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Levitation, holding, and rotation of cells within traps made by high-frequency fields

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Biological cells and other particles can be electrically manipulated by means of negative dielectrophoresis within microchambers whose electrode geometry is of the order of the cell size. Very-high-frequency fields (50 MHz and above) and media of increased relative permittivity are especially suitable for the purpose, as shown by experimental data on levitation and rotation. It appears to be possible to move and rotate cells or particles at will using this technology.

Introduction

Electrical and magnetic field traps have been used in physics by Paul and coworkers for the confinement and investigation of atomic and elementary particles [1,2]. Application of this principle to biological cells was suggested by Pohl [3], but only a few examples of its use exist [4,5]. One reason is that applications of this technique to cells have used positive dielectrophoresis, which results in instability of the cell position. Addition of a quick-acting feedback system can tame this system [6,7], at the cost of some additional complexity.

In contrast to positive dielectrophoresis, negative dielectrophoresis allows stable, contact-free positioning and levitation. Although most applications have involved positioning of air bubbles [8] or low-permittivity particles [9–11], application to the levitation of cells is a promising area. This has very recently been confirmed by the demonstration of cell movement and collection by negative dielectrophoresis [11,12], and by the simultaneous use of positive and negative dielectrophoresis [12].

A second reason for the lack of levitation work with biological systems is the difficulty of fabricating suitable electrode geometries. In contrast to atomic particles in a vacuum, cells are subject to viscous forces, so that their electrophoretic response at the frequencies necessary to prevent electrolysis is very small. The only forces that can be exploited are those that depend upon interaction of induced polarization with the field gradient, requiring strongly inhomogeneous fields [3,13,14]. Such fields are found close to electrodes with a small radius of curvature, therefore the percentage of the chamber volume which is active can be maximised by using highly convoluted electrode forms with small inter-electrode spacings (the dimensions may approach those of the cells).

The above considerations led to the development of the helix chamber [15], the [illegible] and a chamber with castellated electrodes [17,18]. The production of the latter types of chambers requires planar lithographic techniques, first suggested for electrode production in 1960 [19].

This article describes a synthesis based on the above principles. We use high-frequency traps (as described by Paul [1,2]) but with several significant modifications. The traps have the following features:

(1) Extremely small chamber geometry, see also Refs. 16–18. Reduction of scale reduces the heating and convection which can be a problem in conductive media (such as are usual in biology). There is the further advantage in terms of the necessary electronics that modest electrode voltages (as little as 3 V) give sufficient field and inhomogeneity.

(2) Both 4- and 8-electrode types are used for production of multipole and rotating fields.

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Abbreviation: DK, permittivity (dielectric constant).

(3) The cells are trapped by means of the balance between electrical and gravitational forces, as suggested by Jones and associates [6–10].

(4) The electrodes have an easily-fabricated planar form (height 0.5–20 μm).

(5) The exclusive use of negative dielectrophoresis, both to distance the cells from the electrodes and to bring them into stable positions. This combination is not possible with positive dielectrophoresis, because field maxima cannot exist except on an electrode surface [8].

(6) Rather high field frequencies (10 MHz–250 MHz) are used. These exceed the more usual range used for levitation (up to a few MHz [4–6,8,9,12], exceptionally up to 50 MHz [7]), although cellular spin resonance has been reported using frequencies up to 140 MHz [20]. The period of these frequencies is much less than the charging time of the membrane [21–24], which is therefore not subject to the voltage stress induced by lower frequencies.

We will show how use of appropriate electrode geometry and voltages can create effective field-traps, from which a cell cannot escape unless an additional force is applied. It is our aim to show how these open up new possibilities for the manipulation of cells, and also how the measurement of the dielectrophoretic force and other dielectric spectroscopic techniques based on single cells can be improved.

Materials and Methods

1. Production of negative dielectrophoresis

A simple physical picture of living cells is that of a membrane-covered spherical droplet [11–14]. The membrane is a thin insulating layer of low dielectric constant, and the interior is an aqueous electrolyte (some average of the cytoplasm, vacuole sap, and nucleoplasm). Restricting discussion to non-dispersive suspension media (otherwise, see Ref. 14), there are usually two frequency-regions where the dielectrophoretic force is negative (the shaded regions in Fig. 1).

It can be seen that low frequencies give a larger repulsive force: however, this is at the cost of the generation of a large membrane potential, especially in the case of larger cells (radius > 7 μm) *. This, and the fact that, electrolytic bubble formation may occur, causes these frequencies to be unsuitable for electromanipulation. High frequencies (roughly 100 MHz) are more difficult to generate but are much kinder to the cells, because they induce hardly any membrane potential. It is our opinion that the use of frequencies in this region is the most practical method for the movement of living cells.

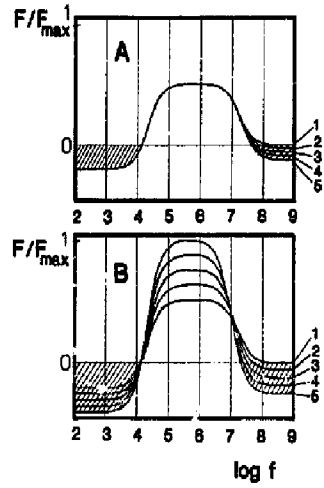

Fig. 1. Dielectrophoretic force (F), normalised against F_{max}, that is calculated to be exerted on a cell in media of various properties. The calculation is according to Ref. 14, taking account of the conductivity and assuming a linear field gradient. The parameters for the reference spectrum (curve 1 in both figures) are:

	Conductivity		DK
Cell interior	0.2	S/m	80
Membrane	$8 \cdot 10^{-7}$	S/m	5
Medium	10^{-2}	S/m	80

Radius = 50 μm
Membrane thickness = 6 nm

(A) Variation of the DK of the cell interior. Curves: 1, 80; 2, 70; 3, 60; 4, 50; 5, 40.
(B) Variation of the DK of the medium. Curves: 1, 80; 2, 100; 3, 120; 4, 140; 5, 160.

2. Production of electric-field traps

Under conditions that give negative dielectrophoresis, particles move towards regions of lower field strength, resulting in field strengths that are denoted by the closeness of the spacing of the field lines. Fig. 2 shows how this effect can be used to give two sorts of traps by application of alternating voltages to a 4-electrode structure.

Fig. 2A, the two phases of an alternating voltage are applied to neighbouring electrodes. Except for the very short zero-crossing periods of the alternating wave, a force is exerted towards the middle of the chamber at all times.

* A low-frequency alternating field of strength E induces a membrane potential of $1.5 \cdot a \cdot E$ in a spherical cell of radius a [21–24]. Therefore in a typical electro-manipulation field of 20 kV/m, the maximum cell radius that will not cause the induced membrane potential to exceed 100 mV is $a = 6.7$ μm.

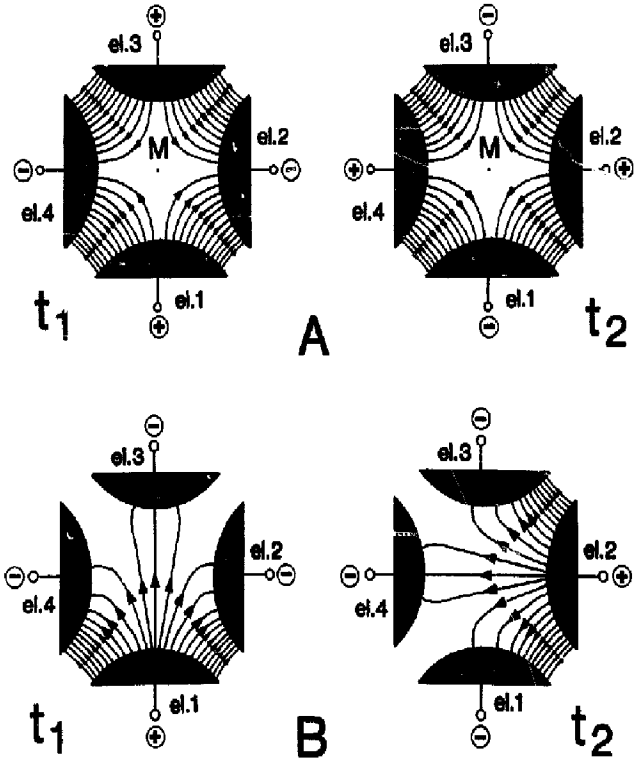

Fig. 2. (A) Field distribution inside a four-electrode chamber, showing the static local field minimum (M) at times t_1 and t_2 during the application of an *alternating* field. (B) Field distribution inside a four-electrode chamber at two instants during a cycle of a *rotating* field. Time t_2 is a quarter cycle after t_1. (The field distributions after subsequent quarter cycles are given by successive rotation of the diagram by 90°).

Fig. 2B, a four-phase generator supplies the electrodes one after the other, so generating a rotating field [25–29]. At any instant the 'cage' is open in two directions, however, the particle will not escape if the period of the field rotation is much shorter than the time constant of particle movement. In this case we can consider time-averaged effects: the average magnitude of the field-strength in the chamber center is much less than that between the electrodes. A time-averaged confinement force is established after one or more cycles (dynamic field trap).

3. Production of measurement chambers

This uses the high-resolution photolithographic and vacuum deposition processes developed for semiconductor production [30,31]. Application to biological cells has been described before [16–18,32]. This technology is limited to planar geometry, with film thickness 0.1–50 μm, and width very approximately 0.3–30 μm, the latter depending very much on the state-of-the-art and scale of the plant. The lower limits of electrode width and gap are important, for reasons discussed in the Introduction.

The chambers used here were fabricated on commercially-available wafers of quartz or silicon. After deposition of a plating base (Cr/Au) a photoresist was deposited and lithographically structured. Electrodes of gold (0.5 or 20 μm thick) were formed by electroplating, and after photoresist removal the plating base was ion-milled away. Arrangements of electrodes for production of quadrupole and octupole fields are shown in Fig. 3. The distance between opposite electrodes was typically 100 μm.

4. Driving voltages

Generation of rotating fields used inter-electrode phase-shifts of 90 degrees in the 4-electrode chamber and 45 degrees with 8 electrodes. The necessary voltages were provided from generators of our own design. Up to 2 MHz, 50 V peak-to-peak (ptp) was available from the complementary transistor pair SD339/SD340 (Halbleiterfabrik, Dresden), at higher frequencies 4.8

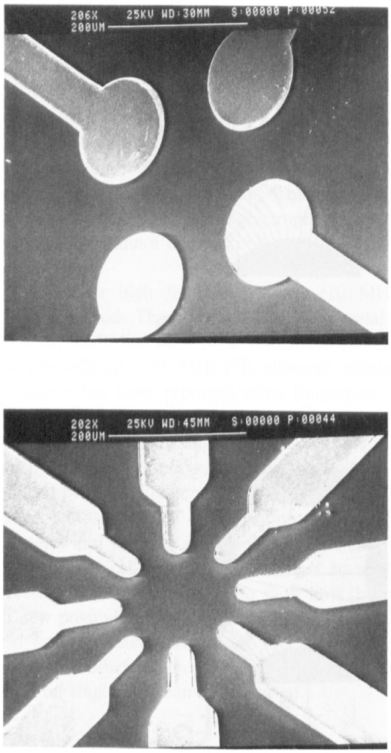

Fig. 3. Scanning electron micrographs of chambers (material Au on Si/SiO_2) used for the production of field traps suitable for biological cells. (A) 4-electrode chamber. (B) 8-electrode chamber.

V ptp was obtained from transistor type KT 913B (from the former USSR).

Quadrupole fields were generated by use either of two phases (with 180° phase-shift) of one of the rotation generators, or else by single-phase drive from one of the following commercial generators. Voltages up to 15 V peak-to-peak (ptp) between 1 kHz and 3 MHz were available from a function generator type HM 8030-4 (Hameg GmbH). Between 10 MHz and 250 MHz a signal generator type SMG (Rohde & Schwarz) with external amplifier based on a GPD 405 hybrid (Avantek) was used to give voltages up to 10 V ptp.

At frequencies above 10 MHz, connections between the generators and electrodes were made with 50 ohm coaxial cable, terminated in 50 ohms at the experimental chamber. This consisted of the 4- or 8-electrode micro-chambers (on a 1 cm × 1 cm wafer) bonded and connected to a leadless ceramic carrier (LCC-68). After insertion into a LCC-68 socket, the assembly could be handled as a microscope slide. The voltages in these 50 ohm systems were monitored with an oscilloscope of 150 MHz bandwidth (Tektronix 2445). The possibility that the voltage on the electrodes was significantly different (due to losses or resonances in the bonding wires and the electrodes) was ruled out by checking the voltages on the electrodes inside the chamber with a 150 MHz oscilloscope probe (Tektronix P6122).

5. Measurement objects and media

We used artificial particles (porous spheres of cellulose disulphate, diameter 50–150 μm) as well as biological cells. The latter were the unicellular peat-bog alga *Eremosphaera viridis*, [33] as well as pollen grains of *Helianthus annuus* (sunflower) and of *Pinus sylvestris* (scotch pine). The salt concentration was adjusted by addition of KCl or $CaCl_2$ to values tolerated by the organisms concerned. The resulting conductivities lay between 10^{-3} and 0.5 S/m. In some cases the permittivity of the medium was increased by the use of 1.5 M glycylglycine [34,35]. The experimental objects were introduced into the chamber with a micromanipulator.

6. Dielectric properties of media and particles

The relative permittivity of the media and the unknown dielectric properties of the cellulose-disulphate particles at frequencies up to 10 MHz were checked by use of an impedance analyser and variable-gap parallel-plate impedance chamber as described elsewhere [36]. In order to prevent sedimentation of the cellulose-disulphate beads, sucrose was added to the medium. Fig. 4 shows that the medium containing 1.5 M glycylglycine and 0.66 M sucrose showed a high and effectively frequency-independent DK of 182 between 1 and 10 MHz, whereas a suspension of the beads at

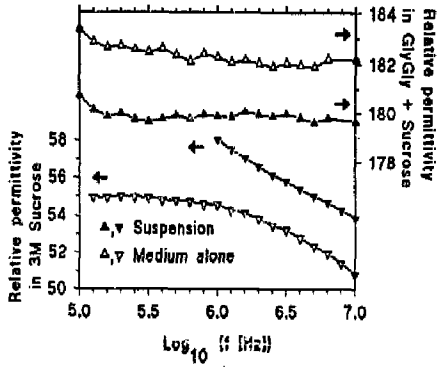

Fig. 4. The permittivity (relative to that of vacuum) of aqueous media and of suspensions of cellulose disulphate beads in these media. The media contained just sufficient sucrose to prevent settling of the particles. The data were measured at 23°C. Upper curves: △, 1.5 M glycylglycine and 0.66 M sucrose (conductivity at 1 kHz, 10 mS/m); ▲, suspension of beads (9.7 mg dry weight per ml). Lower curves: ▽, 3.0 M sucrose (conductivity at 1 kHz, 10 mS/m) alone; ▼, suspension of beads (15 mg dry weight per ml).

9.7 mg/ml (11.6% by volume *) had a DK of 180, also constant over this frequency range. The absence of an appreciable dispersion indicates that the bead conductivity is little different from that of the medium. The mean value of the DK difference was 2.22 ± 0.17 (mean $\pm$ S.D., $n = 11$). Use of the Maxwell mixture equation [37–39] gave a DK value for the beads, assumed to be homogeneous, of 163. This high DK is presumably a consequence of the bead's high porosity (91% *) and the high DK of the medium.

In the absence of glycylglycine, it was necessary to use 3 M sucrose in order to prevent sedimentation. This not only decreased the low-frequency DK to 55 (Fig. 4), but also introduced a dispersion above 1 MHz (not seen in 0.66 M sucrose, which has a much lower viscosity). The 15 mg/ml (18% by volume *) suspension of the beads in 3 M sucrose (Fig. 4) showed a DK that was 2.5–3.0 units *higher* than that of that medium. For the range 3.16–10 MHz, calculations yield a homogeneous-particle DK of 68.6 ± 0.3 (mean $\pm$ S.D., $n = 6$) in this case. This suggests a (partial?) exclusion of sucrose from these particles. The absence of an appreciable dispersion indicates that the bead conductivity is little different from that of the medium.

7. Measurement of negative dielectrophoresis

Consider the excitation of an alternating quadrupole field within a chamber consisting of four electrodes in a horizontal plane. The field extends above the plane of the electrodes, and a particle which shows negative dielectrophoresis experiences an upwards force. At a given sufficient field strength, the locus of points where this force is equal but opposite to the gravitational force has a funnel-shaped form, the central axis of which is a field-minimum for any given height (Fig. 5). Due to the field non-uniformity, a particle on this surface experiences a horizontal (centering) force, so that it cannot come to rest until it arrives at the lowest point of the funnel. At lower field strengths (and for denser particles) the lower part of the funnel rests on the same plane as the electrodes. If the field strength is increased, the lowest part of the funnel increases in height so that the particle is levitated; at the same time the sides of the funnel become less steep so that too much field can result in escape of the particle if this is even slightly disturbed.

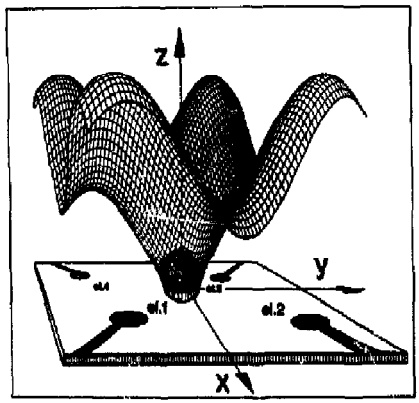

Fig. 5. The quadrupole field trap produced by four electrodes (el.1 to el.4) when excited so as to give negative dielectrophoresis. The gridded area represents the boundary of the trap for a particular frequency, field strength and set of particle properties. On this surface, the Z-component of the negative dielectrophoretic force exactly compensates the sedimentation force (gravity). The horizontal component of this force is towards the central (Z) axis. This means that all particles initially within the funnel are trapped and will eventually come to rest at the bottom of the funnel (assuming some viscous damping to be present). In order to simplify the calculation, it is assumed that the electrodes act as point field sources and that the particle does not disturb the field.

Due to the self-centering action of the trap, only vertical motion needs to be considered. With the arrangement shown in Fig. 5, motion in the positive Z-direction results from a negative dielectrophoretic force. This exists when the effective particle dipole moment p (the difference between the moments of particle and of the medium displaced by it) is negative. The dielectrophoretic force $(p \cdot \nabla)E$, where E is the field strength and ∇ is the Del vector operator, is then directed upwards because the field strength decreases in the positive Z-direction. The interplay with the sedimentation force and viscous drag gives the equation of motion:

$$m \cdot d^2Z/dt^2 = (p \cdot \nabla)E - V(\rho_p - \rho_a)g - 4\pi\eta R \cdot dZ/dt \qquad (1)$$

where the symbols represent: m, R, V: effective mass, radius and volume of the particle; t: time; η: dynamic viscosity of the medium; ρ_a, ρ_p density of medium and particle, respectively; g: gravitational acceleration.

Particles with negative p but positive buoyancy ($\rho_p < \rho_a$) will tend to escape upwards out of the trap, and the positive sign of $(p \cdot \nabla)E$ can only accelerate this process. On the other hand, the dielectrophoretic force acts opposite to negative buoyancy (sedimentive force), and the magnitude of p as well as the field inhomo-

* The wet volume per mg dry weight of these beads, as well as their porosity (the percentage of their wet volume that is accessible to a low molecular weight solute, here KCl) were calculated from dextran-exclusion data. The volumes accessible to blue-dextran (assumed impermeable) and to KCl (assumed permeable) within a pelleted wet volume (0.26 ml) of beads, were found to be 9.6% and 91.5%, respectively. The washed pellet had a dry weight of 19.5 mg.

genecity decrease with increasing Z [8,13]. Therefore a stable position can be attained at some height where:

$$0 = (p \cdot \nabla)E - V(\rho_p - \rho_a)g \qquad (2)$$

The increase in the magnitude of $(p \cdot \nabla)E$ with decreasing Z has two other consequences:

(1) Attempts to trap a positively buoyant particle by means of positive dielectrophoresis will result in instability, unless the electrode voltage is feedback-controlled [6,7].

(2) We can assess the force on the particle by measurement of the levitation height h, which can be done with a vertical-axis microscope because of the narrow depth of field of the optics. The focus travel was assessed with a micrometer adapter (Messtaster type MT12, Heidenhain GmbH, W8225 Traunreut, Germany) which was attached directly to the microscope (Axiotron, Carl Zeiss, W7082 Oberkochen, Germany). The standard deviation of the height measurements was ± 2.1 μm.

Results

1. Alternating-field traps

It was first necessary to show if the second frequency band (near to 100 MHz and above) of negative dielectrophoresis was really shown by cells. This was in some doubt, because the existence of this band was predicted (Fig. 1) on the assumption that the internal conductivity and dielectric constant were constant, which is probably only a rough approximation above 50 MHz [37–39]. It can be seen from Fig. 6 that pollen grains do show two regions of levitation, the higher frequency region starting between 10 and 100 MHz

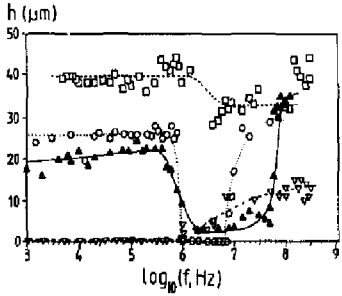

Fig. 6. Height of ascent of synthetic particles and of cells in an ac-field trap of diameter 100 μm as a function of frequency (f) with sinusoidal driving voltages. The voltages are the peak-to-peak values at each electrode, equal to the peak value between opposite electrodes. ▲, Pollen from *Helianthus annuus* $G = 24$ mS/m, $U = 12$ V; ○, pollen from *Pinus sylvestris*, $G = 24$ mS/m, $U = 12$ V; ▽, cellulose-disulphate beads in water, $G = 1$ mS/m, $U = 2$ V; □, cellulose disulphate beads in water, $G = 50$ mS/m, $U = 10$ V.

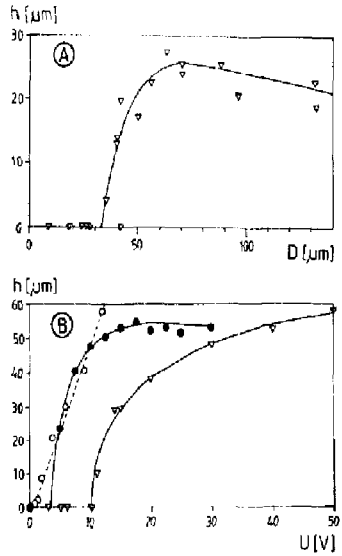

Fig. 7. Height of ascent of synthetic particles and of cells in an ac-field trap of 100 μm diameter: (A) as a function of particle diameter D at constant voltage; (B) as functions of voltage U (ptp value at each electrode). Parameters: (A) ▽, Cellulose-disulphate spheres in water, $G = 1$ mS/m, $U = 12$ V ptp, $f = 60$ MHz. (B): ○, Pollen from *Pinus sylvestris*, axes 62×44 μm, $G = 24$ mS/m, $f = 100$ MHz; ●, *Eremosphaera viridis*, $D = 150$ μm, $G = 24$ mS/m, in 0.5% human serum albumin, $f = 1.2$ MHz; ▽, cellulose-disulphate beads, $D = 31$ μm, $G = 1$ mS/m, $f = 1.9$ MHz. Note that, although the dielectrophoretic force is proportional to U^2, dh/dU in (B) *decreases* at high U. This means that the dielectrophoretic force (at constant U) decreases very rapidly at large h.

and continuing up to at least 300 MHz. The levitation of the cellulose disulphate particles in a medium of high conductivity (50 mS/m) showed less frequency-dependence, although at low conductivity (1 mS/m) only the higher frequency region gave levitation. We were not able to measure levitation of the large algal cells *E. viridis* except in the low MHz range, partly because of their tendency to stick to the electrodes (these cells possess a slime layer.)

Fig. 7A shows how the levitation by a given voltage (and therefore a given field strength and divergence) required a certain minimum size of object. The levitation height increased at first rapidly with particle diameter, only to decrease slowly for very large particles. As expected, a particle of a given size required a threshold electrode voltage before the force of gravity was compensated: Fig. 7B shows that the height of levitation increased rapidly with voltage once the threshold was exceeded, but then slowed as the regions of lower inhomogeneity were reached. Less dense particles such as pollen grains of *P. sylvestris* showed lower threshold

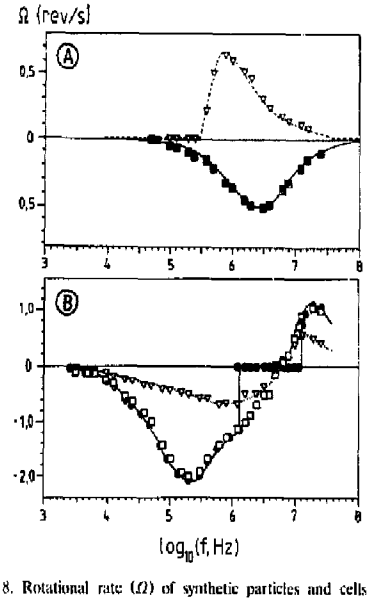

Fig. 8. Rotational rate (Ω) of synthetic particles and cells as a function of the frequency of the rotating field (180 degree symmetrical square-wave excitation). ▽, Cellulose-disulphate beads, $G = 1$ mS/m, $U = 4.6$ V; ■, cellulose-disulphate beads, $G = 25$ mS/m, $U = 4.6$ V. (B) ▽, *Helianthus annuus*, $G = 1$ mS/m, $U = 4.6$ V; □, ●, *Pinus sylvestris*, $G = 24$ mS/m, $U = 4.6$ V.

voltages for levitation, and could be raised to greater heights, than cellulose-disulphate particles or *E. viridis* cells.

The results with the alternating octupole field were similar to those with the quadrupole field. However, the 8-electrode chamber produces a less deeply-indented field barrier, so that it can be somewhat larger without losing the ability to center the cells.

2. Rotating-field traps

Rotating fields were found to be equally effective in the generation of traps. As noted above in connection with Fig. 3, these traps are of the dynamic type: the particle is prevented from escaping by its inertia in the face of a very rapidly rotating field (up to $3 \cdot 10^7$ per second). The particle cannot follow the instantaneous field, instead it integrates the force over many cycles. The result is not only centering and levitation, but also a comparatively slow rotation (limited by viscous forces) in response to particular frequency bands.

It can be seen from Fig. 8 that the rotation stops as soon as frequencies giving positive dielectrophoresis are used, which makes some of the rotation peaks appear sharper than in chambers designed to give a homogeneous rotating field. This is a consequence of the attraction of the particles to the electrodes. This combination of forces can cause very small differences in particle properties to give very large differences in spectra, as demonstrated by the measurements on *P. sylvestris* (lower part of Fig. 6).

3. Combination of alternating and rotating fields

Clearly we can use an alternating field to support (center and levitate) the cell, so that the rotation spectrum can be measured without interference from positive dielectrophoresis. Methods of combining the fields that suggest themselves are:

(a) Simultaneous application of the fields. Each field is applied through its own set of 4 electrodes of an 8-electrode chamber.

(b) Simultaneous application, but using a frequency-selective filter to combine the two sets of driving voltages (which must have distinct frequency ranges).

(c) Sequential application of the (quadrupole or octupole) alternating field with a similar rotating field to a *single* set of electrodes, by means of a rapid 4- or 8-pole switch.

(d) Sequential application by amplitude modulation of both fields.

Preliminary measurements using an 8-electrode chamber and a support field of 144 MHz have shown that possibility (a) requires exceptionally careful adjustment of the high-frequency properties of the chamber, otherwise one or other electrode voltage is disturbed by the presence of a neighbouring electrode.

The use of two levitation arrays of planar electrodes, stacked only so far apart that their effective fields touch (about 200 μm for the present chambers) is a very interesting possibility. This arrangement would permit a fully closed cage to be developed between the two planes, which would be effective for particles with positive or negative buoyancy, and also relatively independent of orientation or of gravity. It has the considerable advantage over a single set of electrodes that increasing the field strength causes the particle(s) inside to be more tightly trapped. Further, use of one of the multiplexing possibilities discussed above allows the cells to be turned about any axis or driven in any given direction.

Discussion

The production of electrode arrays with the same dimensions as cells, or even smaller, allows the use of inhomogeneous fields for a wide variety of cell manipulations. If negative dielectrophoresis is used, no feedback electronics are necessary in order to keep the cells stably suspended, in contrast to earlier methods of cellular levitation [4–7].

The necessary frequencies can be found for almost all cells, or else changes can be made to the conductiv-

ity or permittivity [34,35,38,40] of the suspending medium to create suitable frequency ranges. For biological particles the frequency range above 50 MHz is particularly useful. To use this region it is necessary that the permittivity inside the particles is less than that of the medium, so that regions of minimum field strength can be used to collect them. The opposite case, the collection of more polarizable particles at a field maximum, is only possible on electrode surfaces, which often results in loss of viability.

In order to allow all cells to be collected, it is therefore desirable to increase the permittivity of the medium. This will also have the advantage of increasing the field-induced forces in all cases (see Fig. 1B). Although the majority of solutes decrease the permittivity of water, there are some (amino acids, peptides and proteins) that can increase it [34,35,38,40].

The measurements with 1.5 M glycylglycine in water (relative permittivity 182) showed an increase in levitation height of the particle, showing that the force is indeed increased. The frequency spectra (Fig. 6) can be understood on the basis of the properties of a homogeneous particle. Thus, in the kHz-region the properties are dominated by the conductivities, and the effect of doubling the external permittivity is to simply double the force. At higher frequencies (higher MHz region) the permittivities determine the direction and magnitude of the force, so that not only the doubling of external permittivity comes into play, but also the now much increased permittivity difference.

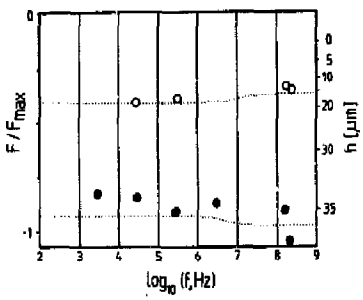

Fig. 9. The frequency-dependence of the calculated dielectrophoretic force (F/F_{max}, continuous line) and of the measured height of ascent (h) of cellulose-disulphate beads two media: ○, water (DK 80); ●, 1.5 M glycylglycine in water (DK 183). The beads were modelled as dielectrically homogeneous spheres. Their porosity results in their DK values changing with that of the medium. In accordance with the impedance data presented in Methods they were given DK values of 69 (in water) and 163 (in glycylglycine). The other parameters were: particle radius 50 µm, particle conductivity: 44.5 mS/m, medium conductivity 50 mS/m. The h axis is expanded towards higher values of h, because as h is increased small changes in h reflect ever larger changes in the dielectrophoretic force (see legend to Fig. 7B).

Fig. 9 shows that it is indeed possible to increase the dielectrophoretic force several times over. It is certain that additives of this form are also useful for cells. There is also the possibility that the dielectric properties of the cell can be changed by adhesion of substances to the cell surface or increase of the internal concentrations. Use of the specific chemical affinities of cell surface receptors represents a new departure in electro-manipulation, although the connection with microstructures has been suggested previously [30,31].

Conclusion

Field traps can be produced above planar electrodes, whilst true cages can be produced by means of 3-dimensional arrangements. The particular advantage of the methods used here to produce the microstructures is the exact reproducibility of the electrode form down to the sub-micrometer range. This allows fields of greater or lesser inhomogeneity to be created, so that the structures can be applied to the widest range of cells and particles. In addition, the possibility of previewing the electrode form at the design stage allows optimisation and flexibility in the response to particular applications.

It is necessary to point out that the small size of, and the negative dielectrophoretic force within, these traps means that it may be difficult to bring cells into them. However, it seems likely that this problem can be solved by the use of further microstructures which induce cell transport by means of travelling waves [32,41,42]. It is therefore possible to control the cell movement automatically, since only electrical signals are required. The combination of linear and circular electrode arrays allows the development of manipulation- and measurement-chambers.

The range of possible applications of this new technique is wide:

(a) transport and confinement of cells in free suspension;

(b) arrangement of suspended cells into groups in order to permit observation of individual cells;

(c) long-term cultivation under electrical field influence;

(d) filters for cells and particles.

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