Dispensing and bio-functionalization of giant unilamellar vesicles on a chip

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Abstract. Giant unilamellar vesicles (GUVs) [1], typically >10 μm in diameter, can serve as interesting models for mimicking biological membranes and furthermore can be usefully leveraged in drug delivery and bio-sensor applications. In this paper, we have demonstrated a chip based vesicle dispensing scheme that utilizes liquid dielectrophoresis (L-DEP) to dispense, precisely positioned GUVs over a range of diameter 30 – 100 μm, a capability that is not readily achieved by either conventional or microchannel technology. These GUVs can also be individually addressed and tailored for a variety of applications such as bio-sensing and cell-on-a-chip.

1. Introduction
Giant unilamellar vesicles or, GUVs [1] are lipid bilayer vesicles typically with diameter in range of 10-100 μm. There is significant interest in the formation and bio-application of GUVs for numerous reasons. Size of GUVs makes them optimal for mimicking cell behavior and creating artificial cell models [2], often known as cell-on-a-chip. GUVs furthermore may be used to encapsulate intact cells and other bio-molecules in a controlled environment [3] and thus utilized for tailored bio-sensing applications [4].

There are very few standard protocols for producing GUVs and the conventional vesicle formation schemes such as ultrasonication and phase inversion methods are not suitable for forming large mono-dispersed lipid vesicles. The two more popular schemes for forming GUVs are: spontaneous swelling and electroformation [1]. Both of these methods require dry lipid powders on solid substrates with specific solvents in order to produce large vesicles. But, these schemes also have limitations in terms of the population and potential screening of the dispensed GUVs. The evidence clearly demonstrates the need for a more compact and integrated, on-chip GUV dispensing scheme that can precisely dispense arrays of large vesicles and furthermore manipulate the GUVs by individually addressing them in parallel, automated steps. We have previously demonstrated that L-DEP based precision emulsion dispensing scheme can be leveraged to dispense large emulsion droplets (dia. ~ 30-100 μm) [5]. In this work, we utilize a simple integration of L-DEP based dispensing [6, 7] and electrostatic droplet manipulation schemes (or, D-DEP) [8] to dispense and manipulate GUVs, encapsulating cell-sized polystyrene microbeads (dia: 10 μm) with bio-functionalized surface. The GUVs are furthermore utilized to perform a standard DNA-bead binding assay to demonstrate the potential features of our chip based dispensing and bio-functionalization of large phospholipid bilayer vesicles.

2. Materials and Methods

2.1 Materials
Two phospholipid samples, namely POPC (1-palmitol-2-oleoyl-sn-glycero-3-phosphocholine) and NBD-PC (1-acyl-2-[6-[7-nitro-2-1,3-benzoxadiazol-4-yl]amino]hexanol]-sn-glycero-3

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phosphocholine), were purchased from Avanti Polar Lipids as 99% pure chloroform solutions. The later (NBD-PC) has a truncated fatty acid chain to incorporate a fluorophore ($\lambda_{ex./em.}$: 460/534 nm; green emission) and was used to facilitate better observation of formed lipid membranes. First a mineral oil dispersion of the two lipid samples was prepared. A combination of 90 $\mu$L POPC and 10 $\mu$L NBD-PC stocks was spread in a pyrex glass bottom and the chloroform solvent was evaporated under air flow to obtain a thin, dry mixture of the two phospholipids. 10 mL of mineral oil (Crystal Plus 70fg; viscosity $\sim$ 12.7 cSt; STE Oil Company) was then added to the glass bottom and the mixture was ultra-sonicated for approximately 60 min. and then left overnight to prepare uniform, stable lipid dispersion in mineral oil.

A 5 bead/nL sample containing 10 $\mu$m diameter polystyrene microbeads and a single-stranded DNA (ssDNA) oligonucleotide with a specific fluorophore (TAMRA$^{TM}$ : $\lambda_{ex./em.}$: 546/574 nm; red emission) sample was also prepared in de-ionized water (conc. $\sim$ 2 $\mu$M).

2.2 L-DEP based dispensing of GUVs

Device fabrication protocols and dispensing of single emulsion droplets leveraging L-DEP has been reported in [9]. A $\sim$ 1 $\mu$L droplet of polystyrene microbead suspension sample (conc. $\sim$ 5 beads/nL) is covered by a comparable sized mineral oil-lipid dispersion sample and placed on one end of the L-DEP electrode scheme (Figure 1(a)). Upon application of an AC voltage (480 Vpp at 100 kHz), an emulsion jet is formed and rapidly propagated to cover the entire L-DEP electrode length [9]. Upon removal of the externally applied voltage, the emulsion jet rapidly disintegrates into precisely positioned single emulsion droplets, containing a lipid monolayer at the aqueous-oil interface (Figure 2(a)). It is important to note that during L-DEP finger actuation, the polystyrene microbeads are positioned at the aqueous-oil boundary and hence coated with functional lipid membrane (Figure 2(b)). This binding of lipid molecules to the bead surface can be enhanced by utilizing specific ligand binding between a biotin molecule (located in lipid bilayer) to avidin (immobilized on the bead surface) [10]. The experimental results of the stable avidin-biotin conjugated DNA-lipid binding will be presented at the conference. At this stage, the L-DEP chip is immersed in lipid-mineral oil dispersion media, partially doped with comparable viscosity silicon oil, to diffuse the outer oil boundary and suspend the uniform monolayer lipid vesicles (vol. $\sim$ 1 nL; dia. $\sim$ 60 $\mu$m) into an oil bath (Figure 2(c)). The bilayer is assembled by introducing a third aqueous phase (viscosity controlled by increasing glycerol concentration) on top of the oil bath. The sinking interface consists of a second lipid monolayer which results in the bilayer formation over the dispensed daughter droplet, encapsulating functionalized microbeads (Figure 2(d), 2(e)).

2.3 ssDNA binding to the functionalized microbead surface

Large fatty acids in the hydrophobic tail of lipid molecules provide a soluble molecule with suitable binding sites for bio-molecules including DNA, RNA and various proteins [3]. In this work, we have utilized an ssDNA oligonucleotide, containing a fluorophore (TAMRA$^{TM}$) to demonstrate binding between lipid molecules and the ssDNA molecule. An integrated electrode scheme was used (see Figure 1(b)), where electrostatic droplet actuation electrodes (or, D-DEP electrodes) are suitably
integrated with the L-DEP electrode scheme, to facilitate transportation of dispensed daughter droplets and vesicles to specific reaction/mixing sites. For this assay, first L-DEP electrode scheme was used to dispense a single array of lipid monolayer vesicles with 5-6 lipid coated beads per droplet. On another L-DEP electrode, arranged in parallel to the first set, a second parent droplet of ssDNA sample (conc. ~ 2 μM) was actuated, under the oil bath. A pair of monolayer lipid vesicle and ssDNA daughter droplet was then transported and mixed using fishbone shaped D-DEP electrode scheme (Figure 1(b)). The mixed vesicle and unmixed droplets are shown in Figure 3(a-f). Since the microbeads have a lipid membrane sitting on their surface, they facilitate binding of ss-DNA molecules. Finally, the GUV is formed from the mixed lipid mono-layered vesicles, as explained earlier in section 2.2.

3. Results and Discussion
The reported experiments demonstrate the versatile applications of GUVs in housing and transporting bi-sensors. In absence of a lipid membrane, DNA or other bio-molecules have a lower affinity to bind to polystyrene bead surface. However, as discussed earlier, the presence of a stabilized lipid molecule at the interface provides sites for interaction between the DNA molecule and the microbead. The results of such a DNA binding assay are shown in Figure 3. Figure 3(a-f) shows unmixed lipid vesicles and DNA daughter droplets on L-DEP electrodes, along with the mixed vesicle (centre). Both green and red fluorescence emission was observed from the mixed vesicle and the surface of coated microbeads was resolvable from the bulk emission resulting from the unbound DNA molecules.
Finally, the second lipid layer was self-assembled [5] to form bilayer vesicles (Figure 3(g, i)) and the bio-functional microbeads were encapsulated inside the vesicles. As a control, 0.1 % of Triton X-100 was added to the first parent droplet (containing microbeads) which resulted in degradation of formed lipid membranes and in this case, minimal to no DNA binding was observed. However, since Triton X-100 was only present in the inner sample, the bilayer formation is unaffected and the GUV was again formed for this droplet (Figure 3(j-l)). This effect has been demonstrated in Figure 3(k, l) where due to minimal DNA binding to bead surface, the microbeads cannot be resolved under either blue (for green emission) or green (for red emission) light exposure and entire signal was recorded from the bulk of the GUV.

4. Conclusion
This work demonstrates that on-chip integrated electrode schemes such as L-DEP and D-DEP can be practically leveraged to achieve precision dispensing and subsequent manipulations of functionalized lipid vesicles, which can either themselves serve as bio-sensor or can be leveraged to create artificial test beds for multiple bio-assays in a rapid, automated fashion. The superiority of vesicular bio-sensors in comparison to solid surface based bio-sensors is yet to be fully confirmed.

5. Future Work
We intend to demonstrate the superiority of vesicle based bio-sensors when compared to the conventional bio-sensors [4] and utilize specific lipid and DNA molecules that can provide stable and selective linkage, such as the biotin-avidin linkage resulting in more stable and irreversible binding of DNA or other bio-molecules for preparing tailored bio-sensors. Quantum Dots can replace microbeads in order to provide enhanced quantum efficiency and more stable photo-response in future bio-sensing applications [11].

6. Acknowledgements
The authors acknowledge the financial support received from National Science and Engineering Research Council of Canada (NSERC) and CMC Microsystems during this work.

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