Electrowetting on dielectric-based microfluidics for integrated lipid bilayer formation and measurement

Jason L. Poulos,[1] Wyatt C. Nelson,[2] Tae-Joon Jeon,[3] Chang-Jin "CJ" Kim,[2,4] and Jacob J. Schmidt[1,4,a)]
[1]*Department of Bioengineering, University of California Los Angeles, Los Angeles, California 90095, USA*
[2]*Department of Mechanical and Aerospace Engineering, University of California Los Angeles, Los Angeles, California 90095, USA*
[3]*Department of Biological Engineering, Inha University, Incheon 402-751, Republic of Korea*
[4]*California Nanosystems Institute (CNSI), University of California Los Angeles, Los Angeles, California, USA*

(Received 19 May 2009; accepted 3 June 2009; published online 8 July 2009)

We present a microfluidic platform for the formation and electrical measurement of lipid bilayer membranes. Using electrowetting on dielectric (EWOD), two or more aqueous droplets surrounded by a lipid-containing organic phase were manipulated into contact to form a lipid bilayer at their interface. Thin-film Ag/AgCl electrodes integrated into the device enabled electrical measurement of membrane formation and the incorporation of gramicidin channels of two bilayers in parallel. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3167283]

Artificially reconstituted lipid bilayer membranes have been used to provide an easily manipulated and highly controllable environment for the study of ion channels at the single molecule level since their development over 40 years ago.[1] Measurements of ion channels in planar lipid bilayers have been pursued for a wide range of applications in biosensing, single molecule mass spectrometry, and DNA sequencing.[2–6] Unfortunately, practical embodiments of ion channel-based devices are limited by the shortcomings of the lipid bilayer scaffold containing the ion channel.

Initially, formation of reconstituted lipid bilayer membranes required the deposition of lipids or a lipid-containing solution over an orifice in an insulating partition separating two electrolyte reservoirs. The resultant bilayers have characteristically high resistance ($>G\Omega$) and support the measurement of ion channels at the single molecule level. However, their characteristic short lifetime and mechanical instability limit any technological applications. Bilayer formation over a mechanically stabilizing support such as a solid surface[7] or a porous hydrogel[8] have been shown to significantly extend bilayer lifetime. However, measurement of solid-supported bilayers is somewhat complicated by their limited volume on one side, preventing dc measurements. Although hydrogel-supported bilayers do not share this shortcoming, they cannot withstand transport and must be created on site by a skilled operator.[9–12]

Although a shippable lipid bilayer membrane platform has been demonstrated,[13] there is also interest in automated on-demand bilayer formation because of potential for highly integrated compact devices with small sample volumes and completely electronic system control enabled by use of microelectromechanical systems fabrication technologies. A number of microfluidic devices constructed have used the traditional Mueller–Rudin bilayer formation method[14,15] and required the creation and manipulation of a solvent bolus within an aqueous-filled microfluidic channel to form a lipid bilayer, a process problematic to automate. Microfluidic devices that lessen this burden have also been explored recently.[16,17]

Recently, a bilayer formation method based on mechanical union of self-assembled lipid monolayers has simplified the process of bilayer formation within microfluidic devices[17–19] leading to the possibility of extremely high throughput.[20,21] An application of this technique by Aghdaei *et al.*[22] used dielectrophoresis (DEP) to drive bilayer formation in a microfluidic device.

Electrowetting on dielectric (EWOD) is an alternative microfluidic driving mechanism by which samples are manipulated solely via electrical signals.[23] EWOD is exceptionally well suited for lab-on-chip applications because highly concentrated electrolyte solutions can be manipulated without joule heating, which can limit the applicability of DEP.[24] In EWOD-driven droplet motion, electric fields are applied locally across a hydrophobic-coated dielectric, increasing the wettability of selected regions of the droplet on the substrate, resulting in droplet motion from induced differential surface tension. This method requires low power and fabrication is simple and scalable, making devices amenable to integration with myriad other on-chip transduction mechanisms.

Here we describe a device that combines the EWOD driving mechanism with on-chip thin-film electrodes for parallel formation and measurement of artificial lipid bilayer arrays. Electrowetting is used to facilitate the contact of separate aqueous droplets immersed within a lipid-containing alkane solution, resulting in functional lipid bilayer membranes able to host ion channels. Furthermore, by integrating fabrication of Ag/AgCl electrodes into the device, our EWOD chip allowed automatic and direct access to droplets for multiplexed electrical measurements. The contacting monolayer method on an EWOD chip with integrated Ag/AgCl electrodes represents an attractive and scalable platform that allows automated formation of lipid bilayers and simultaneous monitoring of ion channels in an array format.

Devices were fabricated from a glass wafer coated with 140 nm of indium tin oxide (ITO) (Tech Gophers Corporation). The ITO layer was patterned and etched using standard

a)Electronic mail: schmidt@seas.ucla.edu.

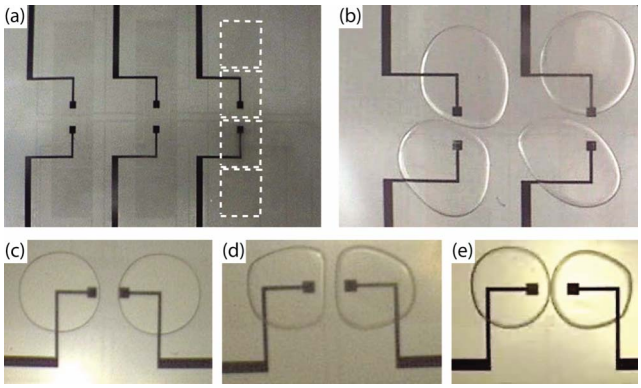

FIG. 1. (Color online) Device structure and droplet movement using EWOD. The device contains three pairs of Ag/AgCl electrodes (black areas) with underlying ITO electrodes (dark gray areas, with the rightmost ITO electrodes outlined in dotted lines) for droplet movement (a). In (b), aqueous droplets surrounded by the lipid-containing organic phase are being positioned prior to an experiment. (A bilayer has formed on the left, while the right droplets are still being moved into position.) Activation of the ITO electrodes causes aqueous droplet movement [(c)–(d)]. After droplet positioning, the electrodes are deactivated and the interfaces of the aqueous droplets relax, forming a bilayer (e). For scale, the length of the square ends of each Ag/AgCl electrode is 300 μm.

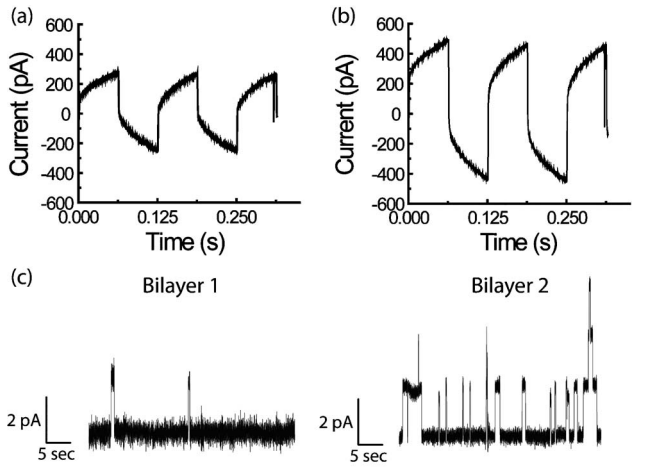

FIG. 2. Electrical measurements of membrane formation and ion channel incorporation. With the initially large droplet separation, the measured background capacitance (proportional to the amplitude of the measured waveform) is large (a), but less than the total capacitance measured upon bilayer formation (b). (c) Gramicidin incorporation was also measured in two bilayers simultaneously (70 mV applied potential).

photolithography processes to create the underlying electrodes, enabling movement of droplets by EWOD.[25] After patterning the ITO, a 1-μm-thick silicon nitride layer was deposited by plasma-enhanced chemical vapor deposition, insulating the ITO electrodes from the rest of the chip above. Next, 300 nm of silver was evaporated and patterned by lift-off to define the electrodes for measurement of membrane and ion channel activity. Finally, 200 nm of Cytop® (Asahi Glass Co.) was spun onto the wafer and patterned using oxygen plasma etching, exposing regions of the silver electrodes. Figure 1 shows a top view of the final device; see EPAPS supplementary material in Ref. 26 for a schematic of the process flow.

Prior to use of the device, 0.5 μl droplets of standard bleach were placed on the exposed silver electrodes for approximately 30 s to create a Ag/AgCl electrode. The device was then rinsed with deionized water and blown dry. Next, 2–5 μl aqueous droplets (1*M* KCl, 10 mM Tris-HCl, 1 mM EDTA, *p*H 8.0) were placed on the exposed Ag/AgCl electrodes. A Cytop®-coated ITO glass plate was then placed on top of the droplets using double-sided tape as spacers such that the gap between the upper and lower hydrophobic coated plates was ~400 μm.

The droplets were moved over the Ag/AgCl electrodes by activating the ITO EWOD electrodes with 30 to 60 V rms at 1 kHz.[27] A solution of 5% 1,2-diphytanoyl-sn-glycero-3-phosphatidylcholine (Avanti Polar Lipids) in *n*-decane (MP Biomedicals), a standard formulation for conventionally formed Mueller–Rudin lipid bilayers, was then pipetted into the side of the device where it easily wetted the Cytop® surface and filled the enclosed volume. To prevent the aqueous droplets from merging during the addition of the organic solvent, the ITO EWOD electrodes were actuated, which immobilized the droplets. For experiments in which ion channel incorporation was measured, gramicidin A (gA, Sigma) was dissolved into the organic phase to a final concentration of 100 ng/ml. The number of ion channels incorporated into the bilayer could be roughly controlled by adjusting this concentration.

After the initial placement of the aqueous droplets and injection of the organic phase, a monolayer of lipid molecules begins to self-assemble at the aqueous-organic interface.[18] This is a time-dependent process; therefore, before final droplet contact was made the droplets were entirely surrounded by the organic solvent lipid mixture for 5 min. If the droplets were brought together without taking this self-assembly time, the droplets fused and no bilayer was formed.

To form a bilayer, the ITO EWOD electrodes were activated to move the droplets toward each other at a speed ~1 mm/s [Figs. 1(c)–1(e)]. During this movement, each water droplet was deformed in the direction of movement, flattening the leading interface. When the electrodes were deactivated, the droplet interface relaxed into a circular shape. The movement and subsequent relaxation were sequentially employed to move the droplets together and into contact, forming lipid bilayers. Specifically, the droplets were positioned so that the relaxation of the droplet interfaces caused the two monolayers to come into contact, while each droplet volume was in contact with a separate individual Ag/AgCl electrode. The device could be used in several different configurations to form multiple individual membranes simultaneously [Fig. 1(b)]. Devices could be cleaned for reuse by soaking and rinsing in acetone, methanol, isopropanol, and deionized water and baking at 200 °C overnight.

The bilayer resistance and capacitance were measured, as well as the conductance of any ion channel incorporated into the bilayer, using Axopatch 200B and DigiData 1322A (Axon Instruments) connected to the Ag/AgCl electrodes. Membrane formation was monitored by measuring the capacitive current flowing in response to an applied 20 mV (peak-to-peak) 8 Hz triangle wave. The constant background capacitance was measured while the droplets were separated in the initial placement of the aqueous droplets on the device [Fig. 2(a)]. Compared to traditional bilayer platforms, the background capacitance was large, a result of the device design and electrode geometry.

When the droplets were moved into contact, the capacitance increased and eventually stabilized at a larger value

[Fig. 2(b)]. The bilayer contribution to this total capacitance was determined by subtracting the background from the capacitance measured when the droplets were in contact. The capacitance of the bilayer in Fig. 1(b) was determined in this way to be 650 pF. The in-plane dimension of the bilayer in Fig. 1(b) was visually estimated to be 450 μm; with the aforementioned vertical gap of 400 μm, a bilayer area of $\sim$0.18 mm^2 would result. This area, with the measured bilayer capacitance, yields a membrane specific capacitance of approximately 0.36 μF/cm^2, consistent with literature values.[28,29] After bilayer formation, the measured resistances were greater than 2 GΩ.

We verified bilayer formation and functionality by measuring the incorporation of the pore forming peptide gramicidin A. Gramicidin A forms a monolayer-spanning monovalent cation-selective channel with a pore diameter of $\sim$4 Å.[30] Gramicidin can be incorporated into artificial bilayers by adding it to the aqueous or organic phase, as it is soluble in both. Here, we add it to the organic phase because bilayer formation is immediately indicated by gA channel measurement. Measurements of incorporated gA were obtained for several hours, limited by the bilayer lifetime. Although we did not extensively explore bilayer lifetimes in this study, those we observed ranged from 2 to 12 h, comparable to those in previous microfluidic devices.[14,31]

We also explored the simultaneous formation and measurement of two bilayers in a single device [Fig. 1(b)]. We measured the bilayers with a custom-built electronic package that automatically switched between each channel at specified time intervals. This was accomplished using 2 SPDT Micromini switches (RadioShack) controlled by an Arduino Diecimila microcontroller (Arduino Inc.). The switches repeatedly connected and disconnected each of the two bilayers to the Axopatch input and output. After bilayer formation, the characteristic dimer formation and dissociation of gA was seen in the measurement of each bilayer [Fig. 2(c)]. The use of additional amplifiers would allow for simultaneous measurement of a larger number of bilayers.[14]

The process of lipid bilayer formation through contacting monolayers is more controllable than conventional artificial bilayer formation techniques because the removal of the solvent between the monolayers preceding bilayer formation is directly achieved mechanically and, as a result, is well suited for use in microfluidic devices. When the aqueous phases are droplets very small sample volumes are obtainable, enabling a high degree of miniaturization and parallelization with minimal reagent needs. EWOD is ideally suited to manipulate these droplets requiring almost no fluidic handling apparatus. An EWOD device with integrated Ag/AgCl electrodes enables measurement of lipid bilayer formation and incorporation of ion channels in an extremely compact device without requirement of an operator. We have taken the first steps toward such a system with the device presented here, capable of parallel creation and measurement of two lipid bilayers and incorporated ion channels. Scaling of this device is straightforward and future work will be directed toward application of this platform to ion channel-based biosensing.

Wyatt Nelson and Jason Poulos contributed equally to this work. This work was supported by NSF Integrative Graduate Education and Research Traineeship (IGERT) through the UCLA Materials Creation Training Program (MCTP) and NSF CAREER award Grant No. 0644442.

[1] P. Mueller, D. O. Rudin, H. T. Tien, and W. C. Wescott, Nature **194**, 979 (1962).
[2] H. Bayley, Curr. Opin. Chem. Biol. **10**, 628 (2006).
[3] R. Capone, S. Blake, M. R. Restrepo, J. Yang, and M. Mayer, J. Am. Chem. Soc. **129**, 9737 (2007).
[4] L.-Q. Gu, O. Braha, S. Conlan, S. Cheley, and H. Bayley, Nature (London) **398**, 686 (1999).
[5] V. A. Karginov, E. M. Nestorovich, M. Moayeri, S. H. Leppla, and S. M. Bezrukov, Proc. Natl. Acad. Sci. U.S.A. **102**, 15075 (2005).
[6] J. W. Robertson, C. G. Rodrigues, V. M. Stanford, K. A. Rubinson, O. V. Krasilnikov, and J. J. Kasianowicz, Proc. Natl. Acad. Sci. U.S.A. **104**, 8207 (2007).
[7] I. Koper, Mol. Biosyst. **3**, 651 (2007).
[8] R. F. Costello, I. R. Peterson, J. Heptinstall, and D. J. Walton, Biosens. Bioelectron. **14**, 265 (1999).
[9] T. J. Jeon, N. Malmstadt, J. L. Poulos, and J. J. Schmidt, BioInterphases **3**, Fa96 (2008).
[10] T. J. Jeon, N. Malmstadt, and J. J. Schmidt, J. Am. Chem. Soc. **128**, 42 (2006).
[11] X. F. Kang, S. Cheley, A. C. Rice-Ficht, and H. Bayley, J. Am. Chem. Soc. **129**, 4701 (2007).
[12] J. W. Shim and L. Q. Gu, Anal. Chem. **79**, 2207 (2007).
[13] T. J. Jeon, J. L. Poulos, and J. J. Schmidt, Lab Chip **8**, 1742 (2008).
[14] B. Le Piouffle, H. Suzuki, K. V. Tabata, H. Noji, and S. Takeuchi, Anal. Chem. **80**, 328 (2008).
[15] M. Zagnoni, M. E. Sandison, and H. Morgan, Biosens. Bioelectron. **24**, 1235 (2009).
[16] N. Malmstadt, M. A. Nash, R. F. Purnell, and J. J. Schmidt, Nano Lett. **6**, 1961 (2006).
[17] K. Funakoshi, H. Suzuki, and S. Takeuchi, Anal. Chem. **78**, 8169 (2006).
[18] L. M. Tsofina, E. A. Liberman, and A. V. Babakov, Nature **212**, 681 (1966).
[19] M. A. Holden, D. Needham, and H. Bayley, J. Am. Chem. Soc. **129**, 8650 (2007).
[20] A. J. Heron, J. R. Thompson, A. E. Mason, and M. I. Wallace, J. Am. Chem. Soc. **129**, 16042 (2007).
[21] J. L. Poulos, T. J. Jeon, R. Damoiseaux, E. J. Gillespie, K. A. Bradley, and J. J. Schmidt, Biosens. Bioelectron. **24**, 1806 (2009).
[22] S. Aghdaei, M. E. Sandison, M. Zagnoni, N. G. Green, and H. Morgan, Lab Chip **8**, 1617 (2008).
[23] J. Lee, H. Moon, J. Fowler, T. Schoellhammer, and C.-J. Kim, Sens. Actuators, A **95**, 259 (2002).
[24] T. B. Jones, K. L. Wang, and D. J. Yao, Langmuir **20**, 2813 (2004).
[25] H.-W. Lu, F. Bottausci, J. D. Fowler, A. L. Bertozzi, C. Meinhart, and C.-J. Kim, Lab Chip **8**, 456 (2008).
[26] See EPAPS supplementary material at http://dx.doi.org/10.1063/1.3167283 for a schematic of the process flow.
[27] U.-C. Yi and C.-J. Kim, J. Micromech. Microeng. **16**, 2053 (2006).
[28] T. Hanai, D. A. Haydon, and J. Taylor, J. Theor. Biol. **9**, 422 (1965).
[29] K. Janko and R. Benz, Biochim. Biophys. Acta **470**, 8 (1977).
[30] D. W. Urry, M. C. Goodall, J. D. Glickson, and D. F. Mayers, Proc. Natl. Acad. Sci. U.S.A. **68**, 1907 (1971).
[31] M. E. Sandison, M. Zagnoni, and H. Morgan, Langmuir **23**, 8277 (2007).