Comparison of ESD and Conventional Methods in Single Cell Spray

Takayuki Umemura[1], Kazuya Tamura[2], Kenta Shirasu[2], Masato Morita[1], Kumiko Nagase[3], Wakako Hamanaka[3], Masatoshi Kakihana[3],
Tatsuo Ohira[3],Norihiko Ikeda[3],Tetsuo Sakamoto[1,2,#]
[1]*Graduate School of Electric Engineering and Electronics, Kogakuin University, 2665-1 Nakano-machi, Hachioji, Tokyo 192-0015, Japan*
[2]*Department of Applied Physics, Kogakuin University, 2665-1 Nakano-machi, Hachioji, Tokyo 192-0015, Japan*
[3]*Division of Thoracic & Thyroid Surgery, Tokyo Medical University, 6-7-1 Nishi-Shinjuku Shinjuku Tokyo-160-0023, Japan*

Various inorganic ions are related to activity in a cell. Behavior of biological activity is traditionally observed by using optical microscope and/or MALDI. However, higher spatial resolution imaging technique is needed to observe organelles in a cell. In such cases, fixation in vacuum becomes quite big problem to maintain original structure in a cell. Rapid freezing seems one of the promising methods. But single cells cannot be treated as section. In this study, we devised a new rapid freezing method by combining electro-spray deposition and metal-touch freezing. In this method, individual cell is sprayed with a droplet emitted from the electro-spray tip, and each cells impact on the cooled metal surface, then frozen rapidly.

I. Introduction

It is known that inorganic ions plays an important role of activity of cells[1)]. In recent years, visualization of constituents has large demands. In fact, both bulk analysis and local imaging analysis techniques have been developed[2)]. However, micro-imaging of organelle has not been realized except fluorescent imaging. In our research group, high lateral resolution Focused Ion Beam Time-of-Flight Secondary Ion Mass Spectrometer (FIB-TOF-SIMS) has been developed. This apparatus has the highest resolution of 40 nm in the world[3)]. It promises element-imaging of organelle in a single cell for various inorganic species simultaneously.

The most important thing when a cell is analyzed with the TOF-SIMS is water in the cell, because inside of the TOF-SIMS is high vacuum around 10^{-6} Pa. It is needed to perform pretreatment for fixation of water with some kind of method. There are some kinds of fixation methods. Rapid freezing is only the method where constituents are hold. In this method, cells are rapidly frozen so as to avoid movement of constituents in a cell and prevent crystallization of water. For rapid freezing, "metal-touch" method is employed[4)]. This method uses a cooled metal block on which a sliced tissue is pressed. In case of individual cells in culture medium, it is very difficult to carry out like a tissue, because cells are small and cannot be separated with the medium. Through such process, crystallization of medium and cytoplasm should be occur[5)].

ct13087@ns.kogakuin.ac.jp

We hit upon a good idea for single cell rapid freezing using electrospray. Electrospray is known as a spray method employing strong electric field. Furthermore, sprayed droplets are charged, and split into smaller droplets during drifting in air. As a result, very small droplets of the solvent can be obtained. In our idea, cells in the medium are electrosprayed, then cells with medium droplets land on a cooled substrate. In this method, each droplet contains small amount of medium and a cell, therefore heat capacity of the droplet is minimized, and rapidly frozen. A standard nebulizer which also generate small droplets was used for comparison with electrospray.

II. Experimental

Experiments of single cell spay were carried out with both electrospray and nebulizer. An ITO plate (20 x 20 mm^2) was used as a substrate in each experiment. 4-(2-hyrdroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) is used as the medium at 0.1 M in water. Cultured human lung cancer cells (A549) were used in the experiment. Densities of the cells in HEPES buffer were three kinds; 0 cell/mL, $1.8x10^5$ cells/mL, and $1.6x10^8$ cells/mL to observe the state of electrospray. After the spray, ITO surface was observed with an optical microscope. Other conditions are summarized in Table1.

Table 1: Experimental conditions

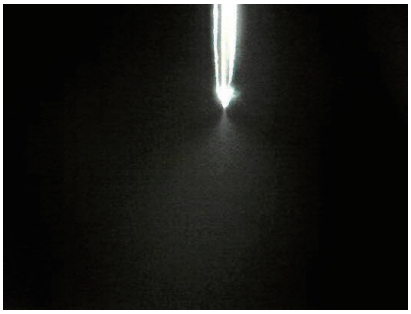
Spray Method	Electrospray	Nebulizer
Meduim	0.1 M HEPES	
Density [cells/mL]	1.8×10^5	1.6×10^6
Distance between spray source to substrate [cm]	5	
Flow rate [mL/h]	0.14	3.30
Spray time [s]	3	

III. Results and Discussion

Figure 1 shows electrospray-tip and sprayed microdroplets. In case of electrospray, cell density at $1.8x10^5$ cells/mL was successful like without cell. However, at cell density of $1.6x10^6$ cells/mL was not stable. This is because of Taylor cone formation at the tip was affected by the excess cells.

In case of nebulizer, the state of spray little depended on the cell density. In this point of view, nebulizer is better than electrospray.

a) Electrospray without cell



b) Electrospray at cell density of 1.8×10^5 cells/mL

Fig.1 Pictures of electrosprays

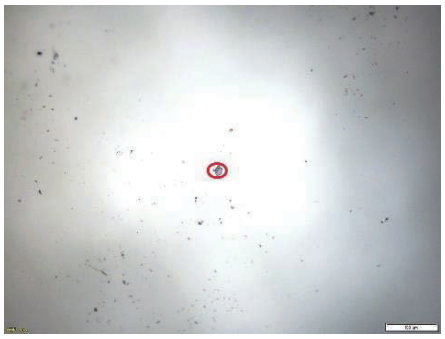


Fig.2 Optical micrograph of the ITO glass after electrospray.

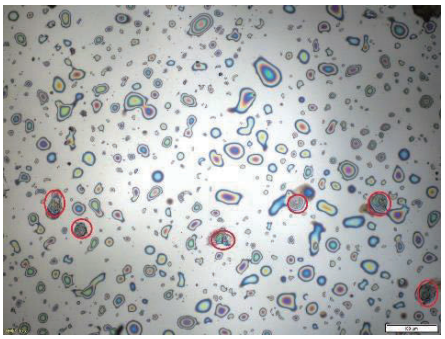


Fig.3 Optical micrograph of the ITO glass after spray using nebulizer.

Figures 2 and 3 show optical micrograph of ITO surface after electrospray and nebulizer, respectively. There was a large difference between the two micrographs. In case of electrospray, droplets were very small whereas they were as large as cells in case of nebulizer. Smaller droplets are suitable for rapid freezing because of small heat capacity. Furthermore, a cell was located with less medium. On the other hand, many cells were viewed in Fig.3, there were also droplets where cell was not contained. In this situation, it will very difficult to identify ice droplet containing a cell in the FIB-TOF-SIMS.

IV. Conclusions

It is concluded that electrospray method is suitable for rapid freezing of single cells. In near future, we will analyze frozen cells using electrospray and the TOF-SIMS. If rapid freezing is successful, yield of reculture will be employed for an indicator. But in electrospray, number of attached cells on the ITO substrate is small. Therefore we should increase the number of cells by modifying electrospray condition and/or spray tip continuously.

References

1 T. Stewartetal et al., BiochimicaetBiophysicaActa, **2502-**11 (2015).
2 K Ogasawara Plant Morphology, **26** ,13-17(2014)
3 T. Sakamoto et al., Applied Surface Science, **255**, 1617 (2008).
4. T.Stewart et al *Biochim. Biophys. Acta - Biomembr.* **1848**, 2502–2511 (2015).
5 祐村 恵彦, 国立大学法人山口大学. “電子顕微鏡観察試料の調製方法 “.特開 2016-85119(2016.5.19)