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Bio-Manipulation Based on Microfabricated Structures

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1. Introduction

Microfabrication techniques based on photo-lithography have found novel applications in micro chemical processes, which are sometimes referred to as micro-total analysis systems (μ-TAS) or lab-on-a-chip (LOC). Through miniaturization and integration, such systems have enabled the chemical analysis and synthesis with small volume of the samples and the reagents, quick reaction, cost reduction, portability, etc. We are now in the stage to go one step further, towards the assays and characterizations at a single-cell or single-molecule level.

A biological cell is not a uniform solution, but has its own structure, such as cytoskeltons or membrane compartments, and functional molecules are arranged therein to optimize their functions. For the understanding and utilization of cellular functions, information not only about its constituents, but also their localization and interaction play an important role. In other words, the assay with temporal and spatial resolution is required.

In order to achieve space-resolved assay, we have to have a method to control the position and the orientation of the object, and apply artificial alternations or stimuli to measure its response. Here exist a need for the manipulation of individual biological object. Their size ranges from several ten μm of cells down to nm of molecules, part of which can be covered by the state-of-art microfabrication techniques.

In this paper, we show some example of our researches on biomanipulation using microfabricated structures.

2. Membrane alternation using micro-structures

There are needs to bring foreign substances into cells. One of the methods makes use of the phenomenon called reversible breakdown of the membrane. A cell consists of a cytoplasm, having physiological saline condition and is a good electrical conductor, enclosed by an insulating cell membrane made of phosphor-lipid bilayer. When an external field is applied to a cell, electrical charging of the membrane takes place, and a voltage across the insulating membrane builds up. When this membrane voltage V_m exceeds the critical voltage V_b (which is known to be about 1 V regardless of the cell type), dielectric breakdown of the membrane occurs and pores are formed. However, if V_m is properly chosen that $V_m \approx V_b$, due to the lateral fluidity of the bio-membrane, the pores spontaneously seal, the phenomenon called "reversible breakdown". When such a breakdown occurs, foreign substances in surrounding medium can be fed into the cells by diffusion (electroporation), or when it occurs at the contact point of two cells, the membranes are reconnected and the cells fuse (electrofusion).

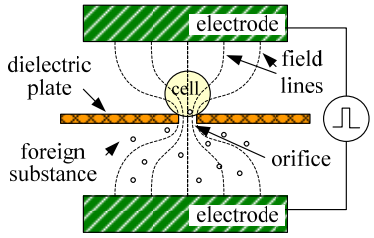
Conventional electroporation or electrofusion is performed in suspension; cell suspension is placed between a pair of parallel-plate electrodes, having typically 1 cm spacing, and the pulse voltage of several hundred volt is applied. In the case of electrofusion, dielectrophoresis is used to bring cells into contact prior to pulse application.

An analytical expression for the membrane voltage is known for a spherical cell of radius a under a uniform field E, which is

$$V_m = \frac{3}{2} a E \cos\theta \qquad (1)$$

where θ is the zenith angle relative to the direction of the external field. Biological cells always have size distribution, and due to the size dependence of V_m in the equation, large cells receive too high V_m and irreversibly destoryed, while the induced V_m is too small to affect smaller cells. In addition, when cells are non-spherical, V_m depends not only upon the cell radius, but also on their orientation, and becomes unpredictable. These factors lead to the low yield of electroporation or electrofusion.

However, such size dependence can be avoided with the use of an insulating barrier with an orifice (or an array of orifices) whose diameter is as small as that of the cells. As biological media have more or less electrical conductivity, the field is dominated by conduction current, and as a result, the field lines cannot penetrate into the insulating barrier, but forms a strong field constriction at the orifice. This means that most voltage drop occurs in the vicinity of the orifice, and the induced membrane voltage V_m becomes approximately equal to the voltage applied to the electrodes. Thus controlled magnitude of V_m can be applied regardless of the cell size or orientation. Also, the field constriction makes V_m insensitive to the location of the electrodes, and they can be placed anywhere in the solution. No precise micro machining is required, just needs an insulating film with an orifice, to realize reproducible reversible breakdown.



Fig,1 Electroporation based on field constriction

Fig.2 is an example of electroporation used for the measurement of the cell response. Here, the response of murine myocytes to the feeding of the substrate for TCA cycle is measured by the fluorescence emitted by NADH in mitochondria. The response is clearly seen, indicating that such a measurement can be a powerful tool to investigate the dynamics (i.e. transfer functions) of cascade reactions involved in cells.

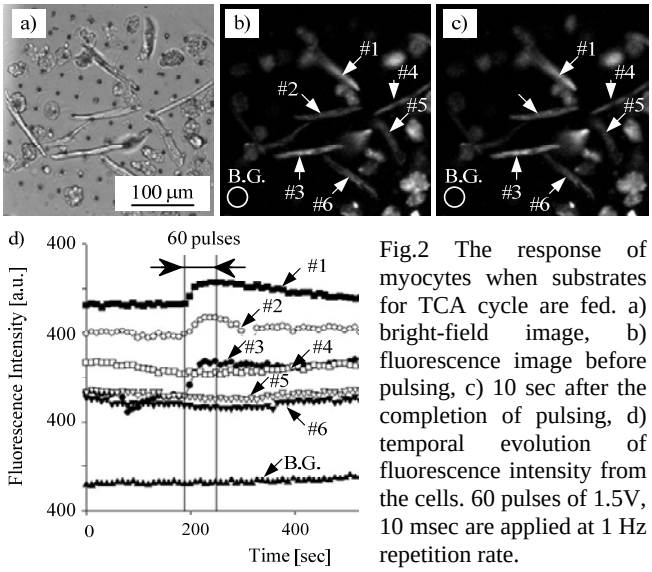


Fig.2 The response of myocytes when substrates for TCA cycle are fed. a) bright-field image, b) fluorescence image before pulsing, c) 10 sec after the completion of pulsing, d) temporal evolution of fluorescence intensity from the cells. 60 pulses of 1.5V, 10 msec are applied at 1 Hz repetition rate.

Fig.3 shows the principle of cell fusion based on the field constriction. As in the case of electroporation, a controllable magnitude of V_m can be applied exclusively at the contact point of the two cells in the orifice, thus leading to reproducible high-yield fusion. Even when a third cell is nearby, it does not take part in the fusion.

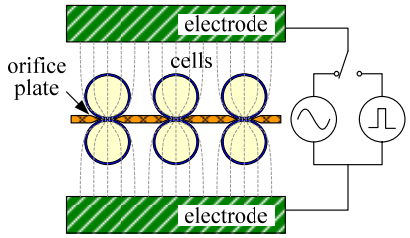


Fig.3 Electrofusion based on field constriction

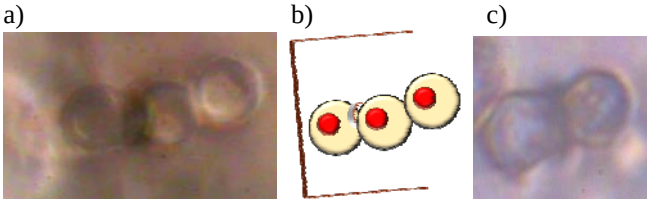


Fig.4 Photo of electrofusion

Fig.4 is a photo of such fusion viewed from a side. Fig.a) is a cell chain consisting of three cells, two cells on one side of the orifice plate, and another on the other side, which is schematically depicted in fig.b). When a pulse is applied, the two cells which are in contact at the orifice fuse, to form a fusant photographed in fig.c). Because we can precisely control the induced membrane voltage, yield of more than 90% is achieved with the method.

3. DNA manipulation with the use of microstructures

An example of DNA manipulation using a microfabricated structure is given in fig.5. It is a pair of microfabricated bobbins, which has narrow waist at the center, as shown in fig.5 a). The DNA molecule is first extended along the substrate surface by electroosmotic flow. Then micro-bobbins are fed, and arbitrarily two of them are picked up by two focused laser beams that are introduced perpendicular to the surface. An elongated particle as the micro-bobbin spontaneously orients itself with its longest axis parallel to the laser beam, i.e. perpendicular to the surface, as depicted in fig.b). Then, they are brought into contact with the extended DNA molecule. When one laser beam is revolved around the other, the DNA molecule is wound around the bobbins, and the tension during winding guides the molecule into the waist of the bobbin. Once wound, the DNA molecule is less susceptible to hydrodynamic breakage, and can be transferred together with the bobbins to any desired location. By reversing the direction of revolution, it is also possible to unwind the DNA.

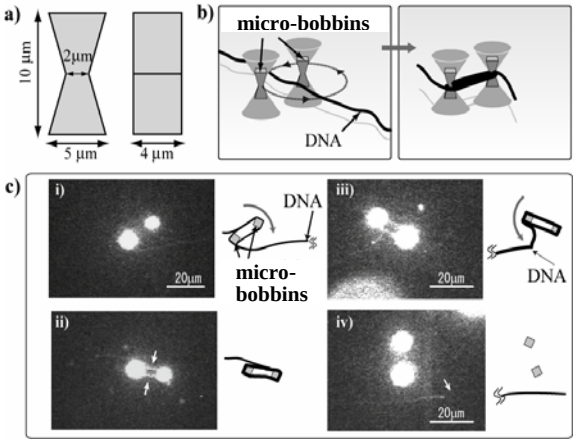


Fig.5 Winding of chromosomal DNA with a microfabricated structure

4. Conclusions

Biomanipulation based on microfabricated structures possesses a large potential for the next-generation bio-nanotechnologies. In particular, the very high-yield electroporation and cell fusion will open a way for regenerative medicine or epigenetic studies through bringing growth, differentiation and initialization factors, or cytoplasm transplant. The handling of DNA will enable a novel space-resolved gene analysis.

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