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RESEARCH ARTICLE

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Detection of the apoptosis of Jurkat cell using an electrorotation chip

Abstract The apoptosis of cells is one of the fields that attract increasing attention in biology today. Usually, the cells are treated with chemicals when detecting apoptosis. It is highly desired to detect apoptosis in a real-time basis. Apoptosis of Jurkat cells was studied using a real-time electrorotation chip. This chip allows the detection of the cell membrane capacitance changes during the course of apoptosis and therefore facilitates the analysis of apoptosis in a real-time basis without involving any chemical treatment.

Keywords apoptosis, electrorotation chip, membrane capacitance

1 Introduction

Cell apoptosis is the programmed cell death decided by gene. Cell apoptosis is one of the fields that attract increasing attention in biology today. As a highly modulated phenomenon, it attracts many biochemists and molecular biologists to research the mechanism controlling cell apoptosis (Gorman et al., 1997). There are varied methods examining cell apoptosis, including morphological examination (e.g. transmission electron microscopy (TEM), scanning electron microscopy (SEM), and flow cytometry) and biochemistry characteristic assay (e.g. propidium iodide (PI) assay, annexin V assay, DNA agarose gel electrophoresis, DNA cleavage assay *in vivo*, and apoptosis protein enzyme (Caspase)-3 assay). But all of these methods need to carry out chemical processing to the cells, and failed to monitor the cell apoptosis in real time.

Electrorotation (ROT) is an emerging technique, which utilizes AC voltage signal to produce rotating electric field on the electrorotation chip. The cell will rotate with the rotating field. Cells having different dielectric properties will rotate in different modes. Therefore, making use of this characteristic, this technique can monitor the cell sensitively (Fuhr et al., 1987; Ameld and Zimmerman 1988; Wang et al., 1993; Zhou et al., 1995). Wang et al. (2002) studied the dielectric properties of HL-60 cells of earlier apoptosis (around the time of the phosphatidylserine externalizing of cell membrane) using dielectrophoresis (DEP) and concluded that the product of the DEP crossover frequency ($f_{\text{crossover}}$) and the cell radius (r) is a function of time, and the cell membrane capacitance (C_m) decreases with time. However, the dielectric properties in the middle and late phases of apoptosis are still unknown at present. This study researched the dielectric properties of Jurkat cells during apoptosis using ROT, computed the cell membrane capacity (C_m) and derived its variety rule. We concluded that cell apoptosis could be monitored in real time by measuring the membrane capacity using ROT method.

2 Materials and methods

2.1 Materials

Cytosine arabinoside and glucose were purchased from Sigma (U.S.). Sucrose was bought from JingXinKe (Beijing, China).

The isotonic working buffer was composed of 8.5% sucrose and 0.3% glucose. The electric conductivity of the buffer was adjusted with adequate PBS to 500 μS/cm, as determined by a conductivity meter (TDSTester, PAKTON Instruments, Vernon Hills, IL).

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2.2 Cells

Jurkat cells (human T-cell leukemia) were purchased from American Type Culture Collection (ATCC). The cells were incubated in a humidified incubator containing 5% CO_2 at 37℃. The RPMI 1 640 medium for the cell culture was supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/L streptomycin. To induce cell apoptosis, cytosine arabinoside was introduced to the cell suspension in the exponential phase at the concentration of 10 μmol/L. The cells were collected and prepared for ROT measure after the addition of cytosine arabinoside for 2, 8, 24 and 48 hours. To obtain dead cells as a reference, 1.0 mmol/L cytosine arabinoside was used. The cell viability was determined by 0.5 g/L trypan blue exclusion. Before performing electrorotation, the cell suspension was centrifuged and washed twice with working buffer.

2.3 Electrorotation and data processing

The electrorotation spectroscopy (the function of the cell rotation speed versus the frequency of the electric field) was obtained with a microfabricated ROT chip containing 4 microelectrodes (Fig. 1). The four sinusoidal voltage with cross and 90° phase shift, which are supplied by a self-made signal generator, are used to drive the ROT chip. Their frequency range is from 1 kHz to 20 MHz. To the cell chamber on ROT chip, 40~50μL cell suspension (2×10^4/mL) was introduced and the chamber was sealed with a glass slide. A microscope equipped with a CCD and a monitor was used to observe the cells. The rotation rates of cells located at the central region were measured at different frequencies with a stopwatch.

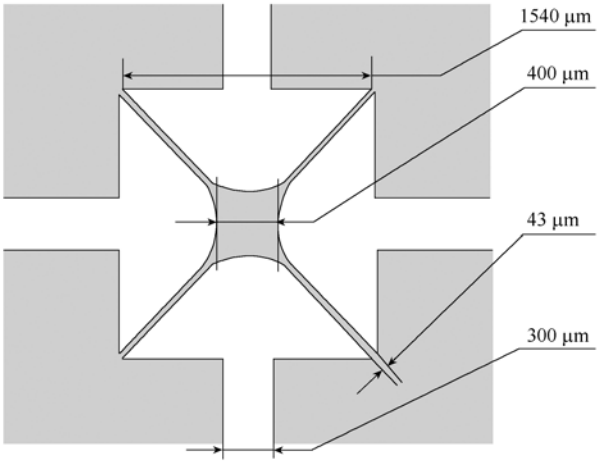

Fig. 1 The electrorotation chip

According to the single shell cell model (Sauer and Schoeg, 1985), the rotation rate of cells (R(ω))in a rotating electric field is the function of the field strength (E), the dielectric constant of the suspending medium (ω_e), the viscosity of the medium (ω), and the Clausius-Mossotti factor ($f(\omega)$) (Arnold and Zimmerman 1988)):

$$R(\omega) = -\frac{\varepsilon_e E^2}{2\eta}\mathrm{Im}(f(\omega)) \tag{1}$$

ω is the angular frequency of the applied field. Im($f(\omega)$) is the imaginary component of the Clausius-Mossotti factor defined as

$$f(\omega) = \frac{\varepsilon_e^* - \varepsilon_e^*}{\varepsilon_e^* + 2\varepsilon_e^*} = \frac{\sigma_e^* - \sigma_e^*}{\sigma_e^* + 2\sigma_e^*} \tag{2}$$

here ε_e^*、ε_e^*（σ_e^*、σ_e^*）are the complex permittivities (conductivities) of the cell and its suspending medium, respectively. The complex permittivity is given by

$$\varepsilon^* = \varepsilon - j\sigma/\omega$$

here ε、σ is the permittivity and conductivity, respectively. Im($f(\omega)$) can be simplified as

$$\mathrm{Im}(f(\omega)) = 3\omega\frac{\varepsilon_e\sigma_e - \varepsilon_e\sigma_e}{(\sigma_e + 2\sigma_e)^2 + \omega^2(\varepsilon_e + 2\varepsilon_e)^2} \tag{3}$$

And the complex permittivity of the cell is defined as (Wang et al., 1994)

$$\varepsilon_e^* = \varepsilon_m^*\frac{\left(\frac{r+d}{r}\right)^3 + 2\left(\frac{\varepsilon_{im}^* - \varepsilon_m^*}{\varepsilon_{im}^* + 2\varepsilon_m^*}\right)}{\left(\frac{r+d}{r}\right)^3 - \left(\frac{\varepsilon_{im}^* - \varepsilon_m^*}{\varepsilon_{im}^* + 2\varepsilon_m^*}\right)} \tag{4}$$

Here ε_m^*、ε_{int}^* are the complex permittivities of the cell membrane and interior, r is the radius of the cell and d is the thickness of the membrane.

Based on the foregoing mathematic model, an optimized parameter algorithm was used to determine the fittest of equations 1-4 to experimental data and thereby to provide estimates for the cellular dielectric properties. This was achieved by minimizing the sum of the squared deviations between experimental ROT rates, R_{exp}, and those predicted from estimated parameters, R_{cst}^-

$$\mathrm{Min}\sum_i [R_{cst}(\omega_i) - R_{exp}(\omega_i)]^2 \tag{5}$$

2.4 Scanning electron microscopy

Untreated Jurkat cells and those treated by cytosine arabinoside were centrifuged, washed twice with 1×PBS and rinsed with 2%~3% glutaraldehyde for 2 hours. The cells were washed and fixed with OsO_4 for 1~2 hours. After that, the cells were successively dehydrated with graded ethanol in the concentration series of 10%, 25%, 50%, 70%, 90% and 100%. Then, the cells were centrifuged and rinsed in 100% ethanol twice, rinsed in amyl acetate for 30 minutes, desiccated with CO_2, sputter-coated with a gold layer and examined in a scanning electron microscopy (JSM-5600LV, JEOL USA, Peabody, MA, USA).

3 Results

3.1 Electrorotation spectroscopy

Figure 2 is a typical ROT spectrum of untreated and treated cells obtained in the frequency range of 1 ~25 MHz. The points are the experimental electrorotation rates at different frequencies, and the solid lines are the best fits using single-shell dielectric model to the experimental data. Apparently, there are distinct differences among the untreated cells, the apoptosis cells, and the dead cells: the peak frequency (f_c) of the anti-field rotation of the apoptosis cells is low while the rotation rate is high; In contrast, the peak frequency (f_c) of the anti-field rotation of the dead cells is high while the rotation rate is low.

3.2 Cell membrane capacitance

Table 1 summarizes the theoretical curve fitting results for the membrane specific capacitance of both cytosine arabinoside-treated and untreated Jurkat cells. After treating for 2 hours, the membrane capacitance changed from 1.15 μF/cm^2 to 1.02 μF/cm^2, but the difference is not distinct (P>0.05). After treating for 8 hours, the membrane capacitance changed to 0.97 μF/cm^2, and there is distinct difference from untreated cells (P<0.02). The difference is more distinct with a longer time after treatment with cytosine arabinoside. The decreased tendency is more apparently demonstrated in Fig. 3.

Table 1 The membrane capacitance of untreated cells and the cells undergoing apoptosis ($\bar{x} \pm s$)and statistical analysis

Time / h	C_m/(μF•cm^{-2})	P
Normal cells (control)	1.15 ± 0.16	
2	1.02 ± 0.22	>0.05
8	0.97 ± 0.20	<0.02
24	0.93 ± 0.25	<0.01
48	0.87 ± 0.08	<0.005

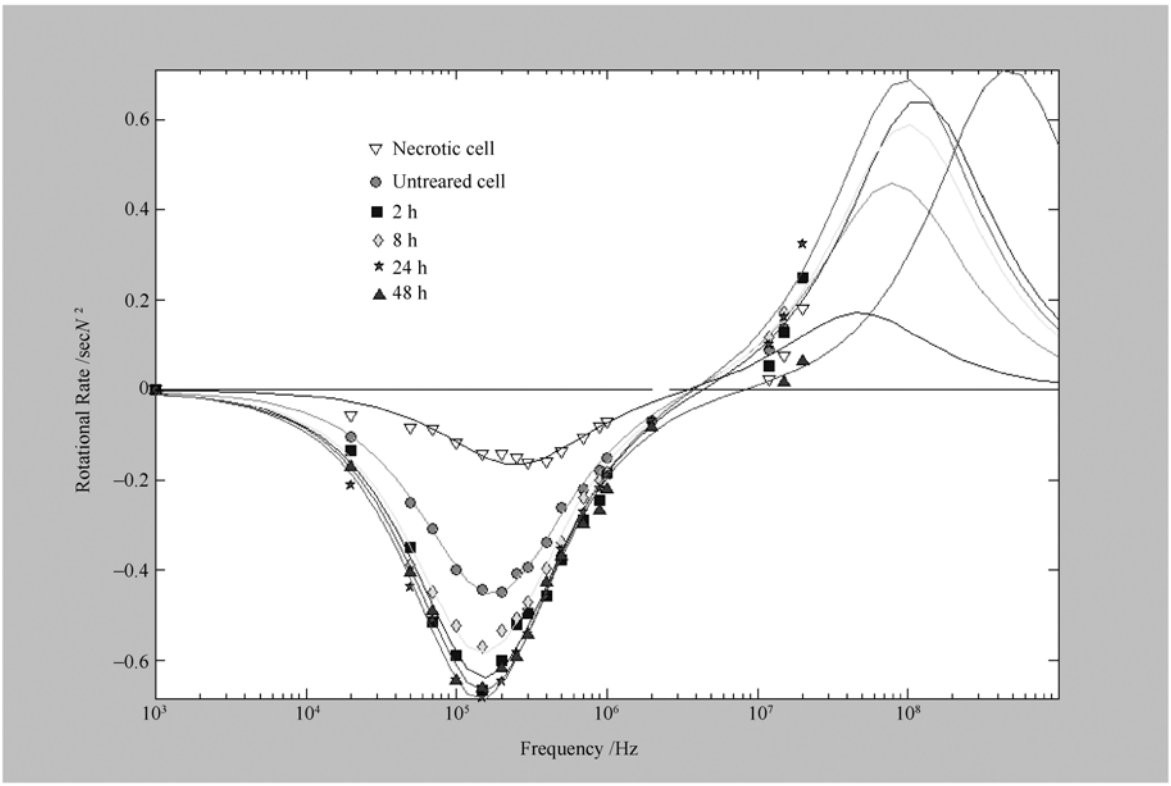

Fig. 2 The electrorotation spectra of normal cells, apoptotic cells treated with cytosine arabinoside for 2, 8, 24, 48 h and necrotic cells

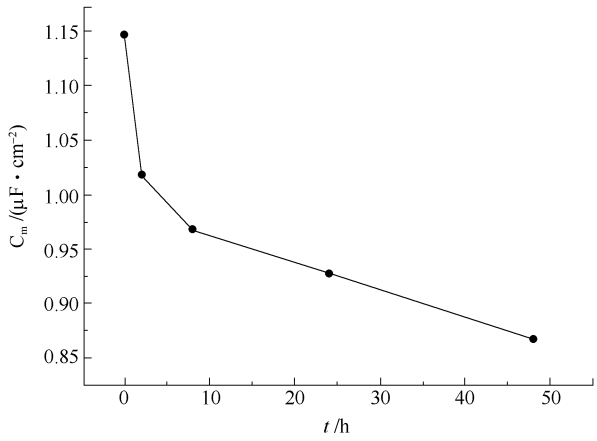

Fig. 3 The curve of the cell membrane capacitance. The dots are the membrane capacitance of normal cells and the cells treated with cytosine arabinoside for 2, 8, 24, 48 h

3.3 Cell morphological changes

The SEM photos of the surface morphology of Jurkat cells are shown in Fig. 4 Compared with untreated cells, the surface morphology of the apoptosis cells have changed strongly. After treatment with cytosine arabinoside for two hours, the microvilli began shedding. With more and more microvilli shedding, the surface of cells became smooth. Small blebs formatted on the cell surface and turned into many small single apoptosis bodies.

4 Discussion

Electrorotation is a phenomenon referred to cell rotation under the influence of a rotational electric field. When a cell is placed into a rotating electric field, a dipole moment is

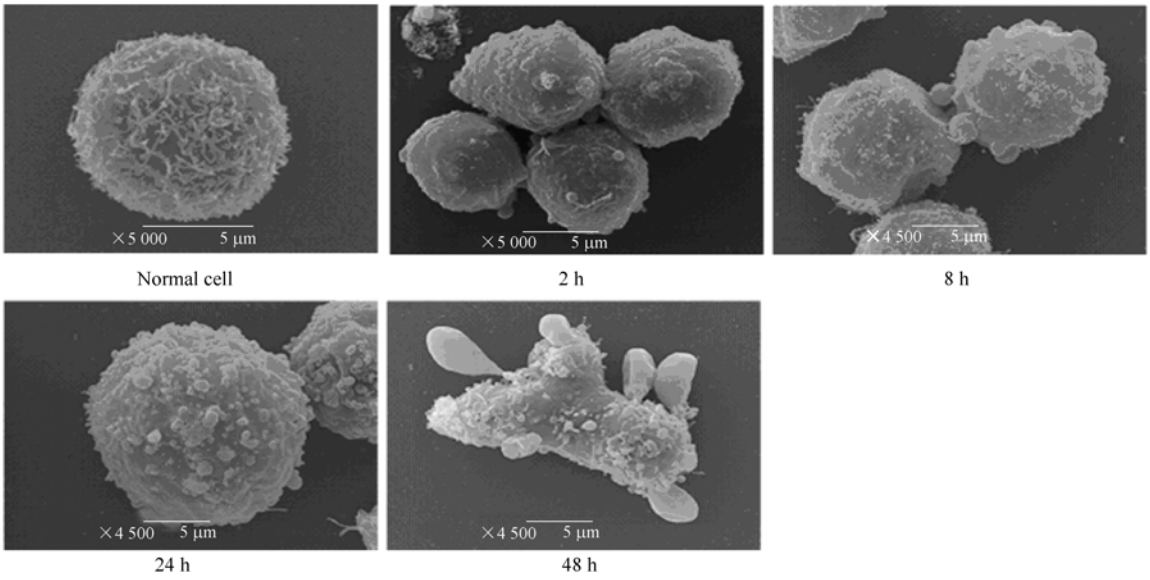

Fig. 4 Observation of Jurkat cell membrane changes with scanning electron microscope

induced. It interacts with the field and causes a torque to be exerted on the cell, resulting in cell electrorotation (Arnold and Zimmerman 1988). The rate and direction of ROT strongly depend on the cell characteristics and the frequency of the applied electric field. The cell dielectric properties can be deduced from these dependencies.

A series of characteristic morphological and biochemical changes of the cell membrane and its interior will arise during apoptosis. Many of these are related to the change of the cell dielectric properties. Therefore, cell apoptosis can be monitored by means of measuring those dielectric changes.

Cytosine arabinoside is a chemical inducing cell apoptosis. During cell apoptosis, there are series of characteristic changes on the cell membrane (Zhang et al., 1995). The low-frequency part of the ROT spectra (anti-field rotation) reflects the membrane dielectric properties (membrane capacitance); while the high-frequency spectra (co-field rotation) reflects the dielectric properties of the cell interior (dielectric permittivity, conductivity). We focused on the low frequency activities of the cells in the present experiment. The morphology of cells during apoptosis changed distinctively, causing the distinct changes on the ROT spectra.

After two hours of cytosine arabinoside treatment, the externalization of phosphatidylserine (PS) caused significant changes of the membrane morphology and the surface structure of Jurkat cells (Wang et al., 2002), resulting in a distinct decrease of membrane capacitance (Fig.3). As time elapsed, the formation of small blebs caused further decrease of membrane capacitance. After 20

hours of cytosine arabinoside treatment, more blebs formed. Apoptosis in small bodies formed after 40 hours. During the progression of apoptosis, the membrane capacitance continually decreased. It can be estimated that the decrease of membrane capacitance reflects the progression of cell apoptosis.

Cell apoptosis, known as the programmed cell death controlled by specific genes, is distinguished from cell death. During apoptosis, the membrane keeps completeness to some degree as judged by trypan blue dye exclusion; while on the condition of cell death, the membrane permeability increases, the cell interior contents leak (Wang et al., 1998), and the cells can be dyed by trypan blue. For the differences mentioned above, there are significant differences in dielectric properties between cell apoptosis and cell death, resulting in their different electrorotation spectra. Compared with normal cells, the rotation rate of apoptosis cells increases and the correlated anti-field rotation peak shifts towards lower frequency. In the case of cell death, there are masses of substance exchanges between the cell and its outer medium due to the high permeability of the membrane. Therefore, the dielectric distinction between the cell and its outer surroundings becomes little, and the rotation rate of the cell decreases. When the membrane is broken, there is no difference between the dielectric properties of the cell and its surroundings, and the cell will not rotate with the applied rotating field. It can be concluded that the ROT responses are in different modes between apoptosis cells and dead cells, and ROT can distinguish them.

Our results are consistent with Wang et al. (2002). They studied the dielectric characteristics of HL-60 cells during the fore-phase apoptosis with dielectrophoresis (DEP) and found that the membrane capacitance decreased during the progression of apoptosis. Here we studied the dielectric properties of Jurkat cells in fore-, middle-, and late-phase apoptosis using ROT. We also found that the membrane capacitance decreased in the fore-phase apoptosis cells. Furthermore, we found that there was a tendency for membrane capacitance to decrease during the progression of apoptosis. The accordance of these two different methods indicates that it is considerable to monitor cell apoptosis by means of measuring the membrane capacitance.

The merits of ROT are significant compared with other apoptosis examining methods. This method does no mechanical harm to the cells during experiment, and chemical process is unnecessary. If the cells are cultured in the chamber on the chip, the whole progression of apoptosis can be monitored in real time. The inherent simplicity makes it ready to realize high-throughput (Cheng et al., 2002) and automation. Compared with the impedance sensing methods, ROT is more convenient in processing suspending cells. The sensitivity of ROT in the present experiment could be further optimized in the future.

Monitoring apoptosis can be utilized to investigate whether a reaction could lead to diseases by causing cell apoptosis, to screen apoptosis correlated genes, and to locate genes accurately into the time phases of apoptosis. Clinically, detecting and monitoring the apoptotic cells in the blood of patients before and during cancer treatment are used to evaluate the effect of treatment and for other diagnostic and prognostic purposes.

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