SINGLE ISOLATED VESICLES IN MICROFLUIDIC TRAPS TO STUDY MEMBRANE PROTEIN KINETICS

Tom Robinson, Phillip Kuhn, Klaus Eyer and Petra S. Dittrich
Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

ABSTRACT

We present a microfluidic platform that is able to trap and isolate single giant unilamellar vesicles (GUVs) in parallel. To demonstrate this, the membrane pore protein α-hemolysin (αHL) was added to GUVs and the kinetics of small molecule release was recorded.

KEYWORDS

GUVs, vesicles, membranes, lipids, membrane proteins, transport, α-hemolysin

INTRODUCTION

Model membrane systems such as supported lipid bilayers and liposomes are valuable tools for studying biological membranes [1]. GUVs (> 1 µm) are particularly useful as they do not require the support of a surface and can be used as cell-mimics owing to their similar size. The disadvantage is the difficulty in handling these delicate objects. A common approach is to allow GUVs with a more dense fluid inside to sediment on a glass surface for imaging. This, however, makes it difficult to supply various fluids without flushing them away. A functionalized surface can solve this problem, but typically requires adding artificial lipids to the membrane. Our approach solves this issue by using a microfluidic device containing hydrodynamic traps that are able to spatially confine up to 60 GUVs. 'Donut' shaped valves facilitate isolation of specific GUVs and separate pressure lines allow up to 8 different experiments in a single chip.

EXPERIMENTAL

Chip fabrication. PDMS was used to fabricate the microfluidic chips that consisted of two layers [2]. The two master molds were made using photolithography as previously described [3]. Briefly, SU-8 2015 photoresist was used to create 20 µm high features on a silicon wafer with a UV light source. PDMS oligomer and curing agent at a ratio of 10:1 was partly cured over the top layer for 3 h at 80 °C. On the lower layer master, the PDMS was spin-coated to a height of 40 µm and cured at 80°C for 1 h. After punching fluidic access holes with a 1.5 mm biopsy puncher, the microchannels were finalized by bonding the PDMS to a glass slide using a plasma oven.

GUV preparation. Unilamellar vesicles were made using the electroformation method [4]. Sphingomyelin, DOPC and cholesterol were dissolved in chloroform/methanol (9:1 v/v) at a concentration of 1 mM. DiI at 1 µM was added and the mixture dried on an indium tin oxide (ITO) coated slide overnight under a vacuum. The lipids were then rehydrated using Millipore filtered water containing 100 µM calcein, and a second ITO slide was placed on top separated by a 1.5 mm silicone rubber spacer. GUVs were produced over 4 hours, by applying 0.7 volts at a 10 Hz and at a temperature of 60°C.

Fluorescence microscopy. Wield-field microscopy was conducted using an inverted microscopy (IX70, Olympus) equipped with a mercury lamp and a 40x/0.65 air objective lens, and images were recorded with an EMCCD (887, Andor). Fluorescence signals were monitored using optical filters sets for DiI (Ex:525/50, Dichroic mirror 560, Em: 566 LP) and (Ex: 455/70, Dichroic mirror 494, Em: 515 LP) for calcein. Optically sectioned microscopy was achieved using a confocal laser scanning microscope (Axiovert 200M, Zeiss) and a 63x/1.4 oil immersion objective lens. Calcein fluorescence was recorded using 488 nm Argon ion laser line, UV/488/543/633 dichroic and a 505 nm long-pass filter.

Chip operation. Following chip assembly, the chip was filled with 2% (w/v) BSA solution in PBS for 30 min using a syringe-pump (neMESYS, Centoni). This coats the channel walls and prevents vesicle rupture upon contact. Before adding the GUV solution, Millipore water was added to the remove the PBS. To operate the PDMS valves, 3 mbar of nitrogen was applied to the upper layer channel using a custom-built pressure control system.

RESULTS & DISCUSSION

The device comprises two PDMS layers; the bottom fluidic layer contains the hydrodynamic traps, while the top layer contains donut-like structures. By pressurizing the top layer, the donuts are lowered into the bottom channel where they surround and isolate the traps in a 600 pL volume (Figure 1). GUVs in an aqueous solution of 100 µM green-fluorescent calcein and introduced into the device at 5 µL/min. This results in a trapping efficiency of > 95% for ~15-20 µm sized GUVs.

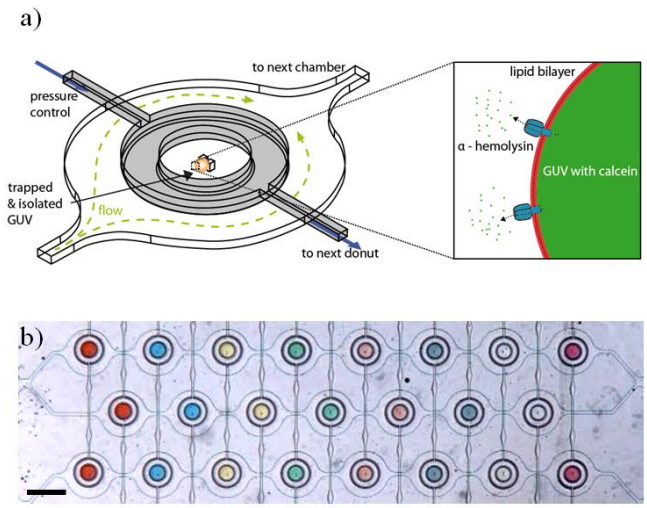

Figure 1: Microfluidic device. a) Schematic showing how a trapped GUV is isolated from surrounding fluid by a donut. Enlargement shows αHL inserted into the GUV membrane resulting in the release of the encapsulated calcein molecules. b) Bright-field image showing 23 of the 60 micro-chambers. Fluidic isolation is demonstrated using food dyes. Scale bar: 500 µm.

Figure 2 shows a single trapped GUV in the surrounding calcein solution and in non-fluorescent aqueous buffer after fluid exchange. The GUVs remain trapped for at least 12 hours without being compromised permitting long-term observations of single vesicles. Moreover, in flow rates up to 50 µL/min there was no visible deformation.

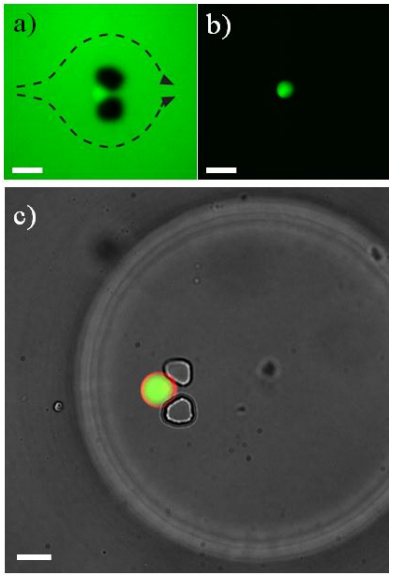

Figure 2. Trapping of GUVs. a) Fluorescence image of a single GUV trapped hydrodynamically by the posts (black). The surrounding calcein solution (green) is diverted around the trap. b) The fluid was then exchanged for water without removing the calcein filled GUV. c) Bright-field image of the donut (grey scale) overlaid with a fluorescence image of the GUV. The membrane is stained with DiI (red). By lowering the donut the GUV is now isolated from the rest of the channel network. Scale bars: 20 µm.

Next, we used the device for biophysical studies and exchanged the solution surrounding the GUVs with 50 µg/ml αHL when the donuts were closed. A single row of donuts was then opened for ~ 2 seconds to expose the GUVs to the membrane pore. Images were recorded as the calcein molecules were released into the chamber (Figure 1a). Figure 3a shows an image series after the addition of αHL, and a clear decrease in the fluorescence intensity is recorded over time (Fig. 3b). With less αHL in the donut (2.5 µg/ml), the calcein was released at a lower rate due to a smaller flux across the membrane. These rates are in the same order as reported by previous studies [5, 6]. GUVs in donuts that remained closed served as a control.

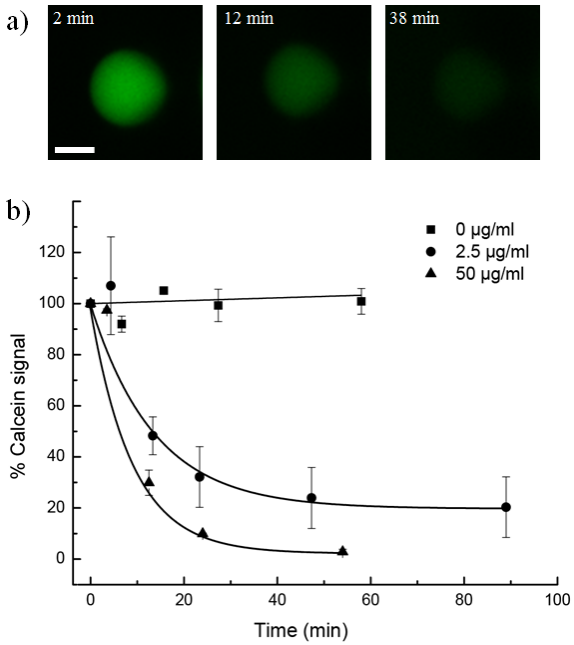

Figure 3. αHL assay. a) Fluorescence images of calcein released from a GUV with 50 µg/ml αHL. Scale bar: 10 µm b) Kinetics of calcein release for 0, 2.5 and 50 µg/ml αHL. The control without αHL showed no calcein release. With 50 µg/ml αHL most of the calcein was released after 25 min, whilst with 2.5 µg/ml αHL only 80% of the calcein was released over 90 min. Averages from 3 GUVs were used and error bars were taken from the standard deviation. All signals were background corrected.

OUTLOOK

Being able to reliably trap and isolate single GUVs and quickly exchange buffer solutions for membrane analysis is of great interest for studies on artificial membranes serving as simple cell models. The approach could be used for a wide range of applications including encapsulated enzymatic reactions, ligand-membrane protein binding, and drug screening.

ACKNOWLEDGEMENTS

This work was funded by the European Research Council (ERC Starting Grant No. 203428, "nµLIPIDS").

REFERENCES

[1] "Lipid Rafts As a Membrane-Organizing Principle," D. Lingwood and K. Simons, Science, 327, 5961 (2010).
[2] "Monolithic microfabricated valves and pumps by multilayer soft lithography", Unger, M., Chou, H., Thorsen, T., Scherer, A., & Quake, S. Science, 288(5463), 113–116 (2000).
[3] "A microchamber array for single cell isolation and analysis of intracellular biomolecules", K. Eyer, P. Kuhn, C. Hanke and P. S. Dittrich, 12, 765 (2012).
[4] "Liposome electroformation" M. I. Angelova and D. S. Dimitrov, Faraday Discussions of the Chemical Society, 81, (1986).
[5] "Stepwise Synthesis of Giant Unilamellar Vesicles on a Microfluidic Assembly Line", S. Matosevic and B. M. Paegel, Journal of the American Chemical Society, 133, 9, (2011).
[6]"Unilamellar vesicle formation and encapsulation by microfluidic jetting", J. C. Stachowiak, D. L. Richmond, T. H. Li, A. P. Liu, S. H. Parekh, and D. A. Fletcher, PNAS, 105, 12, (2008).

CONTACT

Petra S. Dittrich: dittrich@org.chem.ethz.ch