論 説

Micromachines for Cell Manipulation

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1. INTRODUCTION

About a decade ago, the surface-micromachining technology that was compatible with integrated-circuit (IC) was introduced to give birth to this field. Thus, the same technology base that enabled the miniaturization of electrical devices was extended to fabricate micromechanisms and microactuators [1]-[3]. Since then, remarkable progresses have been achieved. Micromotors having a diameter of one-tenth of a millimeter have rotated at more than 10,000 revolutions a minute, and arrays of microactuated micromirrors have projected the pixels of high-resolution images on large screens. These devices have confirmed the feasibility of many advanced device concepts.

Nowadays, commercial products, including accelerometers and gyroscopes, ink-jet printing heads, projection displays and micro fluidic devices have been introduced, and are expanding in numbers and in diversity. The fluidic application is one of the most prospective applications, because the micromachine technology has superior features for improving both sensitivities and through-put of chemical analysis and synthesis. In addition, it provides key functional devices for genetic diagnosis, a market expanding exponentially after all the human genomes have been read. This paper deals with the application of micromachine technology to tools for bio technology.

2. Cell Captured by Immobilized Antibodies

We succeeded in moving and catching cells using an arrayed microholes structure combined with immobilized antibody layer. One microhole caught one cell. The success rate of catching cell was almost 100%. Cells are moved toward microholes with liquid flow into holes, then they meet antibody which is attached around the hole over patterned gold. The cell was then fixed by the antibody. This is the key technology to inject gene into cell using microcapillary array injector [4, 5].

The microsystem is applied for the realization of a bio-microsystem whose purpose is to improve the efficiency of DNA insertion into cells for gene therapy. As the cells are locally micromanipulated, antibodies are used to catch and isolate them onto the active part of the microsystem (one cell for each micromanipulator, hundred of thousands cells having to be treated at the same time). The part of catching and immobilizing cells in the whole microsystem is reported.

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The principle of the whole microsystem is explained on Fig. 1. It has five functions: catch and arrange cells as an array by means of the microholes and of the antibodies immobilized around the microholes (see Fig. 2), insert the DNA by means of the same microholes and of micro-electroporation using microelectrode check insertion by means of the expression of a fluorescent gene coupled to the therapeutic gene, kill non-transfected cells by means of the same microelectrodes but using high voltage, and finally release transfected cells for cultivation [4].

This kind of bio-microsystems is at the interface between micromachine technology and technology related to biology and biochemistry: both technologies have to be fully compatible. In terms of compatibility, the complete cleanliness of the micromachined substrate required by the biology has been first resolved [5].

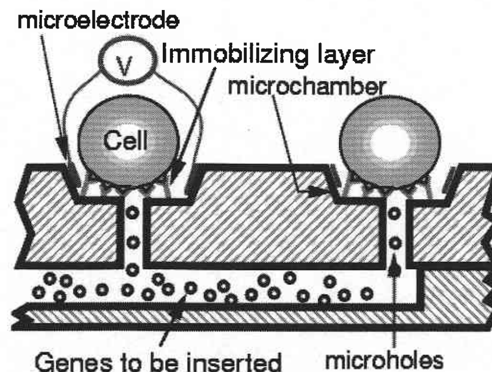

Fig. 1 Principle of the microsystem with the microchambers, microholes, microelectrodes and active layer (immobilizing layer).

We have improved the efficiency of catching and immobilizing cells assisted by a mechanism of aspiration. Cells are moved toward microholes with liquid flow into holes. Then they are immobilized by the corresponding antibodies placed on the gold layer which is deposited around the microholes in order to link antibodies on it via molecules interface. The photograph in Fig. 3 shows that almost all microholes have caught one cell, proving a high success rate after only one aspiration. The photograph in Fig. 4 shows a close view of a cell attached on a microhole and immobilized by the antibodies placed on the gold layer. After a whole night placed in a buffered solution, the cells remain attached, without doing any other aspiration process. This result confirms the principle of immobilizing cells assisted by a mechanism of aspiration. It can be noticed that the microholes insures in fact two functions: to catch cells by aspiration and to insert the DNA once cells are attached above these microcapillaries.

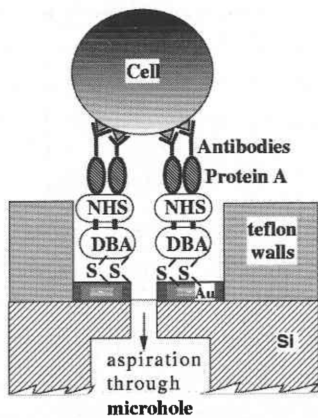

Fig. 2　Principle of the attachment of cells.

Fig. 3　Array of microholes where cells are caught and attached by antibodies placed on the gold layers.

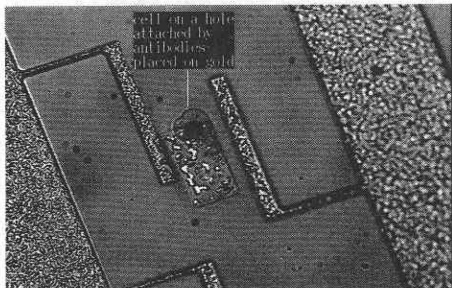

Fig. 4　After one night and no new aspiration: a cell caught on a microhole and immobilized by the antibodies placed on gold.

3. DNA Injection into Cells by Microcapillary Arrays

With the rapid progress in genetic technologies, it is becoming increasingly important to transfer genetic materials across the cell wall to obtain target proteins or to clone cells with desired properties. There are a number of methods to inject DNA into cells, such as a particle gun, some kind of bacteria, micromachined needles [6] and electric-field-enhanced penetration. But few can achieve well-controlled injection. Amount of injection is controlled in a microinjection method but it is a time-consuming operation even by an expert. In order to perform microinjection as a parallel process [7], we have fabricated an array of hollow microcapillaries that can pierce the cell wall and inject genetic materials.

3.1 Fabrication of the hollow microcapillaries

The micromachined injection system is composed of a microcapillary array for injection and a microchamber array for cells trapping. The concept of injection system is illustrated in Fig. 5. This study deals with the microcapillary part because the microchamber array can be made by silicon etching with KOH [8].

Arrays of hollow microcapillaries have been fabricated by silicon bulk micromachining; Fig. 6 shows the process flow. The wafer is thoroughly cleaned by a standard RCA clean, next a protective layer of silicon dioxide against deep RIE etching is grown on the wafer by wet oxidation. Photoresist is then spun on the wafer, and patterned into arrays of circular patterns of 5μm in diameter, and the wafer is placed in buffered HF acid to remove the oxide layer. The patterned wafer is etched as deep as 100μm or deeper by deep trench etching (Plasma Therm Inc., ICP-RIE). Figure 7 shows an example of deep RIE etching, 100μm long and 5μm in diameter. Oxide layer was removed. Whole wafer then was cleaned and oxidized (1 to 2μm)and followed by anodic bonding with processed pyrex glass.

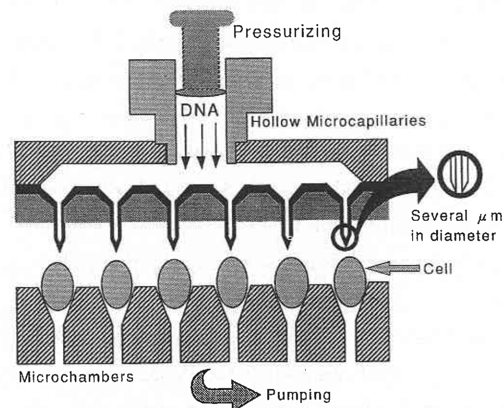

Fig. 5　Concept of a micromachined DNA injection system.

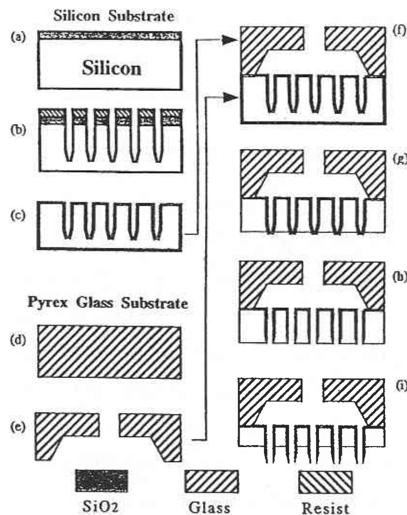

Fig. 6 Process flow of hollow microcapillaries. (a) Silicon is oxidized. (b) Silicon is patterned and etched as deep as 100μm by ICP-RIE. (c) Whole wafer is then reoxidized to 1 to 2μm. (d) Pyrex plass. (e) A pyrex plass is etched and a hole is drilled. (f) Silicon is bonded anodically with the processed pyrex glass. (g) Silicon etching in TMAH solution is proceeded until tips of microcapillaries appear. (h) Tip oxide etching in BHF. (i) Silicon etching in TMAH.

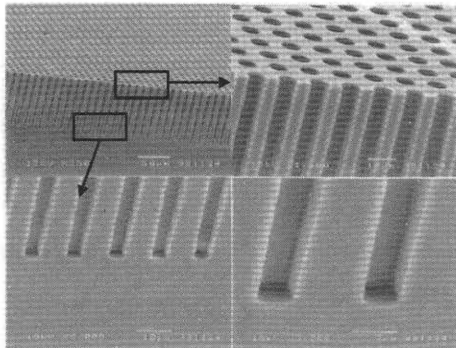

Fig. 7 An example of deep holes etching on a silicon substrate by ICP-RIE, 100μm long and approximately 5μm in diameter.

Backside silicon etching is allowed to proceed untill the tips of microcapillaries appear. Finally, silicon oxide at the edge of microcapillaries is etched in HF acid and the height of capillaries are controlled by silicon etching in TMAH solution. Fig. 8 shows a SEM view of the hollow microcapillaries, which are 30μm in hight, approximately 5μm in diameter at the tip and with 25μm spacing.

3.2 Experimental results

The hollow microcapillaries were applied on Tobacco (BY-2) plant cell conglomerates. The hollow microcapillaries were soaked in ethanol for 1 hour and then dried under ultraviolet rays. A plasmid vector pB221 containing a CaMV35S promoter and a GUS gene was used. A mixture of 50μl DNA in TE buffer was injected into tabacco cell conglomerates with hollow microcapillaries.

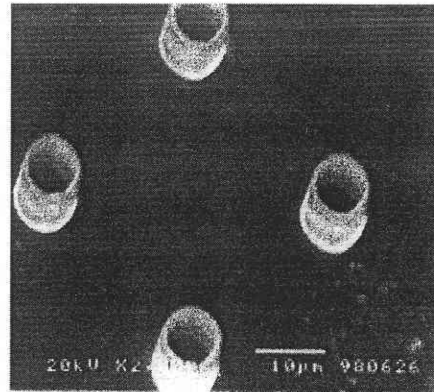

Fig. 8 Top-view of hollow microcapillaries.

Then X-Gluc was performed to evaluate the amount of GUS DNA transferred into the cells. The injection experiment showed positive transient symtoms of blue and high injection level. No noticible degradation of microcapillaries was observed after injection work.

4. CONCLUSION

Micromachine technologies have matured enough for practical applications. In this paper, the applications to bio technolog is described. Biological and chemical micro systems are under intensive development for biomedical products. As an example, basic research on the micro system for genetic therapy and a DNA injection device to cells through arrayed microcapillaries were described.

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