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Dielectrophoresis as a tool for electrophysiological characterization of stem cells

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ABSTRACT

Dielectrophoresis (DEP), a nonlinear electrokinetic technique caused by Maxwell–Wagner interfacial polarization of neutral particles in an electrolyte solution, is a powerful cell manipulation method used widely for various applications such as enrichment, trapping, and sorting of heterogeneous cell populations. While conventional cell characterization and sorting methods require tagging or labeling of cells, DEP has the potential to manipulate cells in a label-free way. Due to its unique ability to characterize and sort cells without the need of labeling, there is renewed interest in using DEP for stem cell research and regenerative medicine. Stem cells have the potential to differentiate into various lineages, but achieving homogeneous cell phenotypes from an initially heterogeneous cell population is a challenge. Using DEP to efficiently and affordably identify, sort, and enrich either undifferentiated or differentiated stem cell populations in a label-free way would advance their potential uses for applications in tissue engineering and regenerative medicine. This review summarizes recent, significant research findings regarding the electrophysiological characterization of stem cells, with a focus on cellular dielectric properties, i.e., permittivity and conductivity, and on studies that have obtained these measurements using techniques that preserve cell viability, such as crossover frequency. Potential applications for DEP in regenerative medicine are also discussed. Overall, DEP is a promising technique and, when used to characterize, sort, and enrich stem cells, will advance stem cell-based regenerative therapies.

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I. INTRODUCTION

Dielectrophoresis (DEP) is a non-linear electrokinetic phenomenon caused due to Maxwell–Wagner interfacial polarization, resulting from an externally imposed electric field when a particle is in contact with a saline solution. If a difference in electrical polarizability between the particle and the suspension medium (saline solution) exists, then both free and bound charges have to be induced at the surface of the particle by the applied field in order to ensure the continuity of the normal component of the total current density across the material interface. This produces a ponderomotive electrical stress on the suspended particles in the imposed electric field gradient, leading to the movement of particles in a specified direction, i.e., toward high-field maxima or low-field minima regions. The applied electrical stress, and thus particle movement, is a function of the morphology and intrinsic dielectric properties of the suspended particles in addition to the externally imposed electric field. Therefore, based on these principles, DEP can be controlled to characterize and manipulate particles in solution, making DEP an ideal biological cell characterization tool. DEP was first utilized to sort stem cells in the 1990s, with the first reported study in 1995,[1] and then subsequently in 1996[2] and 1999.[3] These early studies evaluated hematopoietic stem cells (CD34+). Subsequently, the number of studies doubled to at least 7 (2001–2010) and increased nearly tenfold in the current decade (2011–2020). By 2018, roughly 30 projects/year were investigating the potential of DEP for stem cell research,[4] highlighting a renewed interest in using DEP to characterize and sort stem cells.

This recent interest in DEP is partially driven by the need to characterize different types of stem cells via their dielectric properties, in order to enhance the potential use of stem cells in tissue engineering and regenerative medicine.[5–10] Since the inception of DEP in the 1950s,[11] its widespread use has refined DEP into a powerful tool for applications ranging from separation of live and dead cells[12,13] to distinguishing between various stages of cell differentiation.[5,14] Recent advances in the technique, such as using non-uniform electric fields to manipulate cells, have significantly improved DEP's accuracy, sensitivity, and utility for characterizing and separating cells. Overall, DEP has been successfully used to separate various biological components, including proteins,[15] bacteria,[13] and stem cells[5,7,10] for various applications such as trapping, sorting, and characterization.

DEP has evolved as a powerful cell sorting tool, as it eliminates the necessity of labeling the cells, instead exploiting minute differences in their cellular dielectric properties that occur between different cell phenotypes and genotypes. DEP is sensitive to slight changes within the cell or its membrane, as these subtle differences affect the cellular dielectric properties.[4,14,16] DEP has several advantages over traditional cell sorting methods such as fluorescence activated cell sorting (FACS), as it relies on less tedious sample preparation and results in improved cell viability following separation.[17,18] Additionally, the experimental setup for DEP is relatively simple[19] compared to the other methods of cell sorting, such as flow cytometry, making it an attractive tool for distinguishing between cell populations based on small variations in electrophysiological (dielectric) properties. For example, a simple DEP setup to obtain the dielectric properties consists of a pair of electrodes with appropriate spacing between them that is subjected to an alternating current (AC) electric field to introduce non-uniform electric gradients within a microwell (Fig. 1).

The microwell contains a suspension of the particles of interest that is observed via an inverted microscope to track their trajectory, i.e., toward or away from the perpendicular electrode (high-field maxima region). Because of this simple setup, DEP microdevice fabrication is relatively easy and cost-effective compared to other microfluidic separation techniques. These relatively simple DEP microdevices have been further modified to accommodate a microchannel instead of a microwell for trapping, separating, and enrichment of cells.[20,21]

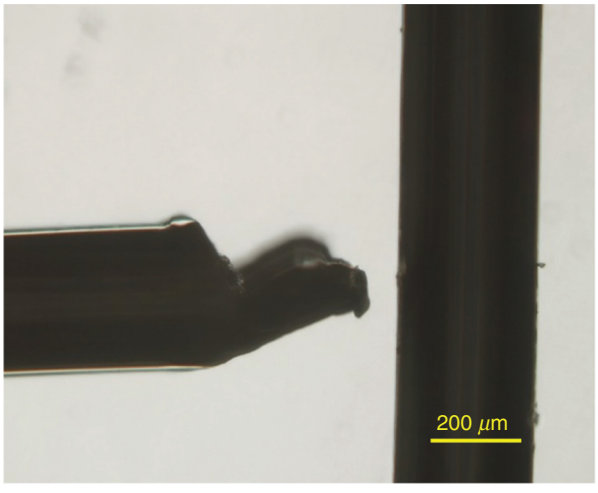

FIG. 1. Sample image showing sealed platinum electrode setup in a 3-mm-diameter microwell with ~75 μm electrode spacing between them.

DEP technology has also been extensively evaluated and refined to provide high levels of accuracy and utility compared to other microfluidic techniques.[22] For example, red blood cells (RBCs) and white blood cells (WBCs) were reportedly separated with 90% efficiency in a high-conductivity DEP suspending medium.[23] Another study reported 99% purity of separated RBCs and WBCs from whole blood.[24] Recently, a novel particle separation technique based on deterministic lateral displacement (DLD) was combined with DEP to increase the sensitivity of sorting particles.[25] Hybrid dielectrophoresis,[26] a technique that is gaining popularity, combines DEP with other passive microfluidic techniques like inertial microfluidics, DLD, pinched flow fractionation, etc., based on the specific applications.[27,28] DEP was also used to artificially create hematons[29] and has several other potential biomedical[17,20,30,31] and industrial applications.[32] Another DEP-based technology is traveling-wave DEP (twDEP), which causes flow of particles in a channel due to the phase-varying non-uniform electric field generated between a parallel electrode setup.[33] twDEP has been extensively studied both theoretically[34–37] and experimentally to develop high-throughput particle sorters.[38,39] However, to date, twDEP applications for characterizing stem cells have been limited and, thus, are not extensively discussed in this review.

Based on these recent advances and potential applications, this review briefly summarizes the theory that enables dielectrophoresis, and then discusses significant research findings regarding the electrophysiological characterization of stem cells. We focus on studies that characterize cellular dielectric permittivity and conductivity using techniques that preserve cell viability, like measurement of DEP crossover frequency. Although the potential of DEP as a stem cell sorting and diagnostic tool has been explored in several recent reviews,[17,40–42] DEP has not been extensively investigated in the context of generating homogeneous cell populations for applications in musculoskeletal tissues. Therefore, the utility and future needs for DEP with a particular focus on musculoskeletal tissues are also discussed.

II. THEORY OF DIELECTROPHORESIS

DEP is a non-invasive, label-free technique that induces motion of particles relative to the medium in which they are suspended due to the gradient of a non-uniform electric field. Particle movement is based on the polarizability and the dielectric properties (permittivity and conductivity) of the cell membrane and cell interior (cytoplasm and nucleus).[6,11] As DEP induces particle motion based on their dielectric properties, it was initially used to separate live and dead cells.[12] In the first decade since its inception, the applications of DEP have been extended to several processes such as cell enrichment, trapping, and sorting.[43] Briefly, when a biological cell (intrinsically nonpolar) is subjected to an externally imposed electric field, an induced dipole moment (m_{eff}) occurs within the cell. The dipole magnitude can be derived as follows,[42] assuming the cell to be spherical in shape and having radius r:

$$m_{eff} = 4\pi\varepsilon_m r^3 pE, \tag{1}$$

where ε_m is dielectric permittivity, E is the applied electric field, and p is the effective polarizability (per unit volume) signified by the Clausius–Mossotti (CM) factor, which is expressed as[17]

$$p = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}, \tag{2}$$

where ε_p^* and ε_m^* are the complex permittivity of the particle and the medium, respectively. Complex permittivity of the particle and the medium can be calculated using the relation

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega}, \tag{3}$$

where $j = \sqrt{-1}$, σ is conductivity of the material, and ω is the angular frequency. However, Eq. (3) is only applicable for bioparticles with a single shell and, hence, may not be suitable for bioparticles with multilayer internal structures. The net DEP force acting is proportional to the product of the induced dipole moment and the field gradient, expressed as[17]

$$F_{DEP} = (m_{eff}.\nabla)E. \tag{4}$$

Combining Eqs. (1) and (4), the DEP force experienced by a particle is given as

$$F_{DEP} = 4\pi\varepsilon_m r^3 p(E.\nabla)E, \tag{5}$$

where ∇ is the gradient operator, which mathematically represents $i\frac{\partial}{\partial x} + j\frac{\partial}{\partial y} + k\frac{\partial}{\partial z}$. This result considers a depolarization factor of 1/3 to account for the fact that a spherical body distorts an external applied field, and that the electric field inside the sphere differs from the outer external field. This is due to the fact that the electric current continuity condition has to be satisfied at the particle/medium interface with the piecewise-defined constant dielectric properties across the adjacent medium phases.

Equation (5) can also be represented by neglecting the imaginary part of p and considering the real part alone as

$$F_{DEP} = 4\pi\varepsilon_m r^3 Re[p](E.\nabla)E, \tag{6}$$

Equation (6) is analogous to Eq. (7), which is another widely used expression with vector transformation on the electric field[9,44]

$$F_{DEP} = 2\pi\varepsilon_m r^3 Re[p]\nabla E_{rms}^2. \tag{7}$$

Dielectrophoretic force can also be commonly written as[45]

$$F_{DEP} = \frac{3}{2}\varepsilon_m \upsilon Re(p)\,\nabla E^2, \tag{8}$$

where υ is the volume of the bioparticle, i.e., stem cell in this article, i.e., $\frac{4}{3}\pi r^3$. Substituting volume in Eq. (8) results in Eq. (7).

Mathematically, p is bound within the limits $-0.5 \le p \le 1.0$. The dielectric properties of the suspending medium (DEP buffer) and the cells determine the value of p, depending on the angular frequency. In general, the medium properties are standardized, and altering the frequency of the applied electric field results in motion of the suspended cells toward the high or low electric field regions. Cells move toward the high electric field (i.e., toward the electrodes) when $p > 0$, termed as positive dielectrophoresis (pDEP). Cells move toward the low electric field (i.e., away from electrodes) when $p < 0$, termed as negative dielectrophoresis (nDEP). Finally, there exists a frequency at which $p = 0$, where there is no noticeable motion of the cells. This is termed as the crossover frequency (f_{xo}) or the zero-force frequency, at which the acting DEP force is zero. This crossover frequency indicates that the real parts of the effective polarizabilities of the cell and the medium are equal to each other and is unique for every cell based on their membrane and cytoplasmic characteristics. By determining this frequency, the dielectric characteristics of cells can be quantified using mathematical modeling, which in turn can be utilized to develop a microfluidic platform for enrichment or sorting of cells via modeling and simulation studies using commercially available software packages, like COMSOL Multiphysics.

There exist two zero-force frequency regions termed as first (f_{x1}) and second crossover (f_{x2}), i.e., transitions from nDEP to pDEP and back to nDEP. Utilizing f_{x1}, i.e., the transition from nDEP to pDEP or vice versa (determined experimentally), the properties of the membrane can be quantified using[6]

$$C_{mem} = \frac{\sqrt{2}\sigma_{med}}{2\pi r f_{x1}}, \tag{9}$$

where C_{mem} is the capacitance of the membrane, σ_{med} is the electrical conductivity of the medium, and r is the radius of the cell. f_{x1} typically occurs in the radio frequency band due to β-dispersion (i.e., frequencies between 0.010 and 0.1 MHz) and is caused by the capacitive charging of the insulating cell membrane in an AC field, and hence, f_{x1} is impacted by cell size, morphology, and membrane properties (Fig. 2). Biological cells produce three types of dispersions under wide frequency bandwidth, based on which they are classified into α, β, and γ regions.[46] These cells are characterized for dielectric properties based on α-dispersion at low frequencies (Hz–kHz), β-dispersion in the radio frequency band (kHz–MHz), and γ-dispersion in the microwave frequency region (>GHz).[47] Quantified C_{mem} can be extended to determine the permittivity of the membrane, which is proportional to its capacitance given by

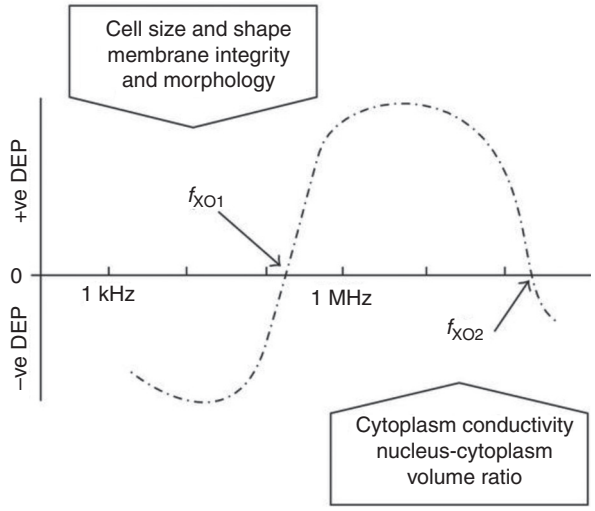

FIG. 2. DEP response exhibited by a viable cell, indicating the first and the second crossover frequencies with the respective dependence on parameters. Reprinted with permission from R. Pethig, A. Menachery, S. Pells, and P. De Sousa, "Dielectrophoresis: A review of applications for stem cell research," BioMed Res. Int. **2010**, 182581,[42] licensed under a Creative Commons Attribution (CC BY) license.

$$\varepsilon_{mem} = \frac{C_{mem} d}{4\pi r^2 \varepsilon_0}, \tag{10}$$

where ε_{mem} is the permittivity of the membrane, d is the thickness of the membrane, and ε_0 is the permittivity of vacuum.

At frequencies >10 MHz, i.e., high frequency range (10 MHz–1 GHz), the contents of a cell's interior (e.g., cytoplasm and nucleus) play a significant role in determining the second crossover frequency (f_{x2}), caused by the interfacial charge relaxation at the bioparticle-medium interface, in which the dominating polarization mechanism changes from conduction current to the displacement current. In order to quantify the dielectric properties of the cell's cytoplasm, f_{x2} is utilized as shown in Eq. (11), as reported by Gimsa *et al.*[48]

$$f_{x2}^2 = \frac{1}{4\pi^2} \frac{1}{\varepsilon_o^2} \frac{(\sigma_m - \sigma_{cyto})(\sigma_{cyto} + 2\sigma_m)}{(\varepsilon_{cyto} - \varepsilon_m)(\varepsilon_{cyto} + 2\varepsilon_m)}. \tag{11}$$

Properties of the cytoplasm (conductivity and permittivity), nuclear envelope permittivity, and nucleus-cytoplasm (N/C) volume ratio play significant roles in determining f_{x2} (Fig. 2).[42] Therefore, each cell phenotype has a unique dielectric property that impacts both f_{x1} and f_{x2}. Characterizing these crossover frequencies, and using the equations described above, can identify the unique dielectric signatures of cells. Dielectric signatures unique to each cell can be utilized to develop sorting microfluidic platforms. The applied non-uniform field causes the target cells to behave differently than the rest of the cell population. These differences in cell behavior allow target cells to be isolated, sorted, or enriched as necessary, depending on the purpose of the application. Though DEP is sensitive enough to detect subtle changes within cells, disadvantages of exposing cells to stronger electric fields, i.e., >10 V_{pp} (peak-to-peak voltage) for longer durations of >30 min include alterations in cellular properties and decreased viability. This threshold potential and time period is typical for electroporation or cell lysis applications and not for sorting or enrichment platforms.[49–52] Cell characterization experiments at stronger electric potentials, i.e., >10 V_{pp} should be run at shorter time durations, i.e., <5-min periods, to maintain cell viability.

III. APPLICATIONS OF DEP IN STEM CELL RESEARCH

Stem cells have been explored for use in tissue engineering approaches and regenerative therapies due to their ability to differentiate into multiple tissue types. However, stem cells require further characterization to improve their clinical potential. Two major challenges that can be addressed with DEP sorting and characterization techniques are (1) achieving homogeneous cell phenotypes from an initially heterogeneous cell population and (2) identifying a specific cell phenotype within a group of stem cells. The unique dielectric properties (permittivity and conductivity) of the cell membrane and cell interior that occur between phenotypes can serve as label-free biophysical markers and may be distinct at different stages of differentiation. These alterations in dielectric properties can be exploited by DEP for cell characterization and sorting. DEP is also an appealing separation technique due to its demonstrated ability to sort cells with a high degree of accuracy and based on subtle dielectric property differences, while also preserving cell viability. This review's focus is limited to a basic classification of stem cells before discussing the DEP characterization technique for these cells.

A. Types of stem cells

Stem cells are undifferentiated cells that have the potential to self-renew and can differentiate into multiple cell lineages.[53] Stem cells exist both in embryos and adults and are classified based on their potency and origin, as summarized in Tables I and II. "Potency" refers to a stem cell's ability to differentiate into different cell types. Based on potency, totipotent stem cells have the highest potential of differentiating into cells of any kind, followed by pluripotent stem cells (PSCs). PSCs are descendants of totipotent cells and can differentiate into cells derived from any of the germ layers, but not the placenta.[54] The germ layers are the three primary cell layers formed during early embryonic development and consist of the endoderm (inner layer), the ectoderm (outer layer), and the mesoderm (middle layer).[55] Multipotent stem cells have a narrower spectrum of differentiation compared to pluripotent stem cells. Multipotent stem cells can differentiate into cells of a closely related lineage. For example, bone marrow contains multipotent stem cells that can give rise to all the cells of blood [e.g., hematopoietic (blood) stem cells (HSCs)],[54] but no other cell types. Oligopotent stem cells have the ability to differentiate into only a few cells, for example, lymphoid or myeloid stem cells that can only replenish other lymph of myeloid cells. Unipotent cells can divide repeatedly to produce only their own cell type and are usually able to proliferate rapidly, but have the least differentiation capacity.[53,54] Muscle stem cells are a representative unipotent cell.[56,57]

In addition to their potency, stem cells are broadly classified into three categories based on their original source: embryonic stem cells, adult stem cells, and infant stem cells, a larger grouping that includes cord blood stem cells.[57] Embryonic stem cells can differentiate into any fully developed cell of the body.[58] In the initial stages of embryonic

TABLE I. Classification of stem cells based on potency (regenerative potential).[53] Reprinted with permission from M. J. Łos, A. Skubis, and S. Ghavami, "Stem cells," in *Stem Cells and Biomaterials for Regenerative Medicine*, edited by M. J. Łos, A. Hudecki, and E. Wiecheć (Academic, 2019), Chap. 2, pp. 5–16. Copyright AIP Publishing LLC.

Type of cell	Characteristic
Totipotent	Ability to differentiate into cell lineages derived from all three germ layers: mesoderm, endoderm, ectoderm (including placental cells).
Pluripotent	Ability to differentiate into cell lineages derived from all three germ layers: mesoderm, endoderm, ectoderm (excluding placental cells).
Multipotent	Ability to differentiate into a limited number of types from germ layer of origin.
Oligopotent	Ability to differentiate into a few types of cells with related functions.
Unipotent	Ability to produce cells of their own type exclusively.

TABLE II. Classification of stem cells based on their source.[53] Reprinted with permission from M. J. Łos, A. Skubis, and S. Ghavami, "Stem cells," in *Stem Cells and Biomaterials for Regenerative Medicine*, edited by M. J. Łos, A. Hudecki, and E. Wiecheć (Academic, 2019), Chap. 2, pp. 5–16. Copyright AIP Publishing LLC.

Stem cells	Source; characteristic
Embryonic stem cells	Blastocysts; pluripotent
Adult stem cells	Adipose tissue/bone marrow/peripheral blood; multipotent
Induced pluripotent stem cells (iPSCs)	Any somatic cell, most commonly fibroblasts, keratinocytes, peripheral blood mononuclear cells; pluripotent
Mesenchymal stem cells	Bone marrow/adipose tissue/skin/peripheral blood, perinatal tissue: umbilical cord blood, amniotic fluid, membrane and placenta; multipotent, non-hematopoietic
Hematopoietic stem cells	Bone marrow/hepatic tissue; multipotent/bipotent
Skeletal stem cells	Bone marrow and local periosteum; multipotent

development, the cells of the zygote are totipotent.[57,59] Once the zygote forms a blastocyst (approximately 7 days following fertilization), these cells become pluripotent.[57] Adult stem cells (also known as somatic stem cells) are harvested from mature tissues. Mesenchymal stem cells (MSCs), HSCs, neural stem progenitor cells (NSPCs), hepatic, epidermal, and pancreatic stem cells are representative of commonly utilized somatic stem cell lines.[57] Induced pluripotent stem cells (iPSCs) are typically derived from adult cells, but are programmed to be embryonic-like stem cells with pluripotent characteristics, and are widely used in drug development and disease modeling applications.[57]

To date, DEP has been used to characterize a number of these different stem cell types. Although stem cells of many different origins and potencies have been isolated and classified, tissue engineering and regenerative medicine applications commonly utilize only a subset of identified stem cells. We next examine existing uses of DEP in tissue engineering and stem cell research and discuss ongoing investigation areas in which expanded DEP use may positively impact regenerative medicine.

B. Recent applications of DEP in stem cell research

Major unmet needs in stem cell research include selecting specific cells of interest from a cell population (e.g., isolation and separation), identifying when cells have differentiated (e.g., characterization), and increasing the number of cells of interest (e.g., enrichment). DEP has the potential to address these major unmet needs in stem cell research. The first stem cell studies utilizing DEP evaluated the CD34+ hematopoietic stem cells.[1–3] Experimental studies using DEP for stem cell characterization and sorting are still at their nascent stages[42] but have received renewed interest throughout the last three decades. DEP has now been applied to characterize, separate, enrich, isolate, and sort different types of stem cells (Table III). In the following section, we discuss the recent characterizations of the dielectric properties of commonly used stem cells, which can in turn be used to further refine DEP to characterize, separate, enrich, and isolate different types of stem cells and progress toward addressing these unmet needs in stem cell research.[60]

1. Hematopoietic stem cells

Hematopoietic stem cells (HSCs) were the first stem cells successfully sorted by DEP.[1–3] Sorting was based on isolation and enrichment of CD34+ cells (a transmembrane protein and marker for human HSCs). These initial studies in HSCs found that DEP was effective for enriching and separating HSCs from a heterogeneous cell population, which consisted of HSCs as well as bone marrow and peripheral blood stem cells.[1,2] Talary *et al.* (1994) reported DEP enrichment of HSCs using 6 V_{pp} and 500 kHz for 10 min where all the cells experienced pDEP, thereby causing them to be trapped.[1] Another study showed DEP was able to isolate human breast cancer cells from HSCs, while also characterizing CD34+ stem cells (HSCs) to obtain their dielectric properties. The dielectric values of CD34+ HSCs reported[3] are provided in Table IV.

A pDEP regime has also been successfully used to create DEP-based artificial micro-environments for HSCs via construction of hematon-like structures [a compact, three-dimensional (3D) spheroid complex from central adipocytes, fibroblastoid cells, and resident macrophages that compartmentalize progenitor cells], while maintaining cell viability.[29] A hematon consists of at least two distinct structures: an inner core of support cells and an outer layer of blood-producing cells. In this study, mouse stromal cells were used as support cells and

TABLE III. Application of dielectrophoresis (DEP) in studying stem cells for various applications such as isolation, characterization, separation, etc. BM-MSCs, bone marrow-derived mesenchymal stem cells; NSPCs, neural stem/progenitor cells; ADSCs, adipose tissue-derived stem cells.

DEP application	Types of stem cells	References
Isolation	Cancer (glioblastoma) stem cells	44
Characterization	Human MSCs	6
Separation	Neural stem cells, BM-MSCs, NSPCs, ADSCs	61,62
Trapping	Mouse NSPCs	7
Enrichment	BM-MSCs, ADSCs	61,63

TABLE IV. Reported dielectric properties of membrane and cytoplasm of human CD34+ cells (HSCs).[3] Reprinted with permission from Y. Huang, J. Yang, X. B. Wang, F. F. Becker, and P. R. Gascoyne, "The removal of human breast cancer cells from hematopoietic CD34+ stem cells by dielectrophoretic field-flow-fractionation," J. Hematother. Stem Cell Res. **8**(5), 481–490 (1999). Copyright 1999 Mary Ann Liebert, Inc.

Cell type	Specific membrane capacitance (C_{mem}) (mF/m^2)	σ_{int} (S/m)	ε_{int}
CD34+ (HSCs)	10.2 ± 1.2	0.71 ±0.11	141.2 ± 28.0

Jurkat cells (human T lymphocytes) that produce blood cells as an outer layer. A frequency of 1 MHz at 20 V_{pp} was used to first layer the bottom with stromal cells using pDEP, followed by adding Jurkat cells to aggregate on the outer layer until the desired height was attained, a process which lasted between 3 and 15 min. The hemispherical structure produced could be used as an artificial microniche for HSCs, and variation of the position and type of cells within the structure could aid in the study of HSC renewal, proliferation, and differentiation.

Cell diameter of human HSCs was estimated to be 8.2 ±1.1 μm and 8.7 ± 1.7 μm using the Coulter electronic counter method and image analysis method, respectively.[64] Accounting for size-based changes in the cell groups is an important consideration for accurately determining the dielectric properties, since f_{x1} is a function of size and shape of the cell.

2. Mesenchymal stem cells

Mesenchymal stem cells (MSCs), the multipotent progenitors of muscle, tendon, bone, and cartilage, are especially promising for musculoskeletal tissue engineering applications due to their ability to undergo differentiation into several musculoskeletal tissue lineages.[65–71] MSCs can be isolated from several sources, including adipose tissue (ADSCs) and bone marrow (BM-MSCs). However, the inherent heterogeneity of MSC populations presents a unique challenge for tissue engineering applications. Precise control over stem cell differentiation and application of a homogeneous and differentiated cell population is crucial to regenerative medicine and tissue engineering. Cells that remain undifferentiated can result in aberrant tissue formation, including ectopic ossification when used in tendon repairs,[72] or form malignant tumors.[73]

Human MSCs (h-MSCs) express a range of biomarkers on their membrane, challenging characterization efforts. Nevertheless, undifferentiated h-MSCs membrane permittivity and capacitance have been quantified to obtain the dielectric properties,[6] which are summarized in Table V. This initial characterization using DEP crossover frequency technique also suggested that treatment of the cells with a polymer (polypeptide) significantly impacted the dielectric properties, compared to the untreated cells. Pre-DEP treatment (such as adding polymers), i.e., as seen in the elastic-like polypeptide polyethyleneimine (ELP-PEI)-treated group in Table V, should be accounted for when using cell-surface biomarkers to characterize cells. This study used electric fields at 10 V_{pp}, while the frequency was swept from 0.01 to 35 MHz for 90 s in a Ti-Au quadrapole electrode setup. The first crossover frequencies for untreated h-MSCs were reported to be 0.62 MHz and 1.3 MHz at 0.03 S/m and 0.10 S/m suspending medium conductivity, respectively, while the ELP-PEI-treated h-MSCs had a reported first crossover frequency of >35 MHz.

TABLE V. Dielectric properties of undifferentiated human mesenchymal stem cells as a function of medium.[6] Reprinted with permission from T. N. G. Adams, P. A. Turner, A. V. Janorkar, F. Zhao, and A. R. Minerick, "Characterizing the dielectric properties of human mesenchymal stem cells and the effects of charged elastin-like polypeptide copolymer treatment," Biomicrofluidics **8**(5), 054109 (2014). Copyright AIP Publishing LLC.

	Human MSCs	
Cell treatment (suspending medium conductivity)	Membrane permittivity	Membrane capacitance (pF)
Untreated (0.03 S/m)	2.0	2.2
Untreated (0.10 S/m)	4.1	4.5
ELP-PEI treated (0.10 S/m)	0.050	>0.13

In another study, a DEP assisted platform was used to separate and enrich BM-MSCs from a heterogeneous cell population consisting of MSCs and human promyelocytic leukemia cells. Using AC voltage of 5 V_{pp} and 30 kHz of applied frequency for 5 min resulted in separation of BM-MSCs with purity, recovery, and enrichment rates of 83.5 ± 7.1%, 29.1 ± 4.1%, and 2.3, respectively, while the viability of cells remained above 90%.[61] h-MSCs and their differentiation products (osteoblasts) were also assessed after continuous flow sorting using DEP to separate undifferentiated h-MSCs from MSCs that had differentiated into osteoblasts. DEP separation achieved 84% purity for h-MSCs and 87% purity for osteoblasts.[9] Cells were viable after sorting and collecting, and followed distinct trajectories during separation based on their differentiation state (h-MSCs or osteoblasts).[9] Finally, the collection efficiency for h-MSCs was high (92%), while 67% was achieved for osteoblasts.[9] Overall, this study showed that DEP can separate osteoblasts from their parent stem cells, although due to the concern of ectopic ossification of MSCs in other applications, such as in tenogenic differentiation,[72,74] a higher purity and collection efficiency would be desirable for musculoskeletal tissue engineering and clinical applications.

DEP is also capable of separating mature musculoskeletal cell populations from stem cells. A recent study used a 3D DEP device fabricated from a laminate of copper and polyimide layers to distinguish between two osteosarcoma cell lines (MG-63 and SAOS-2) and an immunoselected enriched skeletal stem cell fraction (STRO-1 positive cell) of human bone marrow.[8] By using DEP to develop a model that generated the membrane and cytoplasmic properties of the cell populations, significant differences were observed in the cytoplasmic conductivity and specific membrane capacitance of each cell type (MG-63, SAOS-2, and STRO-1), which allowed further sorting of the cell populations.[8] The MG-63 cells exhibited a cytoplasmic conductivity of 0.23 S/m (the lowest of the three cell types) and the highest specific membrane capacitance at 16.0 mF/m^2. The SAOS-2 cells exhibited the highest cytoplasmic conductivity of 0.52 S/m and a specific membrane capacitance of 13.6 mF/m^2. Finally, the STRO-1 positive fraction of human bone marrow cells exhibited a cytoplasmic conductivity of 0.34 S/m and a specific membrane capacitance of 10.7 mF/m^2. This study demonstrates the ability of DEP to separate both mature and stem cell populations, even from a heterogeneous human bone marrow cell

population. Overall, the ability to detect and separate musculoskeletal cell populations will greatly accelerate the clinical application of stem cell-based therapies.

Characterization of cells using DEP is impacted by the cell shape and size, especially the first crossover frequency, when using the DEP crossover technique. Hence, the cell size and its effect should be well understood, since variance in size is thought to be a significant cause of severe vascular obstructions when MSCs are injected in large and small animal models.[75] MSCs had an average cell size (diameter) of 17.2 ± 1.2 μm and remained small and spherical until 4 days of culture before increasing to over 30 μm in diameter by day 7.[76] In terms of morphology, h-MSCs that are cryopreserved have spindle-shaped morphology 1 day after plating. It has been observed that MSCs derived from different sources such as adipose tissue, amniotic tissue, bone marrow, chorionic tissue, liver, and umbilical cord have distinct cell morphologies.[77]

Based on the derived site, MSCs displayed varying differentiation potentials, even though at 1 day in culture, cells from most sources had similar, spindle-like morphology[77] (Fig. 3). The above-reported cell sizes also match prior reporting of the average cell diameter of fractionated MSCs based on the culturing method. Cells cultured in monolayer for six passages had cell diameters ranging from 17.9 μm (small) to 30.4 μm (large).[75] MSCs at passage six varied significantly in size, from 15 to 50 μm, with an average diameter of 26.5 μm. Based on these findings, variations in the size and morphology of MSCs can be exploited by quantifying the DEP first crossover frequency to characterize and sort heterogeneous cell populations.

ADSCs, a subset of MSCs, are promising for stem cell-based therapies due to their availability and relatively easy procurement from adipose tissue.[78] ADSCs have not previously been characterized using DEP, and their dielectric parameters have not been established. However, one prior study explored the potential use of dielectric properties in monitoring ADSC differentiation into osteoblasts and mature adipocytes. With the use of an electric cell-substrate impedance system (ECIS), ADSC membrane capacitance was measured by monitoring time- and frequency-dependent complex impedance at the cell-electrode interface.[79] The cell membrane capacitance of undifferentiated human ADSCs was reported as 1.65 ± 0.07 μF/cm^2, while the membrane capacitance of osteo-induced and adipose-induced cells (4 days after induction) was found to be 1.72 ± 0.10 μF/cm^2 and 2.25 ± 0.27 μF/cm^2, respectively, representing significant differences in their membrane capacitance. Alterations in membrane capacitance present a mechanism by which DEP might be useful for selecting ADSCs from more differentiated progeny. In a different study, undifferentiated human ADSCs had a mean radius of 15.4 μm. However, after seven days of adipogenic induction, the cells exhibited a slightly larger mean radius of 18.8 μm and were further enlarged after 14 days of induction with a mean radius of 20.3 μm.[80] These changes in size also indicate that DEP might be able to detect undifferentiated and differentiated ADSCs based on the differences in their size, since the particle-translating speed due to the action of DEP force is linearly proportional to the radius squared (r^2), by considering the force balance with the Stokes fluidic drag.

Recent studies have assessed the electrokinetic adaptability of ADSCs. Due to the adaptive nature of ADSCs, they had a higher resistance to oxidative stress as examined using oxidative stress-induced senescence and β-galactosidase (SA-β-Gal) assay.[59] Oxidative stress

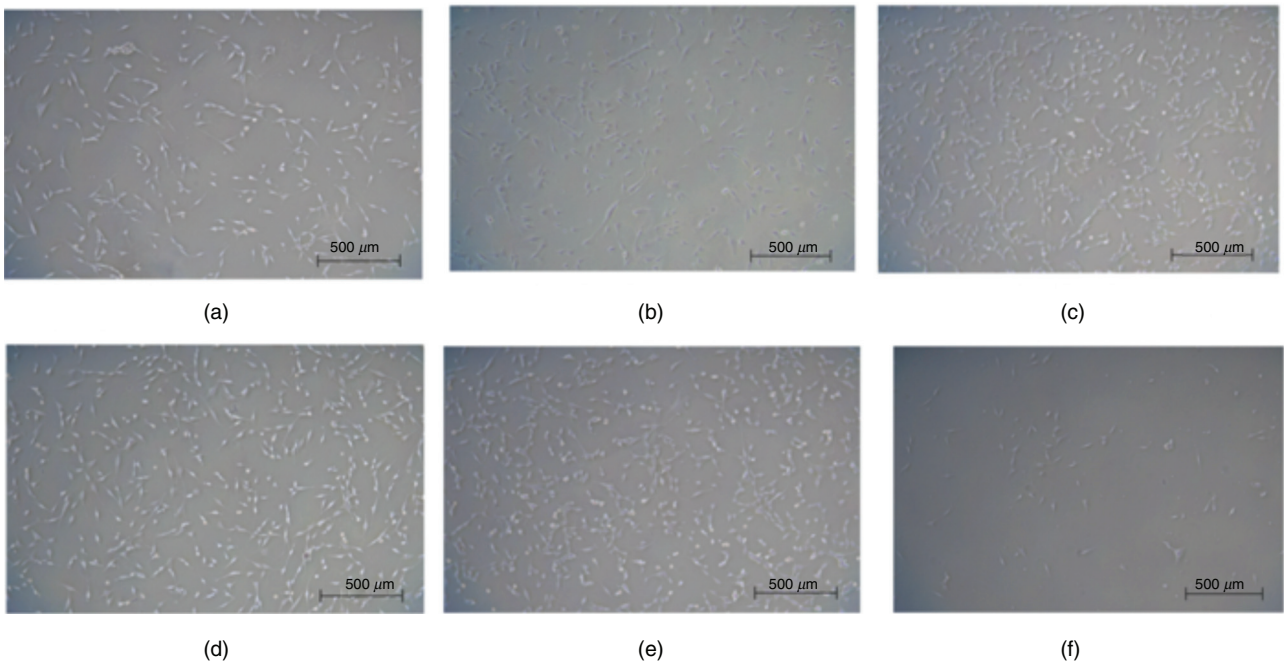

FIG. 3. Phase-contrast images of 1-day-old plated human mesenchymal stem cells that were derived from (a) adipose tissue, (b) amniotic tissue, (c) bone marrow, (d) chorionic tissue, (e) liver, and (f) umbilical cord. Reprinted with permission from E. Schmelzer, D. T. McKeel, and J. C. Gerlach, "Characterization of human mesenchymal stem cells from different tissues and their membrane encasement for prospective transplantation therapies," BioMed Res. Int. **2019**, 6376271,[77] licensed under a Creative Commons Attribution (CC BY) license.

was induced by treating cells with hydrogen peroxide (H_2O_2). This induced oxidative stress was utilized to simulate decline in organ function and cellular aging. After treatment, ADSCs and BM-MSCs did not show any morphological changes (Fig. 4). However, the BM-MSC proliferation rate decreased and 90% of BM-MSCs tested positive for cellular senescence as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay described in Ref. 59, whereas ADSCs remained more potent. Therefore, ADSCs were considered resistant to induced oxidative stress. Additionally, in the same study, ADSCs displayed adaptability to electric fields when exposed to repeated electric stimulation. This potential to adapt to electric fields was characterized by measuring dielectric properties using a DEP traveling wave technique (twDEP). Traveling wave speed and rotational speed were measured at 10 V_{pp} and 8 MHz. The particles travel at different velocities based on their dielectric properties and the frequency of the electric field. Therefore, using twDEP, particles can be characterized based on a plot of traveling wave velocity and frequency (twDEP velocity spectra).[33] Overall, ADSCs displayed slower velocity movement at lower frequency and higher velocity at higher frequency compared to BM-MSCs. The velocity of BM-MSCs remained nearly constant throughout the frequency sweep measurements from 1 kHz to 8 MHz at a fixed voltage of 10 V_{pp}.[59] Taken together, ADSCs display a greater adaptive potential to electric fields compared to BM-MSCs.[59] Furthermore, the viability of ADSCs after DEP-based separation appears higher compared to BM-MSCs,[62] suggesting that DEP-sorted ADSCs might be useful in clinical applications. However, further studies are needed to evaluate the clinical potential of DEP for characterizing and sorting ADSCs and MSCs, but the adaptive response of ADSCs to repeated electrical stimulation and their maintained viability gives ADSCs a significant advantage over BM-MSCs.

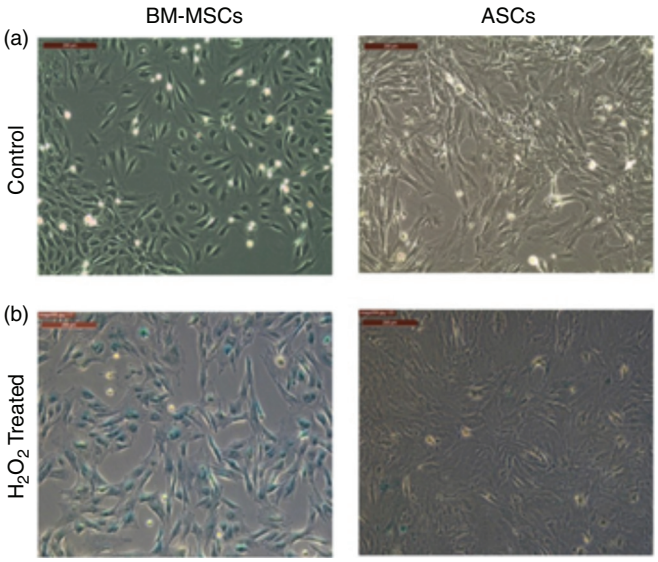

FIG. 4. (a) Control group: bone marrow-derived mesenchymal stromal cells (BM-MSCs) and adipose stem cells (ADSCs). (b) Hydrogen peroxide-treated BM-MSCs and ADSCs to induce oxidative stress, which is known to cause cellular aging and deteriorates organ functioning. ADSCs had higher proliferation than BM-MSCs, after treatment. Reprinted with permission from A. El-Badawy *et al.*, "Adipose stem cells display higher regenerative capacities and more adaptable electro-kinetic properties compared to bone marrow-derived mesenchymal stromal cells," Sci. Rep. **6**, 37801 (2016),[59] licensed under a Creative Commons Attribution (CC BY) license.

DEP has also been evaluated for enrichment of stem cells from adipose tissue using a field flow fractionation technique.[63] Cells isolated from adipose tissue were subjected to an AC electric field of 200 kHz. Frequency was then linearly decreased to 60 kHz over 40 min, and cells were characterized using a processing volume of 1500 μL/min. At 200 kHz frequency, intact cells experienced pDEP, which resulted in separation and trapping, while the damaged cells and cell debris were not retained in the fractionating chamber. Further decreasing the frequency over time resulted in nDEP behavior of intact cells and achieved satisfactory results with up to 14-fold enrichment from initial <2% of NG2-positive cells (pericytes and/or putative progenitor cells), highlighting their potential use in clinical trials.[63] Overall, these studies demonstrated that ADSCs have unique dielectric properties and cell size compared to their differentiated progeny. Additionally, ADSCs can be identified and separated using DEP, indicating that DEP is a useful tool when working with ADSCs and has clinical potential.

Clinical applications require homogeneous populations of MSCs in order to avoid further post-treatment complication, such as tumor formation at the treatment location.[81] Heterogeneity is thought to be one of the causes for such behavior of MSCs used clinically.[81,82] Sorting MSCs into homogeneous cell populations is challenging, as they are difficult to isolate rapidly in large numbers, leading to heterogeneity of differentiated products. Using traditional label-based cell separation and sorting techniques has proven challenging due to great diversity of MSCs based on the primary tissue of origin, the donor age, the isolation method, and the cell culture conditions.[83] Cell source and number of passages are also important considerations in regenerative medicine and contribute to the heterogeneity of MSCs.[83] Another challenge is the behavior of individual MSCs within a heterogeneous population. A single MSC from the population used in a clinical application may proliferate rapidly and give rise to new cells that aid recovery, or turn cancerous and/or die due to nutrient deprivation, DNA and membrane damage, etc.[84] All of these considerations pose challenges to stem cell sorting and highlight the need for next generation label-free DEP sorting technology.

The potency of MSCs presents a further characterization challenge. Although most stem cells used in regenerative approaches are multipotent, the differentiation potential of MSCs derived from different sources varies. In one study, murine MSCs derived from bone marrow (BM), compact bone (CB), and adipose tissue (AT) were separated using FACS and cultured for three passages. All cells retained fibroblastic morphology, but growth was stalled in the BM-derived MSCs.[85] Additionally, FACS analysis of cell markers revealed that the AT- and CB-derived MSCs were positive for CD29, CD44, CD105, and Sca-1 but negative for CD34, TER-119, CD45, and CD11b.[85] From this study, AT-derived cells appeared to have the most potential as a source of MSCs for future musculoskeletal tissue engineering uses based on their growth rate and ability to form colonies. While the distinctions detected by FACS are valuable, a method such as DEP that can separate cells based on characteristics other than membrane markers or tags, while maintaining cell viability, would improve predictions of which cell line is ideal for a specific musculoskeletal tissue application, such as tendon regeneration. In applications outside tissue

engineering, MSC contamination with hematopoietic stem cells during their isolation is a concern that is difficult to mitigate with traditional methods, such as FACS or cell labeling.[86] Future studies using DEP could enhance the homogeneity of isolated MSC populations and facilitate the isolation of MSCs from multiple sources. Overall, improved characterization of MSC differentiation will greatly enhance their potential use in regenerative therapies. More work is needed to elucidate the effects of cell culture and differentiation on MSC morphology, size, and potency, and DEP is a promising tool for improving the clinical potential of MSCs.

3. Neural stem cells

The dielectric properties of neural stem cells (NSCs) have been characterized using impedance measurement.[87] Cell membrane capacitance and conductivity are able to serve as label-free biophysical markers and are distinct for different stages of differentiation. The specific membrane capacitance and cytoplasm conductivity of NSCs were studied as biophysical markers during the differentiation process over 7 days in culture.[87] Undifferentiated NSCs isolated from two different rats of the same species were characterized by measuring their dielectric properties at days 0, 1, 3, and 7 in culture. Specific membrane capacitance was found to be $1.71 \pm 0.45\ \mu F/cm^2$ and $1.74 \pm 0.66\ \mu F/cm^2$ for rats I and II, respectively, and cytoplasmic conductivity was calculated to be 3.21 ± 2.05 S/m and 2.41 ± 1.40 S/m for rats I and II, respectively. Further properties associated with the varying differentiation stages assessed at each time point are presented in Table VI. Undifferentiated cells (day 0) exhibited large differences in cytoplasmic conductivity compared to the differentiating cells, signifying cellular heterogeneities that were possibly due to the differences in culture medium. Throughout differentiation, specific membrane capacitance varied widely as a function of days in culture, indicating changing expression of the membrane proteins (Table VI). Utilizing whole membrane capacitance (i.e., the cell's ability to store electrical energy), separation of rat NSCs using DEP was assessed. Applying DEP to separate cells at the transplantation scale of $\sim 10^9$ cells yielded promising results.[5] Results also suggest that whole membrane capacitance has the potential to serve as a biophysical marker to enrich and separate NSCs.[5]

Cellular dielectric properties can also be used to distinguish undifferentiated NSCs from differentiated cells, while also revealing heterogeneity in the cell population.[7] In another study, direct current-insulator based dielectrophoresis (DC-iDEP) was successfully employed to characterize and distinguish NSCs by measuring the electrokinetic (EK) mobility ratio, a key biophysical property that was observed to be distinct for neural stem and progenitor cells (NSPCs).[88] The EK mobility ratio is the ratio of electrokinetic and dielectrophoretic mobilities, which is determined by the location of capture for a particular device and applied voltage. This ratio reflects the conductivity, radius, and zeta potential of the particles. The EK mobility ratio of NSPCs showed more heterogeneity variance of 3.4–3.9 (standard deviation) than human embryonic kidney cells (HEK 293) with standard deviation of 1.1, supporting the heterogeneity of cells in NSPC cultures. This study highlights the potential of applying DC-iDEP, which does not depend on the frequency of electric field, in successfully identifying and distinguishing desired cells within heterogeneous cell populations. Identifying the fate potential of cells via EK mobility ratio would be ideal. However, limitations in cell transport and single cell bioanalytical methods prevents the above DC-iDEP technique of characterization, i.e., measuring the fate potential of cells.[88]

In addition to characterizing NSCs, DEP has been used to characterize the biophysical properties of differentiated neural cells. DEP is shown to accurately estimate the dielectric permittivity, cytoplasm conductivity, and specific membrane capacitance of mouse hippocampal neuronal and glial cells, providing novel information about these three biophysical properties in two understudied types of neural cells.[19] Overall, DEP is emerging as a useful and relevant tool for obtaining homogeneous populations of desired NSCs for tissue engineering applications. As the technique is refined and novel biomarkers are discovered, the utility of DEP will continue to increase. We next discuss recent DEP advances in high-throughput stem cell sorting.

IV. RECENT ADVANCES IN DEP FOR SORTING STEM CELLS

In addition to the challenges already discussed, another significant hurdle is accurately identifying cells at high throughput, since only a small percent of a given cell population can typically be used therapeutically.[88] DEP has the potential to be a powerful and successful technique for distinguishing and manipulating cells based on their morphological and physiological characteristics, including their size, shape, ratio of cytoplasm to nuclear volume, and dielectric properties, but traditional DEP techniques are challenged by relatively low throughput. Recent modifications to DEP-based devices to improve throughput have combined DEP with hydrophoretic modules (in which an induced pressure gradient and non-uniform fluid flow induce cell motion) and expanded the applicability of DEP to lab-on-a-chip systems, microfluidics, and other experimental setups. Importantly, these modifications have also allowed high-throughput

TABLE VI. Dielectric properties of rat NSCs during their course of differentiation at days 0, 1, 3, and 7 which depict significant difference in the properties at every time point.[87] Reprinted with permission from Y. Zhao *et al.*, "Electrical property characterization of neural stem cells in differentiation," PLoS One **11**(6), e0158044 (2016), licensed under a Creative Commons Attribution (CC BY) license.

	Specific membrane capacitance ($\mu F/cm^2$)		Conductivity of cytoplasm (S/m)	
Time point	Rat I	Rat II	Rat I	Rat II
Day 0	1.71 ± 0.45	1.74 ± 0.66	3.21 ± 2.05	2.41 ± 1.40
Day 1	4.26 ± 1.73	3.44 ± 1.22	3.71 ± 2.26	2.83 ± 1.59
Day 3	2.80 ± 1.71	3.12 ± 2.07	1.19 ± 0.59	1.43 ± 0.73
Day 7	2.65 ± 1.50	3.70 ± 1.81	1.40 ± 0.65	1.22 ± 0.64

separation of stem cells at rates of ~240,000 cells/h,[10] which is much higher than any conventional DEP-based platform (6000–100,000 cells/h), and is higher than commercial techniques like FACS and MACS.[10] Neural stem cells were enriched at a frequency of 184 kHz based on the differences in the dielectric behavior in a hydrophoresis combined DEP platform at a high throughput of 240,000 cells/h.[10] To our knowledge, no therapeutic-based evaluations were reported for stem cells using DEP. However, based on these recent studies, DEP offers a promising way of sorting stem cells for tackling some of the pressing problems in regenerative medicine and neurology.

A. Potential future impact and application of DEP for musculoskeletal tissues

While all musculoskeletal tissues are active research objectives for regenerative therapies using stem cells, tendons have emerged as a relatively understudied tissue that would benefit immensely from expanded regenerative treatment options. Tendons, the musculoskeletal tissues that transfer forces from muscle to bone to enable movement, are frequently injured and heal poorly, resulting in permanent loss of function.[89] Regenerative therapies and *in vitro* tissue engineering approaches for tendons are especially challenging due to the poorly understood characteristics of tendon-specific cells and the limited cell-surface markers needed for FACS-based characterization and sorting. There are only a few distinguishing transcription factors that allow for differentiated tendon cells to be identified.[90–92] Therefore, using DEP to identify, isolate, and enrich populations of stem cells primed for tenogenesis (differentiation toward tendon) would greatly enhance tissue engineering and regenerative approaches to treat tendon injuries.

A promising application of DEP is improved characterization of the cells involved in tendon differentiation and development. A recent study showed that the make-up of tendon progenitor cell populations is heterogeneous. Single-cell analysis of tendon stem/progenitor cells (TSPCs) showed that some cells had active expression of nestin at specific stages of tendon development, and during healing following surgery (Fig. 5).[93] Nestin, an intermediate-filament protein commonly associated with nerve cells, was expressed by some TSPCs, and nestin+ TSPCs displayed enhanced tenogenic capacity and ability to self-renew, compared to nestin− cells.[93] When nestin expression was knocked down using shRNA, TSPCs had suppressed clonogenic capacity and reduced tenogenic potential both *in vitro* and *in vivo*.[93] These results suggest that certain subpopulations of TSPCs may be more primed toward tenogenesis than others, despite all being tendon progenitors, and highlight a potential use for DEP in generating viable pools of nestin+ TSPCs. In particular, DEP methods to determine f_{x2}, which can detect properties of the cytoplasm and possibly distinguish nestin+ intermediate filaments from nestin− TSPCs, should be explored. While it is unclear if nestin expression alone can allow for DEP separation, it is possible that other variations exist between nestin+ and nestin− TSPCs that do allow for DEP-mediated distinction.

Variations between tenogenically differentiating cells may manifest as differences in the transmembrane cell-cell junction proteins, including cadherins and connexins.[94] Embryonic and adult tendon cells contain an array of cell-cell junction proteins including cadherin-11, N-cadherin, connexin-43, and connexin-32.[94–96] Recently, cadherin-11, N-cadherin, and connexin-43 were found to be altered during tenogenesis of MSCs *in vitro*,[97] and changes in the levels of

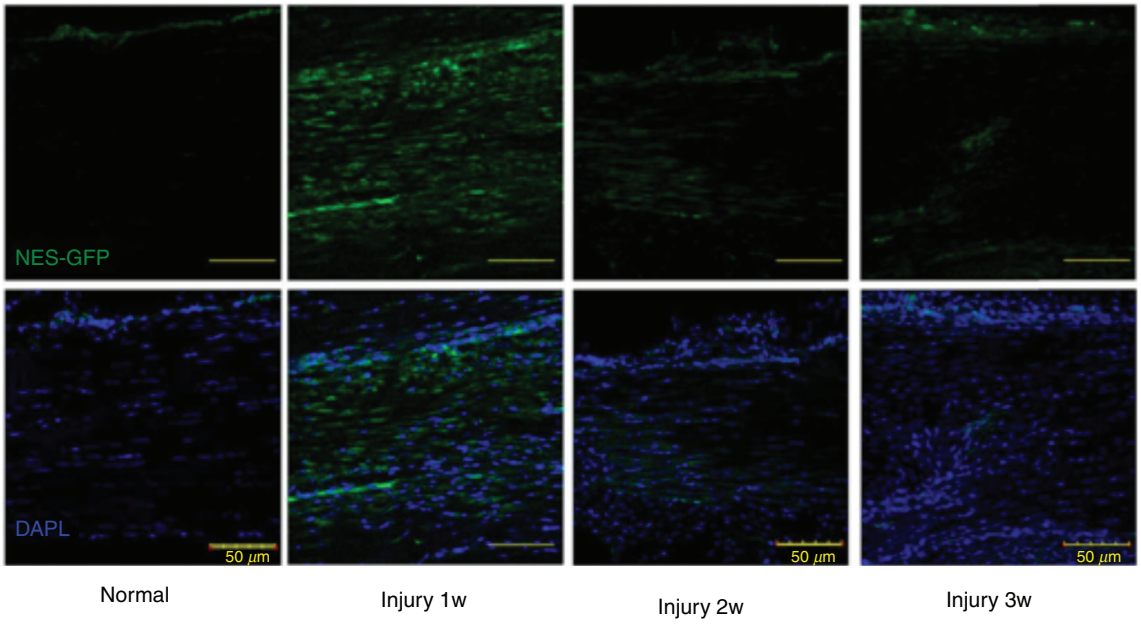

FIG. 5. Nes-GFP expression in normal and surgically injured adult mouse Achilles tendon 1, 2, and 3 weeks after surgery. The presence of Nes-GFP, along with the stem cell markers CD146 and CD105, suggests that endogenous tendon stem cells are activated upon injury and that nestin could be a reliable marker for functional TSPCs. Scale bars = 50 μm. Reprinted with permission from Z. Yin *et al.*, "Single-cell analysis reveals a nestin+ tendon stem/progenitor cell population with strong tenogenic potentiality," Sci. Adv. vol. **2**(11), e1600874 (2016),[93] licensed under a Creative Commons Attribution (CC BY) license.

these proteins may be unique distinguishing characteristics of tenogenic differentiation. Subtle differences in levels of membrane-bound cadherins and connexins would likely result in detectable changes to the membrane specific capacitance of the cells, allowing DEP and specifically characterizations of f_{x1} to distinguish between tenogenic and non-tenogenic cell populations. Taken together, the emerging role of cell-cell junction proteins during tenogenesis provides an additional marker that DEP can utilize to derive optimized cellular precursors for tendon tissue engineering applications.

Beyond cell sorting, DEP may be useful to tendon tissue engineering applications as a method of building and characterizing scaffolds to support cell growth and tendon formation. Aligned 3D nanofibrous silk fibroin-chitosan (eSFCS) scaffolds were fabricated using DEP.[98] The percent-aligned area of the nanofibers in the scaffolds increased as a function of DEP frequency, with 10 MHz resulting in the greatest alignment. Furthermore, fiber size decreased as DEP frequency increased from 100 kHz to 10 MHz, providing a strategy for controlling fiber size.[98] When seeded with human umbilical vein endothelial cells (HUVECs), the HUVECs formed aligned and branched capillary-like vascular structures, indicating the DEP-aligned fiber scaffold was favorable for vascularization. Overall, this study highlights the use of DEP beyond cell sorting and as a potential tool for customizing scaffold properties for applications in tendon tissue engineering.

In addition to sorting and selecting tenogenic cells and fabricating tissue engineering scaffolds, DEP has potential applications in characterizing the cells involved in musculoskeletal tissue injuries. In tendon, the ability to sort cells involved in the injury response may augment clinical treatments of tendinopathies. Tendons have limited healing capacity, and the inflammatory response involves distinct cell types. Embryonic tendon heals scarlessly,[99] and postnatal tendon has been shown to retain some regenerative capacity,[100] but this is lost in early postnatal stages. To examine the roles of distinct cell populations in scar formation, a recent study subjected postnatal day (P) 5 and 50 mice to an Achilles tendon transection. In the P5 mice, pools of scleraxis-positive cells infiltrated the wound and formed a "neo-tendon" that regained native mechanical function, as shown via the lack of scarring and the return to normal gait 28 days after the transection.[100] In the same study, P50 mice with Achilles transections did not have scleraxis-positive progenitors infiltrate the injury, and healing occurred with permanently altered gait and scarring.[100] Rotator cuff tendon injuries are another significant clinical challenge,[101] and like in the Achilles, scar formation in the rotator cuff is associated with distinct cell populations.[102] Specifically, minimal scleraxis or Sox9 expression was found in scar tissue following a rotator cuff tear,[103] whereas stem cell lineages were identified within the scar tissue.[103] These results in Achilles and rotator cuff tendons suggest that identifying, isolating, and enhancing scleraxis-expressing or regenerative cells in an injury site may improve regenerative healing, and highlight potential applications for high-throughput DEP sorting technologies.

Taken together, these studies highlight the need for DEP technologies to select for certain cell populations to enhance tendon healing and regeneration. These recently identified characteristics, including decreases in cadherin levels with tenogenic induction in MSCs,[97] nestin levels in TSPCs,[93] and scleraxis in an injury site,[100] offer potential cellular mechanisms that DEP might able to detect and should be explored in future studies. The ability to separate and enrich cells based on tenogenic markers is highly desirable for tendon tissue engineering applications, and DEP is a promising method for accomplishing this separation in a label-free way. Overall, DEP has the potential to separate cells based on minute variations in physical properties, while preserving cell viability, making it an appealing technique for generating homogeneous populations of stem cells for tissue engineering and regenerative applications.

B. Potential application of DEP in microgravity environments for stem cells

Extended human space flight, such as the duration required for manned missions to Mars, is currently prohibited by the limited understanding of the effects of prolonged exposure to microgravity (μg) on the body, including on stem cells. Cells cultured in microgravity freely float and interact with each other to develop 3D structures.[104] Microgravity is known to induce significant changes in stem cells.[105] Exposure of mouse embryonic stem cells to microgravity resulted in retention of cellular self-renewal markers and inhibited differentiation.[105,106] Different mechanical devices are used to simulate microgravity-like conditions artificially. Clinostat systems are the most widely used method that reduces the impact of gravity by constantly changing orientation.[107] While more research is needed, clinorotation results in flattening of hMSCs due to the changes in functional activity induced by microgravity.[104] Elucidating the effects of microgravity on living tissues and cells is crucial for understanding and mitigating the detrimental effects of microgravity on the human body. Continued space exploration depends on the development of effective ways to minimize the negative effects of microgravity on astronauts, as health problems such as bone loss, muscle atrophy, and cardiovascular and immune system changes are common following extended spaceflight.[107] Additionally, 3D stem cell culture techniques that mimic microgravity improve pluripotency, thus enhancing the use of stem cells for forming artificial organs by inducing and guiding differentiation.[107] Mammalian cells have also been cultured using high aspect ratio vessel (HARV) under μg conditions,[108] but there are several other types of equipment to simulate μg-like conditions, such as a random positioning machine (RPM).[109] Figure 6 depicts human fetal osteoblast cells (hFOB) [Figs. 6(a) and 6(b)] and h-MSCs [Figs. 6(c) and 6(d)]. hFOB cells grown on RPM and HARV (μg conditions) have resulted in the formation of spheroids from adherent cells. hMSCs were then cultured for 7 days under normal gravity and microgravity using RPM, which also resulted in the formation of 3D spheroids, as seen in Fig. 6(d). Cultured 3D spheroids more closely resemble tissue structures found in living organisms, and they are formed by partially negating the effect of gravitation field.[104] Generally, 3D cell culture techniques using stem cells are also known to maintain pluripotency, thus improving the differentiation potential and aiding in the formation of organoids for use in regenerative medicine.[107] Overall, 3D culture techniques have several advantages over 2D monolayer culture and could be combined with DEP to improve precision and homogeneity when differentiating multiple populations of stem cells.[104]

V. CONCLUSIONS

Following its initial discovery, DEP has rapidly evolved as an efficient, accurate, and label-free technique for characterizing and sorting

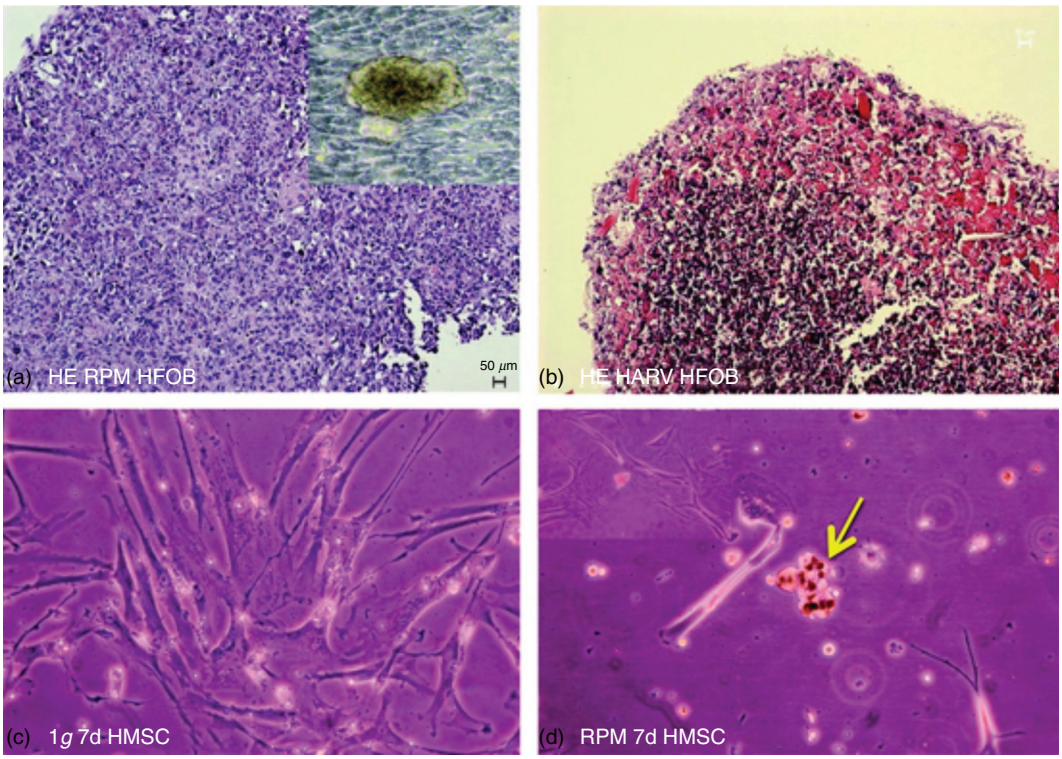

FIG. 6. Images describing the effects of cell culturing at different conditions using a high aspect ratio vessel (HARV) bioreactor and a random positioning machine (RPM). (a) Adherent cells and spheroids grown from human fetal osteoblasts (hFOB) cultured for 7 days on RPM. Spheroids are stained with hematoxylin-eosin (HE) (inset: phase-contrast microscopy–adherent cells and spheroid). (b) Similar 3D tissue grown from hFOB cells on HARV. (c) hMSCs–7 day culture under standard gravity conditions. (d) hMSCs–7 day culture grown on RPM (microgravity) resulting in spheroids (inset: adherent MSCs and spheroid). Reprinted with permission from D. Grimm *et al.*, "Tissue engineering under microgravity conditions–use of stem cells and specialized cells," Stem Cells Dev. **27**(12), 787–804 (2018).[107] Copyright 2018 Mary Ann Liebert, Inc.

cells. Although DEP has been used in the field of stem cell research for some time, there is renewing research interest in DEP for tissue engineering and regenerative medicine applications. Dielectric properties, which serve as biomarkers for label-free sorting and enrichment purposes, are still being studied and recorded for large-scale characterization of many different types of cells. DEP may be particularly useful for tendon research, as there are limited tendon-specific cell markers. While the accuracy and reproducibility of DEP cell characterization require further improvement, DEP has already enhanced research in the field of regenerative medicine. In the past decade, dielectrophoresis has been utilized for a wide variety of applications such as cell characterization, disease detection, cell sorting, and trapping. With the rapid expansion of microfluidics and the ease of manipulating bioparticles of various sizes ranging from nanometers to micrometers, DEP has shown promising results with increased sensitivity, specificity, and cell viability following exposure to high electric currents for limited time periods. Another decade may pass before DEP can be utilized in clinical practice, but DEP continues to evolve as an efficient, label-free, and low-cost technique that may revolutionize the application of stem cells in regenerative medicine.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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