INVESTIGATING THE EFFECTS OF MEMBRANE TENSION AND SHEAR STRESS ON LIPID DOMAINS IN MODEL MEMBRANES

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ABSTRACT

We present a method to apply different forces to model membranes using microfluidic technology. Giant unilamellar vesicles (GUVs) were biochemically immobilized within a multilayer chip using a cholesterol linker. A deformable PDMS membrane allows the application of mechanical pressure to the vesicles. Furthermore, using the laminar flow within the microchannels, shear stress can be applied. To study the effects these forces have on lipid rafts, we used GUVs with phase-separated domains as model and made observations using confocal microscopy. The results indicate that prolonged pressure, and hence increased membrane tension, alters the patterns of the domains. Moreover, the device permits monitoring of domain reorganization after disruption by shear stress. This approach opens up the possibility of studying the effects of simultaneous forces, not only on model membranes but on whole cells.

KEYWORDS

GUVs, vesicles, membranes, membrane tension, shear, lipids

INTRODUCTION

Cells are constantly subjected to external forces from their environment and it is vital that they maintain their integrity and respond with the appropriate cellular process [1]. Lipid rafts are thought to play an important role in maintaining the membrane structure and aiding the function of the membrane proteins responsible for signal transduction [2]. Studying the behavior of lipid membranes in response to external mechanical stimuli is therefore of great interest. GUVs with phase separated lipid domains can be used to study lipid rafts, but handling of such objects and applying forces to them is not straightforward. Here we present a new method, using microfluidic technology, which allows GUVs to be easily handled and subjected to mechanical pressure and shear stress.

EXPERIMENTAL

Chip fabrication. The microfluidic chips were made from PDMS and consisted of a two-layer design requiring two master molds [3]. These were fabricated using multilayer soft lithography as previously described [4]. Briefly, SU-8 photoresist was spin-coated onto two silicon wafers at different speeds (to obtain different heights) and subsequently exposed to UV light through a film mask containing the channel designs. On the upper layer master (20 µm height), PDMS (oligomer to curing agent ratio 5:1) was partly cured for 30 min at 80 °C (thickness of 5 mm). On the lower layer master (50 µm height), PDMS (oligomer to curing agent ratio 20:1) was spin-coated to a height of 70 µm and partially cured at 80°C for 15 min. Fluidic access holes were punched with a 1.5 mm biopsy puncher in both layers. Biotinylated-BSA was then immobilized onto the surface of a glass slide by means of microcontact printing [5], and the microchannels were completed by bonding the PDMS to the glass slide using a plasma oven.

GUV preparation. The electroformation protocol was used to create unilamellar vesicles [6]. The lipids sphingomyelin/DOPC/cholesterol were dissolved in chloroform/methanol (9:1 v/v) at a concentration of 1 mM together with 1 µM DiI, and dried on an indium tin oxide (ITO) coated slide overnight under a vacuum. Millipore filtered water was then used to rehydrate the lipids and a second ITO slide was placed on top, separated by a 1.5 mm silicone rubber spacer. Then 0.7 V at a 10 Hz was applied for 4 hours at a temperature of 60 °C.

Confocal microscopy. Optically sectioned microscopy was achieved using a confocal laser scanning microscope (Axiovert 200M, Zeiss) and a 63x/1.4 oil immersion objective lens. DiI fluorescence in the membrane was recorded using a 543 nm laser, UV/488/543/633 dichroic and a 560 nm long-pass filter. FITC 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, Cetoni). This serves to coat the channel walls and prevent vesicle rupture upon contact. This was followed by PBS to remove the unbound BSA and then re-filled with 0.01 mg/ml avidin in PBS for 30 min. In the final step 7 µM cholesterol-PEG-biotin was flowed through and incubated for 30 min and followed by Millipore water. GUVs were then introduced by gravity and incubated overnight to allow capture by the cholesterol linkers. To operate the PDMS membrane, up to 1 mbar of nitrogen was applied to the upper layer channel using a pressure control system (MFCS-FLEX, Fluigent).

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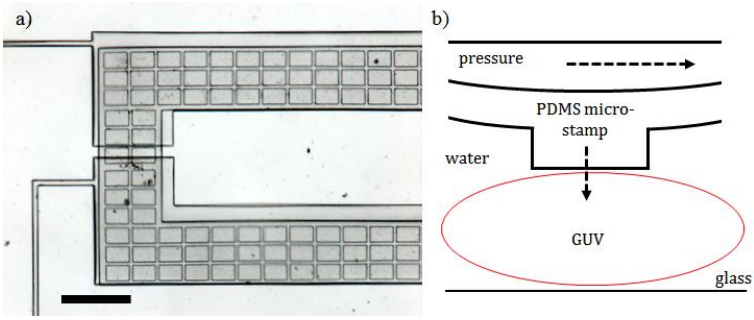

Figure 1: Microfluidic device. a) Bright-field image showing the pressure layer with integrated micro-stamps situated above the fluidic layer. Scale bar: 200 µm. b) Schematic side view as pressure is applied to the top layer and a micro-stamp deforms a GUV

RESULTS & DISCUSSION

The bottom fluidic layer of the chip contains the GUVs while the top layer serves as a control layer for deforming the ceiling of the bottom layer. Structured 'micro-stamps' within the ceiling, can be lowered by applying pneumatic pressure to the top layer (Figure 1). The GUVs are immobilized using a biotin-PEG-cholesterol linker patterned on to the glass bottom of the fluidic channel (Figure 2). This provides the stability for 3-D imaging using confocal microscopy and also serves to spatial confine the GUVs whilst under shear stress from fluid flow.

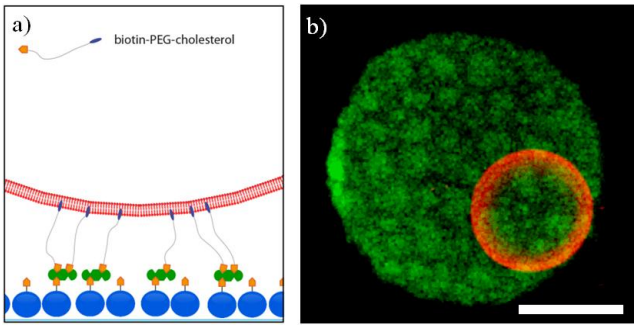

Figure 2. Immobilization of GUVs. a) Biotinylated-BSA, patterned onto the glass surface of the microchannels, is linked to biotin-PEG-cholesterol via avidin. The cholesterol inserts into the membrane to hold the GUV. b) Confocal image of an immobilized GUV with membrane probe DiI (red). Here the avidin is labeled with FITC (green) to visualize the patterned surface. Scale bar: 10 µm.

Figure 3 shows how the GUVs are deformed when subjected to mechanical pressure from the micro-stamps. Using this approach, continued observations of deformed GUVs can be made over long time periods. Deforming a GUV will cause an increase in the tension across the lipid membrane. In this situation it is understood that lipids will sort laterally in order to reduce this tension [7], which is difficult to investigate over long time periods. Here we use phase-separated GUVs and studied the effects of induced tension by applying mechanical pressure. Figure 4 shows a GUV which has been deformed for 45 minutes and the apparent change in the pattern indeed indicates that lateral sorting of the lipids has occurred in order to reduce the tension.

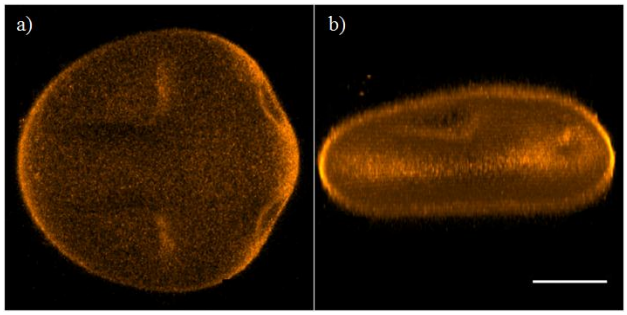

Figure 3. Deformed GUV under a micro-stamp. a) Top view and b) side view acquired using 3-D confocal microscopy with DiI. Scale bar: 20 µm.

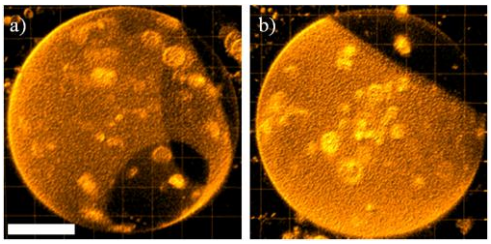

Figure 4. Induced tension by deformation causes lateral sorting of lipids. a) Top view of GUV showing DiI in the disordered phase and two ordered patches (not stained). b) After 45 min the lipids appear to have sorted into one disordered patch to reduce the tension. Scale bar: 20 µm.

The effects of shear stress on the lipid domains can be investigated using the same platform. Here, we take advantage of the laminar flow existing within the channels. As the fluid passes over the surface of the GUVs it disrupts the normal pattern of lipid domains. To image this process, the flow rate is varied and a series of video-rate images is acquired at the surface of the GUV. Figure 5 shows how the lipid domains are disturbed by a high flow rate. Additionally, this allows observations of the dynamics of the domain reorganization when the flow is stopped (Figure 6).

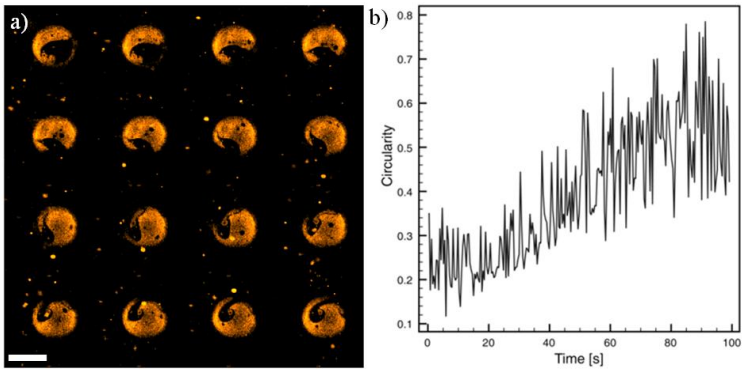

Figure 5. Applying shear stress. a) Shear stress caused by laminar flow in the microchannel. From top left to bottom right: a time series every 0.5 s of the GUV surface showing how the lipid domains are disrupted. Scale bar is 50 µm. b) Reorganization of the lipid domains after disruption by shear stress. When the flow is stopped, image analysis shows how the lipids diffuse across the membrane and the domains become more circular over time.

OUTLOOK

This microfluidic platform offers a new technique for studying the biophysical effects of forces upon lipid membranes. The device also has the potential for studying the mechanosensitivity of membrane bound proteins and whole cells.

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