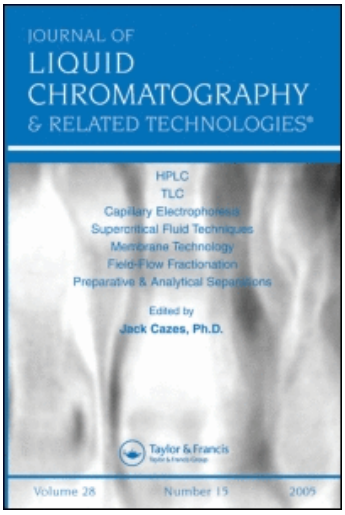

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DEP-FFF: Field-Flow Fractionation Using Non-Uniform Electric Fields

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DEP-FFF: FIELD-FLOW FRACTIONATION USING NON-UNIFORM ELECTRIC FIELDS

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ABSTRACT

Dielectrophoresis (DEP) – the movement of particles in non-uniform electric fields – can be used in combination with Field Flow Fractionation (FFF) to separate particles with differing dielectric properties. An introduction is given to the technique of DEP-FFF and its application to the separation of cells and other particles. The separation of yeast cells using the subtechniques of steric and hyperlayer DEP-FFF is demonstrated. It is shown that the hyperlayer-DEP-FFF techniques have a number of advantages, including an improved separation efficiency and reduced adhesion to chamber walls. The hyperlayer-DEP-FFF separation technique is also independent of particle size and allows the use of higher medium conductivities than for conventional DEP methods.

INTRODUCTION

Field-Flow Fractionation (FFF) is a family of techniques[1,2] in which a force field is applied perpendicular to the flow through a narrow chamber driving the particles towards the chamber wall. Through different factors such as diffusion, steric, hydrodynamic, and other effects the particles with different properties attain different positions away from the chamber wall, and separation of particles is achieved through the different velocities of the particles in the parabolic velocity profile in the chamber. The FFF techniques have various applications in the analysis and separation of industrial, environmental and biological samples, and can separate particles with diameters ranging from a nanometer to over 100 microns.

FFF separation techniques have been used in the separation of various biological materials. Viruses and various biopolymers were among the first to be separated.[3,4,5] The separation of human and animal cells, in particular blood cells, has been demonstrated.[6,7,8] Bacteria have been separated on the basis of mobility and cell size, shape, and density,[9] as have yeast cells grown under different conditions.[10]

Dielectrophoresis (DEP)[11,12] is the movement of particles in non-uniform electric fields. The DEP force is the result of the interaction between the dipole that is induced in the particle when an electric field is applied, and the non-uniformity of the electric field over the particle. The magnitude of the DEP force F_{dep} is given by the equation:

$$F_{dep} = 2\pi\varepsilon_0\varepsilon_m r^3 \, \mathrm{Re}\left(\frac{\sigma_p^* - \sigma_m^*}{\sigma_p^* + 2\sigma_m^*}\right)\nabla E^2 (\mathrm{rms}) \qquad (1)$$

in which ε_0 is the permittivity of free space (8.854×10^{-12} F m^{-1}), ε_m the relative permittivity of the suspending medium, r the (equivalent) radius of the particle, σ_p^* and σ_m^* the complex conductivity of the particle and the medium, and ∇E defines the field non-uniformity. Re stand for "the real part of." The complex conductivity is defined as:

$$\sigma^* = \sigma + j\omega\varepsilon \qquad (2)$$

in which σ and ε are the permittivity of the particle or medium, $j = \sqrt{-1}$ and ω is the angular frequency of the applied electric field ($\omega = 2\pi f$).

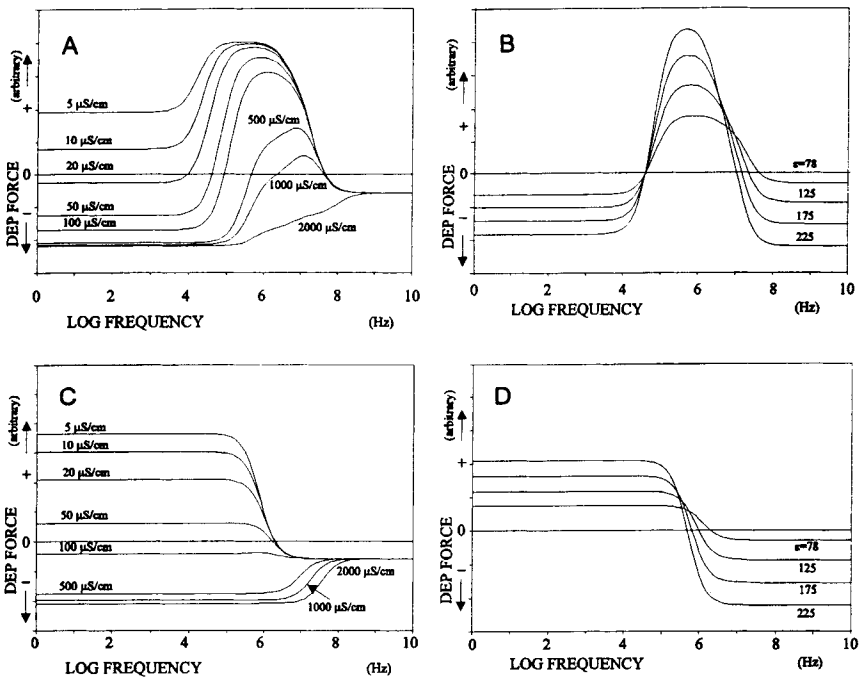

Figure 1. Effect of the medium conductivity and permittivity on the dielectrophoretic spectra of viable and non-viable yeast cells. The spectra were calculated using the multishell model.[20] For the calculation of the spectra of viable cells, the following parameters were used: relative permittivity of the cell interior, membrane and wall 50, 6 and 60, respectively; interior, membrane and wall conductivity 2000, 2.5×10^{-3} and 140 μS/cm, respectively; cell diameter 8 μm, wall thickness and membrane thickness 8 nm and 0.22 μm, respectively. The spectra of non-viable cells were calculated using the same parameters as for viable cells, but with an interior conductivity of 70 μS/cm and a membrane conductivity of 1.6 μS/cm. Figure 1a: viable cells, medium permittivity 78, varying medium conductivity. Figure 1b: viable cells, medium conductivity 30 μS/cm, varying medium permittivity. Figure 1c: non-viable cells, medium permittivity 78, varying medium conductivity. Figure 1d: non-viable cells, medium conductivity 30 μS/cm, varying permittivity.

Both positive DEP (movement towards high field strength regions) and negative DEP (movement away from high field strength regions) are possible, depending on the relative size of the complex conductivity of the particle compared to that of the medium. The phenomenon of dielectrophoresis can be

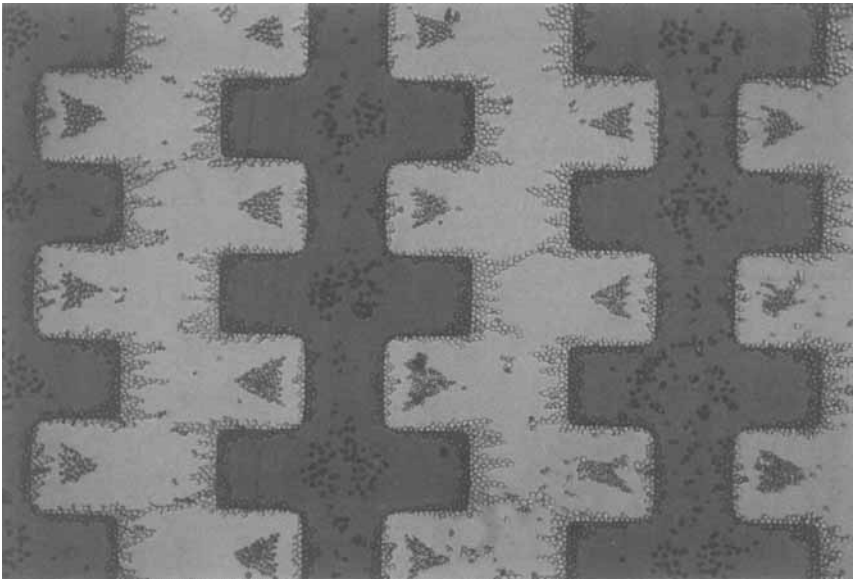

Figure 2. Dielectrophoretic separation of viable and non-viable yeast cells in a system of interdigitated castellated electrodes. The viable cells have accumulated at the electrode edges, whilst the non-viable cells have aggregated in triangular-shaped aggregations between the electrodes and diamond shaped aggregations on top of the electrodes.

observed with charged as well as uncharged particles and in both DC and AC electric fields. In the frequency range in which DEP is usually studied (1 Hz - 100 MHz), the effective complex medium conductivity σ^*_m is relatively constant, but the effective complex conductivity σ^*_p of many particles shows large changes as a function of the frequency of the applied electric field. By measuring the DEP force as a function of the frequency of the applied field, spectra can be obtained that are characteristic for the particle in question. Of particular interest are the frequency spectra of cells, since due to interfacial polarisation at the structures that form a cell (such as the cell wall, cell membrane, etc.) relatively large DEP forces are exerted on cells which are sensitive to small changes in the electrical properties of cellular structures and the suspending medium. As a consequence, efforts are being directed towards the utilisation of DEP forces in the separation of cells, and successful separations to date include the separation of normal and cancer cells,[13,14] stem cells from blood,[15] viable and non-viable cells,[16,17] and Gram-positive and

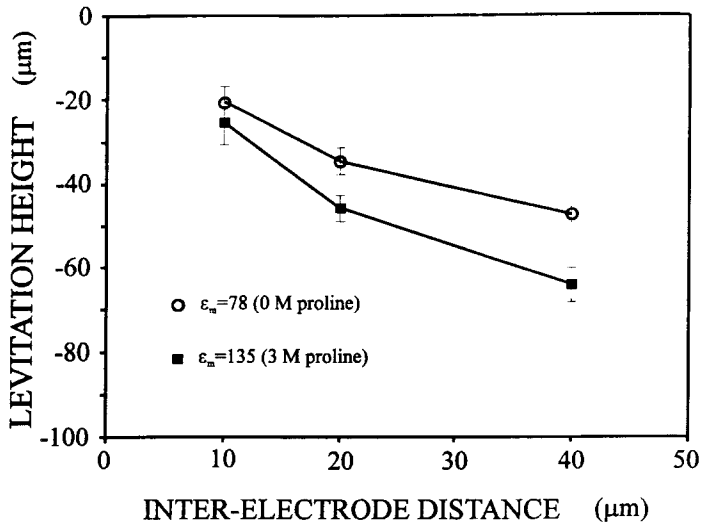

Figure 3. Effect of the relative permittivity ε_m of the suspending medium on the dielectrophoretic levitation height of non-viable yeast cells above interdigitated electrodes of varying spacing. The medium conductivity was 34 μS/cm and the applied voltage and frequency were 5 V pk-pk and 10 MHz, respectively.

Gram-negative bacteria.[18,19] Since the composition of the medium can be changed experimentally, this can be used to advantage in many separations. Figure 1a-d shows the DEP spectra of viable and non-viable yeast cells calculated for different medium conductivities and permittivities. The medium conductivity can easily be changed by the addition of salts, whilst the medium permittivity can be increased by the addition of zwitterions such as glycine or proline (or decreased by for example the addition of sugars).[21]

The combination of A.C. dielectrophoresis with field-flow fractionation techniques (DEP-FFF) is potentially a very gentle and selective method for the separation of cells and other particles. The already existing technique of electric FFF,[4] which can separate particles on the basis of their surface charge, makes use only of uniform DC electric fields, and is limited by electrode polarisation effects.

Although a large number of different designs has been used and proposed[11,12,22] to achieve the dielectrophoretic separation of particles, the most successful designs[12-17] to date make use of wide, long, narrow chambers similar

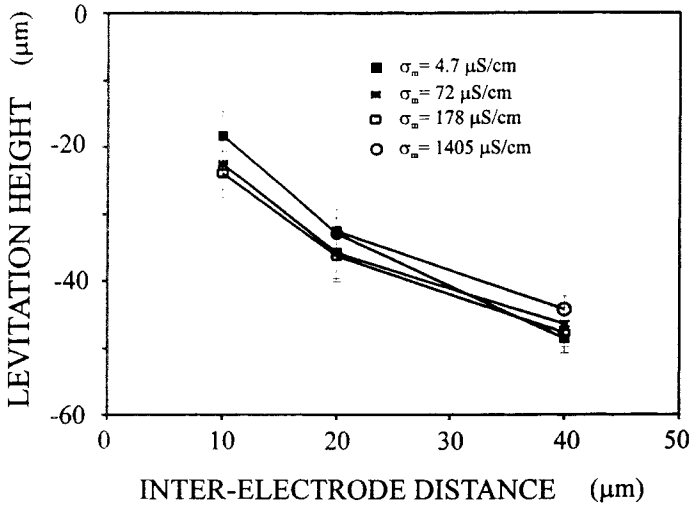

Figure 4. Effect of the medium conductivity σ_m on the dielectrophoretic levitation of non-viable yeast cells above interdigitated electrodes of varying spacing. The applied voltage and frequency were 5 V pk-pk and 10 MHz, respectively.

to FFF separation chambers, but containing large arrays of microelectrodes. The advantage of using microelectrodes rather than macroscopic electrodes is that relatively small voltages can be used to generate the high field gradients needed to observe dielectrophoresis. This not only simplifies the equipment needed to generate the electric fields, but also reduces side effects such as heating. The particles are held at the electrodes in the chamber by a DEP force that is dependent on the electrical properties of the particles and the surrounding medium, the frequency and magnitude of the electric field, and the design of the electrodes. An additional force over the surface of the electrodes, such as hydrodynamic forces, can remove any particles held by the electrodes.

DEP-FFF is an unconventional FFF technique in that the DEP force is inherently non-uniformly distributed over the chamber, not only in the plane of the electrodes/chamber wall, but also across the chamber above the electrodes.[23] Also, because the particles distort the electric field around them and in that way form local field non-uniformities, mutual attraction occurs between particles which can lead to what is called pearl-chain formation. As a consequence, interparticle interactions, involving both mutual attraction by DEP as well as electrostatic interactions between charged particles, can be considerable.[24]

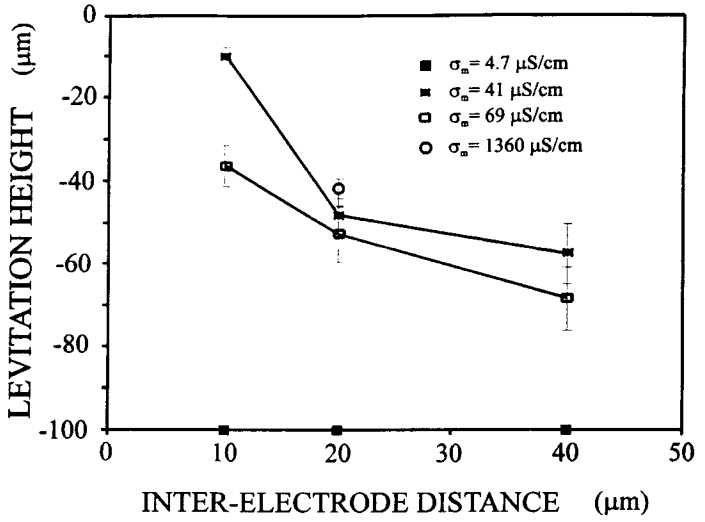

Figure 5. Effect of the medium conductivity σ_m on the dielectrophoretic levitation of viable yeast cells above interdigitated electrodes of varying spacing. The applied voltage and frequency were 5 V pk-pk and 20 kHz, respectively.

The main separation mechanisms in FFF are normal, steric, and hyperlayer modes.[1,2] In normal FFF, the earliest form of FFF, separation is achieved through back-diffusion of the particles away from the chamber wall against the driving force into different velocity regions. Washizu and co-workers[25] described the use of dielectrophoresis in combination with fluid flow through an open chamber with interdigitated sinusoidally corrugated electrodes to separate macromolecules such as proteins and DNA. Although it was not explicitly stated, this situation is very comparable to normal FFF.

Steric FFF is a separation principle that is generally seen in the separation of larger particles in which back-diffusion is negligible. In steric FFF, the driving force actually pushes the particle against the accumulation wall. Because of their size, larger particles protrude further into the channel and are caught in higher velocity streamlines.

Most dielectrophoretic separations of cells to date have used steric FFF. The cells are usually allowed to settle on the electrodes by gravity or attracted to the electrodes by positive DEP, and effectively immobilised in potential

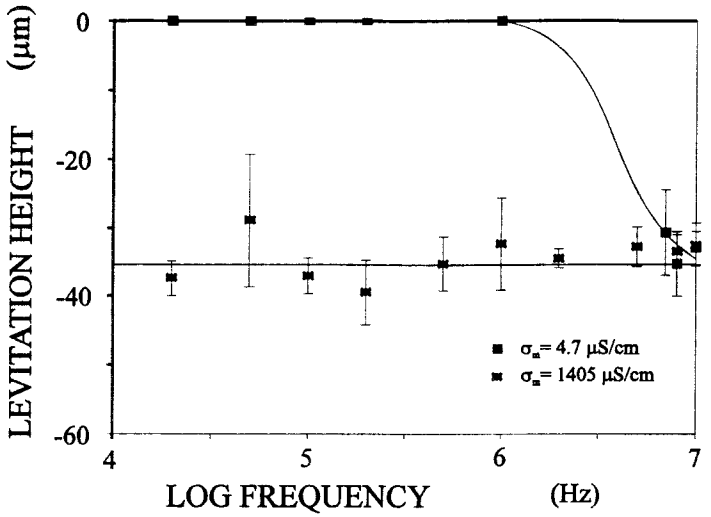

Figure 6. Effect of the medium conductivity and frequency on the dielectrophoretic levitation height of non-viable yeast cells above interdigitated electrodes with an electrode spacing and width of 20 μm.

energy minima[23] near the electrodes by primarily a combination of gravity and electric field forces. Fluid flow through the chamber is then used to apply additional hydrodynamic forces to the particles, and remove those particles that are held less strongly at the electrodes.

In most DEP separations, only positive DEP is used, or a combination of positive and negative DEP.[11-19] The particles are still held at the same plane as the electrodes, resulting in hydrodynamic forces being exerted on the particles similar to steric FFF. In a recent development,[26] negative DEP forces have been used to levitate particles above the electrodes. The DEP force on particles has already been given by Equation (1), whilst the gravitational force F_g on a particle with density ρ_p in a medium of density ρ_m is given by:

$$F_g = \frac{4}{3}\pi r^3(\rho_p - \rho_m)g \qquad (3)$$

in which g is the gravitational constant.

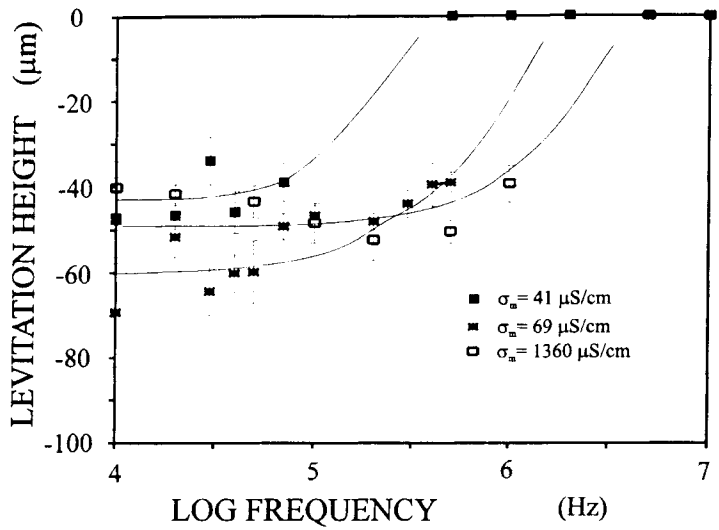

Figure 7. Effect of the medium conductivity and frequency on the dielectrophoretic levitation height of viable yeast cells above interdigitated electrodes with an electrode spacing and width of 20 μm.

Since the DEP force and gravitational force have a similar dependence on the volume of the particle, particles with the same electrical properties and density but with different sizes are levitated to approximately the same height above the electrodes.[26] Negative DEP could thus be used to levitate particles above the chamber wall into different velocity streamlines of the flowing liquid in a similar way as hyperlayer-FFF. In this paper, we will compare the use of steric DEP-FFF and hyperlayer-DEP-FFF in the separation of viable and non-viable yeast cells using media of different conductivities and permittivities.

MATERIALS AND METHODS

Yeast Cells

The yeast used was baker's yeast (*Saccharomyces cerevisiae*) strain R12. The yeast was grown overnight at 30 °C in a medium containing 0.5 % yeast extract, 0.5 % bacterial peptone and 5 % sucrose. Part of the yeast culture was rendered non-viable by heating to 90 °C for 20 minutes. The yeast cells were washed 4 times in deionised water and resuspended in deionised water, dilute

NaCl solutions (to change the conductivity) or concentrated proline solutions (to raise the permittivity). Conductivity measurements were made using an HP4192A impedance analyser using a cell with a cell constant of 1.573 cm^{-1}.

DEP Chambers

For the steric-DEP-FFF experiments, a dielectrophoretic separation chamber was used as described previously.[17,19] The chamber had a length of 30 cm, a width of 2 cm, a height of 200 μm, and contained electrodes of the interdigitated castellated type with a characteristic size of 70 μm.

The electrodes were made by photolithography from gold on a thin layer of chromium and had a thickness of approximately 0.3 μm. Liquid was pumped through the chamber using a Gilson Minipuls 3 pump. For the levitation experiments, interdigitated electrodes (without castellations) with a width and inter-electrode spacing of 10, 20 and 40 μm were used. An AC voltage of 5V pk-pk was applied to the electrodes at a frequency between 10 kHz and 10 MHz using a HP33120A frequency generator. The levitation height was estimated by focusing on either the electrodes or on the particles, and reading off the grading on the calibrated focus adjustment of the microscope (Nikon Labophot-2).

For hyperlayer-DEP-FFF experiments, a chamber was used as described for the steric FFF experiments, but with a length of 12.5 cm and containing electrodes of the interdigitated kind (without castellations) with a width of 40 μm and an interelectrode spacing of 60 μm. The electrodes were observed under a microscope and the speed at which particles travelled over the electrodes was measured at different flow rates and voltages.

RESULTS AND DISCUSSION

Steric-DEP-FFF

Referring to Figure 1a-d, one can expect that at modest conductivity values viable cells (with intact membranes) will show positive dielectrophoresis at a frequency of approximately 5 MHz, whilst non-viable cells show negative dielectrophoresis at this frequency. When a mixture of viable and non-viable cells is placed in a chamber containing interdigitated castellated electrodes, one can see (figure 2), as has been shown previously,[16] that the viable cells accumulate at the high field gradient regions near the edges of the electrodes,

whilst non-viable cells accumulate at the low field gradient regions between and on top of the electrodes (in "triangular" and "diamond"-shaped aggregations, respectively). Since the non-viable cells are held in relatively shallow potential energy wells by a combination of gravitational and DEP forces, these cells can easily be removed from the electrodes (and hence from the viable cells) by fluid flow through the chamber – provided the positive DEP force holding the viable cells is strong enough.

Hyperlayer-DEP-FFF

The use of a combined hyperlayer-DEP-FFF technique would have a number of advantages over steric-DEP-FFF, including overcoming the need for positive DEP, the use of high suspending medium conductivities, making better use of the parabolic velocity profile in the chamber, and the reduction of cell entrapment in the pearl chains formed by DEP.

As a model system, to demonstrate the hyperlayer-DEP-FFF technique, we chose the separation of viable and non-viable yeast cells. To establish the conditions for the separation of cells, the effect of the permittivity and conductivity of the medium, the size of the electrodes, and the frequency of the applied field were investigated.

Figures 3-5 show the effect of the medium permittivity and conductivity on the levitation of viable and non-viable yeast cells above interdigitated electrodes of different electrode spacings. The width of the electrodes was the same as the distance between the electrodes. The results correspond well with the predictions in Figs. 1a-d that the effect of the medium conductivity on the levitation height of non-viable cells at 10 MHz is small, whilst an increase of the medium permittivity increases the levitation height.

The influence of the medium conductivity on the levitation height of viable cells at 20 kHz involves many contributing factors, including the fact that, at this frequency, the conductivity of the cell wall, which is affected by the suspending medium conductivity,[27] plays an important role in the determination of the effective particle conductivity of the cell. The data also show that an increase in the electrode size increases the levitation height. As shown elsewhere,[26] this is a consequence of using a periodic electrode array and can be further understood by the fact that the electric field and the factor ∇E^2 of Equation (1) extend further above the electrodes with increasing interelectrode distance. From these and other data (Rousselet *et al.*, in preparation) it can be concluded that the optimum interelectrode distance is in the region of 40-100 μm.

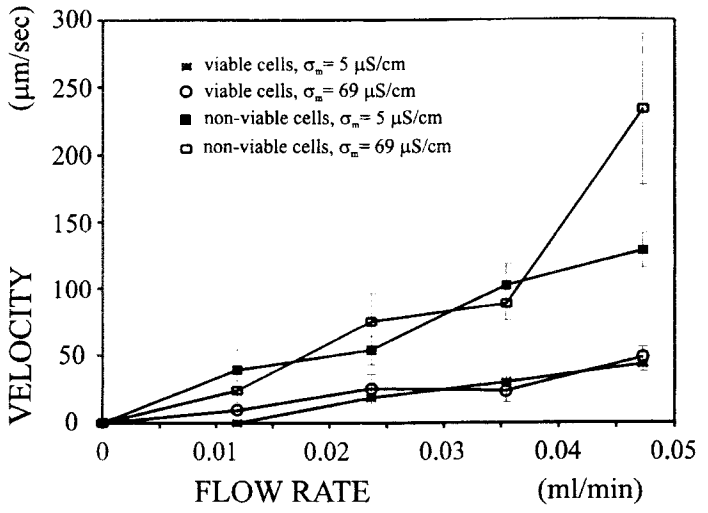

Figure 8. Separation of viable and non-viable yeast cells using hyperlayer-DEP-FFF. The velocity at which non-viable yeast cells (which are levitated high above the electrodes) travel through the chamber is considerably higher than that of viable yeast cells. The applied voltage and frequency were 5 V pk-pk and 5 MHz, respectively.

Figures 6 and 7 show the effect of the frequency of the applied electric field on the levitation height of viable and non-viable yeast cells above the electrodes at different medium conductivity values. The experimental data closely resemble those obtained (Figure 1a-d) from the modelling of the cells, taking into account electrode polarisation effects.[26] Some of the differences observed may be explained by the fact that any changes in the cell wall conductivity have been ignored in the modelling whilst it has been shown before[27] that, because the cell wall of yeast cells acts as an ion exchanger, the dielectric properties of yeast varies with medium conductivity. The data show that, if one is to achieve the most efficient separation of viable and non viable cells, it is best to use a frequency of 2-10 MHz. This conclusion is relatively independent of the medium conductivity.

To achieve the separation of viable and non-viable yeast cells using hyperlayer DEP-FFF the speed was measured at which viable and non-viable cells travelled at different flow rates through a chamber containing interdigitated electrodes. Two different conductivities (5 and 68 μS/cm) and applied voltages (5 and 10 V pk-pk) were used, whilst the applied frequency in

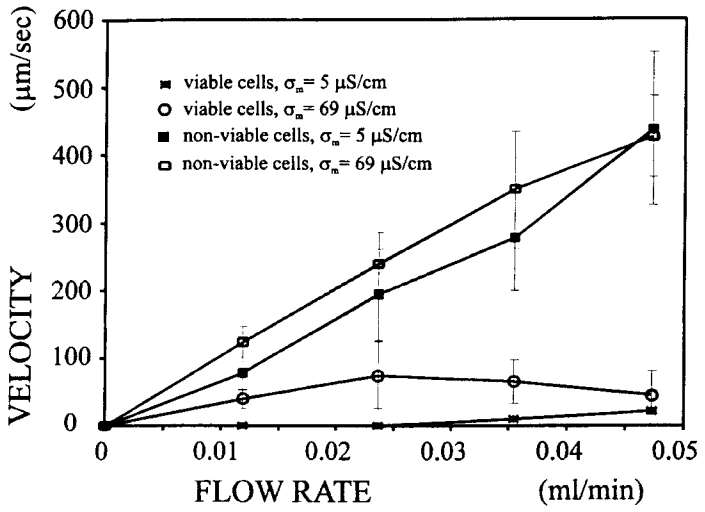

Figure 9. As in Figure 8, but with an applied voltage of 10 V pk-pk.

each case was 5 MHz. The results are shown in Figures 8 and 9. As expected, since the non-viable cells are levitated high above the electrodes, and the viable cells are attracted to the electrodes, the velocity at which non-viable yeast cells travel through the chamber is considerably higher than that of viable yeast cells. Especially at low conductivities and high voltages, the positive DEP force on viable cells was so strong that a considerable number of viable cells remained attracted to the electrodes. For an efficient separation experiment in which positive DEP occurs, it may be necessary to use a pulsed electric field to allow cells to become detached from the electrodes. The cell "mixing" effect that occurs between pulses will also improve the separation. During the levitation experiments, it was observed that near the crossover points from positive to negative DEP the cells could be observed to redistribute themselves over a range of heights near the electrodes. This indicates that it would be relatively straightforward to separate the cells into further fractions. Further work will be aimed at achieving this as well as the separation of other cell types.

CONCLUSIONS

The separation of particles with differing dielectric properties using normal-DEP-FFF, steric-DEP-FFF and hyperlayer DEP-FFF has been

discussed. To demonstrate steric-DEP-FFF and hyperlayer-DEP-FFF the separation of viable and non-viable yeast cells was chosen. The effects of the frequency of the applied field and conductivity and permittivity of the medium was investigated. The separation of viable and non-viable yeast cells is best achieved at a frequency of 2-10 MHz, and for moderate conductivities is relatively independent of the conductivity of the surrounding medium. The hyperlayer-DEP-FFF technique, which was here described for the first time, has a number of advantages over the steric-DEP-FFF technique that is normally used. The hyperlayer-DEP-FFF technique makes better use of the parabolic velocity profile of the fluid flowing through the chamber by pushing the particles to different heights in the chamber, whilst in steric-DEP-FFF the particles stay in the layer near the chamber wall. The levitation height of the particles is independent of particle size, and only dependent on their dielectric properties and density. Also, hyperlayer-DEP-FFF can be performed at high conductivities, adhesion of the particles to the chamber wall is largely reduced as contact with the walls is minimal, and trapping of cells in pearl chains is largely reduced.

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