Extended Abstracts of the 2005 International Conference on Solid State Devices and Materials, Kobe, 2005, pp.452-453

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High-throughput Screening of Mutant Biomolecules Using mRNA Display and Microreactor Array Chips

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1. Introduction

Microarray technology such as a DNA chip and a protein chip is a powerful approach to enable high-throughput (HTP) screening, namely, it has great advantages in feasibility of automated information processing due to one-to-one indexing between array position and molecular function, and also feasibility of large scale parallel processing due to down-sizing and large-scale integration [1]. Conventional microarray chips are, however, applicable only to the screening of the affinity of biomolecules. Biomolecules possess variety of important functions other than affinity, e.g. enzymatic reaction, and they become more and more important in the future biotechnology applications. In this paper, we have studied the HTP screening system of enzymatic activity of mutant aldehyde reductase by the combination of molecular handling technology using magnetic beads, specially designed microreactor array chips, and mRNA display technology.

2. Device Concept

Figure 1 shows the concept of our HTP screening system for enzymatic function. (1) Complex of mRNA and protein (virion) was attached on the bead surface [2, 3]. Mutant proteins can be synthesized by introducing random error in the mRNA before virion synthesis (2) single magnetic bead is filled into each microreactor in the self-assembly manner, (3) Enzyme reaction in each reactor is measured using fluorescent microscopy, and the active enzymes (RNA complex) are selectively collected.

3. Results and Discussion

Figure 2 shows the structure of the microreactor array chip. 10,000 micro-reactors of 6 μm in diameter were patterned on a PDMS (polydimethylsiloxane) film coated on the glass plate covered with nickel. The size of the microreactor was designed to be almost the same with that of magnetic beads for the sake of self-assembly packing [4]. The chip fabrication process is shown in Fig. 3. Magnetic beads were arranged on a microreactor chip in the following procedure. The droplet of aqueous solution that suspends magnetic beads of 4.8 μm in diameter (Dynabeads M-480) at 6.7×10^7 counts/ml was spotted on a microreactor chip, and then magnetic beads were introduced into microreactors with the assistance of the magnetic field applied by sliding a permanent magnet horizontally under the chip for a few minutes. Subsequently extra magnetic beads which located outside microreactors were washed off by distilled water. As shown in Table I, the high beads filling ratio more than 99% was achieved for the magnetic field modification caused by swaying a permanent magnet.

The enzyme activity evaluation in a sub pl microreactor was carried out for the mixture of aldehyde reductase (AKR), D-gluconic acid (substrate), and NADPH (coenzyme). Auto-fluorescence from NADPH excited by i line of the mercury lamp (365nm) was measured to track the following aldehyde reduction reaction; aldehyde+NADPH > alcohol +$NADP^+$. A fluorescent microscope equipped with chilled CCD was used in this measurement as shown in Fig. 4. The lower detection limit of the detection system used in this experiment was 10^6 NADPH molecules/reactor. Hence, the necessary time for the evaluation of enzyme activity is estimated to be approximately 200 min under the condition; AKR concentration: 250 nM (20,000/reactor), substrate concentration 10 mM, NADPH concentration 1mM. Here, NADPH consumption by AKR enzyme reaction is estimated to be 3-5 per minute from the literature. The result obtained in our experiment (Fig. 5) is consistent with the predicted value.

4. Conclusions

In summary, we have presented a HTP screening system for enzymatic functions for the first time. Self-assembly arranging of mRNA-protein complex into microreactor array, and enzyme assay in the sub pl volume microreactor has been achieved. This technology will be applied to the creation of useful biomolecules in the near future.

References

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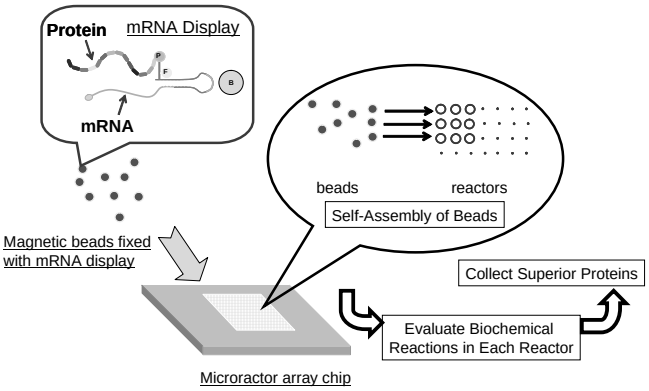

Fig. 1 Conceptual scheme of HTP screening of mutant enzyme.

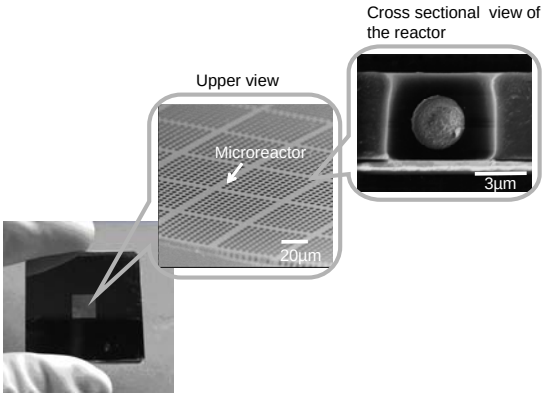

Fig.2 Structure of the microreactor array chip.

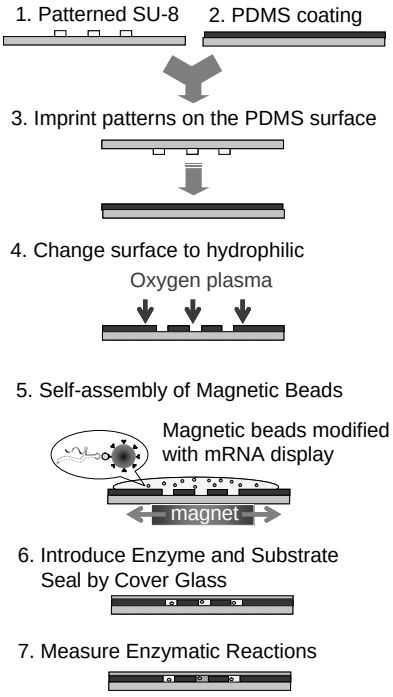

Fig. 3 Fabrication process of the microreactor array chip.

Table I; Beads packing ratio (%) into 10,000 reactors.

	reactor hole diameter				
	3μm	4μm	5μm	7μm	10μm
Electromagnet	17.0	47.1	89.0	95.2	64.4
Rotation	1.8	26.2	29.1	21.6	8.7
Swaying	99.6	98.1	99.0	99.3	97.1

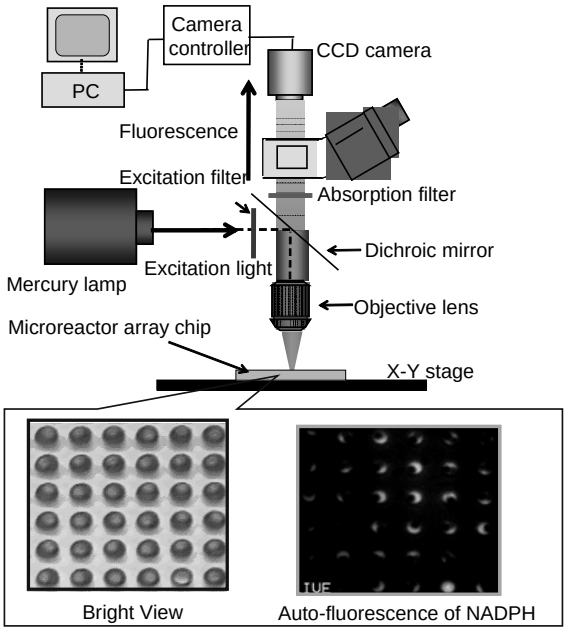

Fig. 4 Microscopic imaging of NADPH auto-fluorescence on microreactor array chips.

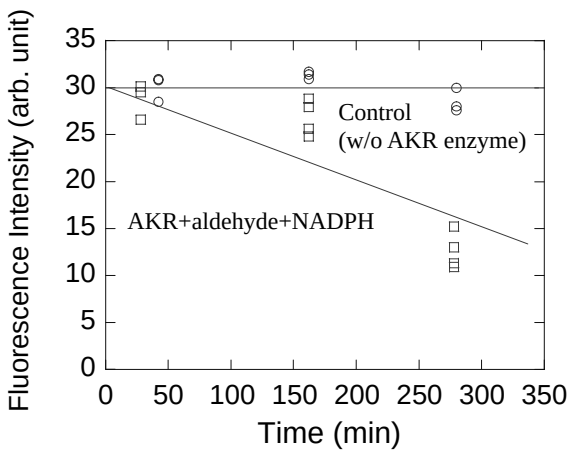

Fig. 5 Fluorescence intensity from NADPH decrease as the enzymatic reaction proceeds over time.