Extended Abstracts of the 2004 International Conference on Solid State Devices and Materials, Tokyo, 2004, pp. 708-709

**P14-5**

# Microreactor Array Chips for High-throughput Function Analysis of Biomolecules Using Magnetic Beads

Y. Hosoi[1], H. Aizawa[2], N. Nemoto[3], and T. Ichiki[1, 4]

[1]Institute of Engineering Innovation, The University of Tokyo
2-11-16, Yayoi, Bunkyo-ku, 113-8656 Japan
Phone/FAX: +81-3-5841-1180 E-mail: ichiki@sogo.t.u-tokyo.ac.jp
[2]Saitama Small Enterprise Promotion Corporation,
3-12-18, Kamiaoki, Kawaguchi, Saitama, 333-0844, Japan
[3]Innovation center for startups, National Institute of Advanced Industry Science and Technology
2F Marunouchi-Mitsui Bldg. 2-2-2 Marunouchi Chiyoda-ku, 100-0005 Japan
[4]PRESTO, Japan Science and Technology Agency, Japan
Kawaguchi Center Building, 1-8, Honcho 4-chome, Kawaguchi City, 332-0012 Japan

## 1. Introduction

High-speed molecular evolution technology is a new biotechnology for the creation of novel biomolecules with high functions [1]. Its core technologies lie in molecular diversity creation and high-throughput screening for molecular functions. For the practical application of high-speed molecular evolution technology, an innovative tool or methodology is required, particularly for molecular screening [2]. One of the promising approaches for attaining high-throughput screening is microarray technology. In the present study, we have developed a new microreactor array chip suitable for application to high-speed molecular evolution technology. The chip has been designed for the feasible positioning of individual magnetic beads in large scale integrated microreactors, and the subsequent parallel evaluation of a variety of biochemical reactions of biomolecules fixed on the beads.

## 2. Chip Fabrication

Figure 1 shows the fabrication sequence of the microreactor array chips. First, a nickel film of 500 nm thickness was deposited on a glass plate of 20 by 20 mm in area. Subsequently 1,000,000 microreactors of 3 μm in diameter were patterned on a 3 μm-thick negative photoresist (SU-8) coated on the glass plate covered with nickel. Finally, the SU-8 surface was exposed to oxygen plasmas for five min, to render it hydrophilic. Micrographs of the fabricated chip are shown in Fig. 2. As shown in Fig. 3, the wettability of SU-8 surfaces is sufficiently reduced by the 5 min oxygen plasma treatment.

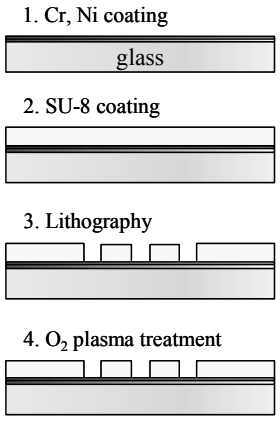



Fig. 1 Microarray chip fabrication process.

Magnetic beads were arranged on a microreactor chip in the following procedure. A droplet of aqueous solution with magnetic beads of 2.8 μm in diameter (Dynabeads M-270) suspended at $6.7 \times 10^7$ counts/ml was spotted onto a microreactor chip, and then magnetic beads were introduced into microreactors with the assistance of a magnetic field applied by sliding a permanent magnet

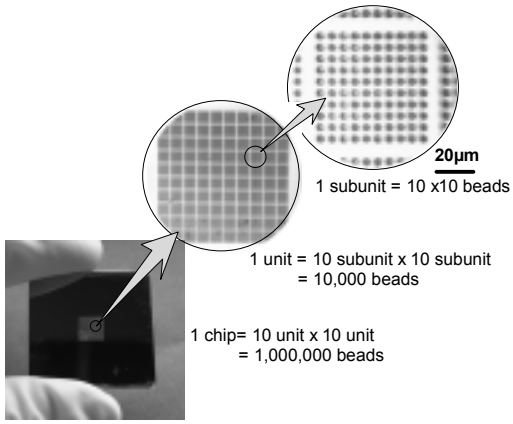



Fig. 2. Micrographs of the fabricated chip.

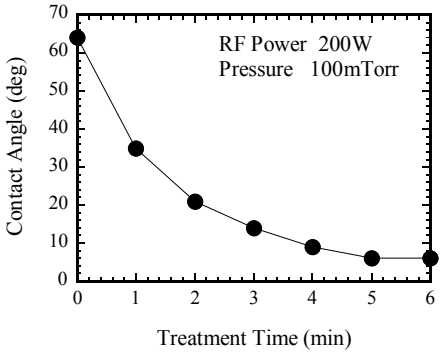



Fig. 3 Water contact angle on the SU-8 surface as a function of the treatment time in oxygen plasma.

horizontally under the chip. Subsequently, extra magnetic beads which are remained outside the microreactors were washed off by distilled water.

### 3. Results and Discussion

The filling ratio, defined as the percentage of microreactors filled with magnetic beads, was examined by optical microscopic observation. Under optimal conditions, the high filling ratio of more than 95% was achieved, as shown in Fig. 4. The microreactor diameters of 3, 4 and 5 μm were examined here. The control experiment using the chip without any nickel film coating resulted in the low filling ratio of less than 3%, and this clearly indicates the significant role of the nickel film. Finally, the model experiment for the affinity assay of biomolecules was carried out using magnetic beads modified with biotinylated PEG (poly ethylene glycol) and target molecules of Texas red-labeled streptavidin, as depicted in Fig. 5. The aqueous solution of streptavidin was dropped onto the microreactor chip filled with biotinylated magnetic beads, and was washed off with the distilled water. Figure 6 shows the micrographs of the microarray chip after washing. The upper and lower figures are a bright field image and a fluorescent image, respectively. As is observed in Fig. 6 (b), a strong red fluorescence from the magnetic beads confirms the capture of the target molecules on the beads.

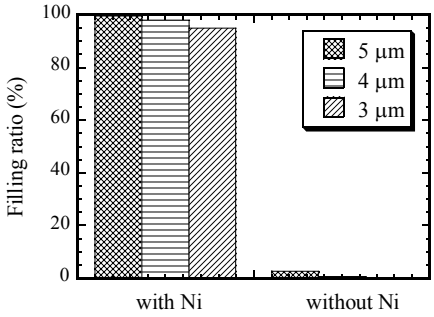



Fig. 4 Comparison of the beads filling ratio between chips with Ni coating and without Ni coating.

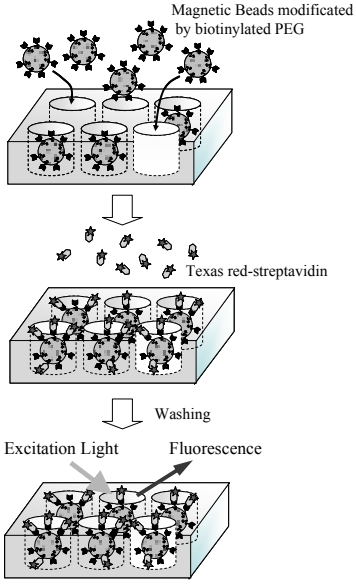
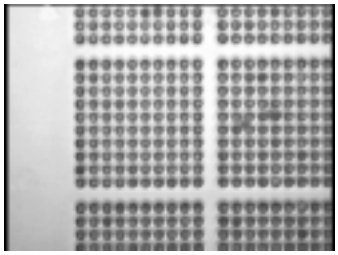


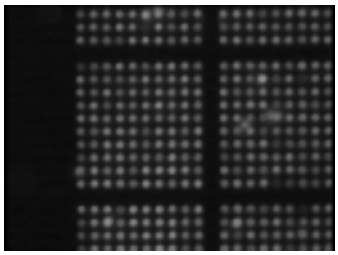

Fig. 5 Procedure of the model experiment for the affinity assay of bimolecules.

(a)



bright view

(b)



fluorescent view

Fig. 6 Microscopic images of biotin-modified magnetic beads in the microreactors.

### 4. Conclusions

In this study, we have developed a novel microreactor array chip for multiplex bead-based assay, and have demonstrated the usefulness of the chip as a molecular screening device that supports high-speed molecular evolution technology.

### References

[1] M. Eigen and W. C. Gardiner, Pure& Appl. Chem., 56, pp. 967-978 (1984).

[2] S. P. Fodor, J. L. Read, M. C. Pirrung, L. Stryer A.T. Lu and D. Solas, Science, 251, pp. 767-773, (1991).