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Full Length Research Paper

Electrical cell lysis using a microfabricated device

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Interest in electrical lysis of biological cells on a microfluidic chip device has increased as it allows for the rapid recovery of intracellular contents without introducing lytic agents. In this research, a new microfabricated device for cell lysis has been developed. The device utilized a microfabricated interdigital electrode array to generate a pulsed electric field with a low voltage, a requirement for effective cell lysis. It is therefore particularly suitable in applications of miniature bio-analysis systems such as lab-on-a-chip systems, where cell lysis is required to obtain the intracellular contents for further analysis. C2C12 cells have been tested using the microfabricated device and the lysis of C2C12 cell was observed using a microscope. The factors which could affect the cell lysis efficiency were investigated such as voltage and pulse duration. The results obtained demonstrated that C2C12 cells could be effectively lysed by the developed device.

Key words: Cell lysis, pulsed electric field, microfabricated device, C2C12.

INTRODUCTION

Cell lysis is an essential step in diagnosis and general bio-experiments in order to extract intracellular materials such as DNA, RNA and proteins. In recent years, different techniques for cell lysis have been developed including physical (Okada et al., 1982; Williams et al., 1980), mechanical (Salonen et al., 2010), chemical (Chen et al., 2006; Mahrus et al., 2005) and electrical methods (Wang et al., 2006). Among these techniques, electrical cell lysis has attracted more and more interest due to its intrinsic advantages. Comparing it with other conventional cell lysis methods, the electrical cell lysis method in which an electric field is applied to the cells is simpler and more suitable for micro-total analysis system (μTAS) technology. Electrical cell lysis has been described in a number of recent reports (He et al., 2007; Lee et al., 1999). However, in previous electrical cell lysis studies, most researchers have applied DC voltages to the electrodes, thus needing a high DC voltage and high power consumption. Meanwhile, the DC voltage applied to the electrode may cause electrolytic effects in the cell suspension and damage the electrodes. In this study, we have designed and fabricated a microelectrode array using microfabrication technology.

The micro scale dimensions of the electrodes can be made to match the dimensions of the cell, for effective cell lysing. Therefore, only a very low voltage was required (V≤3 V). Furthermore, instead of a DC voltage, we applied a pulsed signal to the electrodes, since this showed a higher cell lysis efficiency and eliminated electrode damage.

MATERIALS AND METHODS

Electrical cell lysis principle and modeling

Cells consist of a lipid double-layer membrane with intracellular organelles and cytosolic material. The bilayer structure of a cell membrane is a dielectric. When a cell is exposed to an external electric field, a trans-membrane potential $\Delta\varphi$ is induced. In response to the applied field pulse of magnitude E_0, the voltage generated across the cell membrane of a spherical cell grows exponentially with time (Sukhorukov et al., 2006).

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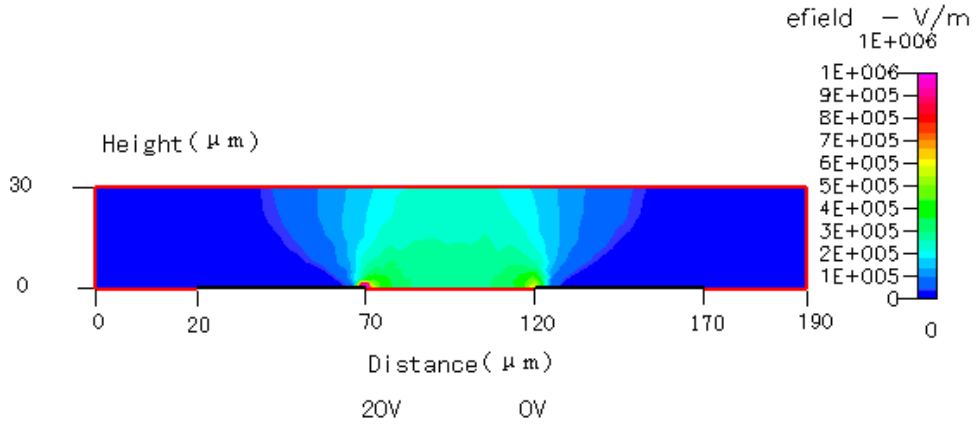

Figure 1. Simulation of the electric field distribution.

$$\Delta\varphi_m(t) = 1.5aE_0\cos\theta(1-\exp(-t/\tau_m)) \quad (1)$$

Where θ is the polar angle measured with respect to field E_0. The time constant of membrane charging τ_m can be approximated by:

$$\tau_m = aC_m\left(\frac{1}{\sigma_i}+\frac{1}{2\sigma_e}\right) \quad (2)$$

Where C_m is the membrane capacitance, σ_i is the intracellular conductivity and σ_e is the conductivity of the external medium.
Previous reports (Kotnik et al., 1997, 1998) have studied how the classical unicellular model acts on the electric field, but ignored the initial cell trans-membrane voltage. However, this is intrinsic to the cells and is also an important measure of cell electrical characteristics. The trans-membrane voltage influences the opening and closing of cell ion passages. It also affects internal cell activities. Therefore, the trans-membrane voltage plays a decisive role in cell physiology. After improving the live cell model, we have:

$$\Delta\varphi_m(t) = 1.5aE_0(f,\delta)\cos\theta(1-\exp(-t/\tau_m)) \quad (3)$$

$$\varphi_m(t) = \varphi_0 + \Delta\varphi_m(t) \quad (4)$$

When $\theta = 0$,

$$\Delta\varphi_m(t) = 1.5aE_0(f,\delta)(1-\exp(-t/\tau_m)) \quad (5)$$

The electric field distribution generated by a microfabricated device has been simulated using CFDRC software (Trial version, CFD Research Corp., AL, USA) which is based on the finite element analysis (FEA) method. In the 2D simulation model, two electrodes with width = 50 μm and gap = 50 μm were used. In the static simulation, 2 V was applied to the electrode, giving the electric field distribution shown in Figure 1. As seen from Figure 1, the electric field varied significantly with location. The strongest electric field is located at the edges of the electrodes where the electric field intensity was up to 9×10^5 V/m. This electric field intensity may be sufficient for cell lysis according to previous studies (Lee et al., 2007).

Cell culture and chemicals

Mouse myoblast cells (C2C12) were donated by the Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University. Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS) were purchased from Sigma Corporation. The 6-well plates were purchased from Millipore Corporation. The C2C12 cells were cultured in a suspension culture in DMEM supplemented with 10% FBS at 37°C under a humidified atmosphere consisting of 5% CO_2 and 95% air. The cells were maintained in logarithmic growth in the 6-well plate with 2 ml in each well and were harvested at a concentration of 2.5×10^5 cells/well for the cell lysis experiments.

Chip device design and fabrication

The device for cell lysis was designed as shown in Figure 2. An interdigital electrode array containing 12 electrodes was used to generate the required pulsed electric field. In order to reduce the operational voltage, the interdigital electrodes, 50 μm wide and 40 mm long were designed based on the simulation results. The interval between two adjacent electrodes was 50 μm. The electrodes were fabricated by a standard microfabrication technique from ITO (indium tin oxide) coated glass using the process shown in Figure 3. We started with a 4-inch ITO conductive glass wafer (CGS Holding Corp., Shenzhen, China) as a substrate and then spin-coated it with a 1.2 μm thick photoresist (AZ 1512). The microelectrode pattern was transferred from the mask to the photoresist layer by UV-lithography. After the development of the patterned photoresist, the sample was etched with an ITO etching reagent (37% HCl: 67% HNO_3: H_2O = 50: 3: 50, volume ratio). After removing the remaining photoresist on the sample, ITO electrodes were obtained on the glass substrate with an optical transparency of 90%.
The thickness of the electrodes was determined by the thickness of the ITO layer; this was known to be 500 nm from the manufacturer's data for the ITO glass.

Experimental setup and cell lysis experiments

The experimental setup for the cell lysis is shown schematically in Figure 4. The cell lysis behavior was observed using an inverted microscope (Nikon, TE2000, Japan) equipped with a 3-Mega-Pixel CCD camera with images recorded on a PC. For the cell lysis

Figure 2. Microelectrode structure.

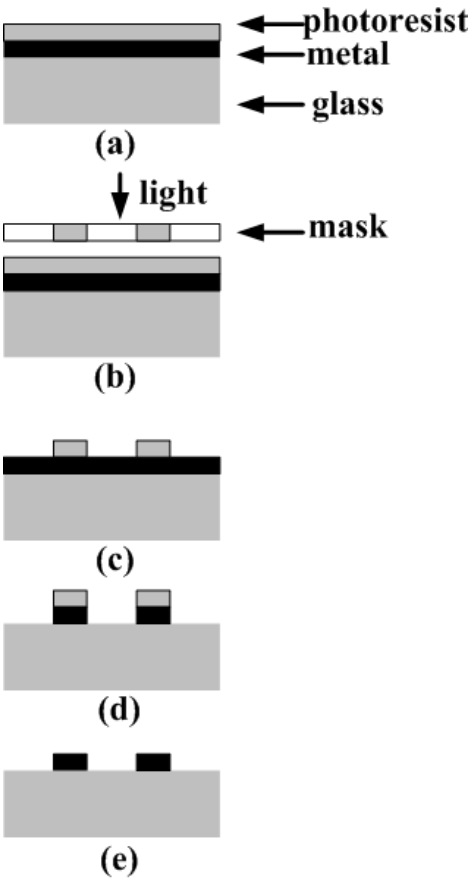

Figure 3. Fabrication process flow for ITO microelectrodes: a) Spin-coating of photoresist on to ITO coated glass substrate; b) transfer of the microelectrode pattern from the mask to the photoresist layer by UV lithography; c) removal of the exposed photoresist in the photoresist development reagent; d) etching of the ITO layer to obtain the microelectrodes; and e) removal of the remaining photoresist.

experiment, 200 µl of the C2C12 cell suspension was pipetted on to the surface of the microelectrode array and a thin glass cover slip placed over the top to prevent evaporation. A home-made pulsed signal generator controlled by a computer was connected to the device to produce a pulsed electric field. The voltage amplitude and pulse duration of the pulse generator can be continuously adjusted in the range; output voltage amplitude: 0 ~ 20 V with 0.1 V resolution; pulse duration: 10 µs ~ 99 s; pulse adjustment time range: 10 µs). To electrically lyse the cells, pulsed signals were applied to the microelectrodes. In order to evaluate the effects of voltages and pulse durations, different voltage amplitudes (0 ~ 4 V) and pulse durations (0.5 ~ 3 m/s) were applied to the microelectrodes.

RESULTS

Cell lysis by the pulsed electric field

Figure 5 shows a typical C2C12 cells lysis process for a pulsed voltage of 2.0 V and duration 3 m/s. Figure 5a shows that the cells before applying the voltage are attracted to the edge of the microelectrode. Figure 5b, c shows that the cell membranes gradually broke up when the 2.0 V pulse voltage was applied. Figure 5d shows the cells after lysing with the electric field treatment for 3 s. It can be seen that the cells were completely broken and only membrane debris was observed under the microscope.

Effects of pulse magnitude and duration on cell lysis

In Figure 6, the cell lysis efficiency is plotted for different voltages and pulse durations. As shown in Figure 6, the efficiency of cell lysis generally increases with increasing applied voltage. For example, for the 1 m/s pulse signal (red curve in Figure 6), there was almost no cell lysis observed when the voltage was 2.0 V. However, when the voltage was increased from 2.0 to 3.0 V, 20% of the cells lysed. Furthermore, when the voltage was further increased to 3.4 V, more than 85% of the cells lysed. On the other hand, we also found that a longer pulse duration (that is 3 m/s) produced a higher efficiency for cell lysis at a given voltage (blue curve in Figure 6).

DISCUSSION

In this work, we have used a microfabricated device to generate a pulsed electric field for cell lysis. Micro scale electrodes have the advantage in that only a low voltage (< 4 V) was required to generate a sufficient electric field for successful cell lysis. Meanwhile, because this relatively low voltage was applied to the electrodes, no significant bubbling or obvious electrode damage was observed in any of the cell lysis experiments. The results obtained also indicated that both the applied voltage and pulse duration had significant effects on the cell lysis performance. Under optimal conditions (voltage = 3 V, pulse duration = 3 m/s), more than 90% of cells lysed. This applied voltage and power consumption were much lower than those reported previously and is thus more favorable for lab-on-a-chip applications (Lee et al., 2007).

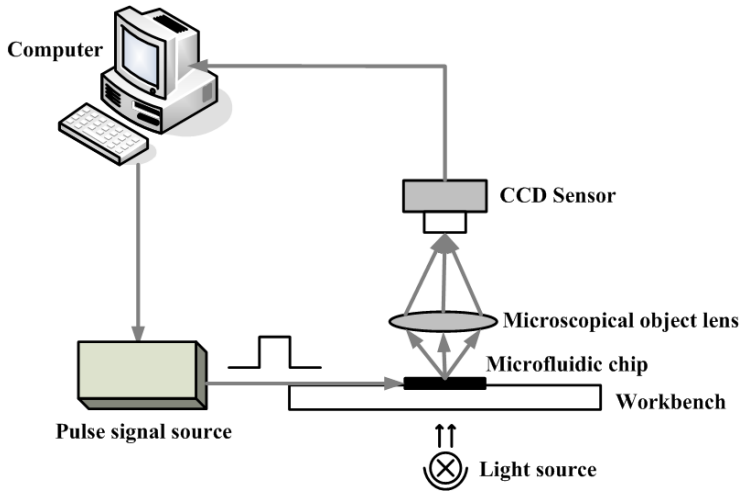

Figure 4. Schematic diagram of the cell lysis system.

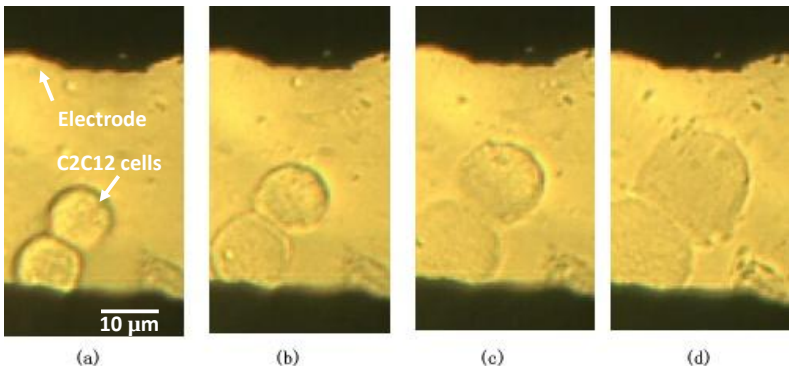

Figure 5. C2C12 cell lysis procedure.

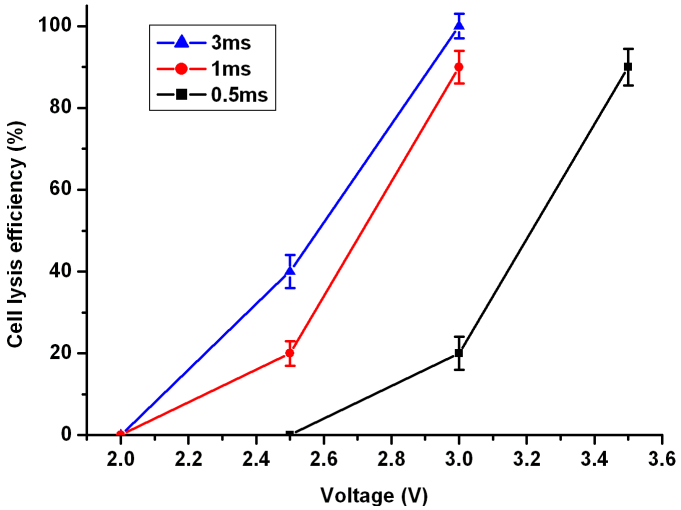

Figure 6. Relationship between cell lysis efficiency and the voltage for three different pulse durations (each data point represents the average value of three independent measurements and the error bar represents the standard deviation of the data from the three measurements).

Conclusions

We have designed and fabricated a microscale device for electric cell lysis; C2C12 cells were successfully lysed by this device. The micro device is effective in lysing cells while operating with more advantageous conditions than conventional systems, namely: low voltage and power.

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