A HIGH-THROUGHPUT MICROFLUIDIC SYSTEM FOR THE SIMULTANEOUS FORMATION OF DROPLET-INTERFACE-BILAYER ARRAYS

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ABSTRACT
The ability to form artificial lipid membranes in a reliable, high-throughput lab-on-a-chip format has the potential to advance membrane protein studies and the development of sensitive molecular biosensors, ultimately impacting on the development of novel and low-cost synthetic approaches to drug screening. Existing methods are limited in terms of their automation, throughput and ease of use. We have developed a microfluidic system that allows the formation, alternation, desired positioning and long-term storage of arrays of droplet-interface-bilayers (DIBs). By encapsulating the desired cocktail of liposomes and metabolites into phospholipid-stabilized water-in-oil (W/O) droplets, hundreds of DIBs were characterized using fluorescence-based assays.

KEYWORDS: Microfluidics, Lab-On-a-Chip, Passive Channel Networks, Droplet-Interface-Bilayers

INTRODUCTION

Over the past decade microfluidics has provided sophisticated methodologies in many areas of science. Scaling down fluidic processes to the micro-scale offers many advantages, some stemming directly from the reduction in sample size - reduced sample volumes, portability, lower costs - and others as a result of the ability to integrate bioassays at this scale. The dimensions of microfluidic structures are comparable to that of cells, making microfluidics a suitable platform for biological research. The creation of biocompatible environments and the laminar flow properties of microfluidic channel networks offer very exciting prospects for the development of automated synthetic biology research on a large scale.
Here, we present a microfluidic device optimised for the formation of droplet-interface-bilayers (DIBs) arrays. The system uses droplet-microfluidic techniques to enable a set of functionalities for the simultaneous formation and storage of hundreds of stable, long-lived artificial lipid bilayers in a miniaturised and fully integrated system.

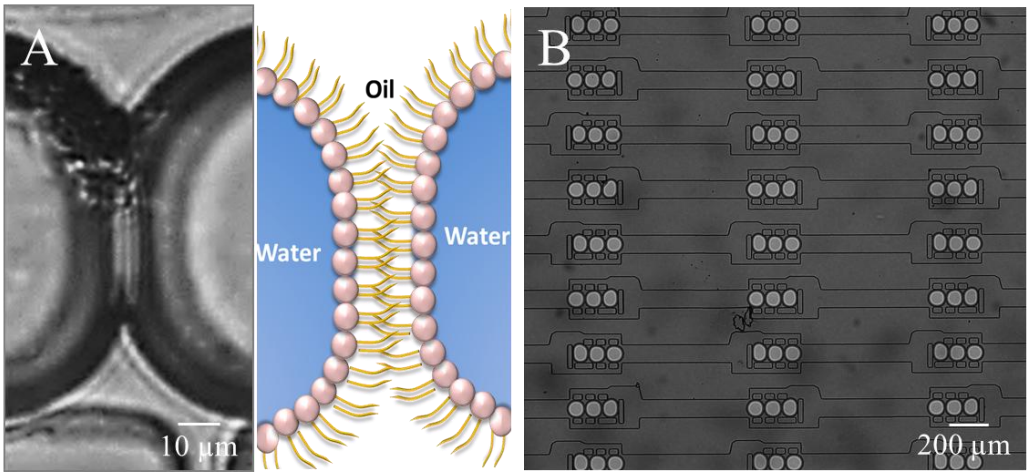

Figure 1:
(A) DIB formation principle. A lipid monolayer self assembles at the oil/water interface, with the hydrophobic lipid tails facing towards the oil and the hydrophilic heads facing towards the water. As the tails of two lipid monolayers come into contact, a droplet-interface-bilayer is formed. Close up image of phospholipid-stabilised contacting droplets forming a DIB (left) and schematic representation of a DIB (right). (B)Example of high-throughput droplet storage within microchannels with DIBs formed between contacting droplets.

THEORY

The principle of DIB formation is simple: when two lipid-stabilised W/O droplets come into contact, the lipid molecules at each interface interact spontaneously forming a lipid bilayer (Figure 1). DIBs are a practical model membrane system [1] that does not require a solid support, thus creating the bilayer in a format resembling that found in nature. Further advantages posed by DIBs are high temporal and mechanical stability, and the ability to form asymmetric bilayers [2].

Several platform have been proposed for DIB formation using electro-wetting on dielectric actuation [3], by manually bringing droplets into contact [4] and via external production of microdroplets followed by syringe pump fluid actuation to bring the droplets into contact [5]. Among these, there have been several examples of DIB production and handling

within a microfluidic platform [6-9]. However, these approaches often lack in throughput, automation or ease of use. The device proposed here aims to overcome many of the disadvantages seen in previous methods.

EXPERIMENTAL

Polydimethylsiloxane (PDMS) devices were fabricated by soft-lithography and consist of a fully integrated, passive microchannel network capable of 3 main functions: droplet production, droplet alternation and droplet trapping (Figure 2). The microfluidic structure comprises a double T-junction (for droplet formation) and a Y-junction (for droplet alternation) followed by a series of optimised microfluidic shift registers [10] for droplet trapping, DIB formation and long-term storage. The register shape enable trains of droplets to be trapped within a pillar structure depending on the balance between the dynamic pressure of the flow and the interfacial tension between the immiscible phases. Outlet 2 (Figure 2A) is used for the extraction of residual air when filling the device. This outlet is subsequently blocked and not used again during normal device operations, thus forcing fluids through the designed passive network of channels. Of particular benefit for reliable DIB formation is the addition of a by-pass channel and arrays of pillars on either side of the main droplet-flow microchannel to divert the continuous phase; this design acting as a pressure relief which slows down droplets, increasing trapping efficiency. With these issues in mind, we have optimised our device design (Figure 2-C&D) to permit a decrease in droplet speed within the registers (where DIBs were formed) without affecting droplet production and alternation. We have used asolectin in our experiments, which is a mixture of lipids at ratios similar to that found in biological membranes. To ensure stable and reliable DIB formation in a high-throughput manner, several parameters were investigated, including lipid concentration, droplet velocity and the shift register geometry.

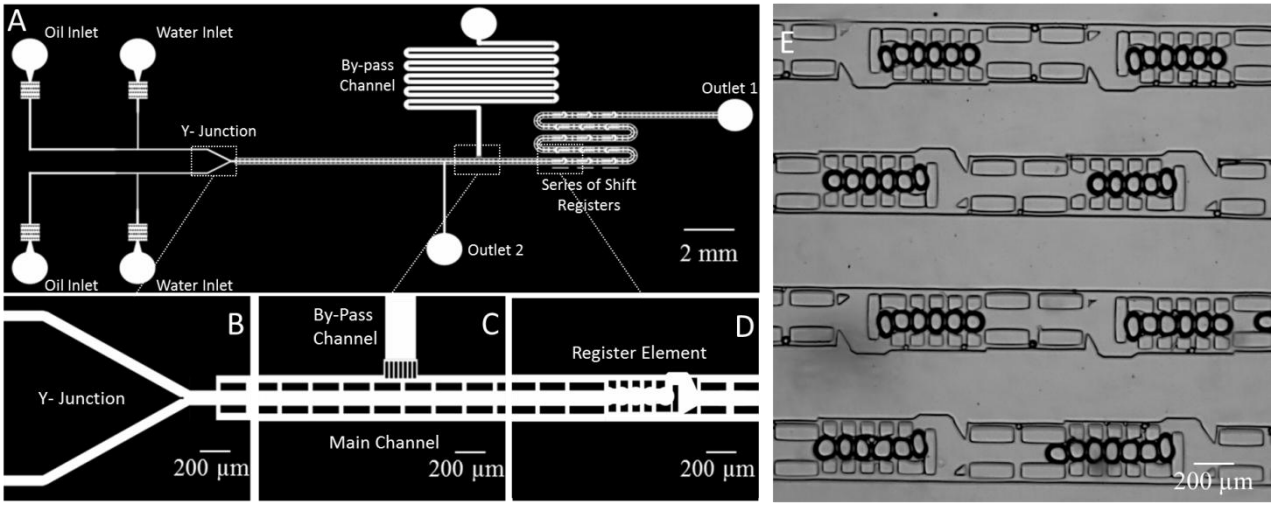

Figure 2:
(A) Drawing of the microfluidic device used. A double T-Junction was used to produce droplets encapsulating different metabolites. A Y-Junction enables droplet alternation (B). The channel structure and a by-pass channel were designed to divert the oil phase and reduce droplet velocity (C). Droplet traps (shift registers) have an optimised pattern to maintain droplets stored in absence of a flow (D). (E) Microscopy image showing high-throughput droplet storage potential within a register array. DIBs were created using 10 mg/ml asolectin.

These studies have allowed us to make an informed decision on the best concentration of lipids to use for long-term droplet trapping and reliable formation of multiple arrays of DIBs.

RESULTS AND DISCUSSION

We have observed that lipid concentration and the speed of droplet collision within the channel network strongly influenced droplet coalescence due to changes in phospholipid adsorption at the water-oil interface. At higher lipid concentrations and lower droplet velocities, droplets became less susceptible to coalescence. However, the increase in lipid concentration decreased the interfacial tension of the droplet interfaces, deteriorating droplet trapping efficiency and storage within the shift registers thus compromising DIBs formation. Moreover, we have validated DIB formation using a membrane permeability assay [11] (Figure 3) based on fluorescent molecule leakage through a lipid bilayer. Carboxyfluorescein, having an additional carboxyl group, takes longer to leak through a DIB than fluorescein. As shown in figure 3, fluorescein molecules permeate through a DIB, making its neighbouring droplets fluorescent. Instead, droplets containing carboxyfluorescein are not seen to leak its content to neighbouring droplets.

Using this system we can generate any number of bilayer networks within a register element. Due to the compartmentalized nature of the droplets, each register element represents an individual experiment providing potential for parallelisation, i.e. studying hundreds of individual assays concomitantly within the same device.

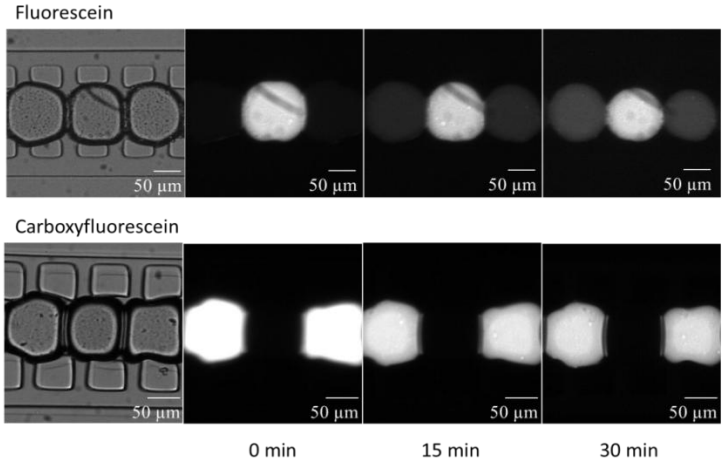

Figure 3:
DIB formation was validated by observing hydrophilic solute leakage across DIB [7]. Fluorescent droplets contained either 250µM fluorescein (top) or carboxyfluorescein (bottom). Phases were hexadecane with 5mg/ml asolectin and a 10mM HEPES, 100mM KCl buffer with 10mg/ml asolectin (extruded liposomes within the droplets). Fluorescein was clearly observed to leak through DIBs, increasing the fluorescent contents of adjacent droplets whilst carboxyfluorescein did not permeate the DIB over the same time period.

CONCLUSION

These miniaturised systems based on DIBs have the potential to be used as sensitive tools for artificial cell mimicry, leading to new approaches for healthcare and for drug screening systems. To the best of our knowledge this is the first fully integrated, passive structure capable of simultaneous DIBs formation and assay parallelisation in a high-throughput fashion.

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