研 究 速 報

Realisation of a Cell Manipulation Bio-Microsystem Using Shadow Mask Techniques

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1. Introduction

As the genetic therapy has made great progress since last years, gene transfection becomes a mayor research topic [1–2]. Beside basic Biological research, the optimizing of the gene insertion system itself, in term of efficiency, is quite important. Using a microsystem for the gene transfer purpose seems to be in fact very efficient [3]. Indeed, it allows to work very locally on cells, thanks to systems having about the same dimensions of them.

The cells can then be locally micromanipulated: we use antibodies 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). But to be sure to reach a very good efficiency of the gene transfection with this method, it is essential to take into account of the compatibility microsystem-biology. For instance, the cleanliness of the technological process for the realization of the microsystem is one of the most important points. The common photolithography step can be used to realise the active part of the microsystem. But it contaminates easily the areas where biological material will afterwards be placed.

That is why a more complicated process but a full compatible one was chosen. The shadow mask technique allow to reach a full compatible process.

In this article, the principle of the microsystem will be first presented. Then the results obtained by separative realization and biological test of each part of the microsystem lead to a discussion about the compatibility between silicon technology and biological technology. Finally, a shadow mask technique solution is proposed to resolve the problem of compatibilities.

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2. Principle of the microsystem

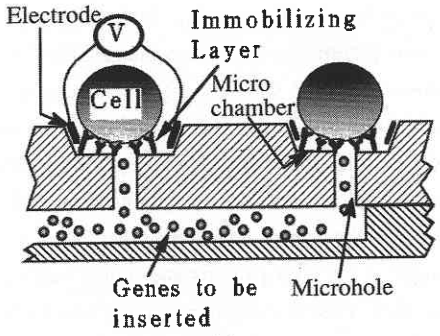
This kind of bio-microsystems is at the interface between micromachine technology and technology related to biology and biochemistry. This bio-microsystem is then multi-function. Its purpose is to be able to i) catch ill cells as an array, ii) treat them in order to insert the appropriate gene, iii)control the insertion (by inserting at the same time a fluorescent gene), iv) eliminate non transfected cells.

On Figure 1 a), the principle of the bio-microsystem is explained [4]. The microchambers have on their edge hydrophobic walls in teflon. These hydrophobic walls allow to push back cells towards the active areas placed inside the microchambers. The active areas are in fact annealed gold patterns: thanks to them the cells are caught on a layer of antibodies which are immobilised on it. The gene transfection is realized thanks to: the microholes and the microelectrodes. The microholes are drilled by ICP-RIE through the wafer and allow the arrival of the gene in the microchamber, where the cells are caught. The microelectrodes are used in two way: for electroporation, helping then for the gene transfection; for killing the non transfected cells, at high voltage.

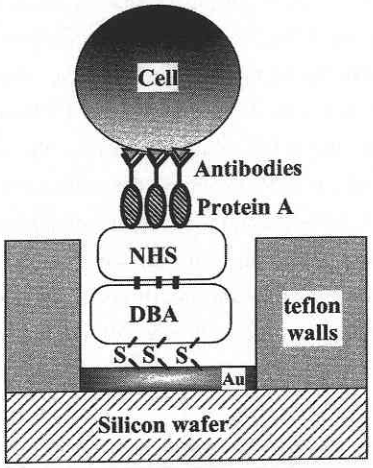
On Figure 1 b), the attachment principle of the cells is shown. Cells attach on a surface only if well oriented antibodies are present. The activated gold is then treated with chemical substances (DBA, NHS) in order to allow the bonding of protein A, able to immobilize in the appropriate orientation the antibodies recognizing specific membrane proteins of the cell.

3. First results and discussion

First of all, each part of the bio-microsystem has been realized individually [4]. It has then been possible to test separatively the

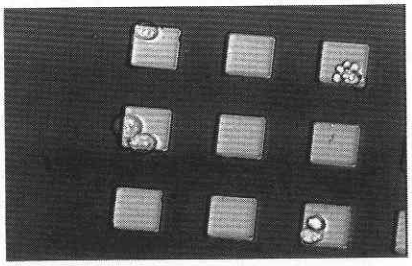


(a)

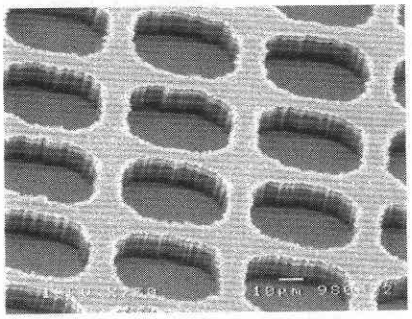


(b)

Fig. 1 a) Principle of the microsystem with the: microchambers, microholes, microelectrodes and active layer (immobilizing layer). b) Principle of the attachment of the cells.



(a)



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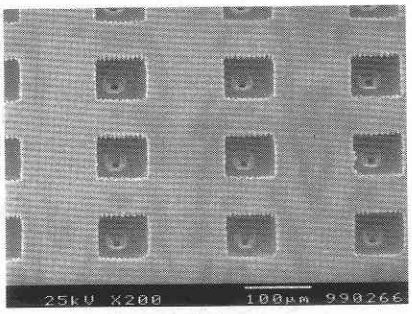

(c)

Fig. 2 Each part of the microsystem is shown to be easily technologically feasible and biocompatible. a) Attachment of cells on gold patterns of 20μm width. b) Microchambers. c) Microholes.

feasibility and the bio compatibility of each part of the biomicrosystem (active surface in gold, microchambers, microholes). On Figure 2 a), the successful attachment of cells on small gold patterns of 20 m width can be noticed. Figure 2 b) and c) present respectively the microchambers and the microholes.

However, the difficulty in the realization of the whole microsystem is to take into account the compatibility between each step of the technological process and the steps of the biological technological process.

For instance, one of the most important step is to realize patterns of annealed gold in a very clean way, to have a good biocompatibility. For that, the patterns have to be realized at the ultimate step of the process, just before beginning the biological process. However, in that case, the commonly used photolithography step is not compatible with the cleanliness required (lithography and organic solvents can pollute the active surfaces) and with the presence of the microholes. Moreover, after depositing gold an annealing is performed at 750 °C, which is no more compatible with the presence of teflon microchambers.

In summary, there must be compatibility of the realization, on the same microsystem, of: microholes, very clean annealed gold patterns and microchambers teflon walls. A solution to have such a compatibility is to use a shadow mask technique with a silicon shadow mask. With a silicon shadow mask, it is possible to deposit by evaporation or sputtering substances according to any kind of shape patterns, without using any organic solution [5, 6].

4. A shadow mask for process compatibility

The principle of the shadow mask technique is presented on Figure 3. The shadow mask consists on two pieces realized by ICP-

RIE: the shadow mask structure and the mechanical alignment structure. The shadow mask structure contains holes in shape of the patterns which have to be printed on the sample. The mechanical alignment structure contains a hole, of dimensions a few microns larger (between 2 and 5μm) than the one of the sample, in which the sample can be easily inserted and mechanically aligned. The two pieces are assembled thanks to the assembly pins (Figure 3 a). They insure a precise alignment between both pieces.

Once the pieces are assembled, the sample is flatten on the shadow mask structure, inside the hole of the mechanical alignment structure (Figure 3 b–c). Then evaporation (or sputtering) is performed through the pattern holes of the shadow mask structure (Figure c). Deposition is performed only on the sample area facing the holes. Wherever else, there is no deposition as it is protected. With this technique, the patterns can be printed with a precision of dimension of 3μm [5]. Moreover, the precision of alignment between the sample and the holes patterns shadow mask has been measured to be less than 10μm [6].

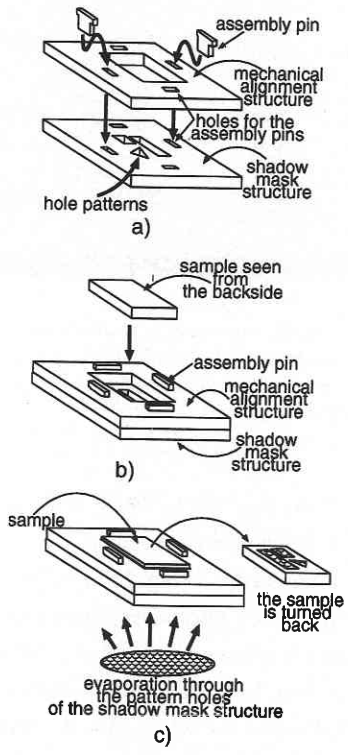

Fig. 3 Principle of the shadow mask technique. a) the mechanical alignment structure and the shadow mask structure are assembled and aligned thanks to pins. b) the sample is inserted inside the hole of the mechanical alignment structure. c) evaporation is performed through the pattern hohles of the shadow mask; the printed patterns can be seen when the sample is turned back.

In the case of the bio-microsystem of this project, the shadow mask technique presents two great advantages. First, as well the gold patterns as the teflon walls can be deposited in a very clean and compatible way on a sample already containing the microholes. Second, thanks to the alignment technique used (mechanical and using pins), the alignment between the microholes, the gold patterns and the microchamber can be done quite precisely.

An example of the gold patterns and teflon walls deposition through a shadow mask is shown on Figure 4 and 5. On Figure 4 b), the fluorescence due to the protein linked on gold is shown, proving the biocompatibility of the shadow mask technique. In what concerns the teflon, because teflon is transparent, a test was imagined in order to be able to reveal the areas where teflon was deposited. The teflon walls are deposited by CVD on an oxidized silicon sample, using RIE. After deposition, the sample is dry etched using RIE, in order to remove the oxide on the areas where teflon was not deposited and to reveal the dimensions of the not deposited areas. The square patterns shown on Figure 5 b) are these areas (future microchambers). By comparison with the initial patterns on the shadow mask (Figure 5 a), the dimensions differs of only 2μm.

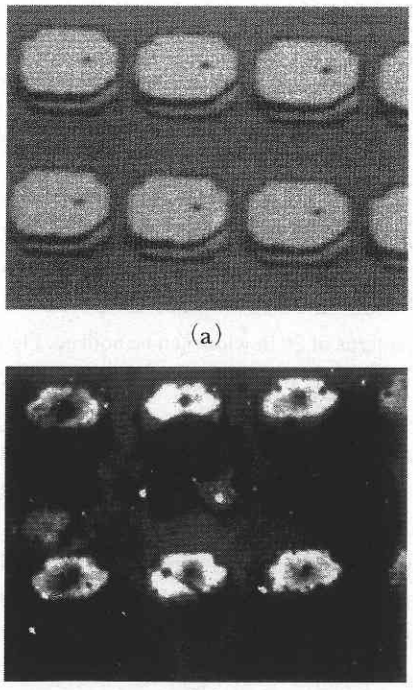

Fig. 4 a) Example of the gold patterns realized by deposition of gold through a shadow mask. b) The biocompatibility of the shadow mask technique is shown, after linking protein A to the substrate, by the fluorescence analysis of the protein A.

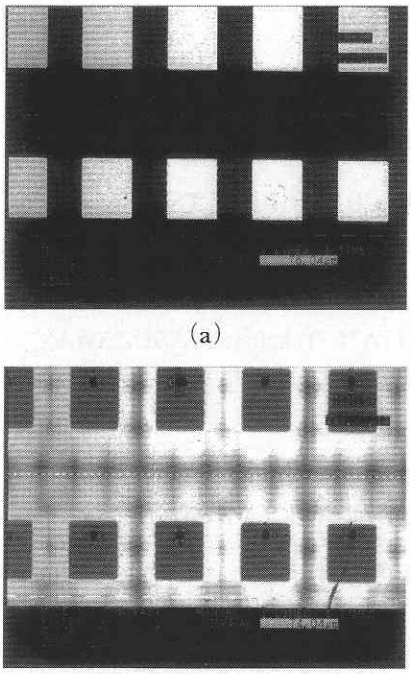

Fig. 5　Example of the microchambers realized by deposition of teflon through a shadow mask. Through the shadow mask (a), teflon is deposited every where on the sample surface, instead on square areas. a) patterns of the shadow mask; b) microchambers with walls in teflon.

5. Conclusions

In order to improve the gene transfection efficiency, an interface biology and silicon micromachining has been developed. This bio-microsystem have on the same sample different parts which processes are not easily compatible one with the other. To overcome the problems of technology and biotechnology compatibility a solution using a shadow mask technique is proposed. The first results obtained show a full biological compatibility and the process of each part of the microsystem are now fully compatible.

Acknowledgments

This project is performed in the frame of LIMMS, Laboratory for Integrated MicroMechatronics Systems, located at Tokyo, a joint laboratory between the Department of Engineering Science of CNRS (CNRS-SPI, France) and IIS/the University of Tokyo. It is supported by the CNRS, the Monbusho (Japanese Ministry of Education) and the JSPS (Japanese Society for the Promotion of Science)

(Manuscript received, December 22, 2000)

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