용액 전도도 및 삼투압 조절된 PBS에서의 위암 세포 전기 분해

김 민수*, 이 국녕**, 조 수형**, 김 병기**, 김 용권*
*서울대학교 전기 컴퓨터 공학부, **서울대학교 응용화학부

Stomach Cancer Cell Lysis in PBS with Conductivity and Osmotic-Pressure Control

Min-Soo Kim*, Kook-Nyung Lee**, Su-Hyung Cho**, Byung-Gee Kim** and Yong-Kweon Kim*
*School of Electrical Engineering & Computer Science, **School of Chemical Engineering, Seoul National University

Abstract - Cancer cell lysis at pulsed DC is realized using micromachined electrodes. In this research, quantitative analyses are performed on cell lysis results. The cell volume increasing at the pulses applied are analyzed in different medium conditions on osmotic pressure and conductivity, and the cell lysis procedures are studied in detail experimentally. Phosphate buffered saline (PBS) is used as the medium. To change the conductivity of PBS, NaCl concentration of PBS is adjusted, and inositol is used with PBS to control the effects of the osmotic pressure to cell lysis performance.

1. Introduction

Cell lysis is a method to obtain intracellular materials by disrupting cells. Commonly used method is a chemical lysis in which detergent, solvent and antibiotics are used as lysis reagents. The electrical method for cell lysis is to use AC or pulsed electric fields (PEF) as electrical shocks to cell membrane. Cell lysis by high electric fields has been tried and researched on macro electroporation system already [1]. Recently, using MEMS technology, the electrodes are micro machined in the order of the biological cells sizes, and it becomes possible to generate high electric fields just at low voltage [2]. Small cells of a few micrometer diameters can be lysed using only several pulses [2]. But, the large cells like Chinese cabbage cells may require many pulses enough to uptake the sufficient medium to be swelled largely. Because the cancer cells are so large compared to the other biological cells such as E. coli, it is considered that more pulses will be required. In this research, we assumed that the medium osmotic pressure and conductivity have high effects on the cell lysis by pulsed electric fields especially in case of large cells. To analyze these effects it is investigated how many pulses are required for each cell to be lysed in different medium conditions.

2. Principles

When a cell is exposed to high electric fields, cell membrane is charged and induced to have potential drop across the membrane. In general, the membrane undergoes electrical breakdown at the potential drop more than 1 V, and have pores formed. If the membrane is discharged, the pores are resealed again. The transmembrane potential drop is calculated as [3]

$$\triangle\psi = fR_{cell}E\cos(\theta) \qquad (1)$$

E is the electric fields intensity, R_{cell} is the radius of a cell and f is a form factor. f is 1.5 in case of spherical cells. θ is the polar angle measured with respect to the direction of the field, as shown in Fig. 1. Sufficient time for a cell membrane to be fully charged can be obtained as [3]

$$\tau = (\rho_s/2 + \rho_c)C_m R_{cell} \qquad (2)$$

ρ_s and ρ_c are the resistivities of suspension medium and cytoplasm, respectively and C_m is membrane capacitance.

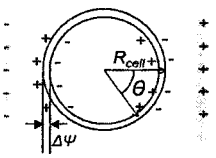


Figure 1. Transmembrane voltage at external electric fields

3. Experimental setup

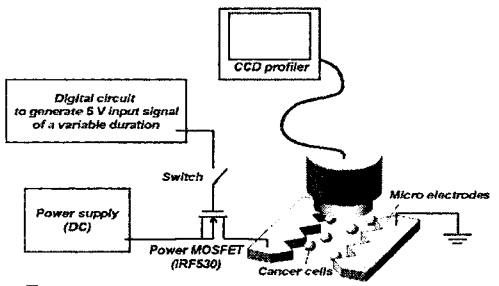


Figure 2. Experimental setup for cancer cell lysis.

The total experimental setup is depicted in Fig. 2. The power supply is connected directly to cell lysis device via a power MOSFET. The pulse of variable duration is made by switching the power MOSFET using 5 V signal of variable duration as a input. The 5 V input signal is generated from the electronic digital circuit composed of TTL logic devices including 74LS123. The duration is controlled between 170 - 1000 μs. In this research, the duration and the magnitude were fixed as 170 μs and 10 V. When applying the electrical parameters of general biological cells to equation (1) and (2), 10 V is high enough for most parts of the cell membrane to undergo electrical breakdown. The charging time is calculated 0.2 μs. 170 μs is considered enough. Microelectrodes are fabricated using micro nickel electroplating as shown in our previous report. The electrodes gap is 50 μm.

4. Experiments and results

4.1 Medium conductivity and cell swelling

To prevent the bubbling by high current flow in the medium, the medium conductivity should be reduced. PBS used as cell suspension medium here is the typical isoosmolar medium of high conductivity for cell culturing. It consists of NaCl 140 mM, KCl 2.68 mM, Na_2HPO_4 8.27 mM and KH_2PO_4 1.76 mM. Among them, NaCl is well-konwn high conductivity compounds. In this research, the conductivity of PBS was lowered by downing the concentration of NaCl. Since the NaCl is contained in PBS at the highest concentration of the other compounds, PBS is totally diluted in water to reduce the conductivity. The conductivity of pure PBS is 2.4 S/m. After dilution of PBS, the conductivity was changed linearly with the NaCl concentration. When the duration and the magnitude of a pulse were fixed as 170 μs and 10 V, the bubbling was initiated at more than 20 mM of NaCl (σ=0.34 S/m). Now, the bubbling in PBS is weaken by reducing NaCl concentration through the dilution of PBS, successfully. On the other hand, PBS is so isoosmolar medium in which osmosis is balanced between in and out of cells, and the cells maintain the original sizes stably with no swelling.

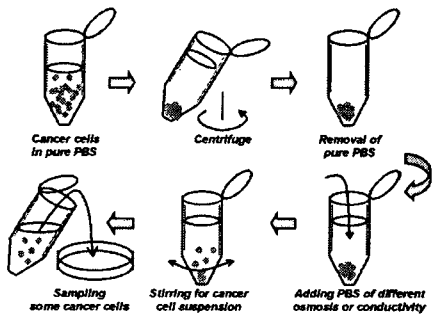


Figure 3. Experimental processes for preparing the newly modified PBS samples and suspending cancer cells in each PBS.

But, in the diluted PBS, most cell swelled largely, and some cells ruptured. This indicates that the osmosis balance has been broken between in and out of cells in the diluted PBS. This is not wanted phenomenon. To investigate the cell self-swelling, we prepared the differently diluted PBS samples, and then suspended the cancer cells in each PBS samples.

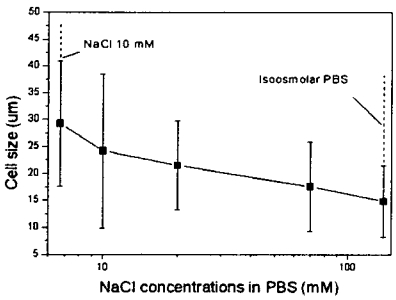


Figure 4. Cell distributions in different NaCl concentrations

Fig. 3 show the procedures of suspending the cancer cells in newly prepared PBS with different conductivities. The cancer cells are observed in microscope. As the NaCl concentrations are reduced, the PBS is getting hypoosmolar and the most cells are increased large size by swelling easily. Fig. 4 indicates the size distribution in different NaCl concentration. The cell distributions are broaden compared to the distributions in pure PBS. The cell's self-swelling is prevented well using inositol in PBS, actively. To investigate the effects of inositol, we readjusted each previously-prepared PBS to have different inositol concentrations.

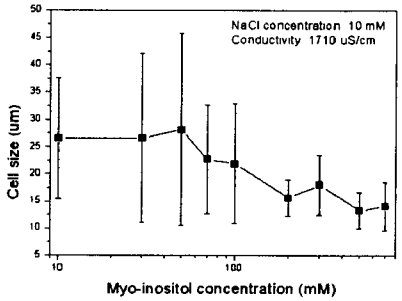
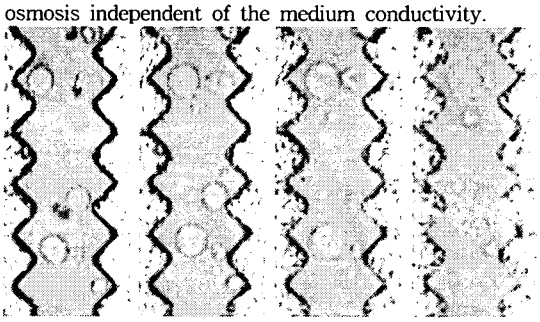


Figure 5. Cell distributions in different inositol concentration.

Inositol is a chemical reagents in powder. After diluting PBS in water to various volume ratios PBS / water (w/w%), the PBS prepared is poured into the inositol-contained falcon tube to desired volume. By this way, we controlled PBS in different osmosis and conductivity, independently. The cells are prepared in the PBS also in the manner as indicated in Fig. 3. In Fig. 4, most cells are very large in size at NaCl 10 mM because of swelling, because the PBS of NaCl 10 mM is already hypoosmolar. But, cell's self-swelling did not occur thanks to the inositol. As shown in Fig. 5, at more than 300 mM of inositol, the cells maintain their sizes similarly to the cell distributions of pure PBS. It was also proved that the inositol has almost no affects on the medium conductivity. It means that it is possible to control the medium osmosis independent of the medium conductivity.



Before lysis 50 pulses 100 pulses 150 pulses

Figure 6. Images of cell swelling at pulsed electric fields applied continually (NaCl 10 mM, (σ=1.7 mS/cm, inositol 100 mM)

4.2 Cancer cell lysis in pulsed DC

A well-known mechanism of cell lysis is if a cell is exposed to high electric fields, the cell membrane is charged to have voltage-drop induced across the membrane. If the voltage drop reaches to 1 V, the membrane have pores by electrical breakdown. The

medium is sucked through the pore, cells swell and rupture. In this research, cancer cell lysis is performed in the PBS prepared previously. It has been already shown that the cells have different size distributions as shown in Fig. 4 and Fig. 5. It is very meaningful to investigate how many pulses are used to lyse each cells of different sizes in different medium conditions. Fig. 6 shows the procedures that the cells swell gradually at the pulses applied and rupture finally. Since the size of cancer cell is so large, many pulses are used other than small cells such as E. coli. Therefore, we investigated the cells distributions in every tens of pulses. It was so difficult to investigate the size increments of all the cells in every pulses. It is investigated that the numbers of pulses used are different in each NaCl concentration; 7 mM, 10 mM and 20 mM, as shown in Fig. 7. As illustrated in Fig. 4, the cells swell well at high concentration NaCl. Likewise, the cell swelling at pulsed electric fields is caused by medium uptake via pores through osmotic pressure. In Fig. 7, Since the PBS of NaCl 7 mM has the highest osmotic pressure among the other two NaCl concentrations, the smallest number of pulses are used to lyse the cells in the PBS of NaCl 7 mM. The total time required for all the cells to be lysed in each NaCl concentrations 20 mM, 10 mM and 7 mM are 27, 17 and 7 ㎳. For the same tests on inositol 100 mM and 300 mM, the special tendencies was not found because the cells distributions in size was not broad. The osmotic pressure differences caused by NaCl concentrations have high affects on the cell lysis performance. As shown in Fig. 8, the critical pulse numbers for lysing 50 % of the cells in each NaCl concentrations; 20 mM, 10 mM and 7 mM are 4, 50 and 100, respectively.

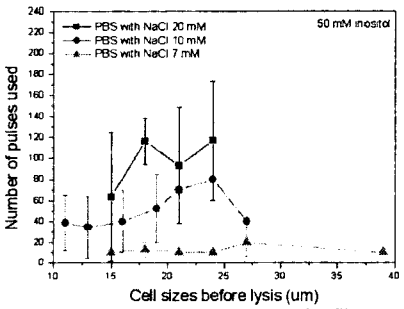


Figure 7. Number of pulses used in each NaCl oncentrations with inositol concentration hold as 50 mM

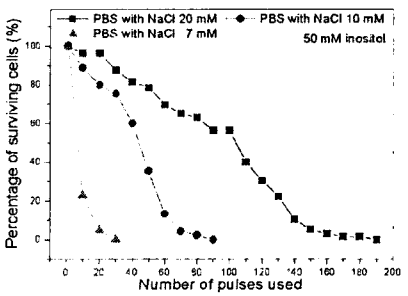


Figure 8. Surviving cells during pulses being applied

With NaCl concentration hold, more pulses are used as much as the inositol concentration is increased, as shown in Fig. 9. Using inositol prevents the cell volume from being increased effectively. According to the triangular symbol line of Fig. 9, the required pulse number for all the cells to be lysed is about 180. In Fig. 5, although the PBS of NaCl 10 mM is hypoosmolar, the cells maintain the cell distribution similarly to that of pure PBS, using inostiol 300 mM. It means that the PBS of NaCl 10 mM (σ=0.17 S/m) is converted from hypoosmolar to isoosmolar using 300 mM inositol. In Fig. 9, the cell lysis by high elecric electric fields was performed in low conductivity but isoosmolar state at NaCl 10 mM and 300 mM inositol. .

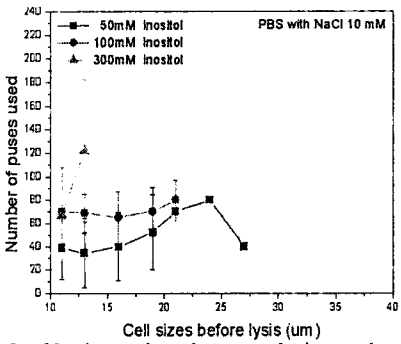


Figure 9. Number of pulses used in each innositol concentrations with NaCl concentration hold as 10 mM (σ = 0.17 S/m)

5. Conclusions

The cancer cells were lysed using pulsed DC in PBS of different osmosis and conductivities. Firstly, the medium conductivity was adjusted. On applying pulses of 10 V and 170 ㎲ to 50 ㎛ gap micro electrodes, the conductivity of PBS was lowered to 0.34 S/m (NaCl 20 mM) at least to prevent the bubbling by high current flow. Secondly, we investigated the different cell size distributions in different concentrations of NaCl and inositol. The cells are differently distributed in different NaCl concentration because of self-swelling. Self-swelling is controlled using inositol, again. In NaCl 10 mM, the cells were preserved stably almost without swelling at more than 300 mM of inositol. Finally, to estimate the cell lysis performance, cell lysis was tested at less than 20 mM of NaCl in PBS with inostiol 50 mM. Especially, the pulse numbers required was investigated. In high concentration NaCl, cell lysis performance was good requiring just small pulses. But, for stable cell lysis with no self-swelling, inositol should be used. In hypoosmolar PBS with NaCl 10 mM, cell lysis was performed stably using inositol 300 mM.

Acknowledgement

The Intelligent Microsystems Program (IMP) that is the project of the "21C Frontier R&D Program" supports this research

[References]

[1] T. Grahl, H. Märkl, "Killing of microorgannism by pulsed electric fields", Applied Microbiological Biotechnology ,vol. 45, pp. 148 157, 1996

[2] S.W. Lee, Y.C. Tai, "A micro cell lysis device", Sensors and Actuators, vol. 73, pp. 74 79, 1999

[3] T. Kotnik, F. Bobanović, D. Miklavčić, "Sensitivity of transmembrane voltage induced by applied electric fields a theoretical analysis", Bioelectrochemistry and Bioenergetics, vol. 43, pp. 285 291, 1997