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Microcompartmentalized cell-free protein synthesis in semipermeable microcapsules composed of polyethylenimine-coated alginate

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We describe microcompartmentalized cell-free protein synthesis in semipermeable microcapsules prepared from water-in-oil-in-water droplets by a rupture-induced encapsulation method. An aqueous solution of template DNA coding for green fluorescent protein and enzymes for the cell-free protein synthesis was aliquoted into water-in-oil droplets using a microfluidic device, and the droplets were transformed into semipermeable microcapsules. Substrates for protein synthesis diffused into the microcapsules through their semipermeable polyion complex membranes composed of polyethylenimine-coated alginate. Cell-free protein synthesis was confirmed by detection of the fluorescence of the synthesized green fluorescence protein in the microcapsules. We also used this microcompartmentalized system to synthesize protein from a single molecule of template DNA encapsulated by limiting dilution.

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[**Key words:** Cell-free protein synthesis; Microcompartmentalization; Semipermeable microcapsule; Microfluidics; Rupture-induced encapsulation]

In order to realize efficient selection of a target from a large population of cells, DNA, protein or small-molecules, a high-throughput screening method is essential. Such method should be economical and involve minimal consumption of expensive and rare biological reagents. The droplet-based micro-compartmentalized reaction, which was originally reported by Tawfik and Griffiths (1), meets these requirements. These investigators developed high-throughput systems for screening enzyme libraries in water-in-oil-in-water (W/O/W) droplets (2–4) and used flow cytometry to screen the enzymes that formed in the droplets. Similar approaches using water-in-oil (W/O) droplets and lipid vesicles have been reported (5–9). Although screening of biomolecules in microcompartments has been successfully demonstrated, improvements in the compartment size uniformity, compartment stability, droplet handling, and substrate supply to the compartments are required to make compartmentalized protein screening practical.

Microfluidic droplet formation is a useful method for preparing uniform droplets, microbeads and microcapsules (10–16). This droplet microfluidics technology provides efficient encapsulation and microcompartmentalization. Recently, the use of droplet microfluidics for microcompartmentalized reactions has attracted attention because uniformly sized compartments can be generated, reaction conditions can be controlled, and substrates can be supplied to the compartments (17–21); therefore, droplet microfluidics is expected to be useful for many kinds of biochemical reactions. For example, Agresti et al. (18) used droplet microfluidics to prepare W/O droplets encapsulating mutated cells and then applied the technology to high-throughput screening of enzymes by means of directed evolution. However, the utility of cell encapsulation method for the synthesis of cytotoxic proteins and proteins requiring long-term maturation is restricted because of the limited cultivation time of the encapsulated cells due to lack of the nutrient supply. In addition, the utility of this method for enzymatic reaction is also restricted due to lack of the substrate supply.

Cell-free protein synthesis is a remarkable method for producing and screening native and mutant proteins in the absence of living cells. The method relies on cell lysates containing the essential components for protein synthesis, such as enzymes, substrates, and template DNA. A screening system involving single-molecule polymerase chain reaction (PCR) and cell-free protein synthesis was developed by Koga et al. (22,23) to screen recombinant proteins by means of directed evolution. A DNA library solution was aliquoted onto microplates at the concentration corresponding to a single DNA molecule per well by means of limiting dilution, and the aliquoted DNA molecules were amplified by means of PCR. The amplified DNA was translated to protein by means of cell-free protein synthesis, and then a protein library with linked genotypes and phenotypes was constructed. Koga et al. (22,23) used this method to screen protein with high enzymatic activity by adding a solution of reaction substrate to the microplate containing the enzyme library.

To number up the library size is protein screening by cell-free protein synthesis, the use of a microfabricated reactor array was proposed (24). In addition, Fallah-Araghi et al. (21) applied droplet

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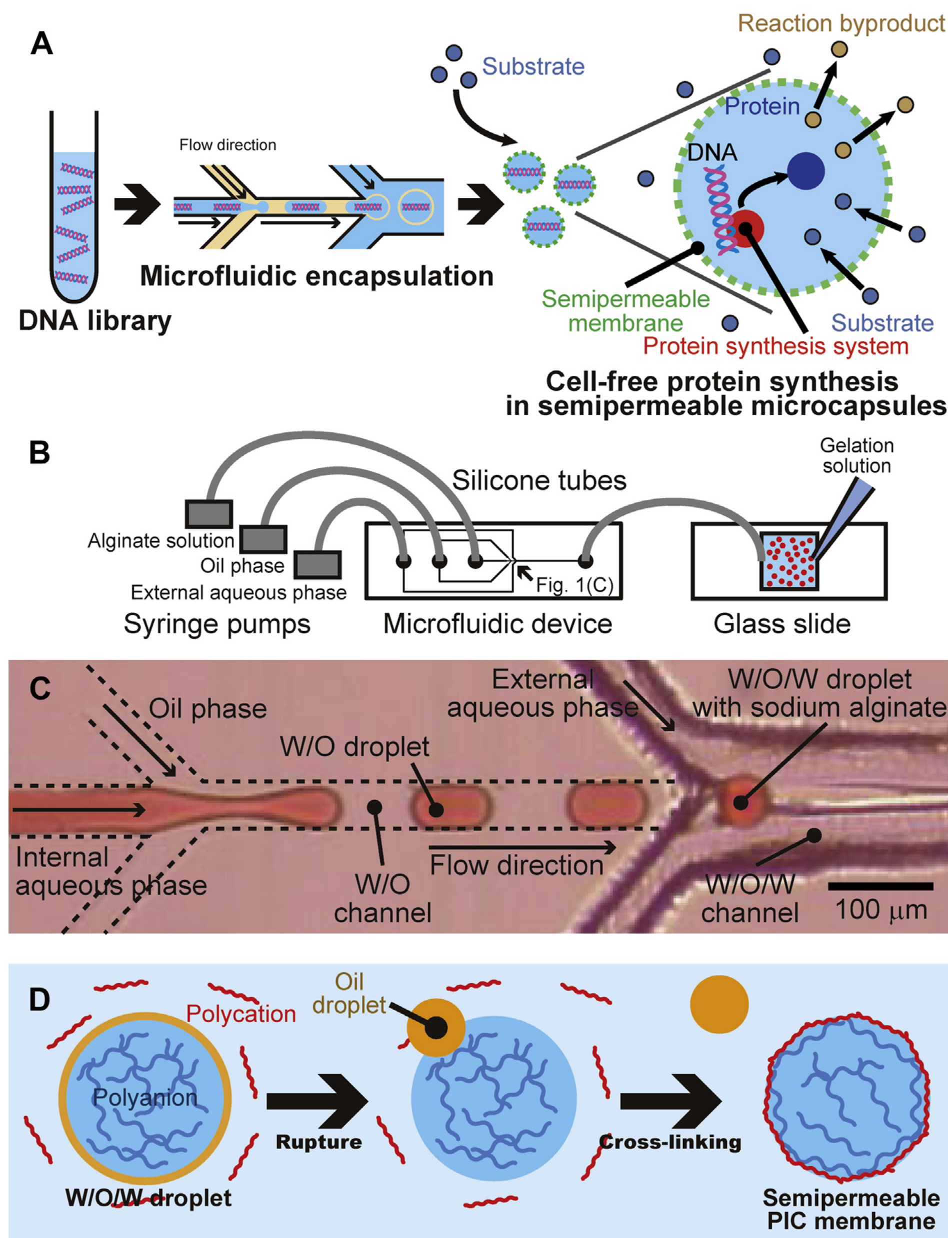

FIG. 1. Microcompartmentalized cell-free protein synthesis in semipermeable microcapsules. (A) Schematic of the experimental procedure. (B) Schematic of the experimental apparatus for preparation of the semipermeable microcapsules. (C) Microscopy image of the formation of W/O/W droplets containing sodium alginate and new coccine dye. (D) Schematic of the formation of the PIC membrane on the W/O/W droplet surface. (B, C) Partially reproduced from Saeki et al. (29) with permission of the Royal Society of Chemistry.

microfluidics to directed evolution by combining single-molecule PCR and cell-free protein synthesis. Although this two-step system works, it comprises many microfluidic processes, including droplet formation, fusion, thermal cycling, incubation, detection, and separation. Ideally, this complicated system would be replaced by a single-step reaction system, that is, direct protein synthesis from a single DNA molecule in small microcompartments. However, the amount of protein that can be synthesized from a single DNA molecule in a cell-free process is small, and detection of such a small amount of protein is difficult, even in small microcompartments.

In contrast, it is known in the macroscopic experiment that the use of semipermeable membranes for continuous cell-free protein synthesis allows for long-duration synthetic reactions and high protein yields (25). Substrates are continuously supplied and low-molecular-weight byproducts are continuously removed through the semipermeable membranes, and proteins can be synthesized at concentrations as high as milligrams of protein per milliliter of solution, which is much higher than the concentrations that can be achieved with batch-mode processes (26–28).

In this paper, we describe microcompartmentalized cell-free protein synthesis in uniformly sized semipermeable microcapsules composed of alginate coated with polyethylenimine (PEI). The semipermeable microcapsules were prepared by a previously reported rupture-induced encapsulation method using a microfluidic device (Fig. 1A) (29). The microcapsules containing template DNA and enzymes were incubated in the presence of substrates, which diffused into the microcapsules through the semipermeable membranes. We also demonstrated direct cell-free protein synthesis from a single DNA molecule encapsulated in these microcapsules.

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