Cell Fission and Formation of Mini Cell Bodies by High Frequency Alternating Electric Field

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ABSTRACT We report the use of high frequency alternating electric fields (AC) to induce deformation of sea urchin eggs, leading to budding of membrane vesicles or fission of cells. Several mini cell bodies can be prepared from a single egg by carefully manipulating the frequency and amplitude of the AC field and the ratio between the interelectrode spacing and the cell diameter, α. α values between 2.2 and 3.5 have been found to be optimal for inducing fission of sea urchin eggs. In a typical experiment, a sea urchin egg (diameter = 75 μm), suspended in a low ionic medium (conductance < 2 mS/m), was located under the microscope between two platinum wire electrodes, separated by a distance of ≈200 μm. A medium strength AC field (<100 V/cm at 2 MHz) was applied to attract the egg to one of the two electrodes via dielectrophoresis. This process took place in a few seconds. The voltage was then slowly increased to ≈1000 V/cm over ≈30 s. The cell elongated and separated into two fragments, the larger one containing the nucleus. When the field was turned off, the mother cell and the daughter vesicle retracted to form spherical mini cell bodies that appear to be stable as assessed by the absence of swelling for the duration of the experiment (≈15 min). This indicates that membranes of these mini cell bodies were not leaky to ions and small molecules. This procedure could be repeated a few times to make several mini cell bodies from a single egg. With practice, several mini cell bodies could also be prepared in a single fission experiment by adjusting the field parameters and the α value. Cell fission is a result of the mechanical stress produced by the AC field. These procedures may be used to prepare mini membrane vesicles for voltage clamp experiments or to perform microsurgical manipulation of cells, embryos, or chromosomes.

INTRODUCTION

Exposure of living cells to oscillating electric fields (AC) can produce many chemical or morphological changes in cells, as diverse as the alignment of cells in chains as a result of electric polarization, translational and rotational motions of cells as a result of dielectrophoresis, cell deformation and shape changes as a result of sheer stresses, induction of membrane pores and membrane fusion as a result of field-induced transmembrane potential, and destruction or lysis of cells as a result of charge repulsion or colloidal osmosis, for example (Cole, 1972; Pohl, 1978; Pliquett, 1968; Foster et al., 1992; Neumann et al., 1989; Zimmermann, 1982; Stenger et al., 1991; Dimitrov et al., 1990; Gass and Chernomordik, 1990; Weaver, 1994; Teckle et al., 1991; Mahaworasilpa et al., 1994; Tsong, 1991). Membrane channels and enzymes may also be activated by the field-enforced conformational oscillations (Tsong and Astumian, 1986; Liu et al., 1990; Tsong, 1992). All of these phenomena are produced by the effects of the electric field. There are other effects that are the result of the current, i.e., the electric energy in the form of Joule heating. Among the known thermal effects are shift in chemical equilibrium, enhancement of reaction rate, and increased membrane fluidity, thermal agitation, and molecular motion (Kinosita and Tsong, 1977; Tsong, 1991). In this communication, we report the splitting of cells into two or several mini cell bodies by exposing cells to alternating electric fields of particular strength and frequency. To our knowledge, this is the first time such a phenomenon has been studied and reported. Previously, heating has been shown to cause protein-free lipid vesicles to deform and, in severe cases, lead to the budding of small vesicles from larger vesicles or to fragmentation of larger vesicles into two or more similar size small vesicles (Dobereiner et al., 1993). However, these thermally induced membrane processes were observed only at high temperatures, and a similar phenomenon has not been shown to occur for cell membranes. These thermally induced membrane processes cannot be manually controlled, and high temperatures will cause denaturation of cellular proteins, DNA, RNA, and supramolecular structures. Thus, the process is unsuitable for cell fission or for the micromanipulation of cells. As shown in this communication, electric manipulation can be done at low temperatures, i.e., <25°C, and has a distinct advantage for studying membrane mechanical strength and for some potential applications.

MATERIALS AND METHODS

To simplify cell manipulation and microscopic observation, we have used sea urchin eggs. Sea urchin eggs are larger than most cells (average diameter, 75 μm) and they are in most cases spherical. Any morphological change can be easily visualized. Sea urchin eggs were harvested from *Arbacia punctulata* by electric stimulation with 12 V/cm AC at 60 Hz. These eggs were washed with and suspended in artificial seawater (Instant Ocean Aquarium Systems, Mentor, OH) that was filtered through a 0.45-μm Millipore filter and supplemented with 10 mM Tris/HCl buffer at pH 8.2. To begin an experiment, the eggs were transferred to and washed three times with an

Received for publication 11 October 1994 and in final form 4 January 1995.

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0006-3495/95/04/1218/04 $2.00

isotonic glucose (0.95 molal) by centrifugation at 200 rpm for 3 min. This procedure reduced the conductivity of the egg suspension from ≈4 S/m (in artificial seawater) to 1–2 mS/m. Low conductivity prevented the sample from severe Joule heating during the experiments with high intensity AC fields. In most cases, experiments were completed in less than 20 min.

To study deformation and fission of single eggs, eggs were mounted on a glass slide and inspected under an inverted microscope (Carl Zeiss, Thornwood, NY). A Wavetek model 148A AC generator that can produce AC fields of various wave forms of up to 20 MHz was used to produce oscillating electric fields for the experiment. Two platinum wires (diameter, 1/32 inch) separated by 150–500 μm were mounted on the glass slide to serve as electrodes. The video system (NC-70 Newvicon camera, Digital Image Processor DSP-200, high resolution monitor HR 1000 Dage-MTI, Michigan City, IN; HR-S5800U VCR, JVC, Japan) was used to record events in real time, and photographs were taken either directly from the microscope during an experiment or from the monitor during later viewing. The voltage and field strength are expressed in peak-to-peak values.

RESULTS

Electric field-induced cell deformation and fission

Preliminary experiments verified that there is a frequency window for the electric field to induce deformation of the sea urchin eggs between 1 and 3 MHz. This value agrees with the predictions of recent electrorheological models (Engelhardt and Sackmann, 1988; Poznanski et al., 1992). At higher frequencies, the susceptibility of a cell to deformation decreased dramatically and, at lower frequencies, the probability of cell electrodestruction increased abruptly.

Fig.1 shows the AC-induced deformation of a sea urchin egg. The egg was exposed to an AC of 2 MHz, with increasing field strength. The frequency of the field, 2 MHz, was chosen as AC at this frequency was previously determined to exert the maximal mechanical force. At first, an egg was brought into contact with one of the electrodes by dielectrophoresis with a low amplitude AC (50–100 V/cm). At this stage, the shape of the egg showed little sign of deformation. This step took less than 20 s and, fission of cells never occurred during the manipulation. The amplitude of the AC was then gradually tuned up to ≈1000 V/cm. As the field strength was increased, the egg began to deform, changing its shape from spherical to ellipsoidal, with the longer axis paralleling the electric field direction (frames *b* and *c*). In frame *d*, the egg elongated to reach both electrodes, the distance between them being 175 μm. The field strength was 1095 V/cm at the electrode surface and 762 V/cm in the middle of the interelectrode space. When the electric field was removed shortly after the egg had formed the bridge, the egg retracted to its original spherical shape. The egg appeared to be stable, and the same exercise could be repeated several times. No swelling was detected for the cell during the period of the experiment, ≈15–20 min. The interelectrode space/cell diameter ratio, α, is a crucial parameter to observe. In this case, α was 2.36. The choice of α value would depend on the mechanical properties of a cell type. For sea urchin eggs, we have found the values between 2.2 and 3.5 to be optimal.

This field-induced deformation of a cell can be used to dissect a cell into fragments. Fig. 2 presents a case for which α was increased to 2.64 to facilitate the fragmentation of

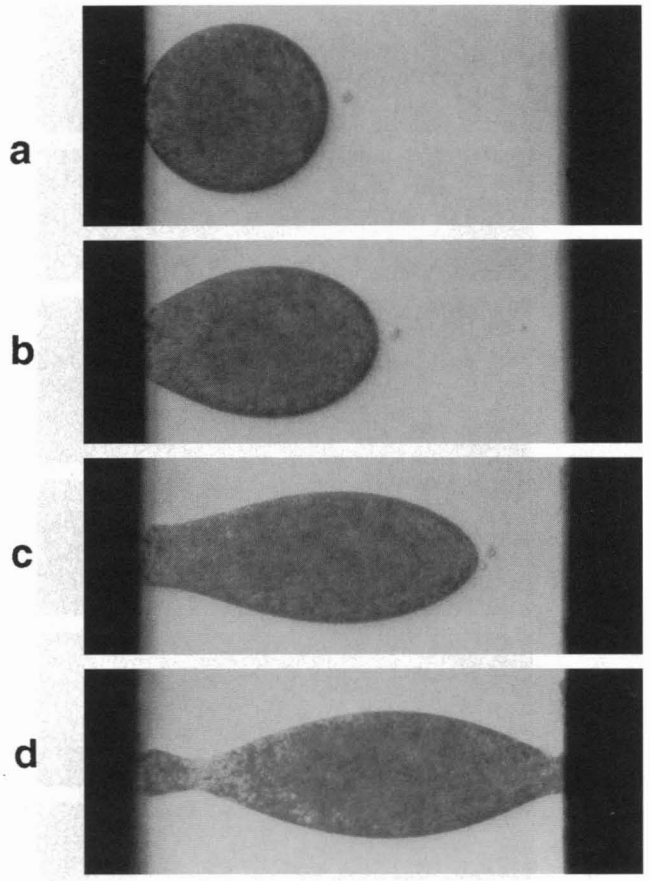

FIGURE 1 Global deformation of *Arbacia puntulata* egg under the AC electric field of 2 MHz. The egg diameter d = 75 μm; the space between the two electrodes l = 175 μm; $\alpha = l/d = 2.36$. The electric field strength was gradually increased from 0 to 1095 V/cm at the electrode surface. Cell deformation was recorded at a time interval of ≈30 s.

cells. The increased space between the electrodes helped to split the cell because of the excessive cell stress due to elongation by the electric field (frames *a* and *b*). After the first cell fission, the field was turned off. Both the mother cell, which contained the whole nucleus, and the daughter vesicle, which contained cytoplasmic fluid and some organelles, retracted to become spherical. Like the previous case, there was no evidence of membrane leakiness in these two mini cell bodies; no colloidal osmotic swelling was observed for as long as 20 min, and the combined volume of the two cell bodies remained constant, i.e., equal to the volume of the original egg. A cell with leaky membranes would swell because of the colloidal osmotic pressure of the cytoplasmic macromolecule (Tsong, 1991; Kinosita and Tsong, 1977). These mini cells were initially connected to neighboring bodies with thin threads of membranes that broke when these mini cell bodies retracted to resume a spherical shape. These thin threads of membranes may contain F-actin, as was shown in a previous study on field-induced membrane protrusions (Gass et al., 1991; Popov et al., 1991). The retraction was due to the surface tension of the cell membrane. When the field was turned on again, the mother cell began to deform and underwent a second fission (frames *d–f*). This process

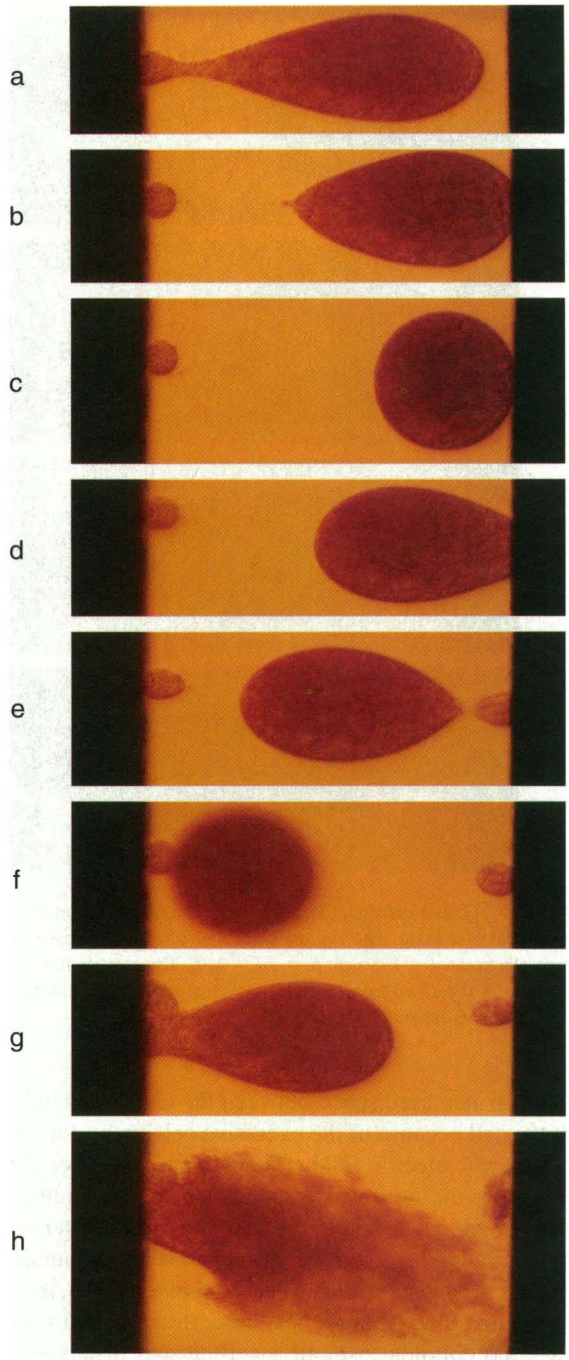

FIGURE 2 Fission of *Arbacia* egg under the AC electric field of 2 MHz. $\alpha = 2.64$. The AC was terminated immediately after the cell split into two bodies (frames *b* and *c*). Electric field strength was increased again (frames *d* and *e*) until the mother cell split a second time. In frame *f*, the egg went out of focus as a result of the gravity but was brought to the electrode by dielectrophoresis and subjected to the AC deformation (frame *g*). In frame *h*, the egg is shown to be completely ruptured by the AC field.

could be repeated several times. Smaller vesicles were more immune to deformation during these manipulations. When the field exceeded a critical value or a cell became unstable after several exposures to an AC field, the mother cell could lyse and become completely fragmented, as shown in frame *h*. The fuzziness of frame *f* simply reflects that the cell was out of focus.

Fission of cells in the absence of electric fields

Fission of severely deformed cells in the absence of electric fields or of the membrane-applied forces showed different properties. Fig. 3 presents such an example. Here a field-deformed cell is shown to split into several mini cell bodies during the membrane relaxation process after the field was turned off. For this process to occur, the cell must be secured tightly to both electrodes when the electric field is turned off. The experiment was performed first by properly manipulating the applied electric field to pull the cell to form an elongated rod-like bridge between the two electrodes. The cell was then tightly bound to the two electrodes. The electric field was then turned off, and the cell began to split into several fragments during the relaxation process. In photo-

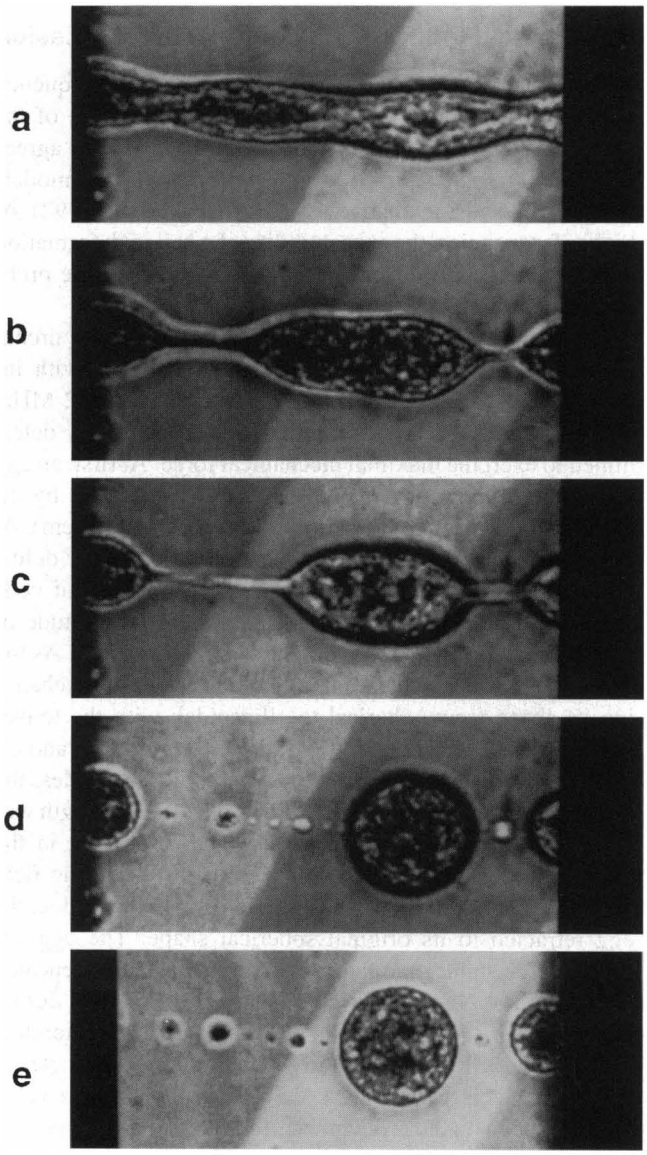

FIGURE 3 Fission of *Arbacia* egg into several mini cell bodies with the AC electric field of 2 MHz. Electrode spacing was 240 μm and $\alpha = 3.24$. After the procedure outlined in Figs. 1 and 2, the electric field was turned off at frame *a*. The severely elongated cell began to retract. Frames *b–e* show the sequence of events, at a time interval of 15 s. Several mini cell bodies were produced from a single egg.

graph a, a cell formed a bridge between the two electrodes separated by 240 μm by the applied AC field. The field was then turned off. The cell began to relax, or retract. In b, two constricted areas appeared. The relaxation continued and, in c, a central body took shape. The constricted area began to thin down to become tubular-shaped connecting mini cell bodies. In d, these connecting tubules broke down, and several mini cell bodies of different sizes were formed. From these photos, it was not possible to judge whether the cytoplasmic content of the cell was also evenly partitioned into these mini cell bodies or whether the nucleus was split into fragments for the larger mini bodies.

DISCUSSION

The data shown above are observations done with single cells. Many cells were undergoing deformations and fissions simultaneously. However, each cell behaved differently. We have not been able to obtain uniform fission of a quantity of cells in a single experiment. This is not surprising as not all cells are uniform in size, age, and stage of development. Properly designed instrumentation and a more thorough evaluation of electric parameters and α value would be required to use this method for large scale treatment of cells to obtain uniform results. At the present time, the method is best for single cell manipulation.

High frequency AC fields have been used to investigate rheological properties of living cells (Furedi and Ohad, 1964; Engelhardt and Sackmann, 1988; Marszalek et al., 1989; Stenger et al., 1991; Poznanski et al., 1992). Global deformation, membrane protrusion, and rupture of cell membranes occur because of mechanical force produced by the AC fields (Evans, 1983; Waugh, 1987; Kaler et al., 1992; Zhelev and Needham, 1994; Kozlov et al., 1988). The use of an AC field to split cells into smaller mini cell bodies or vesicles presents an interesting phenomenon for studying elasticity and mechanical strength of cell membranes. The methods may be applied to preparation of membrane vesicles for channel measurement, to excise selected parts of a cell in developmental research, or to perform microsurgery on neurons or tissue.

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