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Nanoreactors for Biomaterials Synthesis

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Abstract : Microdrops-based nanoreactors are a powerful in vitro compartmentalization (IVC) tool for studying proteins. There has been increasing interest since the last few years, both in the fundamental understanding of on-chip drop generation and control, and in the device development for applications in chemical and biological reactions. Microfluidic technologies have been developed to generate, fuse, split, sort and incubate droplets. Contents of droplets can be mixed and analysed, allowing a range of biological reactions to be performed.

1. INTRODUCTION

Since the last two decades there has been a steady increase in the development of miniaturised chemical and biological analysis system due to the advance of micro-fabrication and microfluidics technologies. Such an advancement has also provided impetus for the development of micro-reaction technologies for chemical and biological synthesis such as the production of low-volume, high-value, costly specialty chemicals, measuring devices in online process optimisation, catalyst screening tools, micro fuel cells, microorganic synthesis/production in the pharmaceutical industry and protein development[1-5]. This article will review the current trend in the development of microdrop-based nanoreaction platforms for biomaterial synthesis and report some of the recent work at CSIRO Australia towards such a system.

2. LAB-ON-A-CHIP NANOREACTORS FOR PROTEIN SYNTHESIS :

IVC refers to cell-like compartments generated artificially as nanoreactors in which protein transcription and translation reactions can occur. The first such system was

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reported[6] using micron-size aqueous droplets dispersed in an oil medium. Such small droplets 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 IVC system, chemically modified amino acids can now be incorporated into proteins[7] so that different or toxic proteins or enzymes can be produced that were previously not possible in biological protein expression systems These advantages have fuelled the increasing effort in the development of droplet-based IVC systems since the last few years[8-14].

With the advance of microfabrication and microfluidic technologies, researchers have started to develop lab-on-a-chip devices for IVC applications which could provide high efficiency and accuracy that the current laboratory protocols cannot achieve. The current progress in the field and the main functionalities required for an IVC chip to perform protein synthesis were summarized[15]. It was pointed out that it should consist of the functions of droplet formation, droplet fusion, droplet incubation, droplet detection, droplet sorting. Other functions may include, depending on the applications, droplet storage, in-drop polymerase chain reaction device, coupling mechanism to analytical instrument such as mass spectrometer, protein crystallization module and so on.

3. DROPLET TECHNOLOGIES FOR NANOREACTIONS :

3.1. Droplet Formation :

Droplet formation is the first step in IVC chip that can form discrete nanoreactors for biochemical reactions. It has been a classical fluid dynamics problem and received a great deal of attention. The two commonly used chip formats for droplet generation are T-junction and flow focusing, as shown in **Error! Reference source not found.**(a). It has been demonstrated that monodispersed droplets/slugs can be formed[15, 16]. An example of the droplet formation is shown in
Figure 1(b).

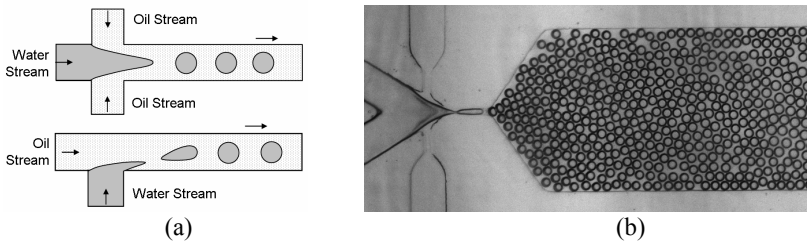


Figure 1 (a) Geometries of microchannel networks for droplet formation. Top: cross channel geometry, bottom: T-junction geometry; (b) A picture of droplets of ~50μm diameter formed from a plastic chip with a main channel of 1 mm and a nozzle size of 50μm.

3.2. Droplet Fusion :

Droplet fusion is required when two or more reagents need to be combined into one drop for biological reactions or detecting products formed. Droplet fusion can be achieved by simply bringing two streams of droplets together and allowing droplets to collide and coalesce, when the flow and material conditions were appropriate. For

more reliable operations, active control is usually required. A number of techniques have been investigated which include electrocoalescence (both AC and DC fields), electrocapillarity, thermocapillarity, thermal control, magnetic beads, and surface-directed and channel geometry-directed methods[17-21]. **Error! Reference source not found.**shows an example of droplet fusion by electrocoalescence.

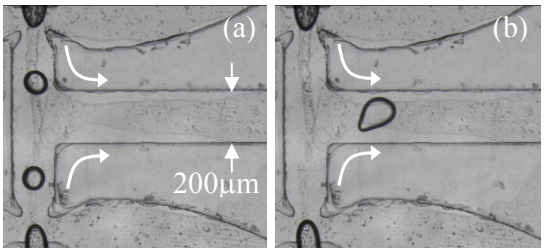
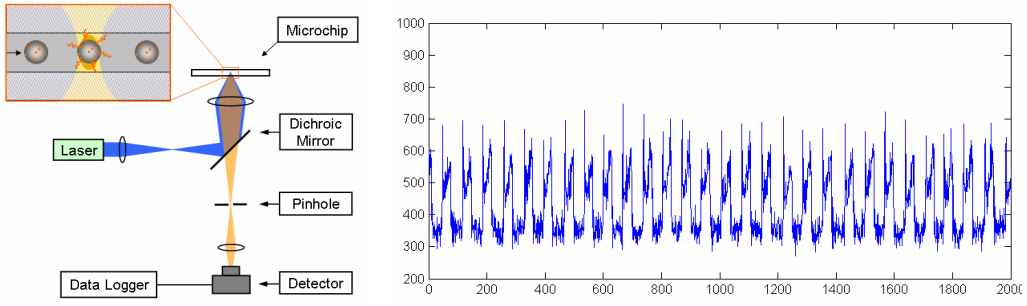


Figure 2 Drop fusion by electro-coalescence technique in a PMMA chip. (a) two droplets just formed independently (b) droplets just fused. Continuous phase: mineral oil with Span80, Dispersed phase: DI water with Tween20. Arrows indicate flow directions. The PMMA microfluidic chip was fabricated in the CSIRO Microfabrication facility in Clayton, Melbourne, Australia**.**

3.3 Droplet Detection :

Once all the reagents are contained in the droplets, biochemical reactions can be performed. The process may require temperature control and this can be achieved by an incubator or on-chip temperature control[22]. The droplets were then screened for products. One of the methods commonly used is the fluorescence-based detection.
Figure **3** shows an optical set-up for screening droplets and an example of the fluorescence signal detected. The detection limit for the system was approximately 50pM. With the droplet size of about 50µm, the average number of dye molecules can be detected is around 2000 per droplet. At such a low concentration, the background noise of the signal needs to be controlled which may include chip material, oil and surfactants in both aqueous and oil phases.



(a) (b)

Figure 3 The optical detection set-up for droplet screening (a) and the fluorescence signal detected from droplets used a biochemical reaction for protein synthesis (b).

3.4 Droplet Sorting and Splitting :

The task of sorting is to identify the droplets of interest and select them for further analysis or storage. The droplets would be selected if the detected fluorescence intensity is above the threshold and directed into a separate fluidic channel. Various forces have been utilised to control the droplet selection such as electrical, electrowetting, magnetic, thermocapillary, hydrodynamics and acoustic forces[23-25]. Droplet splitting is required when droplet size needs to be reduced or the contents of

the droplet need to be divided for different usage. The splitting is mainly achieved by channel branching and obstacles[26]. An example is shown in Figure 4.

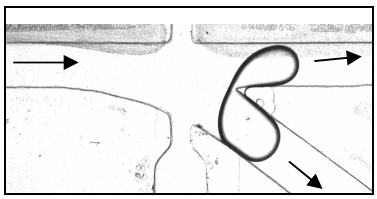


Figure 4 Droplet splitting by channel branching. The PMMA microfluidic chip was fabricated in CSIRO Microfabrication facility in Clayton, Melbourne, Australia. The main channel was of 200x70 μm cross section. Arrows indicate flow directions. The continuous phase is mineral oil while the dispersed phase is DI water.

SUMMARY :

The recent development in microdrops-based nanoreactors as in vitro compartmentalization tool for studying proteins in microfluidic devices is reviewed. Microfluidic technologies have been developed to generate, fuse, split, sort and incubate droplets. Contents of droplets can be mixed and analysed, allowing a range of biological reactions to be performed. Some experimental results were reported to demonstrate these functionalities.

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