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Microfluidic electroporation of robust 10-μm vesicles for manipulation of picoliter volumes

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Abstract

We present a new way to transport and handle picoliter volumes of analytes in a microfluidic context through electrically monitored electroporation of 10–25 μm vesicles. In this method, giant vesicles are used to isolate analytes in a microfluidic environment. Once encapsulated inside a vesicle, contents will not diffuse and become diluted when exposed to pressure-driven flow. Two vesicle compositions have been developed that are robust enough to withstand electrical and mechanical manipulation in a microfluidic context. These vesicles can be guided and trapped, with controllable transfer of material into or out of their confined environment. Through electroporation, vesicles can serve as containers that can be opened when mixing and diffusion are desired, and closed during transport and analysis. Both vesicle compositions contain lecithin, an ethoxylated phospholipid, and a polyelectrolyte. Their performance is compared using a prototype microfluidic device and a simple circuit model. It was observed that the energy density threshold required to induce breakdown was statistically equivalent between compositions, 10.2 ± 5.0 mJ/m^2 for the first composition and 10.5 ± 1.8 mJ/m^2 for the second. This work demonstrates the feasibility of using giant, robust vesicles with microfluidic electroporation technology to manipulate picoliter volumes on-chip.

Keywords: Electroporation; Electropermeabilization; Giant vesicle; Giant liposome; Sample management

1. Introduction

In microfluidics, diffusion and nonuniform flow velocities (particularly from pressure-driven flow) often cause unwanted mixing and dilution, resulting in broad, nonuniform concentration profiles and reaction rates. These effects can seriously degrade device performance when only trace amounts of sample material are available. A design strategy to overcome these challenges is to keep analytes in confined volumes to improve mass transport in the microfluidic device. Immiscible fluids [1] and bubbles [2] have been employed by researchers to isolate analytes and circumvent these obstacles. Each of these methods has its inherent advantages and disadvantages. In this method, giant vesicles (10–25 μm) are used to isolate analytes in a microfluidic environment. Once encapsulated inside a vesicle, contents will not diffuse and become diluted when exposed to pressure-driven flow. To the best of our knowledge, this study presents the first demonstration of giant vesicle electroporation in a microfluidic environment. This study offers a new, practical and simple strategy to handle picoliter volumes in a microfluidic device through autonomous electrical control of biomimetic cell-sized containers.

Rapid electric pulses can be used to enhance the permeability of a cell membrane for the introduction of impermeable molecules into a biological cell through a phenomenon known as electroporation [3,4]. During electroporation, the electric field charges the membrane, changing the electrochemical potential across it [3,4]. As a function of this potential difference as well as other external factors, the pulses can have no effect on the membrane, reversibly open the membrane, or irreversibly open the membrane. Progress has been made toward the application of this technique on individual cells using ultra-microelectrodes [5] and microfluidic platforms for improved control of the reversible permeation of the cell membrane [6–9].

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Giant vesicles (10–25 μm diameter) are of recent interest to explore the rapid chemical kinetics of molecules within a confined nanoenvironment because of their relatively large size and their artificial phospholipid membrane, which closely resembles the membranes of living cells [10–12]. Researchers have demonstrated the utility of giant lipid vesicles through off-chip manipulation techniques such as micro-injection with borosilicate microneedles [10–12] and optical trapping with focused laser beams [13]. Analytes can be loaded into or out of vesicles by electroporation as well, as demonstrated with patch clamp systems [10–12] and with micromanipulator-controlled ultramicroelectrodes [10–12] in non-microfluidic environments.

Various preparation methods of giant vesicles have been reported, including electroformation [14–17], the evaporation of a nonaqueous lipid solution, film hydration, and dispersal in an aqueous buffer [18,19]. We have found previous preparations unsuitable for our microfluidic applications due to problems with reproducibility and vesicle strength under electrical and/or mechanical stress. In this paper, we describe two improved preparation schemes of giant vesicles that we have developed that can withstand electrical and mechanical manipulation in microfluidic devices, and we compare their performance as they are integrated into a prototype platform. These two methods produced a larger fraction of vesicles containing analytes, such as dye, compared to previous published methods [18,19].

The combination of giant robust vesicles with electrically-monitored electroporation is the first step towards a microfluidic platform in which samples are autonomously moved and manipulated through a number of sample loading stations, guided only by electrical feedback. These manipulations include loading analyte into the vesicle, transporting the vesicle between loading stations, releasing analyte from the vesicle, and even fusing of vesicles to combine encapsulated analytes [20] all while overcoming many of the unwanted effects associated with microfluidics. While many of these tasks remain to be demonstrated in order to integrate all of these functions and overcome the limits of pressure-driven flow, we have shown that several of the key steps are achievable.

2. Experimental and analytical methods

2.1. Materials

Three lipids were used: 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine-*N*-[Methoxy(Polyethylene glycol)-2000] (Ammonium Salt) (PEGDOPE), L-α-phosphatidylcholine (Soy—95%) (Lecithin), and 1,2-distearoyl-*sn*-Glycero-3-Phosphoethanolamine (PEGDSPE) (Avanti Polar Lipids, Inc., Alabaster, AL). Alexa Fluor 488 carboxylic acid, succinimidyl ester dye was used to visually verify electroporation (Molecular Probes, Inc., Invitrogen Detection Technologies, Eugene, OR). Other materials were purchased from Aldrich (Sigma-Aldrich Corp., St. Louis, MO). Poly (tetrapropylammonium acrylate) was prepared by adding one equivalent of 0.5 M tetrapropylammonium hydroxide to M_w 16,000 poly(acrylic acid) and removing water under vacuum. 0.4 M poly(Me_4N acrylate) was prepared similarly. $(Pr_4N)_2$ glutarate is prepared by hydrolyzing glutaric anhydride with tetrapropylammonium hydroxide. 75 mM $(Me_4N)_2$ glutarate was made by adding 114 mg of glutaric anhydride to 12.6 mL DI water, then adding 0.72 mL of tetramethylammonium hydroxide, then titrating to pH 7 with the addition of glutaric anhydride.

2.2. First giant vesicle preparation method—PEGDOPE: lecithin

To develop vesicles that are robust enough to withstand a microfluidic environment, preparation methods were developed by modifying Yamashita's method [19]. Initially, a 50 mM lipid stock solution was made by adding 10 mg PEGDOPE and 160 mg lecithin (1:50 PEGDOPE:lecithin) to 2 mL filtered chloroform. A stock dye solution was prepared by adding 1 mL DI water to a vial containing 1 mg (subsequently hydrolyzed) succinimidyl Alexa Fluor 488 carboxylate dye. 120 μL of 1:50 PEGDOPE:lecithin stock solution was then combined with 1.2 μL of the dye stock solution.

100 mg poly(tetrapropylammonium acrylate) was added to 3.2 mL filtered chloroform to make a stock polyelectrolyte solution.

20 μL PEGDOPE–lecithin–dye solution was pipetted into a 4-mL vial, and 1 μL of polyelectrolyte solution was then added.

The chloroform was evaporated by gently blow drying under a nitrogen stream. The open vial was placed under vacuum overnight at room temperature to completely evaporate the chloroform.

After drying, 2 μL DI water was added as a drop on the sidewall of the vial. The vial was capped and placed in a 37 °C oven for 10 min, forming a hydrated film. A capped vial of filtered 75 mM $(Pr_4N)_2$ glutarate in water was also heated at 37 °C for ten minutes.

The vials were then removed from the oven, and 1 mL of 75 mM $(Pr_4N)_2$ glutarate was pipetted into the vial of lipid–dye–polyelectrolyte solution. The capped vial was incubated at 37 °C for 1 h.

2.3. Second giant vesicle preparation method—PEGDSPE: lecithin

Initially, a 50 mM lipid stock solution was made by adding 10 mg PEGDSPE and 160 mg lecithin (1:50 PEGDSPE: lecithin) to 2 mL filtered ethanol. A stock dye solution was prepared by adding 1 mL DI water to a vial containing 1 mg (subsequently hydrolyzed) succinimidyl Alexa Fluor 488 carboxylate dye. 120 μL of 1:50 PEGDSPE:lecithin stock solution was then combined with 1.2 μL of the dye stock solution.

20 μL PEGDSPE–lecithin–dye solution was pipetted into a 4-mL vial, and 10 μL polyelectrolyte solution, 0.4 M poly (Me_4N acrylate) in ethanol, was then added to the vial. The ethanol was evaporated by gently blow drying under a nitrogen