Protoplast Dielectrophoresis in Axisymmetric Fields

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Protoplasts, Dielectrophoresis, Axisymmetric Fields

Pea protoplasts dielectrophoretic coefficients were measured in alternating electric fields of frequency 1 MHz and voltage 5 V applied between two concentric cylindrical metal electrodes of outer and inner radii 0.24 mm and 1 mm, respectively. They do not vary significantly with solution osmolarity, but show a clear expressed maximum in isotonic conditions; the values in 0.4 M, 0.5 M, 0.6 M and 0.7 M manitol solutions are $(6.5, 10.2, 8.8 \text{ and } 5.6) \times 10^{-24}$ A^2s^4/kg, respectively. The average cell radii in those conditions are 14.2 μm, 14.6 μm, 13.8 μm and 13.6 μm. The radii of cell-to-electrode contacts follow the same dependence on the osmolarity as the dielectrophoretic coefficients; they do not depend on the applied voltages up to 18 V. The times of cell approach near to the electrode were too short to be explained by the action only of the dielectrophoretic force; consequently an attractive force appeared at very close approach. These results may be of use in future studies of membrane adhesion and fusion.

Introduction

Motion of cells in nonuniform electric field, called dielectrophoresis [1], has many applications in biology and biotechnology. Cell sorting [1] and electrofusion [2, 3] are two important examples.

Protoplasts are frequently used in cell electrofusion (see, *e.g.*, the review [2]) because due to their large dimensions, they can be observed very well under light microscope. This is especially important when measuring the cell adhesion and fusion kinetics. In addition, protoplasts have important application in genetic engineering of plants.

Recently, we have constructed a dielectrophoretic device, where a nonuniform electric field was created between two concentrical electrodes [4]. The basic advantage of this device is that due to the simple axisymmetric distribution of the electric field, it allows calculation of the dielectrophoretic force by measuring the dielectrophoretic velocities. This method gave dielectrophoretic coefficients of the order of 10^{-25} A^2s^4/kg for human red blood cells [5]. Another advantage of this method and of the method of rotation [6, 7] compared with other methods for investigations of the effective dieletric properties of cells, *e.g.*, by measuring the optical density of cell suspension [8] is that it opperates with single cells.

These measurements were undertaken because of two main reasons:

1. To compare the polarization properties of another type of cells, in particular plant ones, to those of red blood cells. This is part of our programme to investigate the dielectrophoretic behavior of a number of different types of cells.

2. To understand what are the kinetic mechanisms of dielectrophoretically induced cell adhesion to the electrode. The protoplasts are appropriate because of their large diameter.

The ratio of the dielectrophoretic coefficients to the cell volume is the specific effective cell polarization and characterizes the polarization and conductivity of the cell material.

High specific polarizations lead to very close surface approach, respectively to cell adhesion and will facilitate membrane fusion induced by DC pulses [2]. The measurements were carried out in solutions of different osmolarities in order to change the membrane tension, respectively, membrane deformability and cell volume. Those effects are important in kinetics of cell adhesion and in cell polarization.

In this paper we present the first experimental measurements of the protoplast dielectrophoretic coefficients. Some data on dielectrophoretically induced cell adhesion are also shown and discussed.

Materials and Methods

We used a technique for isolation of pea protoplasts similar to that of Hampp and Ziegler [9]. Briefly the isolation is: the leaves were divided into 2 mm strips, which were left in solution of 0.4 M mannitol. 1 mM $CaCl_2$ and 1% cellulase "Onozuka

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R-10" for 5 h. After filtration, the protoplast fraction was centrifuged for 5 min at 200 × *g*. After that they were resuspended in 2 ml of a 0.5 M sucrose solution and 2 ml of a 0.5 M mannitol solution and centrifuged for 10 min at 200 × *g*. The protoplast fraction from boundary layer was washed in 7 ml of a 0.5 M mannitol solution and immediately used for the experiments.

The dielectrophoretic measurements were performed in 0.4 M, 0.5 M, 0.6 M and 0.7 M solutions of D-mannitol from Fluka (M.w. = 182.18). In order to avoid protoplast sedimentation 5% Ficoll (Pharmacia – Sweden) to the mannitol solution was added. Because of the very high molecular weight of the Ficoll (M.w. = 400000) the solution osmolarity does not change appreciably.

The solution viscosity was (3.25, 3.41, 3.63 and 3.87) × 10^{-3} Pa.s, while cell suspension conductivity was (21, 22, 20 and 22) × 10^{-6} S/cm for 0.4 M, 0.5 M, 0.6 M and 0.7 M mannitol concentrations (the solution also contains 5% Ficoll).

The dielectrophoretic device is described in a previous work [4] (see also [5]). Briefly, a platinum wire of radius R_i = 0.24 mm is placed in a hollow metal cylinder (covered with Ni layer) of inner radius 1 mm, outer radius 2 cm and height 5 mm. The remaining part of the device is made from plexiglas. The cell velocities are observed with a phase contrast microscope having a calibrated reticule. We used a sinoidal a c field of 1 MHz frequency and 5V voltage.

The method of calculating dielectrophoretic coefficients from observed velocities is described in our previous work [5]. Briefly, the times of cell passing through certain distances were measured. Then the Stokes' formula was used to evaluate the force F through cell velocities. The dielectrophoretic coefficients α are then calculated by the relation

$$F = -\alpha \nabla (E^2)/2 = \alpha (U^2/r^3)/\ln^2(R_o/R_i),$$

where ∇ is nabla operator, E the electric field intensity, U the applied voltage and r the radial distance.

Results and Discussion

Dielectrophoretic coefficients

Table 1 shows the dielectrophoretic coefficients α for different protoplasts in solutions of different mannitol concentrations. It should be noted that all the cells (10 for each solutions) are different; it is not possible to measure the dielectrophoretic coefficients for one and the same cell in different solutions.

The accuracy of measuring α and R_c is 5 × 10^{-25} A^2s^4/kg and 0.3 μm, respectively. The standard deviation for a population of cells △ α = 1 × 10^{-24} A^2s^4/kg is rather high, due most probably to different cell radii.

As is seen from Table I the dielectrophoretic coefficients for a stohastic cell population are maximum for isotonic solutions (0.5 M mannitol). The maximum for the cell polarization, which can be

Table I. Dielectrophoretic coefficients α × 10^{24} A^2s^4/kg and α/V × 10^{10} $A^2s^4/kg\ m^3$ for cells of radii R_c × 10^6 m.

0.4 M		0.5 M		0.6 M		0.7 M	
α	R_c	α	R_c	α	R_c	α	R_c
7.6	15.0	3.5	15.0	9.8	13.0	11.5	14.0
12.3	14.0	7.0	15.0	8.9	14.0	3.1	12.5
2.0	12.5	5.2	15.0	5.8	14.0	3.5	14.0
6.7	15.0	14.7	15.0	14.3	15.0	3.9	12.5
9.7	14.0	17.9	15.0	14.0	15.0	6.7	14.0
4.9	15.0	8.7	15.0	11.2	12.5	10.1	12.5
6.9	15.0	12.2	15.0	7.6	15.0	4.3	12.5
3.9	12.5	14.2	12.5	6.0	15.0	5.2	15.0
3.1	15.0	6.6	14.0	6.0	12.5	4.8	15.0
7.8	14.0	11.5	15.0	4.5	12.5	2.8	14.0
$\bar{\alpha}$	$\bar{R}_c$	$\bar{\alpha}$	$\bar{R}_c$	$\bar{\alpha}$	$\bar{R}_c$	$\bar{\alpha}$	$\bar{R}_c$
6.5 ± 1.0	14.2 ± 0.3	10.2 ± 1.4	14.6 ± 0.3	8.8 ± 1.1	13.8 ± 0.3	5.6 ± 0.9	13.6 ± 0.3
$(\overline{\alpha/V})$		$(\bar{\alpha}/V)$		$(\bar{\alpha}/V)$		$(\bar{\alpha}/V)$	
5.4 ± 0.8		7.1 ± 1.6		8.2 ± 1.0		5.5 ± 1.0	

represented as α/V (V – cell volume) is, however, shifted to higher mannitol concentrations (0.6 M). It is difficult to establish whether this shift is due to unproper choise of cells to measure or to other reasons. In addition, it is not known whether the dielectrophoretic coefficients change is due only to volume changes, or also to some structural reorganizations. One possible way to partly answer this question is to use only cells with identical radii. The internal differences in structure, however, are dominant and do not allow such comparison. The best way is to use one and the same cell in different solutions. At this stage, however, this is not possible, due to technical difficulties.

The protoplasts dielectrophoretic coefficients are in average about two orders of magnitude larger than those for human red blood cells. The protoplasts volumes are, however, also about 100 times larger than erythrocytes ones. Consequently, the polarization of both types of cells is of the same order of magnitude.

Rate of close approach

Table II shows the experimentally measured times (t_{exp}) for a protoplast to "touch" the electrode starting at a distance of 15 μm from the electrode surface compared to those calculated theoretically. By "touching" we denote the moment when the nearest to the electrode surface part of the protoplast surface is not visually distinguishable from the electrode. It should be pointed out that in most cases up to this moment the protoplast surface preserves its spherical shape. After that moment the protoplast membrane deforms very quickly and the final equilibrium state of adhesion, characterized geometrically by its radius of contact, is formed.

The times (t_{theor}) are calculated by using an interpolation formula [10] valid for the rate of approach of a sphere to a plane solid surface

$$-dr/dt = Fh/6\,\pi\,\mu R_c^2(1 + h/R_c),$$

where t is the time, μ is medium viscosity and $h = r - R_i$ is the separation distance.

It is seen from Table II that in all cases the theoretically calculated times (t_{theor}) are more than one order of magnitude longer than the experimentally measured ones (t_{exp}). This means that at close approach ($h \sim R_c$) the driving force F increase may be due to additional electrostatic interactions, due to electrode surface non-homogenities and/or polarization of material adsorbed on the electrode, as well as additional effects not taken into account by the theory of dielectrophoresis.

Radii of contact

Figure 1 shows a protoplast adhered to the electrode surface. It is seen that the radius of contact is

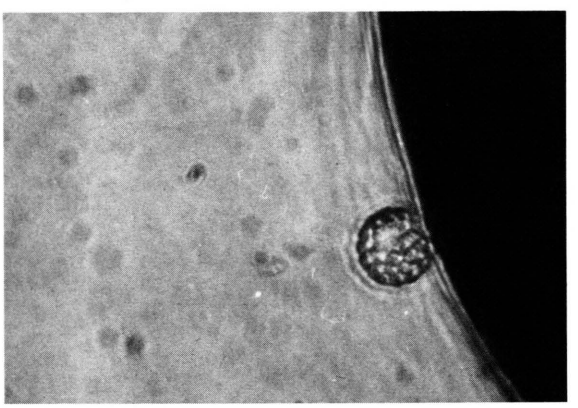

Table II. Times of approach at close separations – t_{theor}(s) and t_{exp}(s).

0.4 M			0.5 M			0.6 M			0.7 M		
R_c[μm]	t_{theor}	t_{exp}	R_c[μm]	t_{theor}	t_{exp}	R_c[μm]	t_{theor}	t_{exp}	R_c[μm]	t_{theor}	t_{exp}
15.0	11.0	1.2	15.0	46.8	1.2	13.0	13.3	1.8	14.0	14.0	1.0
14.0	53.1	2.4	15.0	23.4	6.9	14.0	16.9	3.2	12.5	40.7	2.8
12.5	23.3	2.0	15.0	31.8	1.6	14.0	25.9	1.0	14.0	45.9	1.8
15.0	13.9	1.2	15.0	11.2	1.0	15.0	12.2	0.8	12.5	32.6	1.8
14.0	31.9	3.0	15.0	9.2	0.8	15.0	12.4	1.0	14.0	24.1	2.1
15.0	22.7	3.8	15.0	18.9	1.0	12.5	10.6	1.0	12.5	12.6	1.2
15.0	26.8	2.8	15.0	13.4	1.6	15.0	23.1	1.2	12.5	29.2	2.2
12.5	50.3	2.0	12.5	7.9	1.2	15.0	28.9	2.3	15.0	36.1	2.0
15.0	17.3	2.0	14.0	21.5	1.1	12.5	19.7	3.1	15.0	39.1	2.6
14.0	30.1	3.4	15.0	14.3	1.6	12.5	26.5	2.0	14.0	57.9	3.0

Table III. Radii of contact $R \times 10^6$(m) and contact angles θ for cells of radii $R_c \times 10^6$(m).

0.4 M				0.5 M				0.6 M				0.7 M			
R_c	R	R/R_c	sinθ	R_c	R	R/R_c	sinθ	R_c	R	R/R_c	sinθ	R_c	R	R/R_c	sinθ
15.0	13.5	0.90	0.86	15.2	9.8	0.65	0.65	14.0	10.3	0.74	0.77	11.5	6.1	0.53	0.53
16.0	11.0	0.69	0.68	15.2	7.9	0.52	0.55	13.0	9.4	0.73	0.77	11.5	5.1	0.44	0.44
15.0	14.2	0.95	0.94	16.0	8.2	0.51	0.52	15.3	12.6	0.95	0.92	12.9	7.2	0.56	0.57
17.5	8.5	0.49	0.48	16.0	8.7	0.54	0.54	13.3	7.9	0.59	0.61	12.9	8.6	0.66	0.67
12.5	7.6	0.61	0.64	16.0	11.2	0.70	0.70	14.2	12.3	0.86	0.82	15.0	6.8	0.45	0.49
16.2	6.1	0.38	0.37	16.0	8.2	0.50	0.55	12.5	8.5	0.68	0.63	15.0	7.6	0.51	0.57
18.7	13.7	0.73	0.74	17.0	9.2	0.54	0.55	15.0	9.1	0.61	0.67	13.3	7.8	0.59	0.55
14.0	8.2	0.59	0.58	17.0	8.8	0.52	0.55	13.7	10.3	0.75	0.76	13.3	7.2	0.54	0.55
16.2	8.0	0.49	0.45	15.0	9.8	0.65	0.64	15.0	11.6	0.77	0.76	14.8	10.6	0.71	0.74
14.0	8.1	0.58	0.58	16.0	9.2	0.58	0.59	15.0	12.3	0.82	0.83	14.8	10.8	0.73	0.74

well seen and can be measured with good accuracy. Table III shows measured radii of contact R and macroscopic contact angles θ in solutions of different osmolarity.

It must be pointed out that the macroscopic angles on both sides are different; the values shown in Table III are average. The difference is, however, less than 10% in all cases.

The results have shown that the contact radii depend on the osmolarity in the same manner as the dielectrophoretic coefficients α. Our initial idea was to use the formula for radius of contact in the form [11]:

$$R^2 = FR_c/2\,\pi\,T,$$

in order to evaluate the membrane tension T. As was shown, in the previous section the force F is strongly increased at close approach and generally not known. In spite of this, if we assume that F is 10 times increased compared to that given by the dielectrophoresis theory (10 times because of the 10 time increase in approach times), then the calculated membrane tensions for 0.4 M, 0.5 M, 0.6 M and 0.7 M mannitol solutions are (1.3, 2.7, 1.8 and 1.6) $\times 10^{-1}$ mN/m. These are reasonable values, but probably also reflect elastic resistence due to internal structures.

Another interesting observation following from Table III is that the ratio R/R_c is close to sinθ *i.e.* the protoplast surface is part of sphere. This is rather strange because the membrane deformation should change the spherical shape if the volume is constant. This question needs future theoretical considerations.

The radii of contact and the contact angles do not depend on the applied voltage up to 18 V. If the voltage is further increased (up to 50 V) the protoplast deforms, the contact angle changes, but the contact radius remains the same. In addition, if the field is turned off, the protoplasts do not detach.

These results show that in dielectrophoretically induced adhesion in these conditions short – and long – range intermolecular forces begin to play a determining role after certain moment. Hence the radii of contact do not further depend on the electrical field intensity.

The protoplast polarization (Table I) compared to erytrocytes polarization [5] is high. This may be due to the very high water content of the vacuoles of the protoplasts. This idea may be tested by measuring the specific polarization of vacuoles and comparing it with the polarization of evacuolated protoplasts.

We do feel that the rigorous and detailed interpretation of these and other results needs careful theoretical analysis.

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