BIOPARTICLE MANIPULATION WITH INSULATOR-BASED DIELECTROPHORESIS

Blanca H. Lapizco-Encinas, Tecnológico de Monterrey, Monterrey, NL, Mexico

Abstract

Dielectrophoresis (DEP) is the motion of particles in due to polarization effects in nonuniform electric fields; this electrokinetic transport mechanism has a great potential for the manipulation of a wide array of bioparticles, ranging from biomolecules to microorganisms. Traditionally, DEP has been carried out employing arrays of microelectrodes, however, employing microelectrodes has some drawbacks such as high cost and loss of functionality due to fouling. Insulator-based dielectrophoresis (iDEP) is an attractive alternative, since it employs arrays of insulating structures, instead of electrodes, to create nonuniform electric fields; resulting in inexpensive and robust devices. This study presents the application of iDEP for the manipulation and concentration of different types of bioparticles (from proteins to microalgae). Glass and polymeric microdevices containing channels with cylindrical insulating posts were employed to trap and concentrate bioparticles. A sample of bioparticles was introduced into the microchannel, then; a direct current (DC) electric field was applied across the post array, creating regions of higher and lower electric field intensity, *i.e*. dielectrophoretic traps. Electroosmotic flow was generated to pump the suspending liquid through the microchannel, while the bioparticles were immobilized and concentrated at the dielectrophoretic traps between the cylindrical posts. The dielectrophoretic response of the bioparticles was recorded in the form of videos and pictures. Experiments were conducted varying the electric field, pH and conductivity of the suspending medium, in order to study the effect of these parameters on the dielectrophoretic response of the bioparticles. Successful trapping of the different types of bioparticles was achieved, demonstrating the great potential of iDEP as a technique for bioparticle manipulation.

Introduction

Miniaturization has brought important advantages to bioseparation technology. Numerous fields, including environmental, clinical, pharmaceutical, etc., have beneficiated from the advances of microanalytical systems. There is a growing interest on the development of separation techniques that can applied in microscale. There is a growing interest on the development of separation techniques that can applied in microscale. During the last decade there has been a significant development of microfluidic devices for analytical applications, quality and performance improvements are making it possible for microanalytical systems to become commons aids to numerous different applications.[1] Dielectrophoresis (DEP) an efficient technique with great potential for miniaturization. It has been applied successfully for the manipulation and concentration of a wide array of particles, including bioparticles such as macromolecules and microorganisms. DEP is an electrokinetic transport mechanism produced by polarization effects when particles are exposed to a nonuniform electric field. This novel technique has the potential for achieving concentration and separation on a single step. The majority of the research studies on DEP have used arrays of microelectrodes and AC electric fields to create no uniform electric fields. Electrode-based DEP allows obtaining high electric field gradients employing low applied voltages. However, there are some drawbacks with this approach: high cost of electrode construction, complex fabrication processes and decrease of functionality due to fouling effects, which is a common effect when handling biological samples.

There is an alternative manner of carrying out DEP, insulator-based DEP (iDEP) is a technique where the voltage is applied employing only two electrodes that straddle an insulating structures array. When an electric field is applied across an insulating structures array, the presence of the structures creates regions of higher and lower field strength, resulting in the nonuniform electric field necessary for DEP to occur.[2] Furthermore, if DC electric fields are utilized, and the microdevice substrate is suitable for electroosmosis, then electroosmotic can be employed to pump liquid through the microfluidic system, eliminating the need of a micropump. Additionally, iDEP systems do not lose their functionally despite of fouling effects, which makes them more suitable for biological applications. Moreover, iDEP systems can be fabricated from a wide variety of materials, including plastics, leading to inexpensive systems, increasing its potential for high throughput applications.

Dielectrophoresis, first discovered by Pohl in 1951,[3] is the movement of particles induced by polarization effects in nonuniform electric fields. In DEP the motion of particles is caused due to the unbalanced force of a nonuniform electric field on the induced dipole moment of the particles: one "side" of the dipole is in a weaker field than the other, causing the particle to be pulled electrostatically along the electric field gradient, generating a net movement of the particles.[4] The dielectrophoretic force acting on a spherical particle can be represented as:

$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left(f_{CM}\right) \nabla E^2 \qquad (1)$$

where ε_m is the permittivity of the suspending medium, r is the radius of the particle, E is the local electric field, and Re(f_{CM}) is the real part of the Clausius-Mossotti (CM) factor:

$$f_{CM} = \left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right] \qquad (2)$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and the medium, respectively. The complex permittivity is related to the real permittivity by $\varepsilon^* = \varepsilon - (j\sigma/\omega)$ where σ and ω are the conductivity and angular frequency of the applied electric field, respectively, and $j = \sqrt{-1}$. Depending on the relative magnitude of ε_p^* and ε_m^*, the dielectrophoretic force acting on a particle can cause it to move toward or away from the high field region, these two effects are called positive and negative DEP behavior, respectively.[3,5] In many practical systems, at frequencies below 100 kHz, the CM factor can be approximated in terms of the real conductivities.[6]

$$f_{CM} = \left[\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m}\right] \qquad (3)$$

DEP is of second order in the applied electric field; at low applied electric fields, low electric fields gradients are produced, which leads to negligible dielectrophoretic force; therefore, the applied electric field has to be high enough for DEP to become significant. The two regimes of iDEP are *streaming* and *trapping* DEP. Streaming DEP occurs when the dielectrophoretic force overcomes diffusion but does not overcome the electrokinetic flow. Trapping DEP is the reversible immobilization of particles in dielectrophoretic traps and it occurs when the DEP force overcomes both diffusion and electrokinetic flow (as well as

pressure-driven flow if present).[2,7,8] The electrokinetic flow, which is proportional to the electric field, comprises the effects of electroosmosis and electrophoresis on particle motion relative to a fixed channel. Electroosmotic flow is the movement of liquid when an electric field is applied in a microchannel or a capillary. For electroosmosis to occur it is necessary that the channel or capillary walls posses a net charge in the presence of an electrolyte.

This study presents the application of iDEP for the manipulation and concentration of different types of bioparticles (from proteins to microalgae). Glass and polymeric microdevices containing channels with cylindrical insulating posts were employed to trap and concentrate bioparticles. A sample of bioparticles was introduced into the microchannel, then; a direct current (DC) electric field was applied across the post array, creating regions of higher and lower electric field intensity, *i.e*. dielectrophoretic traps. Electroosmotic flow was generated to pump the suspending liquid through the microchannel, while the bioparticles were immobilized and concentrated at the dielectrophoretic traps between the cylindrical posts. The dielectrophoretic response of the bioparticles was recorded in the form of videos and pictures. Experiments were conducted varying the electric field, pH and conductivity of the suspending medium, in order to study the effect of these parameters on the dielectrophoretic response of the bioparticles. Successful trapping of the different types of bioparticles was achieved, demonstrating the great potential of iDEP as a technique for bioparticle manipulation.

Materials and Methods

A schematic representation of the experimental setup is shown in Figure 1. Experiments were conducted employing different microchannels made from glass and plastic, all microchannels had a similar layout. Each microchannel had an inlet and outlet reservoir and contained an array of cylindrical insulating posts, the post diameter and arrangement varied from channel to channel. In order to prevent particles from crashing against the posts and plugging the system, “dove-tail” geometry was used for the first row of posts on both sides. A 3000 V high voltage sequencer, model HVS448 (LabSmith, Livermore CA) was used to apply electric fields by employing platinum-wire electrodes with a diameter of 0.3048 mm. An inverted epifluorescence video microscope for microfluidics, model SVM340 (LabSmith, Livermore CA) was employed to record the dielectrophoretic response of the bioparticles in the form or videos and pictures. A 4X microscope objective was used for all experiments. Both, the high voltage sequencer and the microfluidics microscope require the use of a personal computer for their operation.

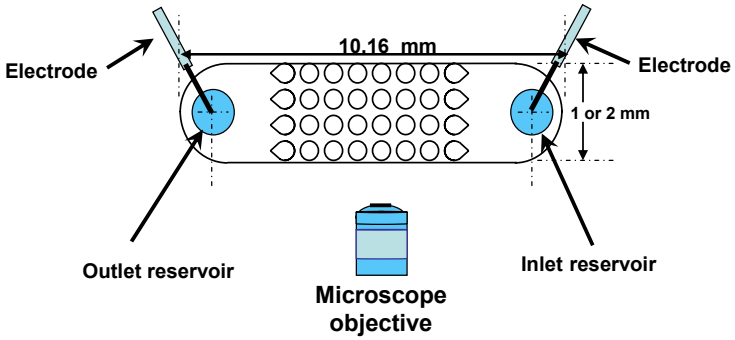


Figure 1. Experimental set-up for iDEP experiments.

Different suspending mediums were prepared, in order to study how the properties of the suspending medium affect the dielectrophoretic response of the bioparticles. The

suspending mediums were prepared with the DI water solution with adjusted pH and conductivity. The pH of this solution was adjusted to a value of between 6 and 9 by adding NaOH. The conductivity of this solution was adjusted to a value between 25 and 115 μS/cm by adding K_2HPO_4

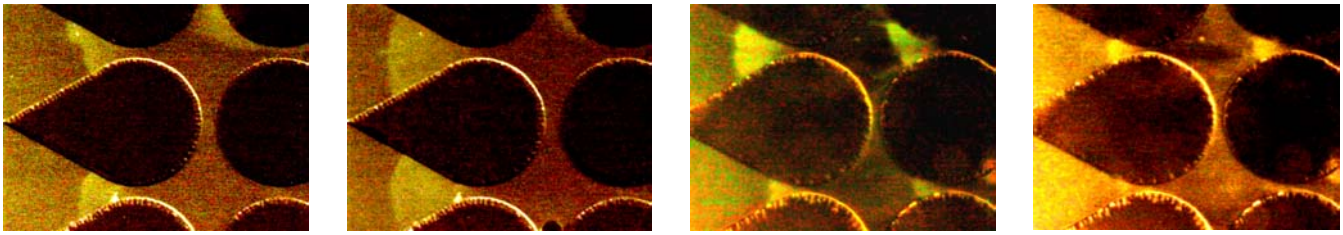
A variety of bioparticles were employed for the iDEP experiments: bovine serum albumin (BSA) particles (MW 66 kDa, Sigma, Toluca, Mexico) were dyed with fluorescein isothiocyanate, λex = 492 nm, λem =518 (Sigma, Toluca, Mexico) following a standard protocol provided by the dye manufacturer. Microalgae *Chlorella kessleri (UTEX#*: 397*)* having a diameter of 5 μm was utilized for iDEP experimentation, these particles were dyed with the DNA-intercalator fluorescente dye syto 11, λex = 508 nm, λem =527 (Invitrogen, Carlsbad, CA, USA). *Saccharomyces cerevisiae* (ATCC: 24858) yeast cells having an approximate diameter of 5 μm were also concentrated with iDEP, yeast cells were visualized without the aid of a fluorescent dye. Finally, live and dead suspension-adapted chinese hamster ovary (CHO-S, Invitrogen, Carlsbad, CA, USA) cells were manipulated with iDEP. These cells have an approximate diameter of 20 μm, and were prepared following standard cell growth protocols. In order to differentiate live form dead cells; live cells were not labeled and dead cells were labeled with trypan blue (Sigma, Toluca, Mexico).

Different types microdevices were employed, glass microdevice made from Schott D263 glass were employed with the protein BSA and microalgae, these devices were manufactured following standard wet-etching techniques. Glass microchannels were 10.12 mm long, and 10-□m deep. Plastic microdevices made form a plastic called Zeonor by employing standard photolithography and replication processes including hot embossing and injection molding, were kindly donated by Sandia National Laboratories, these plastic devices were used with yeast and CHO cells. Plastic microchannels were 10.12-mm long and 75-μm deep.

Results and Discussion

The main objective of the present manuscript is to describe the results that our research work has achieved with iDEP, in order to demonstrate some of the potential applications of this novel technique. One field that can be beneficiated by iDEP is downstream processing of valuable biomolecules, such as proteins. Insulator-based DEP was tested as technique for the concentration and manipulation of proteins, Figure 2 shows the results obtained with BSA particles. The dielectrophoretic trapping of protein particles is strongly influenced by the magnitude of the applied DC electric field. Figure 2a shows the dielectrophoretic response exhibited by protein particles when the applied was 900V/cm, by increasing the field to 1200 V/cm the immobilization of BSA particles increases significantly (Figure 2b). These results are in agreement with Eqn. (1), since the magnitude of the dielectrophoretic force is function of the magnitude of the applied electric field. Additionally, the effect of the suspending medium conductivity can be observed by comparing Figures 2a and 2c. From the figures it can be seen that by increasing the conductivity of the suspending medium to 100 μS/cm (employing a pH of 8 and almost the same electric field), protein particles show stronger dielectrophoretic trapping, since protein bands appear brighter. The conductivity of BSA particles has been reported as a maximum value of 25 μS/cm[9], *i.e.* the CM factor of the protein particles is negative under the operating conditions employed here (σ_m from 25 to 100 μS/cm). These results show that increasing the suspending medium

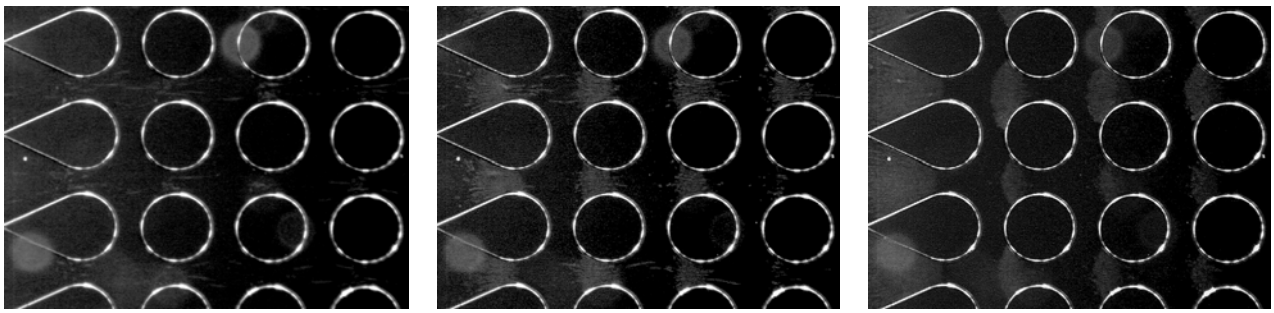
conductivity increases the magnitude of the negative CM factor increases (Eqn. 3), leading to an increase in the dielectrophoretic force. The suspending medium pH also affects the dielectrophoretic response of protein particles. By comparing figures 2a and 2d one can see that increasing the ph of the suspended medium produces less dielectrophoretic trapping, this is due to an increase in electroosmotic flow, which makes it difficult for dielectrophoresis to dominate the system.



(a) E= 900 V/cm pH=8, σ_m=25 µS/cm | (b) E=1200 V/cm pH=8, σ_m=25 µS/cm | (c) E=950 V/cm pH=8, σ_m=100 µS/cm | (d) E=900 V/cm pH=9, σ_m=25 µS/cm

Figure 2. Dielectrophoretic response of protein of particles (shown green-yellow) inside a glass microchannel with cylindrical insulating structures, flow direction is from left to right, post diameter is 440 µm, arranged 520-µm center-to-center, and microchannel was 2-mm wide.

Another potential application of iDEP is for the concentration of microorganisms, such as microalgae. A sample of water containing a dilute concentration of these algae cells could be processed with an iDEP device, in order to concentrate the microalgae for further laboratory tests. Standard laboratory techniques for microalgae concentration are extremely time consuming. A technique that can concentrate microalgae rapidly has great potential applications. Figure 3 shows the results obtained with microalgae cells, these are relative large particles (5 µm in diameter) compared to BSA particles (5 nm in diameter)[9]. From these results we can observe that iDEP devices have to potential to be used as pre-concentrators of microorganisms.



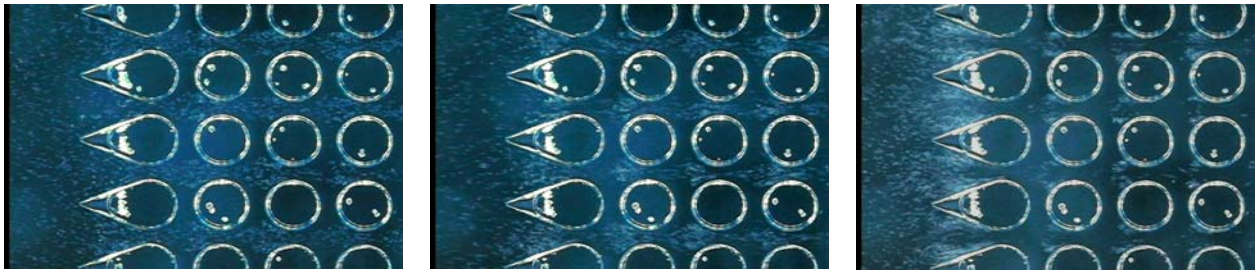
(a) E= 350V/cm pH=6, σ=100 µS/cm | (b) E=800 V/cm pH=6, σ=100 µS/cm | (c) E=1000 V/cm pH=6, σ=100 µS/cm

Figure 3. Dielectrophoretic response of microalgace cells (shown light gray color) inside a glass microchannel with cylindrical insulating structures, flow direction is from left to right, post diameter is 200 µm, arranged 250-µm center-to-center, and microchannel was 1-mm wide.

Figures 3a shows microalgae cells flowing under electrokinetic flow, when a field of 350 V/cm is applied, the dielectrophoretic force is not strong enough to overcome electrokinetics, and thus, microalgae cells are not trapped. By increasing the field to 800 and 1000 V/cm, Figures 3b and 3c, respectively, microalgae cells are dielectrophoretically trapped. Microalgae cells are

exhibiting negative dielectrophoresis, which is caused by the presence of the cell membrane.[10] This means that the cells are repelled form the regions with higher electric field gradient. Comparing Figures 3b and 3c, one can see that when the applied electric field is increased, the band of trapped microalgae is repulsed in a stronger manner from the region of higher electric field gradient. These results are in agreement with Eqn. (1).

In order to extend our work to other types of microorganisms, the potential of iDEP for the concentration of yeast cells was explored. Yeast cells having an approximate diameter of 5 mm were also manipulated with iDEP, but this time, a plastic microdevice was employed. The objective here was to test the functionality of polymer-based devices for iDEP applications. Polymers are inexpensive materials that if used with iDEP open the possibility of this type of particle concentrators for high throughput applications. Figure 4 shows the results obtained with yeast cells.



(a) E= 200 V/cm pH=6, σ=115 μS/cm

(b) E=400 V/cm pH=6, σ=115 μS/cm

(c) E=600 V/cm pH=6, σ=115 μS/cm

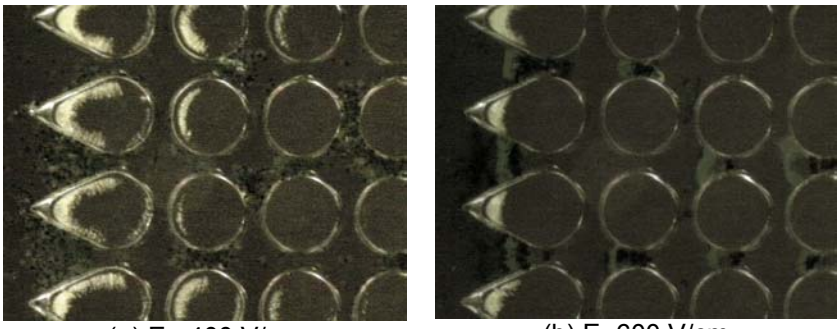
Figure 4. Dielectrophoretic response of yeas cells (shown white) inside a plastic microchannel with cylindrical insulating structures, flow direction is from left to right, post diameter is 150 μm, arranged 200-μm center-to-center, and microchannel was 1-mm wide

Figure 4a, shows that applying a field of 200 V/cm does not produce dielectrophoretic trapping, increasing the applied field to 400 and 600 V/cm (Figures 4b and 4c, respectevely) produces negative dielectrophoretic immobilization of the yeast cells, where the magnitude of the trapping is a function of the magnitude of the applied field.

Finally, the technique of iDEP was tested for applications in the production of biopharmaceuticals. CHO cells are one of the most employed cells lines in the biopharmaceutical field, where significant effort is devoted to cell growth protocols. The objective was to explore the potential of iDEP as a cell screening method for the separation of live and dead CHO cells. The separation between live and dead cells can be used to optimize cell growth protocols by inoculating only with live cells.

Live and dead CHO cells were manipulated with iDEP, live cells were not dyed, and dead cells were distinguished with trypan blue. Figure 5a shows that applying a field of 400 V/cm does not produce a differentiation between live and dead cells. Increasing the applied field to 600 V/cm (Figure 5b) produces a distinction between live and dead cells. The ongoing goal of this set of experiments is to be able to selectively concentrate the live CHO cells. The results in Figure 5b demonstrate that live and dead cells exhibit different dielectrophoretic behavior, which could be exploited to achieve the desired separation. The differences in

dielectrophoretic behavior should be due to changes in cells membrane properties when the cell dies.[10]



(a) E= 400 V/cm
pH=8, σ=25 μS/cm

(b) E=600 V/cm
pH=8, σ=25 μS/cm

Figure 5. Dielectrophoretic response of live (light gray) and dead (black) CHO cells, inside a plastic microchannel with cylindrical insulating structures, flow direction is from left to right, post diameter is 200 μm, arranged 250-μm center-to-center, and microchannel was 1-mm wide

Conclusions

Manipulation of four different types of bioparticles (BSA protein particles, microalgae, yeast and Chinese hamster ovary cells) employing the technique of insulator-based dielectrophoresis (iDEP) was presented. It was shown that this novel technique has great potential for a wide array of applications, from the concentration of valuable macromolecules to the manipulation and screening of microorganisms. All bioparticles exhibited negative dielectrophoretic behavior when they were immobilized inside a microchannel containing an array of cylindrical insulating structures. Microdevices made from glass and plastic were employed, demonstrating that iDEP can be carried out employing different materials. Additionally, the results showed the effect that operating conditions have over the dielectrophoretic response of the bioparticles. Successful trapping of the different types of bioparticles was achieved, demonstrating the great potential of iDEP as a technique for bioparticle manipulation.

Acknowledgements

The author would like to acknowledge the financial support provided by CONACYT grant No. CONACYT-CB-2006-53603 and *Cátedras de Investigación* (CAT161 and CAT162) of *Tecnológico de Monterrey*. The author is thankful to Sandra Ozuna, Israel Martínez, Roberto Gallo and Héctor Moncada for carrying out the experimental work. The author is thankful to Blake Simmons and Sandia National Laboratories for the kind donation of plastic microdevices.

References

1 Dittrich P.S., Tachikawa K., Manz A., (2006), "Micro Total Analysis Systems. Latest Advancements and Trends," *Anal. Chem.* 78(12), pp.3887-3907.

2 Cummings E.B., Singh A.K., (2003), "Dielectrophoresis in Microchips Containing Arrays of Insulating Posts: Theoretical and Experimental Results," *Anal. Chem.* 75(18), pp.4724-4731.

3 Pohl H.A., (1951), "The Motion and Precipitation of Suspensoids in Divergent Electric Fields," *J. Appl. Phys.* 22(7), pp.869-871.

4 Müller T., Fiedler S., Schnelle T., Ludwig K., Jung H., Fuhr G., (1996), "High frequency electric fields for trapping of viruses," *Biotechnol. Tech.* 10(4), pp.221-226.
5 Jones T.B., (1995), "Electromechanics of Particles," Cambridge University Press, USA.
6 Markx G.H., Dyda P.A., Pethig R., (1996), "Dielectrophoretic separation of bacteria using a conductivity gradient," *J. Biotechnol.* 51(2), pp.175-180.
7 Cummings E.B., Singh A.K., "Dielectrophoretic trapping without embedded electrodes," (2000), in Mastrangelo CH, Becker H (Editors), Proceedings of SPIE: Conference on Microfluidic Devices and Systems III, Santa Clara, CA, vol. 4177, pp. 164-173.
8 Cummings E.B., "A Comparison of Theoretical and Experimental Electrokinetic and Dielectrophoretic Flow Fields," (2002), in 32nd AIAA Fluid Dynamics Conference and Exhibit 2002, American Institute of Aeronautics and Astronautics, St. Louis, Missouri, vol. paper 2002-3193,, pp. 1-17.
9 Zheng L.F., Brody J.P., Burke P.J., (2004), "Electronic manipulation of DNA, proteins, and nanoparticles for potential circuit assembly," *Biosens. Bioelectron.* 20(3), pp.606-619.
10 Lapizco-Encinas B.H., Simmons B.A., Cummings E.B., Fintschenko Y., (2004), "Insulator-Based Dielectrophoresis for the Selective Concentration and Separation of Live Bacteria in Water," *Electrophoresis* 25, pp.1695-1704.