VISUALIZING THE GROWTH AND DYNAMICS OF LIQUID ORDERED DOMAINS DURING LIPID BILAYER FOLDING IN A MICROFLUIDIC CHIP

Eric L. Kendall, Chenren Shao, and Don L. DeVoe
Department of Mechanical Engineering
University of Maryland, College Park, USA

ABSTRACT

Here we present a microfluidic platform enabling continuous optical monitoring of the on-chip synthesis of bilayer lipid membranes (BLMs) by a monolayer folding process. The thermoplastic devices (fig. 1A) employ re-sealable elements supporting dried lipid films that are rehydrated in situ by microfluidic perfusion, enabling the controllable formation of lipid-laden air bubbles that are sequentially delivered across a supporting aperture in a thin, on-chip partition film (figs. 1B and 1C). This technique represents a unique microfluidic adaptation of traditional Montal-Mueller membrane formation.(1, 2) Because lipid monolayers defined by the bubbles are delivered independently to either side of the aperture, the lipid composition of each monolayer can be tailored to achieve asymmetry in the resulting bilayer, allowing the on-chip membranes to more accurately mimic the composition of biological cell membranes. Simultaneous electrical and optical characterization of asymmetric BLMs is demonstrated here, with confocal microscopy used to image bilayer formation during the monolayer folding process, revealing the growth and dynamics of asymmetric liquid ordered domains within the bilayers.

KEYWORDS

Lipid membranes, BLMs

INTRODUCTION

Most reports of BLM formation in microfluidic devices employ a solvent-based method where a plug of lipid-solvent solution is delivered to a small aperture and induced to thin into a membrane.(3) This technique cannot produce a membrane with asymmetric leaflet composition. Asymmetric droplet-interface bilayers have been demonstrated in a microfluidic environment,(4) however, access to each leaflet is restricted by the compartmentalized nature of the droplets. The device reported here supports independent control of leaflet composition as well as rapid perfusion of reagents to each buffer channel.

Imaging lipid phase behavior is of great importance to the understanding of cell membrane biophysics. Typically these studies are undertaken using giant unilamellar vesicles (GUVs),(5) which are inherently symmetric in composition, or supported bilayers,(6) which are often an imperfect model of lipid phase behavior due to interaction between the lower leaflet and the underlying solid surface. We demonstrate the ability to image lipid monolayers (fig. 1D) as well as symmetric and asymmetric bilayers in a system unaffected by surface interactions. Furthermore, we demonstrate the ability to image the formation and dynamics of both symmetric (fig. 1E) and asymmetric (fig. 1F) lipid domains starting from the moment of bilayer formation. High resolution fluorescence images of bilayer folding in our device reveal that some elements of liquid ordered domain formation may begin during the lateral monolayer compression that occurs during the folding process. Other dynamics also observed, such as large scale vortex currents within the newly folded bilayer, have implications in any work involving bilayers formed by the Montal-Mueller method.

EXPERIMENTAL

Chip fabrication and bilayer formation: Microfluidic channels were directly milled into PMMA sheets using a CNC milling machine. Top and bottom halves of the chip were separated by a thin PVDC film and thermally bonded at 90 °C/500psi for 5 min. Before chip assembly, at the point of intersection between top and bottom channels, an aperture of diameter 60-100 µm was formed by piercing the PVDC film with a hot needle using a previously described apparatus.(7) The aperture is pretreated with a small quantity (1-5 µl) of hexadecane in hexane before assembly. Ag/AgCl electrodes were prepared by treating clean silver wire with bleach for several hours. Electrodes were held in Upchurch Nanoport fittings. Membrane components were dissolved in hexane, and 10~20 µL volumes were pipetted onto the tips of 4 mm diameter nylon screws which were then dried in a vacuum chamber.

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

Bilayers were formed by sweeping two air bubbles through two chambers containing dried lipids, including fluorescently tagged lipids, each forming a lipid-laden air-water interface. The trailing surface of these channel-filling lipid-laden bubbles was swept sequentially across their respective sides of the aperture, each leaving behind a single monolayer of lipids that zip together to form a bilayer.

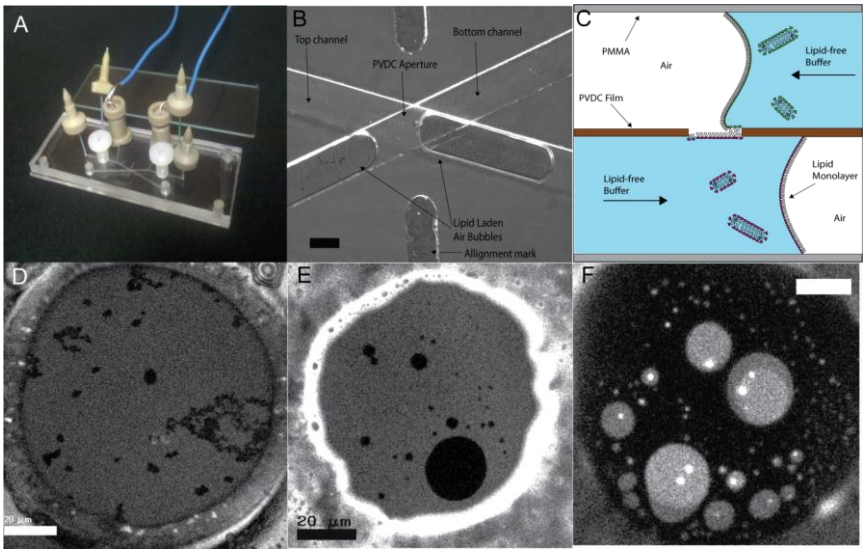

Figure. 1 (a) A fabricated thermoplastic microfluidic membrane chip, next to a standard 75x25 mm microscope slide for scale. (b) Brightfield image of discrete lipid-laden air bubbles approaching the membrane formation site. (c) Side view diagram of the asymmetric monolayer folding process. (d) Confocal fluorescence image of a DPPC/PEG-40-stearate monolayer with gel-phase domains rich in DPPC. (e) Confocal fluorescence image of a bilayer composed of 1:1:1 mixture of DPPC:DPhPC:cholesterol, where dark regions indicate liquid ordered domains rich in DPPC/cholesterol. (f) Asymmetric domains of liquid disordered brighter DiPhPC rich lipid surrounded by darker DPPC and cholesterol rich liquid ordered phase. Scale bars in (d) and (e) are both 20 µm, scale bar in (f) is 10 µm.

RESULTS AND DISCUSSION

Typically, the quality of membranes formed by a monolayer folding process is determined by monitoring the membrane capacitance. Bilayer membranes discussed in this work had measured capacitance values of 0.7 µF/cm^2 or greater, in good agreement with published data for membranes formed near the solvent-free limit.(8) The evolution of membrane capacitance to its equilibrium level matches well with fluorescence images of the process, indicating that a single bilayer immediately forms as the second lipid-laden bubble interface sweeps across the aperture. Excess lipid material associated with each bubble surface is swept downstream in the perfusion channel. The ease with which this platform may be used for imaging lateral phase separation, confirms, in combination with the capacitance data, that a single bilayer may be formed. Symmetric bilayer systems display two distinct fluorescence intensities with very little background fluorescence in the darker regions. Asymmetric bilayers, which may contain asymmetric, unregistered domains, may display three or more distinct fluorescence intensities.(9)

Fluorescence imaging of bilayer formation using a phase-separating lipid mixture reveals some previously unreported dynamics, including domain formation before completion of bilayer folding. The lateral movement of the second lipid laden bubble in this system applies a compressive force on the previously formed monolayer. This can induce phase separation in the previously formed leaflet which is then apparent in the completed bilayer seconds later. Likewise, this lateral movement of fluid in the micro-channel can induce the formation of recirculating vortices within the newly formed lipid bilayer. These large scale motions within the lipid bilayer, visualized by the tracking of newly formed lipid microdomains, are important to consider in any model of lipid phase behavior in folded asymmetric bilayer systems.

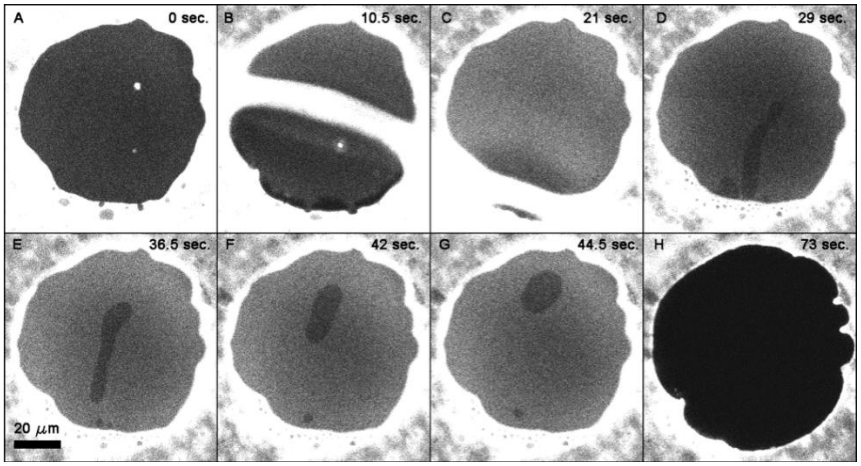

Figure 2. Confocal fluorescence images showing (a) lipid monolayer with air in the upper channel, (b, c) monolayer folding in progress, with lipid laden bubble-water interface clearly visible, (d) liquid ordered domains forming immediately upon completion of folding, with domain distortion resulting from shear forces during membrane formation, (e, f, g) domain recovery, and (h) induced bilayer rupture revealing low background fluorescence.

In addition to the novelty of the bilayer folding technique for facile on-chip BLM formation, we believe this to be the first demonstration of asymmetric membrane formation in a microfluidic chip. The technology described offers unique capabilities to examine and control membrane formation, and promises to be a valuable tool for biophysicists interested in lipid phase behavior in asymmetric bilayers.

REFERENCES

1. Montal M & Mueller P (1972) Formation of Bimolecular Membranes from Lipid Monolayers and a Study of Their Electrical Properties. (Translated from English) *P Natl Acad Sci USA* 69(12):3561-3566 (in English).
2. White SH, Petersen DC, Simon S, & Masaoyafuso (1976) Formation of Planar Bilayer Membranes from Lipid Monolayers - Critique. (Translated from English) *Biophys. J.* 16(5):481-489 (in English).
3. Zagnoni M (2012) Miniaturised technologies for the development of artificial lipid bilayer systems. (Translated from English) *Lab Chip* 12(6):1026-1039 (in English).
4. Hwang WL, Chen M, Cronin B, Holden MA, & Bayley H (2008) Asymmetric droplet interface bilayers. (Translated from English) *Journal of the American Chemical Society* 130(18):5878-+ (in English).
5. Baumgart T, *et al.* (2007) Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. (Translated from English) *P Natl Acad Sci USA* 104(9):3165-3170 (in English).
6. Sackmann E (1996) Supported membranes: Scientific and practical applications. (Translated from English) *Science* 271(5245):43-48 (in English).
7. Hromada LP, Nablo BJ, Kasianowicz JJ, Gaitan MA, & DeVoe DL (2008) Single molecule measurements within individual membrane-bound ion channels using a polymer-based bilayer lipid membrane chip. (Translated from English) *Lab Chip* 8(4):602-608 (in English).
8. Gross LCM, Heron AJ, Baca SC, & Wallace MI (2011) Determining Membrane Capacitance by Dynamic Control of Droplet Interface Bilayer Area. (Translated from English) *Langmuir* 27(23):14335-14342 (in English).
9. Collins MD & Keller SL (2008) Tuning lipid mixtures to induce or suppress domain formation across leaflets of unsupported asymmetric bilayers. *Proc. Natl. Acad. Sci. USA* 105(1):124-128.

CONTACT

Don L. DeVoe, +1-301-405-8125, ddev@umd.edu