CHIP BASED ASSEMBLY OF VESICULAR BIO-SENSORS USING QUANTUM DOTS AS BIO-PROBES

R. Prakash* and K. V. I. S. Kaler**
University of Calgary, CANADA

ABSTRACT
Lipid bilayer vesicles when assembled with specific bio-probes or markers can serve as bio-sensors with potential utility in micro-biotechnology, serving as tailored bio-sensors, drug-delivery agents and mimicking cellular behavior. We report on a Surface Microfluidic (SMF) chip based vesicle dispensing scheme to rapidly dispense variable sized large vesicles (diameter: 30-150 μm) labeled with Quantum-Dot based fluorescent bio-probes for chip based bio-assays.

KEYWORDS: Surface microfluidics, Liquid-dielectrophoresis, Quantum dot, Bilayer vesicles, Bio-sensors.

INTRODUCTION
Lipid bilayer vesicles can be spontaneously assembled, often using off-chip methods such as ultrasonication, phase inversion, electroformation and microchannel technology. However, most of these schemes are very limiting for on-chip applications and often require complex steps with large volume of solvents. We have previously demonstrated that Liquid Dielectrophoresis (L-DEP) based Surface Microfluidic (SMF) chips can rapidly dispense multitude of lipid vesicles of uniform size and structure. This work furthers the applicability of SMF chips in assembling variable sized giant vesicles which can be leveraged to transport Quantum Dot labeled bio-probes and develop vesicular bio-sensors for tailored biodetection [4].

EXPERIMENTAL
The bio-samples used in this work are shown in Figure 1. The two biotinylated phospholipid samples were purchased as 99% pure chloroform stock solution from Avanti Polar Lipids (USA). Qdot®-525 streptavidin conjugate, comprised of a biotin-binding protein covalently attached to the core shell, was purchased as 1 μM stock solution from Invitrogen, USA. NAP®-5 Column from GE was used to re-suspend the Qdot® in low conductivity TRIS-MES buffer (pH 7.5). The two oligonucleotide samples were supplied by Integrated DNA Technologies, USA. Lipid dispersion-in-mineral oil was prepared off-chip and utilized to form an array of single emulsion droplets, subsequently leading to formation of monolayer/bilayer lipid vesicles, as reported in [1]. The oligonucleotide concentrations used during experiments lied in the range of 10-30 ng/μL.

The two different electrode schemes utilized in this work are shown in Figure 2(A, B). Scheme 1 (Figure 2(A(i-iv))) consists of two uniform L-DEP electrode pairs integrated with electrostatic droplet transport electrodes (D-DEP) whereas scheme 2 incorporates continuous tapered and pinched L-DEP electrodes integrated with D-DEP electrodes, to rapidly assemble vesicles and bio-droplets in the size range of 30-150 μm and subsequently manipulate them for bio-assays (Figure 2(B(i-iii))).

Monolayer and bilayer vesicles were formed using L-DEP actuation of an aqueous sample, encapsulated in the two lipid dispersion media. Vesicles assembled using Lipid 1, when viewed under fluorescent microscope (λex = 230 - 400 nm), exhibited a ring shaped green fluorescence (λem = 525 nm) emanating from the vesicle membrane (Figure 3(a-c)). In contrast,
such a fluorescent ring was absent when Lipid 2 sample was used to form vesicles (Figure 4(b)). These observations confirm selective anchoring of Qdot® to specific binding sites (biotin heads) in the vesicular membrane.

RESULTS AND DISCUSSION

Three bioassays were conducted to demonstrate the applicability, target specificity and detection range of the chip based vesicular bio-sensors. In Assay1, oligonucleotide, S1 was mixed off-chip with Qdot® to prepare a binary mixture containing Qdot® bound oligo bio-probes. Upon L-DEP actuation of this aqueous sample-in-Lipid 1-mineral oil dispersion, the streptavidin linker in the Qdot® molecules allows the Qdot®-S1 bio-probe to anchor itself selectively to the biotin heads in the lipid membrane (Figure 3(d, e)). D-DEP actuation is then used to add a complementary oligonucleotide (P1 with IOWA-FQ™ quencher at 5'-end) daughter droplet, to the functionalized monolayer (Figure 2(a, c, e)). The mixing results in hybridization of complementary base pairs in the bio-probes and dominant quenching of anchored Qdots (Figure 3(f, g)).

Two additional assays were also conducted to verify the selectivity and specificity of the binding between the marker and the Qdot® bio-probe. In Assay2, Lipid 2-in-mineral oil dispersion was used and the Qdot-oligo bio-probes did not anchor on the membrane (Figure 4(a, b), resulting in quenching of bio-probes only within the vesicle (Figure 4(c, d)). In Assay3, electrode scheme 2 was utilized, where a variable size vesicle dispensing scheme (Figure 2(B)) was used to demonstrate that by controlling the quantity of the oligonucleotide P1 (quencher) in the dispensed variable volume reagent daughter droplets, selective quenching of bio-probes anchored either on the vesicle membrane (with excess amounts of quencher; see Figure 5(b,
e) or, encapsulated within the vesicle (with scarce amounts of quencher; see Figure 5(c, f)) can be achieved. Subsequently, in each assay, a second lipid layer was spontaneously assembled on the monolayer vesicles to secure the bio-probes on/within a more stable bilayer membrane structure (Figure 3(c, e, g), 4(b, d), 5(d-f)).

**Assay2**

*Figure 4: Assay2 results: (a, b) Bright and fluorescent snapshot of unmixed vesicle with bio-probes encapsulated in the vesicle and not anchored on the membrane surface (absence of fluorescent ring); (c, d) Quenched, mixed vesicle showing quenching of encapsulated bio-probes within the vesicle (QE ~ 75%).*

**Assay3**

*Figure 5: Assay3 results: (e, f, g) Bright images of an unmixed vesicle and two mixed vesicles, mixed with different quantities of the quenching oligonucleotide P1; (h) Fluorescent image of unmixed vesicle with unquenched, anchored bio-probes; (i) Fluorescent image of mixed vesicle with excess of P1-quencher (conc. of anchored bio-probes quenched with QE ~ 75%); (j) Fluorescent image of mixed vesicle with scarcity of P1-quencher (encapsulated bio-probes quenched (QE ~ 70%)).*

**CONCLUSION**

The work demonstrates advance capabilities of our SMF chip for precision dispensing and assembling of vesicular bio-probes and tailored bio-sensors targeting, genomics/proteomics, cell tracking/marking and other biomedical and biotechnology applications. Three quantitative bioassays were conducted to demonstrate that the chip based scheme is capable of detecting specific marker sites embedded in the vesicle membrane and is therefore suitable for applications such as ultralow concentration viral/microbial detections (in pM range) which is comparable to the existing conventional detection schemes.

**ACKNOWLEDGEMENTS**

The authors acknowledge the financial support from National Science and Engineering Research Council, CANADA, CMC Microsystems during the course of the presented research work.

**REFERENCES**


**CONTACT**

*R. Prakash, tel: +1-403-220-2299; prakash@ucalgary.ca; **K. V. I. S. Kaler, tel: +1-403-220-5809; kaler@ucalgary.ca.*