Lab-on-a-chip in Vitro Compartmentalization Technologies for Protein Studies

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Abstract In vitro compartmentalization (IVC) is a powerful tool for studying protein–protein reactions, due to its high capacity and the versatility of droplet technologies. IVC bridges the gap between chemistry and biology as it enables the incorporation of unnatural amino acids with modifications into biological systems, through protein transcription and translation reactions, in a cell-like microdrop environment. The quest for the ultimate chip for protein studies using IVC is the drive for the development of various microfluidic droplet technologies to enable these unusual biochemical reactions to occur. These techniques have been shown to generate precise microdrops with a controlled size.
Various chemical and physical phenomena have been utilized for on-chip manipulation to allow the droplets to be generated, fused, and split. Coupled with detection techniques, droplets can be sorted and selected. These capabilities allow directed protein evolution to be carried out on a microchip. With further technological development of the detection module, factors such as addressable storage, transport and interfacing technologies, could be integrated and thus provide platforms for protein studies with high efficiency and accuracy that conventional laboratories cannot achieve.

Keywords In vitro compartmentalization · Lab-on-a-chip · Microdrop · Microfluidics · Protein evolution · Protein studies

1 Introduction

In vitro compartmentalization (IVC) refers to cell-like compartments generated artificially as reaction chambers in which protein transcription and translation reactions can occur. In biology, cell walls confine networks of chemical reactions so that they can proceed in isolation from the rest of the environment, but toxic proteins are unable to be produced in living systems. Biological degradation systems have the advantage that they remove misfolded proteins from the environment. In IVC, chemically modified amino acids can be incorporated into proteins, expanding the number of variations now available [1] so that different or toxic proteins or enzymes can now be produced that were previously not possible in biological protein expression systems. Tawfik and Griffiths [2] described a system using micron-size aqueous droplets dispersed in an oil medium in which individual gene sequences were proximal to the enzyme variant they encoded. The emulsion droplets in IVC systems may range in size from a few to a few tens of micrometers. The volume of the droplets is ($10^4$ to $\sim 10^{10}$ times) smaller than that used in a typical transcription/translation reaction ($20 \mu L$). Such small droplets ($\sim 500$ million $10 \mu m$ drops per milliliter of sample) provide the opportunity to physically contain one copy of DNA, in an environment containing the components for transcription and translation into protein, so that the synthesized protein is in the same droplet as the DNA that it encodes. In conjunction with versatile controls, these droplets are an ideal means of compartmentalizing biochemical and genetic assays. These advantages have fueled the increasing effort in the development of droplet-based IVC systems in the last few years [3–11].

The advances in microfluidic technologies provide unique opportunities for IVC development. Instead of the conventional methods of preparing emulsions such as homogenizers, stirrers or extruding devices, which produce only polydisperse droplets, microfluidic chips can be used for microdrop formation and control. It has been demonstrated that monodispersed droplets/slugs can be formed and manipulated reliably on-chip for various