CHIP BASED UNILAMELLAR VESICLE FORMATION AND DISPENSING USING DIELECTROPHORESIS

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ABSTRACT

Vesicles, also known as liposome, are bilayers of lipid molecules enclosing a fluidic media. Vesicles can be formed by spontaneous assembling of phospholipid bilayer separating two different solvent media. Alternatively, vesicles can be formed as solid supported membranes. Such vesicles have potential in understanding the structural and other properties of cellular membranes and utility in targeted drug delivery systems and bio-sensors. In this paper, we have demonstrated an L-DEP based on-chip, vesicle dispensing scheme capable of dispensing a population of identically sized, large unilamellar vesicles and furthermore deposition of lipid bilayer onto polystyrene microbeads in order to produce bio-functional membranes.

KEYWORDS: Surface Microfluidics (SMF), Lipid vesicles, emulsion, Dielectrophoresis (DEP), Lab-on-chip (LOC)

INTRODUCTION

Lipid vesicles are important bio-particles, serving as drug delivery agents [1], artificial cells [2], chemical bioreactors [3] and potentially molecular biosensors [4]. Our work focus on the formation and dispensing of identically sized large unilamellar vesicles in a rapid and controlled fashion by leveraging dielectrophoretic forces, utilizing a surface microfluidic (SMF) device. This approach provides an alternative, less cumbersome scheme of forming vesicles in the micron size range, circumventing the need for on/off-chip pumping required in pulsed microfluidic jetting method [5].

EXPERIMENTAL

The lipid samples used in this investigation were prepared using the procedure outlined in Figure 1. The lipids were purchased as 99% pure chloroform stock solution and used as-is, from Avanti Polar Lipids.

To prepare the lipid dispersion, combination of 10 μL of 10 mg/mL POPC stock and 1 μL of 1mg/mL NBD-PC stock was placed in a Pyrex glass bottom where the chloroform was evaporated under airflow to obtain a thin, dry lipid film.

10 mL of mineral oil (Crystal Plus 70fg; Viscosity: 12.32 cSt) was added to this dry film and the suspension was sonicated in an untra-sonicator for 40 minutes and then left overnight for reliable dispersion formation. The SMF chip, comprised of patterned planar metal (150 nm Aluminum) electrodes coated on top with a dielectric layer (450 nm Si 3N4), deposited on a passivated silicon wafer. The chip was rendered hydrophobic by spin coating (~ 0.1 μm) of Teflon® AF (Figure 2(a)). To prepare the emulsion, Liquid-Dielectrophoresis (L-DEP) actuation methodology was used [6]. A 1 μL aqueous parent sample (25% glycerol by volume), covered by a droplet of the dispersion media was placed on one end of the L-DEP electrodes (Figure 2(b)).

Figure 1: Schematic representation of lipid sample preparation method. The stock lipid samples used were purchased from Avanti Polar Lipids as 99% pure chloroform stock solutions and the mineral oil from STE Oil Company.

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RESULTS AND DISCUSSION

On application of AC voltage (500 Vrms @ 100 kHz), an emulsion jet emerged from the parent droplet and rapidly (typically within ~5-10 msec) conveyed over the electrodes. Upon removal of the electric field, jet disintegrated to form sub-nanoliter sized emulsion daughter droplets (Figure 2(b), 3(a)). The formation and break-up of the jet is explained in [6]. The resulting precision emulsion droplets contained lipid monolayer at their water-oil interface (Figure 2(c), 3(a), 3(b)). The outlined procedure of forming emulsion droplets is far more precise and controllable than any of the comparable on-chip or off-chip methodology, eliminating the need for sedimentation. The outer cover of these emulsion droplets was then diffused in a comparable viscosity dispersion media (doped with silicone oil) bath resulting in stable lipid monolayer formation which encapsulates the aqueous solution within the silicone oil bath (Figure 2(d), 3(c), 3(d)). Finally, to assemble the second lipid layer, these droplets needed to be transferred through an interface oil-aqueous phase boundary. To achieve this, a high viscosity glycerol solution (50% by volume-in De-ionized water) was placed on top of the diffused oil layer, forming a lipid layer at the interface which gradually sank towards the chip surface (Figure 2(e), 2(f)). Upon observing this arrangement after roughly 30 minutes, it was clear that the heavy aqueous media now contained the aqueous daughter droplets, confirming that a very stable lipid bilayer has been formed and the formed bilayer prevents loss of shape and volume of these micro-sized droplets (Figure 2(f), 3(e)). To demonstrate formation of bio-functional and solid supported lipid membrane, the aqueous glycerol sample was loaded with fluorescent polymer microbeads (diameter: 0.5 μm; λ_em = 570-590 nm) and the experimental scheme shown in Figure 2 was repeated. The process resulted in encapsulation of the microbeads by lipid membranes deposited onto them during bilayer vesicle formation (Figure 2(f), 3(f), 3(g), 3(h)) which are very stable, solid-supported membranes with potential bio-functionality.

CONCLUSION

The DEP based SMF scheme successfully dispensed arrays of lipid vesicles, identical sized and precisely positioned, for subsequent bio-functionalization, targeted drug delivery, artificial cell models and bio-sensing utility.

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Figure 3: Micrographs showing the formed lipid membranes and vesicle. (a) formation of emulsion jet and dispensed array of single emulsion droplets over a typical L-DEP electrode scheme; (b) Bright field and fluorescent images of dispensed single emulsion droplet pair (monolayer at interface); (c, d) Bright field and fluorescent image of stable lipid monolayer in oil bath; (e) fluorescent image of two formed lipid bilayer vesicles separating the two aqueous media; (f, g, h) Bright field and fluorescent images of red fluorescent beads (size 1.5 μm) encapsulated by supported lipid membrane.

REFERENCES

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