Header: Artificial Tissues (A Atala and JG Hunsberger, Section Editors)

It's Electric: When Technology Gives a Boost to Stem Cell Science

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Abstract

Purpose of review: Advanced technologies can aid discoveries in stem cell science in surprising ways. The application of electrokinetic techniques, which use electric fields to interrogate or separate cells, to the study of stem cells has yielded important insights into stem cell function. These techniques probe inherent cell properties, obviating the need for cell type specific labels.

Recent findings: Analysis of a variety of stem cell types including hematopoietic, mesenchymal and adipose-derived, neural, and pluripotent stem cells by electrokinetic techniques has revealed fate specific signatures of cells. Distinct inherent cell properties are sufficient for their label free enrichment without causing cell damage or toxicity.

Summary: The successful application of label free techniques to the analysis and sorting of stem cells open new avenues for exploring the basic biology of stem cells and optimizing their use in regenerative medicine applications.

Introduction

Stem cells are of great interest due to their potential to expand understanding of basic developmental processes and to induce repair of damaged tissue in regenerative medicine approaches. Stem cells proliferate, or self-renew, over an extended period and differentiate to form the final mature cells of a tissue. During the differentiation process, more committed progenitor cells with limited proliferative ability are formed that then generate fully differentiated cells. Current approaches to study stem and progenitor cells and their differentiated progeny usually employ labeling with an antibody on the cell surface or genetically with a reporter construct that identifies differentiation along a particular lineage. However, new methods are needed to assess the differentiation process since specific and robust labels are lacking for many stem cell populations. Further, minimal manipulation of cells is preferred in regenerative medicine applications, so analysis and sorting methods that do not require cell labeling are advantageous.

Microdevices using a variety of separation techniques have been developed to identify or sort cells without the use of labels and have been covered in several reviews (e.g. (1)). Electrokinetic technologies can detect inherent cell electrophysiological properties without the use of labels. A particularly promising electrokinetic technology for the analysis and separation of stem cells is dielectrophoresis (DEP), in which non-toxic inhomogeneous electric fields induce cell movement. Those interested in the current state of the DEP field, with an eye toward newly developing trends, are directed to a recent eloquent review by Pethig (2).

Progress in the application of DEP to stem cells is on a rapid pace; a search of stem cell DEP studies identifies 2 publications in the 1990s, about 7 in the 2000s, and at least 30 in the 2010s, which is not yet a complete decade (3). Sections below describe the advantages of DEP as a platform for use with stem cells, advances made in stem cell research by using DEP for analysis and sorting of hematopoietic, mesenchymal and adipose-derived, neural, pluripotent, and other stem cells, adaptation of DEP devices to improve applicability to stem cell studies, and the future of label free techniques for stem cells. The literature reviewed herein clarify both the advantages of applying this technology to the study of stem cells and the novel findings that are opening new avenues of exploration in the stem cell field.

Theory and Advantages of DEP

Dielectrophoresis, first introduced by Pohl in the 1950s (4), is the induced motion of polarizable particles when placed in a non-uniform electric field. The time-averaged DEP force ($\vec{F}_{DEP}$) acting on a polarized particle (with spherical shape) can be expressed as (5, 6):

$$\vec{F}_{DEP} = 2\pi\varepsilon_{m} R^{3} Re[f_{CM}]\nabla \vec{E}^{2}$$

where ε_{m} is the surrounding medium permittivity, R is the particle radius, $\vec{E}$ is the electric field strength, and $Re[f_{CM}]$ is the real part of Clausius-Mossotti factor, which describes the relative values of the polarizability of the particle and medium. The sign of $Re[f_{CM}]$ is a function of the frequency of the applied electric field (ω) as well as the dielectric properties of the cell and medium and determines the behavior of cells in a non-uniform electric field. Figure 1A shows the plot of $Re[f_{CM}]$ with respect to the applied electric field frequency for a cell (6, 7). For negative values of $Re[f_{CM}]$ ($-0.5 \leq Re[f_{CM}] < 0$), cells experience negative DEP (nDEP) and are repelled from high electric field regions. When $Re[f_{CM}]$ is positive ($0 < Re[f_{CM}] \leq 1$), cells are attracted toward high electric field regions and thus experience positive DEP (pDEP). However, at two specific applied frequencies, termed the first and second cross-over frequencies (f_{c1} and f_{c2}), the cells experience no induced DEP force due to the transition in their polarity.

In high frequency DEP (>10 MHz), cell dielectric properties are mostly affected by the cytoplasm and nucleus. In contrast, at low frequencies (<1 MHz), membrane properties such as membrane capacitance and conductance primarily dictate cell behavior, with capacitance dominating (8). For viable mammalian cells, specific membrane capacitance (C_{mem}) can be approximated as (9):

$$C_{mem} = \frac{\sigma_{m}}{\sqrt{2\pi f_{c1} R}}$$

where σ_m is the electrical conductivity of the medium. As the majority of DEP-based techniques utilize frequency ranges lower than 1 MHz, the differences in cells' C_{mem} values have mainly been exploited for cell characterization and sorting (10, 11).

Measurement of C_{mem} can be achieved by different methods such as DEP and

impedance sensing ($C_{mem} = -\frac{1}{\omega^2 (\frac{1}{C_{mem}})}$), where Z_{mem} is the specific membrane impedance of the cell (12). Figure 1B shows $Re[f_{CM}]$ for two cells having identical dielectric properties except for C_{mem}. Due to the difference in C_{mem}, there exists a frequency range at which one type of cell experiences nDEP while the other experiences pDEP. Most DEP-based methods have taken advantage of such distinct differences in DEP response to sort cells based on C_{mem} (Figure 1C).

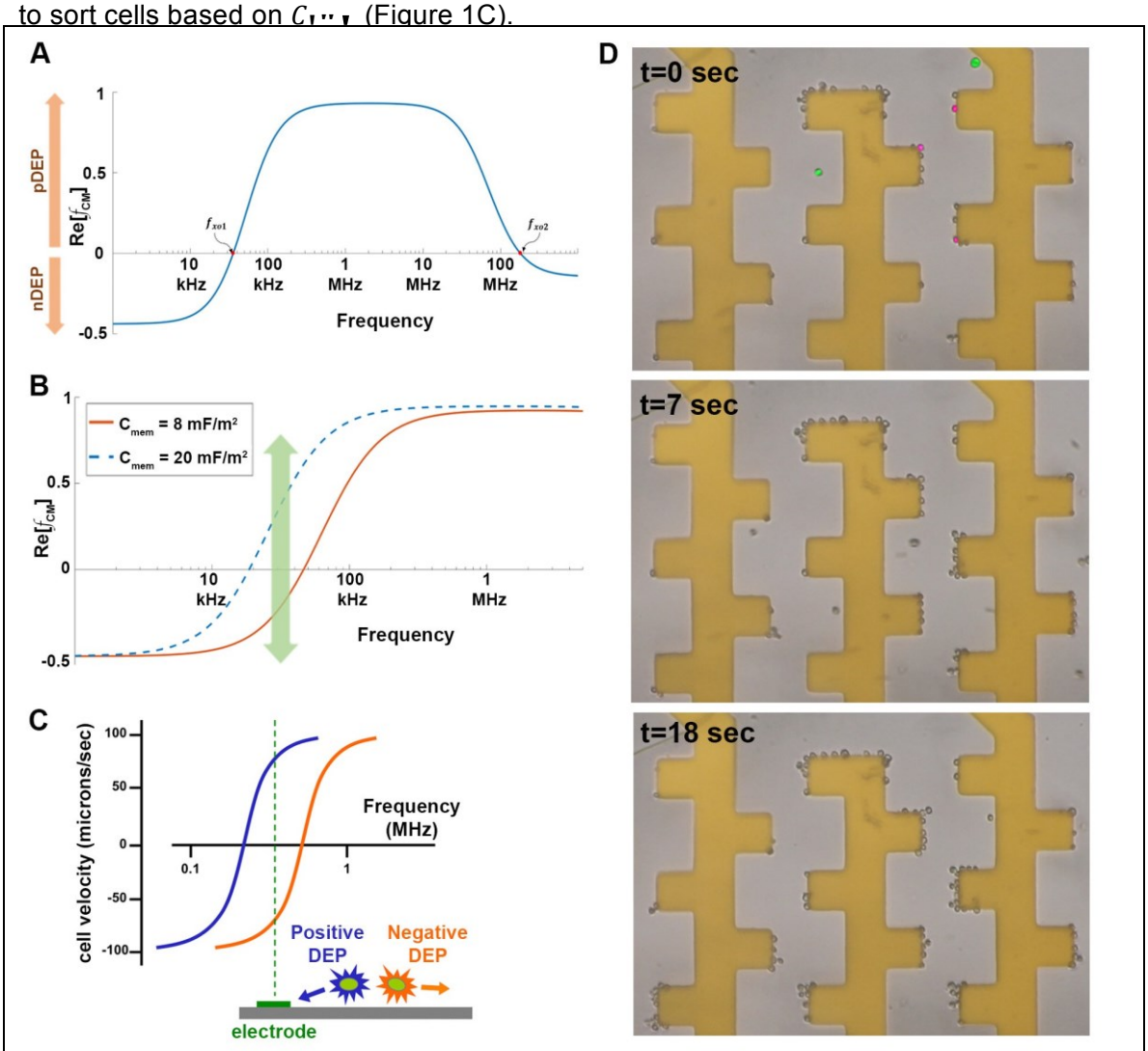

Figure 1: **(A)** Plot of the real part of the Clausius-Mossotti factor ($Re[f_{CM}]$) with respect to the frequency of the applied electric field for a cell modeled with the single-shell model (parameters obtained from (13)). Based on the sign of $Re[f_{CM}]$, cells will experience different motions (nDEP or pDEP) in a non-uniform electric field. At crossover frequencies, f_{xo1} and f_{xo2}, the cells experience no DEP force. **(B)** $Re[f_{CM}]$ for two cells with different membrane capacitance values; the difference in membrane capacitance results in distinct DEP responses at certain frequency ranges. **(C)** Schematic depicts the differential responses of two unique cell types in DEP. Since

the cells have different frequency responses and membrane capacitance values, a frequency can be chosen (denoted by dashed green line) at which one cell is in pDEP and the other in nDEP, providing a force for separating the two cell types. (Reprinted from (14), with permission from John Wiley and Sons). **(D)** Still images from video (Supplemental video S1) show E12 mouse neural stem cells in a microfluidic DEP trapping device with frequency set to 100 kHz such that some of the cells experience pDEP and are attracted to electrode edges (electrodes in gold) while others in nDEP pass by (to aid visualization of cells, some cells in pDEP in first panel are colored pink while those in nDEP are green) (From (15) with permission from John Wiley and Sons).

DEP exploits inherent cell physical properties to distinguish different types of cells and thus requires no cell labeling (Figure 1D). Since cell behavior in DEP at lower frequencies is dependent on membrane physical properties as well as the total amount of membrane, which is affected by cell size, DEP can distinguish similarly sized cells as long as their membrane properties significantly differ. The beauty of DEP lies in the fact that even subtle cell features, such as cell membrane morphology and integrity, affect overall cell dielectric properties and can lead to distinct behavior in DEP (2, 10). For example, membrane capacitance and cell behavior in DEP distinguish normal and malaria infected red blood cells (16), stimulated and unstimulated Jurkat cells (17), breast cancer cells expressing different amounts of the neu oncogene (18), and oral cancer cells differing in adhesion and tumorigenicity (19). Considering these advantages, there has been growing interest in the use of DEP to characterize, manipulate, and separate different types of cells. This is shown by the over 400% increase in DEP publications since 2000 (2). As described further below, stem cell biology is an important area of research in which DEP has intrinsic advantages in distinguishing cells based on their dielectric properties such as membrane capacitance.

Hematopoietic stem cells

Analysis:

DEP has been useful for analysis of cells in the hematopoietic stem cell (HSC) lineage. DEP-based cell characterization revealed differences in the membrane capacitance values of the six mature leukocyte subpopulations, suggesting that a combination of membrane capacitance and cell size would be sufficient to isolate these cells without the use of labels (20). Aggregates of lymphocytes, stromal cells, and osteoblasts were formed using DEP to generate an *in vitro* HSC niche analogous to that found in bone

marrow (21). Mouse embryonic stem (ES) cells that can form HSCs were introduced into the *in vitro* niche, suggesting that DEP is a viable method for generating complex cellular structures to recreate *in vivo* cellular architectures as found in stem cell niches (21).

Sorting:
The earliest publications showing sorting of stem cells focused on the enrichment of HSCs from blood or bone marrow (22, 23). Cells were collected from patient samples of peripheral blood stem cell harvests or bone marrow and red blood cells were removed by centrifugation prior to analysis of the remaining cells by DEP. Cells separated by DEP were analyzed by flow cytometry for expression of CD34, which is a cell surface marker of HSCs. The CD34-positive cells were enriched 6-fold by DEP (23). Subsequent plating of DEP-sorted cells in colony forming assays showed that the sorted cells were viable and able to proliferate at a level expected for CD34-positive cells (22). Thus, these early studies indicate that HSC inherent properties are sufficient for their enrichment by DEP and set precedence for the utility of DEP in stem cell applications.

Mesenchymal and adipose-derived stem cells

Analysis:
Like many stem cell populations, mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs) lack adequate biomarkers, making label free techniques particularly useful. As described above, membrane capacitance can be derived from either DEP measurements or impedance sensing since there is an inverse relationship between capacitance and impedance. Many MSC and ADSC studies have utilized impedance sensing as well as DEP for label free analysis. Since MSCs and ADSCs similarly differentiate into adipogenic and osteogenic cells, they will be considered collectively here.

Baseline values have been established for undifferentiated human MSCs (total membrane capacitance ~2.2 pF in 0.030 S/m conductivity medium and ~4.5 pF in 0.10 S/m conductivity medium)(24) and ADSCs (impedance ~450 $\wedge$ and capacitance of 1.65 ± 0.07 μF/cm2)(25). Altering the cell surface of human MSCs with a polymer shifts membrane biophysical parameters measured by DEP, indicating that DEP at lower

frequencies reliably measures properties of the plasma membrane rather than internal structures (24).

Dynamic changes in cell membrane biophysical properties during ADSC and MSC differentiation have been assessed by treating cells with specific media to either induce adipogenesis or osteogenesis. Impedance sensing reveals that differentiation of human ADSCs to adipocytes is associated with a decrease in impedance (increase in capacitance) while formation of bone cells causes an increase in impedance (decrease in capacitance)(25, 26). Also, adipogenic differentiation of a mouse 3T3-L1 preadipocyte cell line resulted in a decrease in the measured impedance as the cells differentiated (27). Treatment of these cells with an inhibitor that blocks differentiation affected both the impedance measurements and the formation of lipid droplets used as a measure of adipogenesis, suggesting impedance as a specific marker of differentiation (27). Similarly, differentiation of human MSCs is associated with decreased impedance during adipogenesis (26, 28) and increased impedance during osteogenesis (26, 28, 29). Importantly, exposure of human MSCs to the electric fields required for impedance sensing had no effect on cell viability (28).

The shifts in impedance associated with human MSC differentiation occurred well ahead of detectable changes in traditional live cell assays used to identify differentiating cells. For example, alkaline phosphatase staining did not distinguish osteogenic cells until 7 days of differentiation, whereas impedance differences were identified after 1-2 days of differentiation for both osteogenic and adipogenic lineages (25, 26, 28). Impedance signatures also reflect extracellular influences on differentiation since collagen induced more robust osteogenesis than laminin, and MSCs differentiated on collagen showed bigger impedance shifts than those on laminin (26). Shifts in the impedance of human MSCs during osteogenic differentiation were detected for cells in a 2D monolayer culture as well as those in 3D aggregates assessed by a capillary impedance measurement system (29). The differentiation capacity of human MSCs and ADSCs changes with increasing cell passage and is detected by DEP (30). $Re[f_{..}]$ measured by DEP decreased with increasing cell passage over a range of frequencies (10^4-10^6) for both human MSCs and ADSCs (30). Osteogenic differentiation (measure by alkaline phosphatase activity) decreased with increasing passage number (30). Thus, $Re[f_{..}]$ and osteogenic fate potential are positively correlated for human MSCs and ADSCs,

indicating that stem cell fate potential is revealed by cell behavior in DEP.

While these studies consistently report a decrease in impedance with adipogenesis and increase with osteogenesis, two studies report the opposite pattern. An early study showed human MSC-derived adipocytes had an increase in impedance compared to undifferentiated cells (31). Another study analyzed bone-marrow derived skeletal stem cells (SSCs), which are a subpopulation of MSCs defined as Stro-1-positive and CD146-positive cells whose differentiation potential is limited to the skeletal lineage (32, 33). SSCs exhibited a decrease in impedance, which was quantified in terms of opacity and measured at high and low frequencies, during osteogenic differentiation (34). Additional studies will be necessary to clarify whether there are inherent differences in the biophysical characteristics of cells that differentiate from SSCs compared to those from MSCs or ADSCs.

Analysis of undifferentiated MSCs and ADSCs indicate additional utility of electrokinetic techniques for stem cell characterization. High frequency DEP velocity measurements are sufficient to distinguish rat ADSCs from bone marrow-derived MSCs (35). The membrane capacitance values of human SSCs were lower than those of two human osteosarcoma cell lines, suggesting that it might be possible to distinguish and separate healthy stem cells from cancerous cells (36). However, it may be difficult to enrich SSCs from other bone marrow cells since they appear to be similar in biophysical properties (34). Taken together, numerous studies now provide evidence that impedance and membrane capacitance serve as viable early biomarkers for MSC identification and monitoring of differentiation to distinct lineages.

Sorting:

The distinct biophysical properties of undifferentiated cells (MSCs and ADSCs) and their differentiated progeny suggest they can be separated with label-free techniques. Human ADSCs were sorted from subcutaneous adipose tissue using DEP field-flow fractionation to maximum levels of enrichment of ~12-15 fold for NG2-positive and ~4-5 fold for nestin-positive ADSCs (37). This demonstrates that DEP is sensitive enough to discern intrinsic differences in cellular properties within a cell population and enrich a given subset of cells. A DEP-based microdevice was used to separate a mixed population of human MSCs and differentiated osteoblasts (38). Cell collection efficiency and purity

were maximized at a low fluid flow rate and yielded enrichment of MSCs and osteoblasts from a 1:1 mixture (50% each cell type) to 86% MSCs and 65% osteoblasts (38). These studies demonstrate that inherent cell biophysical properties can be utilized to separate undifferentiated stem cells from more differentiated cells in DEP.

Neural stem cells

Analysis:

An important issue relevant for the use of DEP to analyze and separate cells is whether exposure to DEP electric fields affects the cells. This is particularly true for sensitive cell populations such as neural stem cells. Neural stem cells grown in culture contain undifferentiated stem cells as well as progenitors linked to the final differentiated cells of the central nervous system (neurons, astrocytes, and oligodendrocytes), and are thus referred to as neural stem/progenitor cells (NSPCs). Experiments tested effects of DEP on human and mouse NSPCs by exposing cells to AC electric fields across a range of frequencies and times (39). Cells were assessed for survival, proliferation, and differentiation potential. Short-term DEP exposure (less than 5 minutes) had no effect on NSPC survival, proliferation, or differentiation (39). Moreover, NSPC proliferation and differentiation were not altered by any length of DEP exposure (up to 30 min). Long-term exposure (> 5 min) to frequencies near the crossover frequency decreased survival of NSPCs, with a maximum of ~30% cell loss after 30 min, while long-term exposure to other frequencies had no effect (39). A different study assessed the effects of AC and DC electric fields on human NSPCs and found significant increases in activated caspase 3 and cell death in DC, but not AC, electric fields (40). NSPCs exposed to AC fields retained the ability to differentiate into neurons while cells in DC fields did not. These studies found AC DEP is not harmful to NSPCs at short exposure times, thus providing critical information for the design of experiments involving analysis or sorting of NSPCs by DEP.

DEP can be used to detect NSPC fate potential *before* differentiation and discernable marker expression in the cells (14). Cells isolated from the developing mouse cerebral cortex at an early developmental stage (embryonic day 12, E12) at which most NSPCs generate neurons were compared to those from a later stage (E16) when NSPCs form

more astrocytes. The E12 neurogenic NSPCs experienced pDEP at higher frequencies than did E16 astrogenic NSPCs (14). Analysis of differentiated cells showed that neurons experience pDEP at higher frequencies than astrocytes, suggesting that the undifferentiated NSPCs begin to take on characteristics similar to those of the differentiated cells they will eventually form and these characteristics are detectable by DEP.

Measurements of membrane capacitance and conductance by DEP showed that the neurogenic/astrogenic fate potential of both mouse and human NSPCs is reflected in membrane capacitance, but not conductance, values (41). Whole cell membrane capacitance of human NSPCs measured by DEP ranged from 5-13 picoFarad (pF), which is within the range of 5-23 pF reported for rat NSPCs in patch clamp studies (42-44), validating this approach for measuring cell electrophysiological properties (41). The membrane capacitance values of NSPC populations generating more astrocytes were higher than those of NSPCs that preferentially form neurons (41). As human NSPCs are passaged in culture, their ability to generate neurons decreases as their membrane capacitance values increase, showing this measure dynamically reflects fate potential (41). E14 hippocampal rat NSPCs analyzed by impedance sensing exhibited an increase in membrane capacitance as cells differentiated into astrocytes (45). Human NSPCs differentiated in two distinct conditions - one in a medium containing noggin to encourage neuron formation and the other BMP4 to stimulate formation of astrocytes - showed lower capacitance values for cells differentiating in neuronal medium and higher values for those in astrocytic medium (40). Thus, higher membrane capacitance values are linked to astrogenic fates and lower values to neurogenic fates in the neural lineage.

The cellular characteristics contributing to membrane capacitance are not well understood, but for NSPCs may involve cell surface glycosylation. Based on biophysical theory, whole cell membrane capacitance should be impacted by plasma membrane surface area and thickness. Cell membrane microdomains such as ruffles, microvilli, or other morphologies that increase membrane roughness are expected to alter membrane capacitance by increasing cell surface area (46). While NSPCs that have distinct membrane capacitance values do not differ in size as measured by phase contrast microscopy, they may vary in membrane microdomains not visible by phase contrast that could increase cell surface area (15, 41). A cellular process that modifies the

surface of the plasma membrane and contributes to membrane microdomains is glycosylation, by which carbohydrates able to store charge are added to plasma membrane proteins and lipids. Treatment of NSPCs with agents that modify cell surface glycosylation alters their frequency response in DEP (15). Glycosylation may contribute to membrane capacitance in other stem cell lineages since differentiation of MSCs to adipogenic and osteogenic lineages is associated both with changes in membrane capacitance (as described above) and glycosylation (47, 48). Thus, the interaction of membrane capacitance, cell fate, and cell surface glycosylation may have relevance for many stem cell lineages.

Sorting:

NSPCs have been sorted using a variety of DEP-based approaches (11). As shown for other stem cell lineages, differentiated (neurons) and undifferentiated (NSPCs) cells can be separated by DEP (49). However, DEP has also been used to enrich undifferentiated cells on the basis of fate potential. NSPCs differing in neurogenic/astrogenic fate potential display distinct behaviors in DEP and vary in membrane capacitance even though the cells do not differ in size, suggesting that they could be enriched by DEP (15, 41). Mouse NSPCs were separated into distinct frequency bands and differentiation of the separated cells showed that NSPCs forming astrocytes were enriched in lower frequency bands while cells generating neurons were isolated at higher frequencies (15). In a follow up study, low frequency sorts were used to enrich NSPCs biased toward forming astrocytes from a heterogenous population of mouse NSPCs using either a large capacity electrode array (LCEA) device with a microfluidic channel or a simple device with electrodes in the base of a well, showing that sorting of fate-biased cells from NSPCs is robust across multiple DEP platforms (50). Cells sorted by DEP retained their enrichment over multiple passages post-sorting, enabling the generation of 10^9 cells for further study (50). The ability to expand cells after sorting and maintain enrichment is an advantage since many DEP-based sorting devices are fairly low throughput and generate relatively few sorted cells. These studies show that the inherent properties of NSPCs biased to form either neurons or astrocytes upon differentiation are sufficient for their enrichment without the use of cell type specific labels in DEP and provide a means to study cell fate in the neural lineage.

Pluripotent stem cells

Analysis - Differentiation:

Changes in cell electrophysiological properties detectable by label free techniques occur during the differentiation of pluripotent stem cells into several distinct lineages. Impedance sensing distinguishes undifferentiated Oct4-expressing P19 mouse embryonal carcinoma pluripotent cells from differentiated cells, some of which express the neuronal marker MAP2 (51). Single cell analysis of mouse embryonic stem (ES) cells in an impedance sensor revealed an increase in membrane capacitance as the cells underwent differentiation, although this study did not assess the types of differentiated cells formed (52). Differentiation of human ES cells into either MSCs or trophoblast cells was associated with shifts in DEP crossover frequencies and increases in membrane capacitance (53). In contrast, differentiation of human ES cells into hepatocytes was accompanied by a decrease in membrane capacitance, although the change in capacitance was of smaller magnitude (1.1-fold) than those observed between undifferentiated ES cells and either MSCs (3-fold) or trophoblast cells (1.6-fold)(53, 54). Differentiation of human induced pluripotent stem (iPS) cells to ectodermal lineages resulted in higher capacitance values measured by impedance sensing than those for cells forming mesendodermal lineages (55). There are clear differences in the membrane capacitance values of undifferentiated pluripotent stem cells and their differentiated progeny. The magnitude and direction of the shift in capacitance varies depending on the type of differentiated cell formed, suggesting specific electrophysiological properties may define distinct types of cells generated during pluripotent stem cell differentiation.

Analysis - Patterning and embryoid body formation:

DEP devices have been designed to pattern stem cells, particularly pluripotent cells since the size and shape of embryoid bodies generated during initial stages of differentiation can affect the types of final differentiated cells formed. Rapid clustering of pluripotent cells by pDEP enables control of embryoid body size since the number of cells attracted can be controlled by modulating DEP electrode geometries. In one study, induced pDEP forces were utilized to target mouse ES cells to microwells (56). Live/dead assays indicated that the vast majority of the patterned cells were viable, indicating the lack of toxicity with this approach. ES cell differentiation in patterned embryoid bodies was assessed using 7a mouse ES cells with GFP under the control of a

constitutive promoter and Bry-ES cells expressing GFP from the brachury promoter to mark cells of the mesodermal lineage (57). Cells were clustered at castellated DEP electrodes then encapsulated in a Puramatrix peptide-based hydrogel (57). Differentiation of the ES cells after clustering generated brachury-expressing mesodermal derivatives, showing pluripotent stem cells can be aggregated and differentiated in DEP devices to enable more control over the process of embryoid body formation and differentiation (57). Mouse ES cells were patterned by DEP to enable controlled formation of aggregates of increasing size that were then encapsulated in PEG hydrogels (58). Live/dead cell assays showed no decrease in viability of the DEP-exposed cells and this approach generated cell aggregates in a 3D structure. A different type of DEP device was used to induce clustering of mouse ES cells over 15 seconds to form 3D spherical embryoid bodies with high cell viability in photo-polymerizable methacrylated gelatin hydrogels (59). The cells in the DEP-formed embryoid bodies differentiated, as shown by reduction of the stem cell marker nanog. In a different approach, a DEP device designed to generate precise pairs of cells yielded patterned pairs of mouse ES cells and 3T3 fibroblasts, enabling single cell level analysis of cell-cell interactions (60). These studies show a variety of DEP-based approaches can be used to rapidly and reproducibly pattern pluripotent stem cells to control embryoid body formation and cell-cell interactions.

Other stem cell types

Sorting:

Other types of stem cells can be identified in DEP, including potential cancer stem cells and muscle stem cells. PC3 human prostate cancer cells contain ~15% of cells expressing ALDH, which is considered a marker for tumor initiating cells and may indicate cancer stem cells. Analysis of PC3 cells by DEP indicates that complete trapping of the ALDH-positive cells occurs at lower frequencies than those needed to trap ALDH-negative cells (61). Sorting by DEP yielded populations of ALDH-positive and negative cells, and only the positive cells were able to generate spheres, suggesting tumor initiating ability. These data suggest it may be possible to identify and enrich cancer stem cells from tumor samples containing a heterogeneous population of cells with DEP. Analysis and sorting of muscle cells by DEP indicate the utility of this technique for distinguishing undifferentiated cells from their more differentiated progeny,

as shown by the separation of C2C12 myoblasts and more differentiated myotubes (62). In further analysis, myoblasts and myotubes were found to vary significantly in size, which could contribute to their efficient sorting by DEP (63). DEP analysis and sorting is of use for multiple stem cell populations, and continued investigation of additional stem cell types with DEP will help to drive the field forward.

Adaptation and evolution of DEP for stem cells

Over the past few years and with advances in microfabrication and microfluidic technologies, a variety of techniques have been proposed for DEP (64, 65). Generally, methods for DEP can be classified into two main categories: electrode-based DEP and insulator-based DEP (iDEP). Electrode-based DEP utilizes an AC electric field with embedded microfabricated electrodes inside a fluidic channel to create non-uniform electric fields. Metal electrodes (2D (64, 66) and 3D (67-70)), doped silicon (71), liquid electrodes (72), carbon electrodes (73), and doped PDMS (74) are among the main electrode-based DEP microfabrication materials. On the other hand, in iDEP designs, the electrodes are placed remotely and the non-uniform electric field is created by spatially dispersed insulating structures inside the microchannels (75). Both DC and AC electric fields have been used in iDEP. While AC DEP utilizes both spatial variation and frequency-dependent components of DEP, DC DEP relies only on spatial variation.

In stem cell research, electrode-based DEP using 2D metal electrodes has been the most widely used technology for characterization (14, 53) and sorting (22, 23, 37, 38, 49, 50, 62, 76). In addition to the conventional planar interdigitated electrode configuration (14, 37-39, 50, 53, 76), other geometries such as castellated (15, 22, 23, 49), quadrupole “funnel” (62), and saw-shaped (77) electrode arrays were also used for 2D-based DEP. In general, glass slides with patterned electrodes were then used as the substrate for the main separation chamber, most often constructed from polydimethylsiloxane (PDMS). Some designs also used Teflon (76), glass (23, 37), or SU-8 (62) for the separation channel. Studies of tumor initiating cells utilized an iDEP device based on contactless DEP (cDEP). In cDEP, the electrodes (in this case a conductive liquid) are placed in two side microchannels that are separated from the main chamber by a thin PDMS layer (61).

As for operation, the majority of characterization and sorting methods have been based on “trap and release” protocols. However, continuous sorting of stem cells has been also reported by using arrays of oblique planar interdigitated electrodes (38, 62) as well as DEP field-flow fractionation (76). As an alternative to 2D electrode-based DEP, a few groups focused on the use of 3D electrode-based DEP to sort and characterize stem cells. Wang et al. (78) proposed the use of vertical sidewall electrodes for manipulation of mouse neural stem cells. This DEP device (68), equilibrates the cells at specific lateral positions along the width of the microchannel. The 3D DEP-Well chip, developed by Hoettges et al. (79), has been adopted to characterize different types of stem cells based on their membrane capacitance (36, 41). In this method, each well’s perimeter is covered by thin rings of evenly spaced electrodes to generate the DEP electric field. By passing the light through the wells at each different applied electric field frequency, the light disruption in the well caused by the cells’ behavior (induced movement in response to the electric field) could be measured and used to show cells’ DEP spectra.

In designing and evaluating DEP devices for stem cell sorting, there are key aspects that should be considered. An ideal DEP device should sort cells at as high throughput as possible and with high separation efficiency and purity. For clinical applications, optimal throughput on the order of over 1 million cells/hr would help to eliminate the need for post-sorting expansion of stem cells (11). Many DEP-based microfluidic sorters, which are mainly 2D electrode-based designs, still need at least an order of magnitude improvement in throughput to reach this scale. Although scaling up the dimensions of electrodes is one possible option for increasing throughput in 2D electrode-based DEP designs, there exists an upper limit. Using impedance measurements at different frequencies, Simon et al. showed that increasing the length and width of electrode arrays results in reduction of electrical impedance of the arrays and consequently reduced electric field strength (50). Thus, optimizing the electrode array configuration is required to maintain a sufficiently strong electric field. Another intrinsic limitation associated with 2D electrode-based designs is separation chamber height. Due to the exponential decay of the electric field with distance, separation chambers in these designs did not exceed $100\ \mu m$ in height. In this aspect, 3D electrode-based DEP designs are advantageous as the electric field strength does not vary across the microchannel height. However, fabrication complexity and high cost of these designs still limit their applicability. To address such challenges, a low-cost and high throughput 3D

electrode-based cell sorter has been fabricated with laminate drilled to form electrode-bearing wells. This device is capable of sorting 150,000 cells/sec, and could be adopted for stem cell research (80). A key barrier to achieving the maximum possible separation efficiency and purity is heterogeneity in cell size and membrane capacitance (49, 53). However, as described above, many stem cell populations include cells of interest that vary in capacitance. Continued design of DEP devices optimized for use with stem cells will drive exciting progress in the stem cell field.

The future of label free techniques for stem cells

As one of the most promising label free techniques, DEP is revolutionizing the use of stem cells for basic and therapeutic purposes. Eventual clinical utility is suggested by the fact that DEP has been successfully applied to many human stem cell populations. As cell phenotype can be correlated with inherent properties such as membrane capacitance and cell size, DEP-based cell analysis platforms can rapidly identify stem cell populations in research and clinical settings. DEP can be used as a tool for cell manufacturing processes to remove cells with unfavorable attributes and harvest beneficial cells prior to transplant. This approach would greatly improve stem cell transplants by allowing researchers and clinicians to work with uniform populations of cells. DEP-based techniques for stem cell therapeutics require scaling up from current devices (as described above), but post-sorting expansion of stem cell populations can generate clinically relevant numbers of cells (50). Another exciting possibility is a closed and sterile DEP-based system that could sort/purify cells to be immediately transplanted back into patients with minimal manipulation. This type of disposable device that does not involve cell labeling would face fewer regulatory hurdles than more complicated sorting systems. Cell patterning and sorting capabilities of DEP can be employed to build tissue organoid systems from human stem cells. These can be used for drug screening and to understand developmental processes involved in forming human tissues. Similar approaches could be used for personalized medicine and point of care diagnostics, in which patient-specific cells could be isolated, analyzed, or built into screening platforms to assist clinicians in determining the best treatment plan. In summary, the future looks bright for new and exciting discoveries enabled by the application of label free technologies such as DEP to stem cells.

Conclusions

In conclusion, label free techniques provide real time continuous monitoring of stem cells and can identify cells biased to specific cell fates. Cell electrophysiological properties measured by DEP and impedance sensing, such as whole cell membrane capacitance, serve as biomarkers of stem cell fate and differentiation. The application of label free technologies to stem cells has led to novel insights regarding the regulation of stem cell fate, and continued progress will push forward our understanding of basic stem cell biology. Development of next generation DEP-based separation devices should focus on optimizing parameters for stem cell separations that can generate enriched cells for transplant to treat human injuries and diseases. The increasing number of studies using technological advances such as DEP for analysis and sorting of stem cells will continue to drive progress in stem cell science and development of cell-based therapeutics.

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Compliance with Ethical Standards

Conflict of Interest
Mohammad Aghaamoo and Tayloria Adams declare that they have no conflict of interest. Abraham P. Lee has a patent Microfluidic device for cell and particle separation issued. Lisa A. Flanagan has a patent U.S. Pat. No. 7,964,078 issued.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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