SINGLE CELL-LIPOSOME FUSION FOR DELIVERY OF MOLECULES INTO THE CYTOSOL

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ABSTRACT

We developed a microfluidic device for adjacent positioning of cells and vesicles to analyze the fusion of the natural and artificial membranes. Additionally, this allows the delivery of different types of vesicle-encapsulated molecules into single cells.

KEYWORDS
liposomes, fusion, single cells, lipid

INTRODUCTION

The fusion of lipid membranes is an essential process in nature, e.g. during endo- and exocytosis or viral infection. [1] The ability of artificial lipid membranes to fuse with the cell membrane is also exploited in a process called lipofection, which is routinely used to transport membrane-impermeable molecules, such as DNA, into the cell. However, mechanistic studies to understand the fusion processes are challenging, because of the difficulty to bring a defined number of liposomes in close contact to the cell, and to induce the fusion at a predefined time. Besides the fundamental understanding, these studies help to improve the typically poor lipofection efficiency, which could ultimately lead to novel, cell-targeting therapeutic strategies, e.g. against cancer. Here, we propose a novel method to fuse cell and vesicle membranes.

EXPERIMENT

The microfluidic device fabricated from PDMS and covered by a glass coverslip facilitates both the trapping of single cells in an integrated array of 576 geometric hurdles, [2] and immobilization of small unilamellar vesicles (SUVs) below the cells on discrete spots (Figure 1).

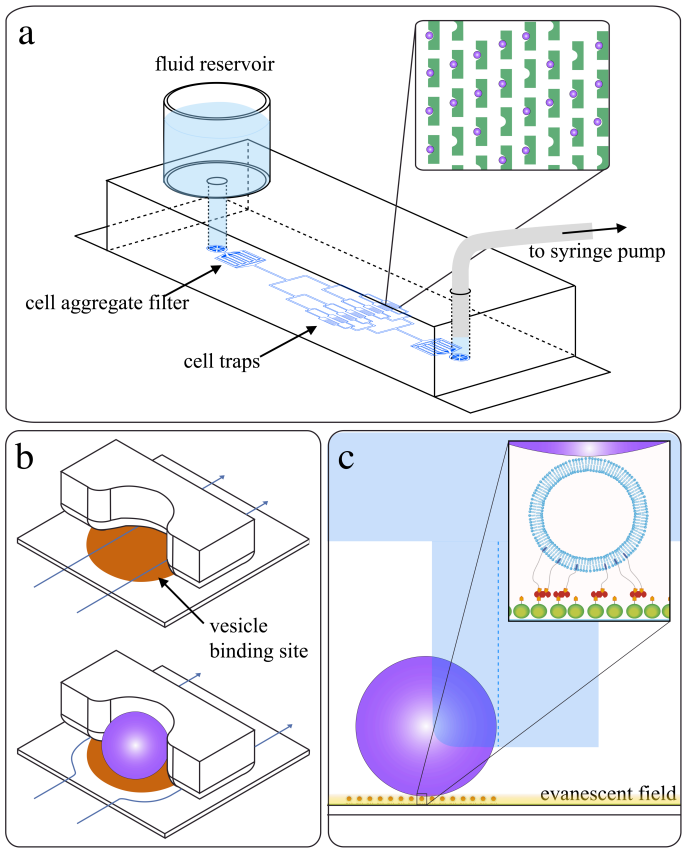

Figure 1. Schematic of the microfluidic device for liposome-cell fusion. a) Device with 8 microhurdle arrays, each allows trapping of 72 cells. All fluids are driven through the chip from the fluid reservoir via suction. b) Liposomes are immobilized on spots (shown in orange) on the glass surface under the cell traps before the cells (shown in green) are trapped. c) Side-view of the trapping site. The inset shows a magnification of the molecules used for liposome immobilization (bBSA;avidin;biot-PEG-chol).

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Before the microchip is assembled, the glass plate is patterned with biotinylated BSA (bBSA) by microcontact printing to produce binding sites for the vesicles. After bonding, the channels are subsequently flushed with (*i*) BSA to block the remaining surface, (*ii*) with avidin that binds to the bBSA, and (*iii*) with biotin-PEG-cholesterol.

Finally, SUVs encapsulating 100 mM calcein were prepared from DOPG and DOPE (17/83 mol%, ca. 5 mol% R18). supplied at low flow rates and incubated for a few minutes, which enabled convenient and efficient immobilization on the designated areas due to the insertion of cholesterol into the lipid membrane.

Next, cells in suspension (CHO or U937) were captured in the designated cells traps directly above the vesicles (Figure 2).

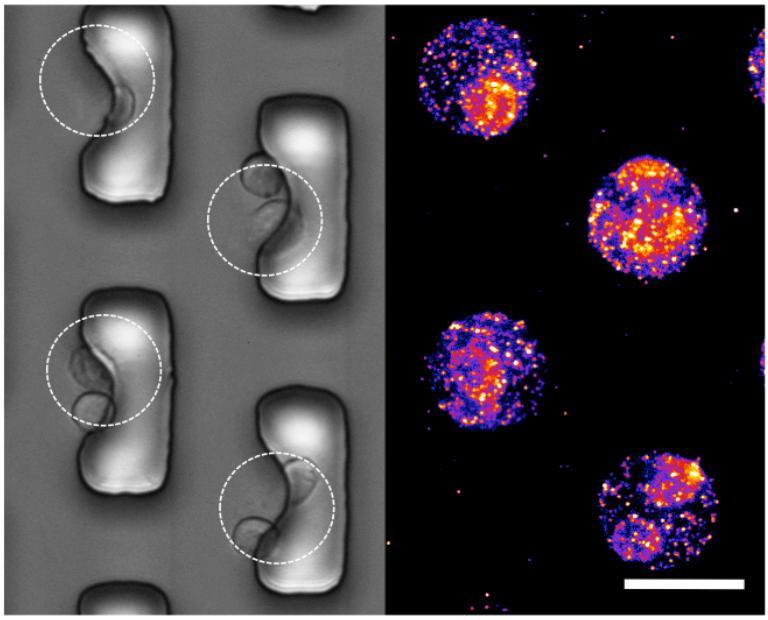

Figure 2. The membrane dye R18 diffuses into cells after fusion. Left: Brightfield image of trapped cells. White lines indicate where the liposomes are immobilized. Right: Wide-field fluorescence image of R18 in liposomes and in cell membranes after fusion (10 min pH 5, followed by 20 min at pH 7.4 for diffusion of R18 dye). Scale bar: 30 µm.

RESULTS

We studied the immobilization and fusion of cells by using total internal reflection fluorescence (TIRF) microscopy and wide-field microscopy.

A fluorescence-dequenching assay with two fluorophores was employed to visualize the process (Figure 2, 3). The red-fluorescent hydrophobic R18 was used to observe the fusion of the lipid membrane, and green-fluorescent hydrophilic calcein was used to prove the release of the vesicles' content into the cells.. The fusion of the vesicle and cell membrane was induced by changing the pH from 7.4 to 5 and is mainly driven by the high DOPE content as reported before. [3] Indeed, the fluorescence intensity of both fluorophores increased after the pH drop, which proved the fusion of the cell membrane with the vesicle membranes.

The fusion process occurred within a few seconds, while the diffusion of the fluorescent dyes into the cell membrane (R18, Fig. 2) and into the cytosol (calcein, Fig. 3) proceeds on a longer time scale (minutes).

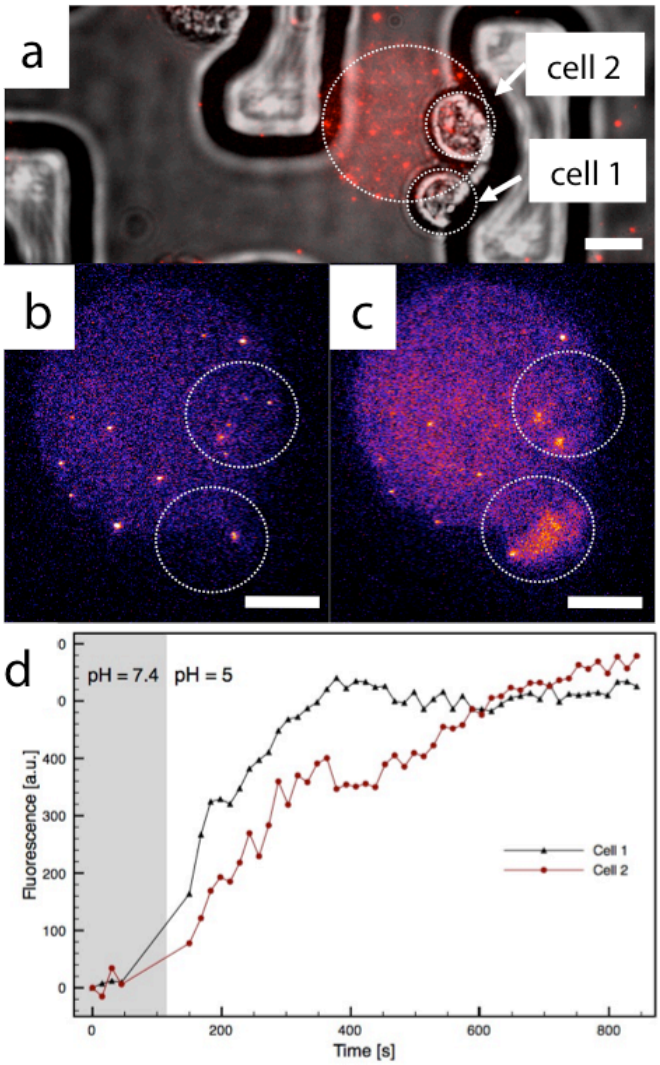

Figure 3. Fusion of liposomes at low pH. a) Here, the cell trap is occupied by two cells, both are in contact with the liposome spot visualized by calcein-fluorescence. b) and c): TIRF images of calcein fluorescence at (b) pH 7.4 and (c) pH 5. Calcein is released into cells and dequenched after the pH drop. d) Calcein fluorescence intensity of the two cells shown in a). After the pH is dropped from pH 7.4 to pH 5, the fluorescence intensity increases because calcein diffuses into the cytosol indicating the successful fusion. All Scale bars: 10 µm

CONCLUSIONS

This method facilitated the transport of hydrophobic (R_{18}), hydrophilic (calcein) and amphiphilic (lipids) molecules from vesicles into single cells and hence, allows for kinetic and mechanistic studies. Moreover, it can be easily applied to study fusogenic processes of viruses and cells.

ACKNWOLEDGMENT

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REFERENCES

[1] "Membrane fusion," W. Wickner and R. Schekman, Nature Structural & Molecular Biology, 15(7), 658–664 (2008).
[2] "Dynamic single cell culture array," D. Di Carlo et al., Lab on a Chip, 6(11), 1445-1449 (2006).
[3] "Biophysical characterization of anionic lipoplexes," S.D. Patil et al., Biochimica Et Biophysica Acta, 1711(1), 1-11 (2005)

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