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REVIEW ON THE DEP CELL MANIPULATION

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ABSTRACT
MEMS based microfluidic devices hold the promise of combining a small analytical laboratory onto a chip-sized substrate (lab-on-a-chip) to identify, immobilize, separate, and purify cells, bio-molecules, toxins, and other chemical and biological materials. Compared to conventional instruments, microfluidic devices would perform these tasks faster with higher sensitivity and efficiency, and greater affordability. Dielectrophoresis (DEP) is one of the enabling technologies for these devices. The device can be easily fabricated using the existing microelectrode photolithography techniques. Cell manipulation is achieved by controlling the applied frequency, to change the direction of the movement of the cells. The design of the electrodes, the choice of the suspending medium, and the applied peak voltage can be pre-determined to optimize the operation of the device. A comprehensive review on the DEP on microchips has been conducted in this paper, which successfully demonstrated the manipulation (separation, transportation, trapping, and sorting) of various biological particles.

Keywords: Cell manipulation, DEP, Microchip, Microelectrodes.

1. INTRODUCTION

The term dielectrophoresis (DEP) was first introduced by Pohl [1], which involves the creation of forces on polarizable particles to induce movement in nonuniform electric fields (usually AC electric fields). The magnitude and direction of the DEP forces will depend on several factors, including the frequency of the AC electric field, the conductivity and permittivity of both the cells and the medium where the cells are suspended, and the gradient of the electric field. The gradient of the electric field depends on the geometry of the device and the number of microelectrodes used. In addition, the high electric fields used in the DEP generate a large power density (Joule heating) in the suspending medium. Due to the high nonuniformity of the electric field (and thus the power density), a temperature gradient occurs, which results in gradients of conductivity and permittivity. The former produces free volume charge and the Coulomb force, while the latter creates dielectric force. These two forces cause the medium to flow (called electrothermal effect) and give rise to an effect of the DEP of bioparticles. DEP forces can be used to characterize, handle and/or manipulate microscale and nanoscale bioparticles. This can include sorting, trapping and separating cells, viruses, bacteria, DNA and the like. DEP-based devices offer several key benefits over traditional (inertial) methodologies, which render DEP particularly attractive for bioagent manipulation, including: low power consumption, high viability, high efficiency, high resolution separations, high degree of adaptability for varying threats, and applicability to viruses and toxins [2]-[5].

2. THEORY

When a dielectric particle is suspended in an electric field, it will polarize [1]. The basic dielectrophoretic effect is demonstrated in Fig. 1 and 2, in which electrodes are used to generate an inhomogeneous electric field. The dipole moment induced in the particle can be represented by the generation of equal and opposite charges (+q and –q) at the particle boundary. The magnitude of the induced charge q, is small, equivalent to around 0.1 % of the net surface charge normally carried by cells and microorganisms, and can be generated within about a microsecond [6]. As the electric field E is nonuniform so resulting force $E.\delta q$ on each side of the particle will be different. Thus, depending on the relative polarizability of the particle with respect to the surrounding medium, it will move either towards the inner electrode or towards the outer electrode. Since the direction of force is governed by the spatial variation in field strength, the particle will always move along the direction in which the electric field increases by the greatest amount. Depending on the characteristic spatial and temporal features of a nonuniform field, the particle may undergo conveyance namely translational motion or rotation, or both.

The DEP force F_{DEP}, acting on a spherical particle of radius r suspended in a fluid of absolute dielectric permittivity ε_m is well established in literature [7] and is given as follows:

$$F_{DEP} = 2\pi r^3 \varepsilon_m \alpha \nabla E^2 \qquad (1)$$

where, α is the effective polarizability of the particle which varies as a function of frequency of the applied field and on the dielectric properties of the particle and surrounding medium.
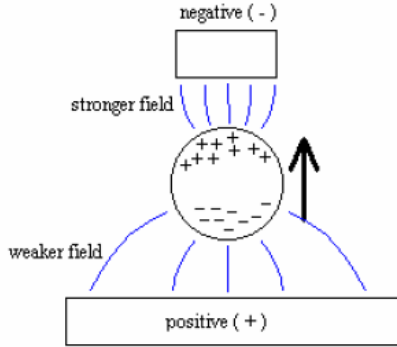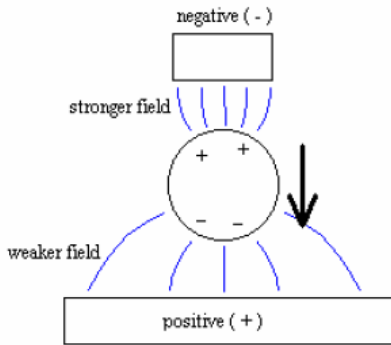

Fig 1. A schematic of p_{DEP} [8]

Fig 2. A schematic of n_{DEP} [8]

A positive α leads to an induced dipole moment aligned with the applied field and a negative α induces dipole moment aligned against the field. The magnitude of the induced dielectrophoretic force decays exponentially with distance from planar electrode surfaces [9-12].

3. DIFFERENT DEP METHODS

3.1 Traveling Wave Dielectrophoresis

In the TWD force acts in the direction parallel to the plane of electrodes are shown in Fig. 3. Particles moved by traveling waves can be directed large distances across the electrode array and diverted to different parts of the array for collection. Traveling electric fields are generated by interdigitized, parallel electrodes. Electrodes are energized with three or more periodic signals, usually sine or square-wave. The most common arrangement is to use four signals phased 0^0, 90^0, 180^0, 270^0, and then 0^0 again, and so on. The need to provide a number of different, independent signals presents problems when providing power to the electrodes, to which bus-bar arrangements above or below the electrodes are a common solution [13,14].
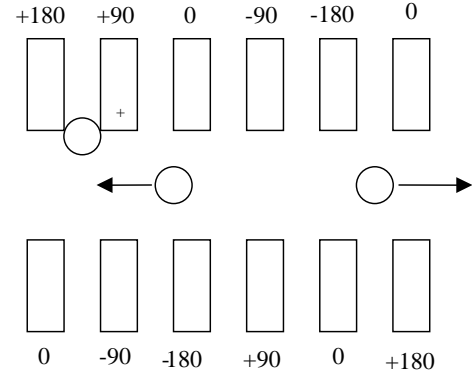

Fig 3. An electric wave travelling from left to right, resulting motion induced by cells and a cell trapped by p_{DEP}.

The application of traveling electric fields to the dielectrophoretic manipulation of particles was first demonstrated by Batchelder [15], who used phased electric fields to impart linear motion. Masuda and others [16, 17] first demonstrated that travelling electric fields could be beneficial to induce controlled translational motion of bioparticles including red blood cells and lycopodium particles, and suggested that such devices could be used to separate particles. However, they used low-frequency electric fields in their experiment. First demonstration of high-frequency (asynchronous) travelling fields was by Fuhr and co-workers [18] who used them to manipulate pollen and cellulose cells. Later work [19, 20] showed that by changing the frequency of the travelling field, it is possible to switch from conventional to the TWD to enhance separation of biocells. The traveling wave provides the effect of pumping the suspending solution, without the need to actually move the solution itself.

Goater and co-workers [21] fabricated a microelectrode device using photolithography and laser ablation, which combined the electrokinetic effects of the TWD and electrorotation. The device was used to concentrate and then determine the viability of Cryptosporidium parvum oocysts. In 2002, a device was built for trapping, concentrating and quantifying polystyrene microbeads [22], demonstrating the concentration capabilities on Saccharomyces cerevisiae. In this device, the electrodes were integrated into a passive sensor/actuator, which was plugged directly into a motherboard. Through the motherboard, a software tool running on the host PC allowed the control and the automation of the actuation and sensing operations. In addition, in 2002, a planar electrode geometry was introduced [23] that consisted of two AC fields exhibiting antiparallel field gradients and demonstrated its application in the manipulation and characterization of DNA molecules. Recently, a combined TWD and DEP levitation of tumour cells on a single PC-controlled printed circuit board (PCB) was developed and claimed to have fabricated the first fully integrated system without the need for external fluid control [24].

3.2 Dielectrophoretic – Field Flow Fractionation

The DEP-FFF is a cell separation technique that relies on an electric field perpendicular to the direction of flow and separation as shown in Fig. 4. The separations are performed in a low-viscosity liquid (typically an aqueous buffer solution), which is pumped through the separation channel. The FFF controls the relative velocity of particles by forcing particles towards the wall of the channel. Particles with high charge density pack closer to the wall and move more slowly compared to particles of lower charge density that form a more diffuse cloud and move more quickly through the channel [25]. Currently there are several constraints in order to separate cells by the FFF, these are: the process cannot handle a difference in molecular weight or diameter of the samples; a sample selective perturbation of the samples toward one wall; and a laminar velocity profile that results in a different average velocity of each constituent of the sample.
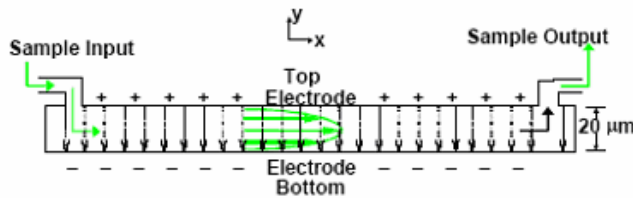

Fig 4. Schematic of the DEP-FFF process [25]

In the DEP-FFF, particles are separated according to a combination of their effective polarisability and density [26. 27]. In contrast to the other DEP separation methods, where cells remain on the same plane and are either eluted or remain trapped, the DEP-FFF exploits the velocity gradient in the flow profile to achieve highly selective separation. Recent examples of applications include the separation of latex particles [28, 29] and blood cells [30]. However, due to randomness, the particles travel at a Gaussian-shaped distribution, where two subpopulations often overlap. Thus, the separated subpopulation often contains residue of other subpopulations. This is an area, which needs improvement.

3.3 Electrorotation

If a polarizable particle is suspended in a rotating electric field, the induced dipole will form across the particle and should rotate in synchrony with the field. However, if the angular velocity of the field is sufficiently large, the time taken for the dipole to form (the relaxation time of the dipole) becomes significant and the dipole will lag behind the field. This results in a non-zero angle between field and dipole, which induces a torque in the body and causes it to rotate asynchronously with the field; the rotation can be with or against the direction of rotation of the field, depending on whether the lag is less or more than 180^0. Speed of rotation of a particle is not only directly proportional to the speed of the applied rotating field, but is also dependent upon the frequency at which a particular polarisable particle exhibits a maximum dielectrophoretic force upon it [31].
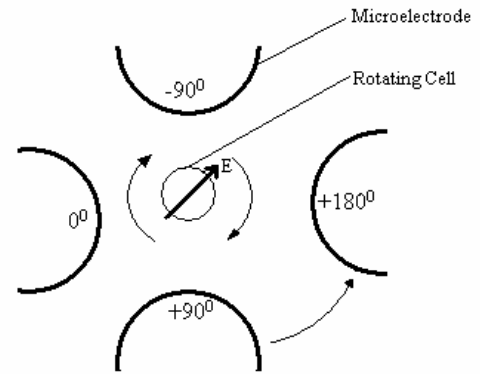

Fig 5. A schematic diagram of a polarizable particle suspended in a rotating electric field generated by four electrodes with 90^0 phase.

Subjected to an electric field, the induced polarization tries to align with the electric field intensity. A rotating electric field can be generated by means of constructing a phase-varying nonuniform electric field, as first described in 1982 in the pioneering work of Arnold and Mischel [32, 33] as shown in Fig. 5.

The electric field vectors and magnitude distribution at the center of the spiral electrode array is shown in Fig. 6 in the electrorotation (EROT) process. This figure is in the instant when electrodes 1, 2, 3 and 4 are energized with 1V rms sinusoidal voltages of phases $30^0, 120^0, 210^0$ and 300^0, respectively. The field strength in the two darkly shaded regions is less than 2.5 kV/m and that within the region of lighter shading has a value in the range 2.5 to 5.0 kV/m. The field strength within 1 μm of the electrode edges exceeds 100 kV/m [34].

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The theoretical and experimental aspects of the EROT have been developed in several laboratories and it has been revealed to be a sensitive method for manipulating cells. Andrew and co-workers [34] describe a lab-on-a-chip based microelectrode device, fabricated using photolithography and laser ablation, which uses electrorotation to concentrate and then separate cells. The microelectrodes consisted of a seed layer of chromium coated with a 0.1 μm layer of gold, deposited by evaporation onto a glass substrate, and were structured using standard printing, photoreduction and etching techniques. They reported a requirement for reliable EROT measurements is that the rotating electric field should be as homogeneous as possible but did not investigate any change in field homogeneity by change in electrode geometry.
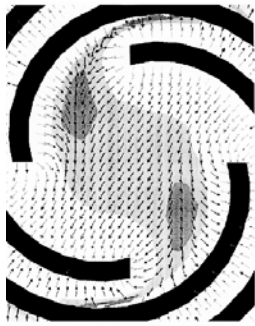
Fig 6. Electric flied in electrorotation [34]

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By using optical techniques to observe the speed of rotation of particles within the EROT chamber, together with particle size measurement and knowledge of the applied electric field strength, it is possible to characterize the particles. Techniques including electrorotational light scattering [36, 37], and impedance measurement when the particles are in the non scale diameter [38].

4. IMPLEMENTATION OF THE DEP

4.1 Particle separation

Differences in the dielectric properties of cells manifest themselves as variations in the dielectrophoretic force magnitude or direction, resulting in separation of cells. Since then a broad range of cell separation techniques have been demonstrated including yeast, cancer cells and bacteria [39, 40], the separation of a mixture of Herpes Simplex and Tobacco Mosaic viruses into two distinct populations, the separation of latex spheres of different sizes, or of similar size but different surface treatments [9, 41] the separation of 93 nm diameter latex spheres according to small variations in surface charge [42], separation of blood cells from bacteria [43].

As shown in Figure 7, working in the region where the Clausius-Mossotti function (Re[K(w)]) is 0 for live cells and 1 for dead cells it is possible to manipulate the cells. In this case, the dead cells will experience a positive DEP, whereas the live cells will not ex-perience any force and consequent separtion of bio cells.
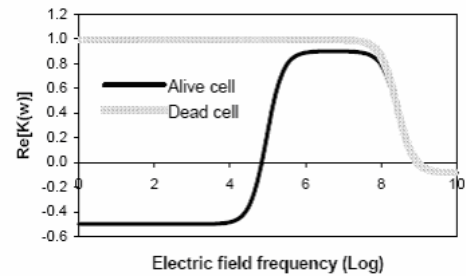

Fig 7. The Clausius-Mossotti function for dead and alive cells [8]

The major disadvantage is that the particles are localized at the electrodes after separation, and flushing needs to be performed to collect the separated particles. Tunable electrostatic filter achieves molecular sorting more efficiently [8].
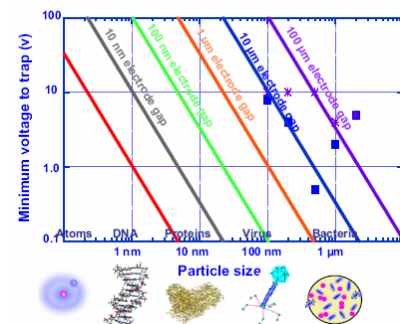

Fig 8. The figure shows how the minimum size of trapped particles (latex beads in this experiment) varies with the applied bias [55]

Currently electrodes are fabricated on standard glass microscope slides [54]. Generally the electrode arrays were patterned using standard photolithography and wet etching techniques. Fig. 8 shows the optimum electrode gap for different size of cells.

4.2 Particle Transport

The DEP can be used to transport cells. Cells are moved in a traveling electric field energized with a four-phase signal [44]. There is no need to pump liquid along the device in order to produce horizontal motion [45,47]. With the present technology cells can be moved only in two-dimensional direction as shown in Fig. 9 [46].\If careful manipulations of the amplitudes of the potentials on the electrode are possible, it will 'steer' the motion of particles across the array.
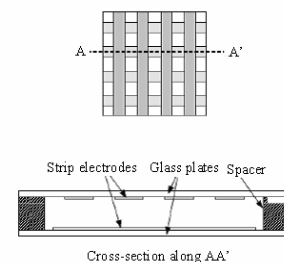

Fig 9. Cell transportation in a grid electrode device using the DEP [46].
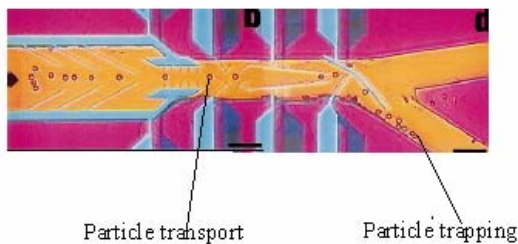
4.3 Particle Trapping

Fig 10. Particle sorting and trapping system [48]

Another important application of the DEP is the non-contact trapping of single cells as shown in Fig. 10. There are different methods such as quadrupole microelectrode structures [31, 49, 50]. It was later proved that the minimum radius is proportional to 1/3 of the trap width and the gradient of the electrical field [31]. In the quadrupole microelectrode structures an open top and gravity is responsible for the downward force holding the cell on the surface. Cells with near neutral buoyancy are less likely to be held in the trap by gravity. A closed trap can be made using two polynomial electrodes placed one above the other, to produce an octopole [51]-[53]. Still to trap only one single cell is a challenging task. It is also important to note that particle trapping is not suitable when the cells are experiencing the pDEP. The DEP force will pull the cells away from the center and immobilized it at the electrodes.

5. FUTURE OPPORTUNITIES

The development of microtools for effective sample handling and separation in micro and nano volumes remains a challenge. The most significant issues still needs to be resolved are optimize design, microfluidic control, interfacing. The next phase of the research in DEP would most likely be focused on the integration of these individual manipulation techniques to form a complete MEMS based lab-on-a-chip, where DEP can be used to transport and separate cells. To authors' knowledge, the techniques demonstrated so far used cells having identical physical sizes. There is still no study to prove their capability in treating cells having different order of sizes (e.g. blood sample).

The fundamental challenge in the advancement of nanotechnology is the development of precision tools for the manipulation of macromolecules in solution phase. There exist a number of possibility down which AC DEP can be developed to meet the needs of nanotechnology more readily. These include the design of electrodes for trapping of smaller single cell, increasing the precision to which cells may be trapped, and examining the extent to which cells may be maneuvered within the trap by alteration of the electric field.

Review of literature indicates that there is no previous work conducted on the optimization of the geometry and position of microelectrodes in cell manipulation technique on lab-on-a-chip devices, which may significantly enhance the effectiveness of cell manipulation and consequently will open a new opportunity for future research.

6. CONCLUSIONS

1. MEMS based DEP manipulation technique could assure fast and low cost biological diagnostics. Compare to the conventional laboratory analysis methods, very small quantities of test sample and reagent could be used in MEMS based Lab-on-a-chip.
2. Proven manufacturing technologies enable the fabrication of microelectrode arrays on insulating substrates, allowing for the development of cost effective, mass-produced, sterile, disposable diagnostic devices.
3. Lack of moving parts in the microscale DEP process assured a promising technology for cell manipulation.
4. The considerable issues in the microscale DEP process are the high voltage requirement, the direct electrical-to-fluid contact, the ionic strength, and to the pH of the solution. Consequently, it is difficult to make it into a generic impetus method. For example, liquids with high ionic strength suffer from excessive Joule heating and it is therefore difficult or impossible to pump biological samples such as blood and urine.
5. One potential problem with the pDEP is that dielectric particle can cause damage, as they are attracted to the electrodes. Less stress is induced, especially in living cells, when the particles move toward field minima (negative DEP). Miniaturization allows such field minima to be created on a scale suitable for handling single, small objects, such as viruses and nanometer particles.

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