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Shear-Driven Motion of Supported Lipid Bilayers in Microfluidic Channels

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Abstract: In this work, we demonstrate how a lateral motion of a supported lipid bilayer (SLB) and its constituents can be created without relying on self-spreading forces. The force driving the SLB is instead a viscous shear force arising from a pressure-driven bulk flow acting on the SLB that is formed on a glass wall inside a microfluidic channel. In contrast to self-spreading bilayers, this method allows for accurate control of the bilayer motion by altering the bulk flow in the channel. Experiments showed that an egg yolk phosphatidylcholine SLB formed on a glass support moved in a rolling motion under these shear forces, with the lipids in the upper leaflet of the bilayer moving at twice the velocity of the bilayer front. The drift velocity of different lipid probes in the SLB was observed to be sensitive to the interactions between the lipid probe and the surrounding molecules, resulting in drift velocities that varied by up to 1 order of magnitude for the different lipid probes in our experiments. Since the method provides a so far unattainable control of the motion of all molecules in an SLB, we foresee great potential for this technique, alone or in combination with other methods, for studies of lipid bilayers and different membrane-associated molecules.

Introduction

An approximately 5-nm-thick membrane is all that separates and protects the interior of a cell from the surrounding environment. The core structure of a human cell membrane is a lipid bilayer: a two-dimensional fluid in which the embedded membrane-associated components can diffuse.[1] One of the most common model systems used to study the properties of cell membranes is supported lipid bilayers (SLBs).[2−4] Due to their random motion, the membrane constituents of an SLB will generally strive to distribute themselves evenly within an enclosed part of the lipid bilayer.[5,6] However, there are several situations in which a nonuniform concentration profile of different membrane components is desirable. For example, recent work suggests that the function of membrane proteins is critically dependent on the lipid environment.[7] Another situation that requires a nonuniform concentration profile is for separation of membrane-associated components within the SLB.[8,9] Despite this, methods of varying the spatial distribution of different types of molecules in an SLB are few and rely primarily on differences in the charge of the membrane constituents.[9−12] An exception is the self-spreading approach,[8,13,14] in which a dried aggregate of lipids is hydrated, followed by spontaneous spreading of the SLB over the support. Although this technique can be applied to move uncharged molecules, it is not possible to control the drift velocity or the direction of motion of the SLB and its components using this approach.

We here present a technique for inducing and controlling the motion of an SLB and its constituents in a well-defined fashion that does not require the SLB to be charged. In this method, an SLB is formed on the walls of a microfluidic channel. By applying a bulk flow inside the channel, the shear force of the bulk flow on the bilayer will, if sufficiently high, move the SLB at a velocity that scales linearly with the bulk flow rate. The motion of the SLB and its constituents can therefore be accurately controlled by modulating the bulk flow, in contrast to self-spreading bilayers, where the velocity of the spreading bilayer front is nonlinear and decreases with the distance traveled by the SLB.[13,14] The method described in this paper thus offers a novel way of controlling the motion of an SLB and enables establishment of concentration gradients of different membrane components in the system, based on the molecules structure and their interaction with the support and the lipid bilayer. An additional advantage with this method is that the membrane-associated components can be analyzed in their native environment, the lipid bilayer, without the need of extracting the

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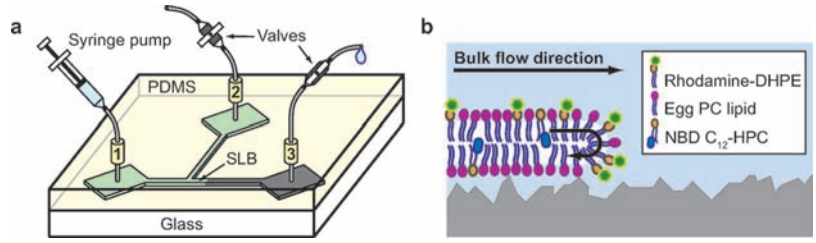

Figure 1. (a) The microfluidic device used to drive the SLB in the direction from 1 to 3 using a bulk flow in the channel. (b) Illustration of the behavior of the moving SLB and its constituents in the microfluidic channel under an applied bulk flow. The bilayer is driven over the glass support in a rolling motion, such that the upper and lower leaflets of the bilayer have different drift velocities.[13]

molecules, which is a complex procedure that can potentially alter the structure of the investigated molecules.[15] Altogether, this method opens the way for new means of studying and separating membrane-solubilized molecules in an SLB, such as different types of lipids, peptides, and membrane-associated proteins.

Principle of the Method. Microfluidic devices made of polydimethylsiloxane (PDMS) have previously been used to selectively form an SLB on specific regions of a solid support.[16,17] In the present work, a microfluidic channel was created by bonding a PDMS replica to a supporting glass slide.[18] The microfluidic setup is schematically depicted in Figure 1a.

An SLB is formed on the glass floor of the channel by allowing a vesicle solution to flow between the inlet at 1 and the outlet at 2 (Figure 1a). The vesicles are adsorbed on the glass surface and subsequently fuse to form a planar SLB.[17,19] After replacing the vesicle suspension with a buffer solution, an apparently flawless, stationary SLB was formed in the left-hand part of the device. The bilayer consisted of egg yolk phosphatidylcholine (egg PC) lipids with a small fraction (~1 wt %) of fluorescent lipid probes, either rhodamine-DHPE (Lissamine rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphatidylethanolamine) or NBD C_{12}-HPC [2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine], to visualize the motion of the SLB using epifluorescence optical microscopy.

Results and Discussion

The bilayer and its constituents could be moved along the channel in the direction from the inlet at 1 to the outlet at 3 by applying a bulk flow of buffer solution in the channel, with the outlet at 2 closed (Figure 1a). The flow of the bulk solution in the channel will result in a drag force acting on the SLB, causing the SLB and its components to move, as schematically depicted in Figure 1b. Typical experimental results illustrating the appearance and motion of the bilayer front are shown in Figure 2.

The Velocity of the SLB and Its Constituents. To determine the drift velocity of the fluorescent lipid probes in the SLB, a spatially confined area of the fluorescent molecules inside the SLB was photobleached, thus creating a spot with a lower intensity than the surrounding SLB (Figure 3a). The drift velocity of the molecules was then determined by measuring the position of the center of the bleached spot as a function of time (Figure 3b). In addition, the diffusion coefficient of the bleached lipid probes could also be determined by recording the recovery of the fluorescent lipid probes.[20] A detailed description of the drift velocity determination is given in the Supporting Information.

Both the drift velocity of the bilayer front and the velocity of the individually labeled lipids were seen to scale linearly with the bulk flow in the channel. At a bulk flow rate of 200 μL/min, corresponding to a maximum bulk velocity of 0.4 m/s in the channel, the drift velocity of rhodamine-DHPE was 0.5 μm/s (Figure 3b), whereas the drift velocity of the bilayer front was only 0.2 μm/s (Figure 2a,b).

Since the shear force on the SLB from the bulk flow is highest at the center of the channel, it could be expected that the bilayer front would become progressively more parabolic as a function of time. Instead, the bilayer front has a constant curvature, for a fixed bulk flow, with all parts apparently moving with the same velocity. A plausible explanation to this behavior is the self-spreading forces acting at the intersection of the bilayer−glass−water phase. At a certain curvature of the bilayer front, the net motion in the direction of the bulk flow is balanced by these self-spreading forces, resulting in a constant curvature of the bilayer front. Note that this curvature will depend on the magnitude of the bulk flow.

A convenient way of characterizing the drift velocity, v, is to use the quantity v/Q, which was seen to be independent of the bulk flow rate, Q, for the bulk flows used in the current experiments (100, 200, and, for NBD C_{12}-HPC, also 400 μL/min); i.e., the drift velocities were seen to scale linearly with the bulk flow rate. The measured values of v/Q for rhodamine-DHPE, NBD C_{12}-HPC, and the bilayer front are given in Table 1.

From Table 1 it can be seen that the bilayer front moves at approximately half the average velocity of the rhodamine-DHPE molecules. Furthermore, only a single mobile fraction could be observed when using rhodamine-DHPE as the fluorescent lipid probe (Figure 3a) and the bleached area remained circular during fluorescence recovery (the difference between the lengths of the semiminor and the semimajor axis of the bleached area were typically on the order of 2% or less during the time of measurement). These observations can be explained by a rolling motion of the SLB, in which the bilayer front moves at the average velocity of the upper and the lower leaflets of the bilayer (Figure 1b).[13] For an almost stationary lower leaflet, this means that the upper leaflet must move at twice the velocity of the

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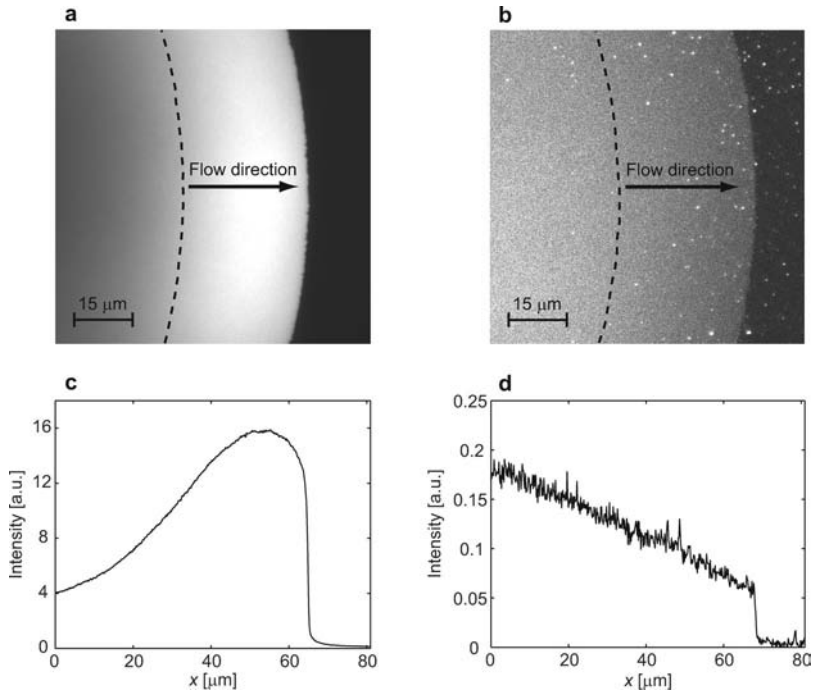

Figure 2. The motion of an advancing SLB front driven from left to right in the microfluidic channel using a bulk flow of 200 μL/min, with (a) rhodamine-DHPE and (b) NBD C_{12}-HPC as the fluorescent lipid probe in the bilayer. The dashed line indicates the position of the bilayer front 150 s before the current frame. The intensity of the fluorescent light along a line through the center of the channel is shown in parts c and d for the SLB containing rhodamine-DHPE and NBD C_{12}-HPC, respectively. The intensities were normalized to the equilibrium intensity of the SLB far away (>500 μm) from the bilayer front. Note that $x = 0$ does not correspond to the initial position of the bilayer front, which is ~1 mm to the left of the bilayer front in the current experiments.

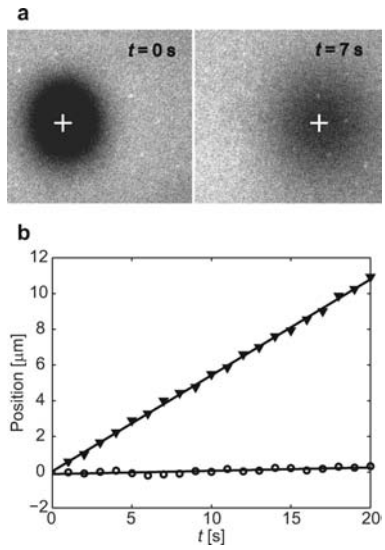

Figure 3. Illustration of the use of photobleaching to determine the drift velocity of the fluorescent lipid probe rhodamine-DHPE in the SLB. (a) The white cross indicates the center of mass of the bleached spot. (b) The position of the center of the bleached spot, relative to the position just after photobleaching, is plotted as a function of time, with ▼ being motion parallel to and ○ being motion perpendicular to the bulk flow direction. The solid lines are linear fits to the data points.

Table 1. Measured Drift Velocities for the Bilayer Front and the Two Fluorescent Lipid Probes Rhodamine-DHPE (One Mobile Population) and NBD C_{12}-HPC (Two Mobile Populations)[a]

	n	v/Q (m^{-2})	$\langle v/Q\rangle_z$ (m^{-2})[b]	$\langle v/Q\rangle_z/\langle v_{\text{rhodamine-DHPE}}/Q\rangle_z$
rhodamine-DHPE	6	156 ± 4	123 ± 4	1
(NBD C_{12}-HPC)$_1$	14	80 ± 5	63 ± 4	0.52 ± 0.04
(NBD C_{12}-HPC)$_2$	14	20 ± 5	16 ± 4	0.13 ± 0.03
bilayer front[c]	3	62 ± 3	62 ± 3	0.50 ± 0.03

[a] All values in the table are given as the mean value ± one standard deviation, obtained from n measurements. [b] $\langle ... \rangle_z$ is the average value over the width of the channel due to the spatial distribution of the bulk shear force.[21] [c] The velocity of the bilayer front is with rhodamine-DHPE as the fluorescent lipid probe. The velocity of the bilayer front was on average slightly higher, v/Q = 70 m^{-2}, when using NBD C_{12}-HPC as the fluorescent lipid probe.

bilayer front, as was observed for rhodamine-DHPE. Therefore, the observation of only a single rhodamine-DHPE population in the current experiments indicates that rhodamine-DHPE is preferentially located in the upper leaflet of the bilayer. Quenching experiments were also made (see Supporting Information for details), with KI as quencher of rhodamine-DHPE,[22] indicating a strong asymmetry in the distribution of rhodamine-DHPE between the two leaflets of the bilayer, with about 95% of the lipid probes being in the upper leaflet. The observation that charged lipid probes may distribute highly asymmetrically between the two leaflets of the SLB has previously been noted.[23]

The NBD C_{12}-HPC molecules showed a markedly different behavior. Two fractions with different mobilities were observed, one moving at roughly 50% and one at 10% of the drift velocity of rhodamine-DHPE (Table 1). Despite this, the bilayer front moved at approximately the same velocity for both fluorescent lipid probes. The fact that two velocities were observed with NBD C_{12}-HPC indicates that this molecule is located in both leaflets of the bilayer and that the drift velocity of the upper leaflet is significantly higher than that of the lower leaflet. This is in agreement with the results obtained from the rhodamine-

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DHPE experiments discussed above. However, the fast population of NBD C_{12}-HPC, associated with the upper leaflet of the bilayer, only moved at half the drift velocity of rhodamine-DHPE. An explanation of this observation could be that rhodamine-DHPE molecules experience a substantially higher drag force from the bulk liquid due to the protruding headgroup, which is larger than that of NBD C_{12}-HPC and the nonlabeled lipids in the SLB. However, in additional experiments with the fluorescent lipid probe NBD-PE [*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine], which has a smaller headgroup than rhodamine-DHPE, the drift velocity was observed to be almost identical to that of rhodamine-DHPE, within the experimental accuracy of the experiments (~5% difference). This points toward the conclusion that rhodamine-DHPE molecules move at the same velocity as the lipids in the upper leaflet, whereas the NBD C_{12}-HPC molecules experience a stronger coupling to the opposite leaflet, leading to a reduction in the drift velocity of the lipid probes in the upper leaflet of the bilayer in the latter case. Irrespective of the interactions responsible for this observation, which is currently under investigation by our group (see Supporting Information for a model representing the drift velocity of a membrane-associated component), a general conclusion is that any difference in the frictional coupling experienced by various membrane components, from the bilayer or from the surrounding environment, will lead to different drift velocities of the components. This, in turn, would allow the technique to be used as a chromatograph, either alone or in combination with electromagnetic fields, for the analysis and separation of membrane-associated molecules.

Lipid Amplification/Depletion at the Bilayer Front. An additional illustration of how the method can be used to manipulate the concentration of different membrane-associated components is shown in Figure 2a,b, where rhodamine-DHPE molecules are observed to accumulate at the bilayer front, whereas NBD C_{12}-HPC molecules are depleted at the front of the moving bilayer. These two effects were also seen at the other bulk flow rates used in the experiments, but with an increasing accumulation of rhodamine-DHPE at the bilayer front at higher bulk flow rates. This corresponds to a concentration of rhodamine-DHPE at the front of the bilayer that is more than 2 orders of magnitude higher than the concentration of NBD C_{12}-HPC at the same position.

The accumulation of rhodamine-DHPE is in agreement with the previous observation that rhodamine-DHPE molecules are preferentially located in the upper leaflet of the bilayer (see above). The front of the advancing SLB will then behave like a selective one-dimensional filter that prevents rhodamine-DHPE molecules from reaching the bottom leaflet of the SLB (Figure 1b). Since the upper leaflet moves faster than the bilayer front, rhodamine-DHPE molecules will accumulate at the front of the SLB. The established concentration gradient will depend on the equilibrium between the convective transport to the bilayer front and a diffusive transport away from the front as well as repulsive electrostatic interactions between the rhodamine-DHPE molecules. Together, these effects result in a larger concentration gradient at higher bulk flow rates. In contrast, NBD C_{12}-HPC molecules were previously argued to be located in both leaflets of the bilayer and have an average drift velocity that is lower than that of the lipids in the SLB (see Table 1 and the discussion above). This, in turn, leads to depletion of these molecules from the front of the moving bilayer.

Similar observations of accumulation and depletion of different lipid probes near the front of a moving bilayer have been made in previous studies on self-spreading bilayers.[13] The phenomenon was there argued to arise from the spreading forces creating a surface pressure gradient in the SLB, resulting in the lipid density being lowest at the front of the bilayer. The lipid probes would then redistribute in the SLB due to a preference to be at a position with a low/high lipid density. However, in the current experiments, it is not a surface pressure gradient that drives the motion of the SLB but a constant shear force from the bulk flow. This is confirmed by the fact that the bilayer stops moving, and the intensity gradient gradually disappears, when the bulk flow is turned off, making a lipid density gradient less likely to be the explanation of the current observations.

It should be noted that the extent of accumulation and depletion of the fluorescent lipid probes at the front of the bilayer is strongly dependent on the drift velocity of the SLB, which can be accurately controlled by changing the magnitude and direction of the bulk flow in the channel. This is a clear advantage compared to self-spreading bilayer approaches, where the drift velocity is determined by the constant self-spreading forces and decreases with the distance traveled by the front of the bilayer.[13,14]

Conclusions

The potential of using bulk-flow-controlled motion of SLBs to analyze and separate different membrane-associated components is obvious from our results. However, it may also be possible to form SLBs with exciting new properties using this technique. Future investigations will focus on driving SLBs over surfaces with grooves or holes to span such structures with a homogeneous lipid bilayer. Other exciting possibilities include moving the SLB over substrates that do not promote self-spreading or spontaneous bilayer formation by vesicle adsorption and subsequent rupture. The well-controlled shear force of the bulk flow in the technique presented here could also be used to examine the properties of lipid bilayers by relating the shear force to the drift velocity of the fluorescent probes in the SLB. Apart from the possibilities of controlling the motion of lipids and other membrane-associated components in an SLB, the method has the additional advantage that it can be combined with numerous other applications based on micro/nanofluidics, including electrophoresis, for separation of membrane-associated constituents. We therefore foresee great potential for this technique, alone or in combination with other methods, for separation and bioanalytical applications, where it is vital to be able to manipulate the lipid bilayer and its constituents in a controllable and uncomplicated way.

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Supporting Information Available: An experimental section and detailed information about how the experiments and the analysis of the data were performed, as well as a model describing the drift velocity of a membrane-associated component. This material is available free of charge via the Internet at http://pubs.acs.org.

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