electrode arrays for biochemical sensors. *Sens. Actuators B Chem.* 49:73–80
81. Gibbs WJ. 1958. *Conformal Transformations in Electrical Engineering*. London: Chapman & Hall. 219 pp.
82. Chang DE, Loire S, Mezic I. 2003. Closed-form solutions in the electrical field analysis for dielectrophoretic and travelling wave inter-digitated electrode arrays. *J. Phys. D Appl. Phys.* 36:3073–78
83. Albrecht DR, Sah RL, Bhatia SN. 2004. Geometric and material determinants of patterning efficiency by dielectrophoresis. *Biophys. J.* 87:2131–47
84. Matsue T, Matsumoto N, Uchida I. 1997. Rapid micropatterning of living cells by repulsive dielectrophoretic force. *Electrochim. Acta* 42:3251–56
85. Simmons B, Lapizco-Encinas B, Shediac R, Hachman J, Chames J, et al. 2004. Polymeric insulator-based (electrodeless) dielectrophoresis (iDEP) for the monitoring of water-borne pathogens. *Micro Total Anal. Syst. 2004*, *Malmo*, *Sweden*, pp. 171–73. Cambridge, UK: R. Soc. Chem.
86. Voldman J, Toner M, Gray ML, Schmidt MA. 2002. A microfabrication-based dynamic array cytometer. *Anal. Chem.* 74:3984–90
87. Voldman J, Toner M, Gray ML, Schmidt MA. 2003. Design and analysis of extruded quadrupolar dielectrophoretic traps. *J. Electrostat.* 57:69–90
88. Schnelle T, Hagedorn R, Fuhr G, Fiedler S, Muller T. 1993. 3-Dimensional electric-field traps for manipulation of cells—calculation and experimental verification. *Biochim. Biophys. Acta* 1157:127–40
89. Schnelle T, Muller T, Fuhr G. 2000. Trapping in AC octode field cages. *J. Electrostat.* 50:17–29
90. Reichle C, Muller T, Schnelle T, Fuhr G. 1999. Electro-rotation in octopole micro cages. *J. Phys. D Appl. Phys.* 32:2128–35
91. Albrecht DR, Tsang VL, Sah RL, Bhatia SN. 2005. Photo- and electropatterning of hydrogel-encapsulated living cell arrays. *Lab Chip* 5:111–18
92. Gray DS, Tan JL, Voldman J, Chen CS. 2004. Dielectrophoretic registration of living cells to a microelectrode array. *Biosens. Bioelectron.* 19:1765–74
93. Taff BM, Voldman J. 2005. A scalable addressable positive-dielectrophoretic cell-sorting array. *Anal. Chem.* 77:7976–83
94. Suehiro J, Pethig R. 1998. The dielectrophoretic movement and positioning of a biological cell using a three-dimensional grid electrode system. *J. Phys. D Appl. Phys.* 31:3298–305

88. This paper introduces the very successful opposed octopole, along with one of the initial reports of cell positioning in cell-culture media.

93. This paper presents a new addressable trapping architecture whose interconnect requirements scale much more favorably than conventional addressing approaches.

95. Fiedler S, Shirley SG, Schnelle T, Fuhr G. 1998. Dielectrophoretic sorting of particles and cells in a microsystem. *Anal. Chem.* 70:1909–15
96. Muller T, Schnelle T, Fuhr G. 2000. Microdevice for cell and particle separation using dielectrophoretic field-flow fractionation. *J. Liquid Chromatogr. Relat. Technol.* 23:47–59
97. Manaresi N, Romani A, Medoro G, Altomare L, Leonardi A, et al. 2003. A CMOS chip for individual cell manipulation and detection. *IEEE J. Solid-State Circuits* 38:2297–305
98. Das CM, Becker F, Vernon S, Noshari J, Joyce C, Gascoyne PRC. 2005. Dielectrophoretic segregation of different human cell types on microscope slides. *Anal. Chem.* 77:2708–19
99. Chiou PY, Ohta AT, Wu MC. 2005. Massively parallel manipulation of single cells and microparticles using optical images. *Nature* 436:370–72

98. Peter Gascoyne's group introduces electrosmear, a new way to separate cells based on electrical phenotype.

99. This paper describes an opto-electronic approach to generating nonuniform electric fields, potentially obviating the need to fabricate internal electrodes.

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