

Reconstitution of the protein synthesis system on a glass microchip

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Abstract

The field of synthetic biology aims to understand the mechanisms of biological systems by designing and constructing artificial biological systems from molecular parts. One of the ultimate goals of the approach is to develop an artificial cell that is under the control of scientists. Although the creation of a bacterial cell controlled by a chemically synthesized genome was accomplished in recent years (1), the construction of a self-replicable artificial cell by organizing essential purified biological macromolecules remains to be achieved (2). Such bottom-up approach is an alternative way for the construction of minimal cells to the top-down approach utilizing natural living cells, which can be helpful for us to explore the boundary between life and non-life.

Here, we reconstituted the protein synthesis system on a glass microchip. By integrating the PURE (Protein synthesis Using Recombinant Elements) system (3), a reconstituted cell-free protein synthesis system composed of purified factors and enzymes responsible for the gene expression in *Escherichia coli*, on a glass microchip, we performed the GFP synthesis with continuous flow in a microchip for the prototype verification.

Biotinylated DNA template encoding the gene for GFP was immobilized on streptavidin-coated sepharose beads with diameter of 34 μm . Prepared beads were introduced into a Y-shaped microchannel in a glass microchip with a 10- μm height dam structure fabricated by 2-step HF wet etching method (Fig. 1) (4). By introducing the components of the

PURE system into this microchip by syringe pump, fluorescent intensity of the recovered solution was demonstrated to be higher than the solution before introduction, indicating that the GFP was successfully synthesized on the microchip.

Because the device has an ability to comprise DNA molecule within it, which can be transcribed to RNA or translated into the protein, the results indicate that it has a potential to be a container of reconstituted life with genetic information. Furthermore, all the macromolecules of the PURE system, tRNA, ribosome, translation factors, RNA polymerase, and several enzymes, consist of RNA and proteins, which can be synthesized by the PURE system, the study sheds light on the way of reconstituting self-replication inside the device, which is crucial for the construction of artificial cells.

- (1) Gibson, D. G. *et al.*, (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329: 52-56.
- (2) Forster, A. C. and Church, G. M. (2006) Towards synthesis of a minimal cell. *Mol. Syst. Biol.* 2: 45.
- (3) Shimizu, Y. *et al.*, (2010) Cell-free translation reconstituted with purified components. *Nat. Biotechnol.* 19: 751-755.
- (4) Sato, K. *et al.*, (2004) Microchip-based enzyme-linked immunosorbent assay (microELISA) system with thermal lens detection. *Lab Chip.* 4: 570-575.

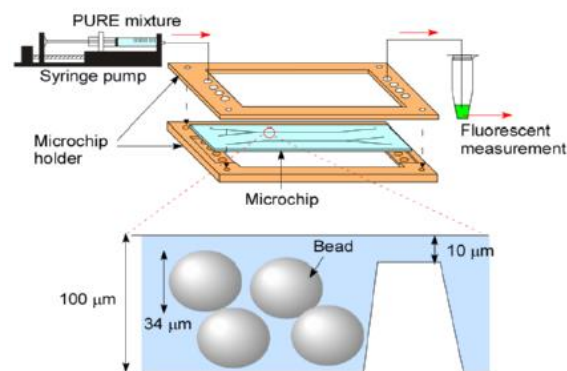


Fig. 1. Design of a microchip