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Isolation of Rare Cells through their Dielectrophoretic Signature

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Abstract

The isolation of rare cells using their dielectrophoretic signature is becoming a popular tool for medical diagnostics, individualized medicine, and anti-bioterrorism. This unique property varies between cell types due to differences in cell size and structure, including membrane morphology and composition.

Keywords: Dielectrophoresis; Biomicrofluidics; Contactless dielectrophoresis; Cancer early diagnosis; Rare cell isolation; Cancer stem cells; Circulating tumor cells; Bioelectrical; Bioelectrics

Introduction

There are various situations in which it is desirable to separate bioparticles, such as DNA, viruses, bacterial or mammalian cells from background [1]. Here, we focus on the isolation of rare mammalian cells, from cell mixtures or body fluids for cancer detection and individualized medicine using electrophoresis (DEP) [2]. Isolating rare cells is challenging, mostly due to their low number compared to background cells. For example, in screening for circulating tumor cells (CTCs) to detect cancer, there are only a few CTCs per mL of blood, which includes approximately a billion red blood cells and a million white blood cells. Specifically, it has been reported that there are less than 5 CTCs per 7.5 ml blood, detectable using immunomagnetic labeling in patients diagnosed with metastatic breast cancer [3]. Moreover, in some applications, rare cells are similar in size with background cells, which make rare cell isolation difficult.

Existing cell sorting approaches, such as fluorescence activated cell sorting (FACS) [4], magnetic activated cells sorting [5], and chemically functionalized pillar-based microchips [6], have shown promise as techniques that isolate rare cells, based on known receptors expressed on the surface of the membrane. For example, cancer stem cells (CSCs) are a rare population of cancer cells that have the ability to transplant a new tumor from an existing one. CSCs are thought to be responsible for the metastatic properties of tumors. In the case of pancreatic cancer, less than 1% of cancer cells expressed positive to specific biomarkers and showed significantly high tumorigenic potential, indicating they are CSCs [7]. Although a powerful technique, biomarker-based methods rely upon surface marker expression labeling, which is time-consuming, and requires special training to implement. The use of biomarkers also requires a prior knowledge, which is not yet available for all cell types. Moreover, irreversible binding of particles to the cell membrane can permanently disturb the functionality of the cell during isolation, making it difficult to do post-studies [8].

Dielectrophoresis (DEP) is an alternative, noninvasive technique that eliminates extensive sample preparation (no antibody labeling, one needs only to prepare a single sample) and provides a high selectivity for separating rare cells. DEP is the motion of a polarizable particle in a suspending medium due to the presence of a non-uniform electric field [9]. As opposed to other techniques that rely on information at the membrane surface, DEP can noninvasively sort populations through differences within the interior of cells, as well as their exterior. DEP has been used for the isolation of several rare cell types including separation of human breast cancer cells [10-16], human leukemia cells [17], cervical carcinoma cells [18], and malaria-infected cells [19] from blood, HL-60 cells from peripheral blood mononuclear cells [20], human breast cancer cells from normal T-lymphocytes and from CD34+ hematopoietic stem cells [21,22], enrichment of CD34+ stem cells from bone marrow and peripheral blood [23], and from peripheral blood stem cell harvests [24], separation of neuroblastoma cells from glioma cells [25], colorectal cancer cells from normal epithelial cells [26], osteoblast-like cells from a heterogeneous population [27], and putative stem cell population from adipose tissue derived cell suspension [28]. Cell isolation has been done through both batch separation [11-14,16-28], in which the rare cells population is trapped due to positive DEP force, while the background cells pass through the microdevice without trapping, and continuous separation [10], in which rare cells continuously separate from background cells.

In conventional DEP techniques, metallic microelectrodes with various geometries, such as interdigitated [11,13,14,20-22,24,28], castellated [12,17,23,29], oblique [10], spiral [19], circular [18,25], ring shape [27], and wedge shape [26], are patterned on a microfluidic device using conventional lithography techniques. Our group developed a DEP-based technology known as contactless dielectrophoresis (cDEP), which replaces metallic electrodes by fluidic electrode channels, filled with a high conductivity fluid [30,31]. Our approach with cDEP capitalizes on the sensitivity of traditional DEP, while eliminating challenges such as bubble formation, electrode delamination, expensive fabrication, and electrode-sample contamination. The absence of electrochemical contamination makes cDEP an ideal "isolate-and-culture" platform to investigate the biological processes of a target cell type, especially rare cells, in a sterilized environment. cDEP has been used to isolate prostate CSCs from normal cancer cells [32], cancer cells from blood cells [15,16], live cells from dead cells [33], and different stages of breast cancer cell lines [34].

In general, differences in dielectric properties of cells come from differences in their size, viability, membrane integrity and morphology,

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surface protrusions, fine surface features, cytoskeleton structure, and internal composition, such as nucleus to cytoplasm (N/C) ratio [35]. The cell membrane specifically acts like a dielectric between two conducting media. Similar to a capacitor, the specific capacitance of a cell depends on the cell membrane's surface. Smoother cells have lower specific membrane capacitance, C_{mem}, in contrast to cells which have complexities on their membrane such as protrusions, blebs, folding, and microvilli [36]. The C_{mem} is reduced if there is an increase in the membrane thickness or change in the intramembrane components, which affects the cell polarizability. Interfacial polarization happens due to the movement of positive and negative charges towards the cell sides facing the electrodes, depending on the location of electrodes, and causes forming aggregation of interfacial charges [37]. The interfacial polarization of a membrane surface can be affected by nucleus, endoplasmic reticulum, and mitochondria, as well as cell insulating bodies and structures, such as protein cytoskeleton and lipid membranes [35]. These characteristics have enabled researchers to isolate cells by exploiting differences in their membrane properties, such as membrane protrusions [38] and membrane conductivity [39], as well as the membrane skeleton [38,40].

Here, we present some of the important applications of DEP for enriching and isolating rare cells.

Cancer Cells from Blood Cells

Isolating cancer cells from blood cells is essential in early cancer detection and monitoring therapeutic outcomes [41]. There are several differences in surface morphology and size of cancer cells and blood cells, which make it possible to isolate them utilizing DEP [10-14,16-18]. For example, using a membrane-specific area parameter, φ, one can quantify subtle variances between cells due to differences in membrane morphology [13]. φ is the ratio of the actual membrane area to the membrane area that would be required to cover a smooth cell with the same radius, and can be used to quantify the amount of surface foldings and protrusions, as well as features such as microvilli, villi, ruffles, ridges, and blebs [13], on the membrane. A smooth cell has a membrane capacitance of 9 mF/m^2 [42]. For normal cells, such as T-lymphocytes, φ has been reported to be close to 1, however for cancerous cells having more surface protrusions, φ can be up to around 4 times higher [43]. DEP can also be combined with other cell sorting techniques, such as multi-orifice flow fractionation (MOFF) [10], to isolate cancer cells from blood cells, by taking advantage of higher throughput of MOFF and higher sensitivity of DEP [10].

Different Stages of Cancer Cells

It has also been demonstrated that cancerous and normal cells have different electrical properties. Oral squamous cell carcinomas have distinctly different electrical properties than more normal keratinocyte populations [44], and non-cancer-derived oral epithelial cells [45]. In another study, it was shown that normal, pre-cancerous, and cancerous oral keratinocytes have distinct electrical properties [46]. Additionally, transformed and non-transformed rat kidney cells [47], malignant human breast cancer epithelial cells and benign breast epithelial cells [34,48], and healthy and infected erythrocytes have been reported to have different electrical signature [49]. We demonstrated that different stages of breast cancer cell lines can be sorted based on cells dielectric properties [34]. We also showed that C_{mem} increases in mouse ovarian surface epithelial (MOSE) cells as the stage of malignancy advanced from 15.39 ± 1.54 mF/m^2 for a non-malignant benign stage to 26.42 ± 1.22 mF/m^2 for the late, aggressive stage [50]. These differences are the result of morphological differences due to changes in the membrane capacitance, associated with the complexity in the surface morphology, specifically, the density of complex features and cytoskeleton structure.

Stem Cells and Non-stem Cells

Stem cells are rare cell populations with the ability of self-renewal, as well as generating mature tissue cells through differentiation. Isolating, characterizing, and manipulating these cells have a wide range of applications, including regenerative medicine and cancer treatment. It has been shown that dielectric properties of stem cells are different from differentiated cells [51]. Using DEP, the progenitor cells were isolated from the stromal vascular fraction, which includes cell debris, erythrocytes, and nucleated cells, based on differences in their density, morphology, and size [52]. Moreover, it has been reported that the specific membrane capacitance of breast cancer cells and hematopoietic CD34+ stem cells are different, 23.0 ± 7.1 and 10.2 ± 1.5 mF/m^2, respectively, and these cells can be isolated accordingly, using dielectrophoretic field-flow-fractionation (DEP-FFF) [22].

Cancer Stem Cells and Normal Cancer Cells

CSCs can be considered as the most relevant cancer therapeutic target, and their isolation is the first step towards understanding their role in the pathogenesis and progression of cancer to improve specific cancer therapies [53]. Up until recently, no one has studied whether there are bioelectrical differences between CSCs and their counterpart. Our group showed that prostate CSCs have different dielectric properties in comparison to non-CSCs, which can be used for enrichment [32].

Stage of differentiation changes the cells protein cytoskeleton, which consequently changes the mechanical and electrical properties of cells at different stages of differentiation [35]. As an example, the differentiation of the erythroleukaemia cells changes the cell surface characteristics of the surface from having numerous microvilli and filopodia to appearing smooth and discoid, which are characteristics of mature cells [40]. Differentiation also increases the integrity of the spectrin in cytoskeleton and increases structural protein content of the membrane, causing a decrease in the membrane leakage and membrane fluidity [40].

Cells' organelles, e.g. endoplasmic reticulum, nucleus, and mitochondria, change during differentiation of cells and can affect their DEP properties. As an example, differentiation, transformation, and tumorigenicity of cells make a significant change in mitochondrial membrane potential [54]. Carcinoma cells have higher mitochondrial membrane potential than normal cells [55-57]. Based on their mitochondrial membrane potential, cells can be sorted as CSC>cancer cells>normal epithelial cells [54]. Moreover, N/C ratio decreases by cell differentiation and maturity [8], and changes the effective conductivity and permittivity of cells. For instance, limbal epithelial stem cells have a higher N/C ratio (=0.82 [58]) than peripheral corneal epithelial cells (=0.17 [59]), [8]. Human embryonic stem (hES) cells have typically higher N/C ratio than ordinary somatic cells, which affects their dielectric properties and consequently, can be used to separate these cells.

In summary, the differences in dielectric properties of cells which stem from dissimilarities in cells size, morphology, nucleus/cytoplasm ratio, nucleic acid content, surface charge, and charged cytoplasmic molecules, can be used not only to monitor changes of cells, but also to electrically isolate rare cells from biosamples. Utilizing DEP eliminates the need of labeling cells through their membrane proteins, which saves time and expense. Cells can be used after DEP isolation for

culturing and molecular analysis. This has crucial applications, such as early cancer detection, performance monitoring of cancer therapy, regenerative medicine, and cell characterization for individualized medicine.

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