The theory of dielectrophoresis and its applications on medical and materials research

Xiao Zhong1*, Yuan Sun2#, Chen Kang1, Gang Wan4

1 Molecular Design Institute, Department of Chemistry, New York University, NY 10003, USA
2 Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210, USA
3 Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA
4 Texas A&M University Libraries, College Station, TX 77845

Abstract: Dielectrophoresis (DEP) utilizes the polarizability of dielectric colloidal scale objects to drive their motions under a non-uniform AC electric field. It enables a non-invasive, reversible approach to manipulating colloidal particles. DEP requires particles to have the size within the range between hundreds of nanometers to tens of micrometers and a dielectric constant opposite to the liquid medium, which then could allow relatively sophisticated manipulation of a wide variety of colloidal scale objects, such as cells, microorganisms, polymeric colloids, and many more. Such unique ability has made DEP a great tool for applications in fields such as biomedical research and materials science. For example, DEP can be applied to sort cells based on their different dielectric constants and morphologies. It could also be applied for cell viability monitoring and stem cell fate prediction. Provoking colloidal crystallization is another good example of the DEP application. Unlike small molecules, the unique “colloidal scale” renders these particles visible by optical microscopy. As a result, the combination of DEP and optical microscopy has become a powerful tool for materials scientists to investigate the phenomenon of colloidal crystal nucleation and growth directly. In this review, recent works regarding DEP’s application on 2D colloidal crystallization will be discussed in detail. This review is intended to provide DEP’s critical functions on two different research fields and demonstrate interdisciplinary potential of DEP.

Key words: Dielectrophoresis; cell sorting; stem cell; lithography; colloidal crystallization.

Correspondence to: xz389@nyu.edu / # Authors contributed equally to this study

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Introduction

Dielectrophoresis (DEP) is a phenomenon that a force can be generated and applied to a dielectric particle when the particle is placed in a non-uniform electric field (1,2). The so-called dielectrophoretic force (DEP force) is originated from the electric polarization of the particles due to the dielectric nature. More specifically, when a dielectric particle is suspended in a liquid medium and a non-uniform AC field is applied, an electric field gradient will be generated which could align all the electric dipoles in each individual particle, making the whole particle as a big electric dipole (3,4). Unlike electrophoresis, DEP does not require the particles to carry any native charge. The only requirement for the particles is to be dielectric (5). The force exerting on individual particles can be described quantitively as shown in equation 1.

$$F_{DEP} = 2\pi r^3 \varepsilon_m \Re \left\{ \frac{x_p - x_m}{(x_p + 2x_m)^2} \right\} \nabla |E|$$

This equation assumes the particle is perfectly spherical, with a radius of r, complex dielectric constant of \(\varepsilon_p\), and is suspended in a liquid medium with a complex dielectric constant of \(\varepsilon_m\), under electric field of E. According to this equation, the sign \(\frac{x_p - x_m}{(x_p + 2x_m)^2}\) of the DEP force is depending on the sign of, which is famously named as the “Clausius- Mossotti factor”. The factor normally has a value within the range of -0.5 to 1.0. In the case when the number is larger than 0, the phenomenon is called positive DEP; particles would usually be pulled toward the electrodes, whereas in the case of negative factor, negative DEP force pushes particles to the central plane of a channel. As a result, such difference in movement could be utilized as the driving force for particle sorting as well as other biological applications (6-12). Also, due to the frequency-dependent nature of the dielectric constants, there exists a frequency that gives the Clausius-Mossotti factor zero. This frequency is named as the “crossover frequency” of a specific material. Practically, the difference of crossover frequencies is widely used as particle sorting mechanism. The following sections will give a discussion of several cases where DEP is used in biological studies. We will also cover a case where DEP is employed for provoking colloidal crystallization (13), which is a hot and new direction in DEP research field.

Cell sorting by DEP

The ability to sort different types of cells, i.e. cell sorting, has a broad range of applications in biological research and clinical operations (14-16). For example, during the bone marrow transplant, the cancer cells in bone marrows often require to be filtered out before the bone marrow can be used. Traditional methods for such need are usually based on the difference in cell density, specific interactions, etc, which are often inadequate and inefficient due to some inherent properties of the particles such as purity issue, sorting speed etc. Therefore, a fast, effective method for cell sorting is always in urgent need. Dielectrophoresis has enabled researchers to investigate...
this issue from a totally new perspective - the dielectric affinity of cells. Cells, in this case, can be viewed as micrometer-scale inhomogeneous particles. With different types of organelles in each cell as well as various morphologies, the dielectric affinities of different types of cells are expected to be distinctive, thus providing a possibility to sort cells with higher accuracy. For instance, Becker and coworkers sorted cancer cell out from blood cells based on their difference in dielectric affinity (17). Firstly, they used a single AC field generator to apply sinusoidal AC fields with 90-degree phase difference (Figure 1). Under this configuration, a rotating field could be generated at the very center of the electrodes, which would enable electrorotation of cells. Then, the frequency spectra for each cell could then be recorded and analyzed (Table 1).

Based on different dielectric parameters of the cells, the respective crossover frequencies could be calculated, which then can serve as guidance for sorting out cells. Figure 2 shows a typical experiment of sorting MDA 231 cancer cells out from blood cells. Parallel electrodes with concave configurations were applied and a mixture of cells was loaded to the channel. Calculated electric field with certain predetermined frequency was applied such that only MDA 231 cells could be trapped near the electrodes. To wash away the blood cells, a fluid flow was used and applied from left to right aiming for sweeping away the blood cells. Finally, MDA 231 cells were successfully separated from the blood cells. Such technique shows great promises on numerous biomedical applications that involve the use of cells.

Sorting and discrimination of stem cells by DEP

One of the emerging applications of DEP is on the research of stem cells. Human embryonic stem cells present great potentials on drug screening, disease modeling, and cell therapies for regenerative medicine. However, the broad ranges of their capacities as well as the heterogeneous cell population often make it difficult to study the precise in vitro behaviors. Thus, there exists a need to separate a certain type of stem cells from many others. DEP separation technique based on different crossover frequencies of cells has drawn a great amount of attention from research community recently. For example, Muratore and coworkers reported myoblast multipotent progenitor cells sorting by DEP crossover frequencies (18). As mesenchymal stem cell progenitors derived from muscle, myoblast cells are essential for regenerative medicine including cell transplantation. To obtain a homogenous population of such cells, DEP was adopted to sort out C2C12 myoblast cells from myotubes and GFP-expressing MRC-5 fibroblast cells with efficiency over 95%. As shown in Figure 3A, a “fishbone” quadruple configuration of electrodes was designed with each fishbone containing a top and bottom electrode. When a mixture of cells flow over the chamber, a certain AC field frequency would deflect one type of cells and allow them to exit from the central “funnel” (Figure 3 bottom). Experimentally determined crossover frequency values were compared and supported by modeling using MATLAB (Figure 4). The resulted sorting efficiencies were over 95%, suggesting excellent accuracy of these crossover frequencies. In conclusion, crossover-frequency-based DEP cell sorting has great potential on stem cells research and will greatly facilitate the study of regenerative medicine.

Manipulation of colloidal crystallization by DEP

Aside from decades of DEP studies on biomedical research, the last decade has witnessed some new application trends of DEP, one of which is the colloidal crystallization provoked by DEP force. In the field of crystalliza-

<table>
<thead>
<tr>
<th>Cell type</th>
<th>(C_{\text{specific}} \text{ mF/m}^2)</th>
<th>(\sigma_{\text{int}} \text{ S/m})</th>
<th>(\varepsilon_{\text{int}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA231</td>
<td>26±4.2</td>
<td>0.62±0.073</td>
<td>52±7.3</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>11±1.1</td>
<td>0.76±0.058</td>
<td>64±5.9</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>9±0.80</td>
<td>0.52±0.051</td>
<td>57±5.4</td>
</tr>
</tbody>
</table>

Table 1. Dielectric parameters for MDA 231 cells, T lymphocytes, and erythrocytes. Adapted from ref. 17 with permission. Copyright 1995 National Academy of Sciences of the United States of America.
tion, tremendous amount of research efforts have been devoted on the structural determination of molecular single crystals, which is crucial for the complete understanding of its properties and applications. The detailed structural information of protein and small organic molecules can be beneficial for applications such as drug design and development. However, because of the small size, molecular scale crystal structural determination and the study of crystallization mechanism often rely on indirect methods such as X-ray diffraction (XRD) and In-Situ Atomic Force Microscopy (In-Situ AFM) (19,20). Although amorphous molecular aggregates have been well characterized by light scattering and a recently developed holographic technique (21-24) directly analyzing crystalline structures remains a major challenge. Using colloids as mimics of molecules is a promising approach to understand the crystallization and engineering of metamaterials with desired properties by design. Since last decade, numerous research progresses on provoking colloidal crystallization using DEP force have been reported. Taking the advantage of the reversible control of DEP on colloids, 2D crystals could be induced and directly visualized by optical microscopy. One of such example is discussed by Collins and coworkers here in detail. A custom-built microfluidic chamber with two electrodes connected to an AC electric field was used to enable the movement of poly(methyl methacrylate) (PMMA) colloids. The contrast of dielectric constants between the medium-water (78.6) and particle-PMMA (2.8) allowed the particles to form 1D chains that were parallel to the electric field direction, followed by the formation of 2D crystals in the central region of the channel (negative DEP). Electric field strength was carefully chosen such that each particle could move smoothly near the bottom of the channel (Figure 5A). If the electric field was too strong, particles would float on top of the channel and 2D crystallization could not be easily achieved (Figure 5B).
To further investigate the crystallization behaviors, microscopic features fabricated by photolithography were introduced to the channel, which enabled the study of packing symmetry near physical confinements (Figure 5C and D). Since the features were made of photoresist that has similar dielectric constant with the colloids, they were acting as traps both physically and electrically. Such traps could capture 2D crystallization locally, even at high electric field strength. Interestingly, such traps enabled 2D crystallization with non-traditional symmetries. Figure 6A shows 2D hexagonally packed crystals trapped between two dumbbell-shaped features. Surprisingly, when the electric field strength was tuned to 750 V/cm, the packing symmetry switched to square packing and could form 2D hexagonal packing again once the electric field was turned back to 200 V/cm. Such 2D phase transition enabled by DEP was illustrated by models and the packing switch was tracked by Fast Fourier Transforms (FFT) using ImageJ (Figure 6C and D).

This work has demonstrated the potential of using DEP in the study of crystallization. The DEP field could be viewed as a reversible virtual confinement, while the traps were actual confinements. The development of novel lithographic techniques and colloids would potentially further advance the study of colloidal crystallization (25-34). Such study could not only shed light on the understanding of molecular crystallization, but also be applied as a sophisticated tool to building advanced structures.

**Summary and outlook**

To conclude, there have been numerous research efforts on using DEP in the biomedical field ever since theoretical studies of dielectrophoresis. This review is by no means aiming to provide a comprehensive overview of all the applications with DEP. For example, the use of travelling-wave DEP on bioparticle manipulation is not included for discussion here. Instead, we are trying to provide a glimpse on this blossoming field in this review. Looking forward, new directions on the biomedical field using DEP are emerging. For example, DEP could potentially be used as a drug discovery and delivery tool if drugs can be prepared to form nanoparticles (35-38). The drug particles could then be purified and monitored by electric field using their dielectric properties, and may even be released in a controllable fashion. A review by Pethig provides an excellent explanation and outlook in this topic (38).

In addition, a transition from biomedical application to crystallization studies is emphasized in this review. Taking the advantage of the “visible” size by optical microscopy, this In-Situ colloidal crystallization technique is a promising direction and worth future research efforts. With the development of novel lithographic techniques, more sophisticated colloidal structures are expected to be assembled and further facilitate other fields such as photonics and metamaterials.

![Figure 5](image1.png)

**Figure 5.** Optical micrographs of the working region of DEP cells containing 1.3 μm spherical colloids dispersed in water in the absence (A, B) and presence (C, D) of dielectric posts at electric field strengths of E = 200 and 750 V/cm. Adapted from ref. 12 with permission. Copyright 2015 American Chemical Society.

![Figure 6](image2.png)

**Figure 6.** (A, B) Optical micrographs and corresponding finite Fourier transforms of colloidal crystals confined within a trap at E = 200 and 750 V/cm, respectively. (C) Overlay of particle positions in cavities at E = 200 V/cm (red) and 750 V/cm (blue). (D) Intensities of the (11) reflection of hexagonally packed crystals (red) and the (10) reflection of square-packed crystals (blue) in the FFT images of a colloidal crystal confined in a trap (left y-axis) as the applied electric field strength is switched between 200 and 750 V/cm in 12.5 s intervals (right y-axis). Adapted from ref. 12 with permission. Copyright 2015 American Chemical Society.
References