

ARTIFICIAL LIPID BILAYERS IN A MICROFABRICATED SYSTEM

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Abstract

A microfabricated silicon device for studying artificial lipid bilayer systems is described. The experimental apparatus employed enabled the simultaneous acquisition of electrical and optical data. Reconstitution of channel-forming proteins into a artificial lipid bilayer formed across an aperture in the silicon device is demonstrated.

Keywords: Micromachining, Lipid Bilayers, Ion-Channels

1. Introduction

Single molecule detection techniques are becoming an increasingly powerful tool in the field of biophysics [1]. In combination with artificial bilayer lipid membrane (BLM) systems, they have been employed to study the behaviour of reconstituted membrane proteins [2,3]. This abstract describes a microsystem that enables the simultaneous recording of electrical and optical measurements from such an artificial BLM structure. The microsystem should facilitate the acquisition of functional, kinetic and pharmacological information from membrane proteins and could be employed for use in a drug discovery platform.

2. Device Fabrication

The experimental apparatus is centred around a microfabricated silicon device. This consists of a well etched through a silicon wafer, at the base of which is a silicon nitride membrane, through which a 50µm diameter aperture is etched (Figure 1). BLM formation occurs within this aperture.

The silicon devices were fabricated from double-polished, silicon nitride coated, n-type Si(100) wafers (supplied by Edinburgh Microfabrication Facility, Edinburgh, UK). The fabrication process was as follows: an aperture was first defined in the silicon nitride film on the backside of the wafer by photolithography and reactive-ion etching in an Oxford Plasma Technology BP80 system (Plasmatech, Bristol, UK), using a C₂F₆ plasma (100 W, 15 mT and 20 ml min⁻¹ C₂F₆) and a photoresist etch mask. The upper area of the well was then similarly defined on the front-side of the wafer. To etch through the bulk silicon and create the suspended silicon nitride membrane, the wafer was immersed in a 4.5M KOH solution at a temperature of 80°C for approximately 8 hours. After dicing the wafer into individual devices, the exposed regions of silicon were then electrically insulated by coating the devices on both sides with 100nm PECVD silicon nitride.

3. Experimental Methods

The experimental set-up employed for the simultaneous electrical and optical recording of BLMs is illustrated in Figure 2. Using adhesive, the silicon device was first bonded over a fluidic channel etched in a glass substrate and a glass well was then bonded over the upper surface of the silicon chip to create the upper and lower fluidic reservoirs. The bilayer was imaged using a fluorescence microscope with a x60 water immersion objective lens and electrical activity was recorded using Ag|AgCl electrodes, which were inserted into the fluid reservoirs on both sides of

the aperture and were connected to a patch clamp amplifier (Industrial Development Bangor, Bangor, UK).

To form a BLM across the silicon nitride aperture, the lower glass flow cell was first filled with electrolyte solution (1M KCl). 1-2 μl of 1 mg ml^{-1} 1,2-diphytanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabama, USA), diluted with n-decane, was then pipetted over the aperture at the base of the silicon well. Once the lipid had spread over the silicon nitride membrane, the upper glass well was filled with the electrolyte solution and a BLM subsequently formed by spontaneous thinning. If the lipid had not thinned within a few minutes of adding the electrolyte, the fluid in the glass well was agitated so as to speed up the thinning process.

Channel-forming peptides were reconstituted into BLMs using two different methods: by directly injecting into the upper fluidic reservoir peptides (such as gramicidin) that will spontaneously incorporate into a BLM and by using vesicle fusion methods. Gramicidin was dissolved in chloroform to 10 ng ml^{-1} and 1-2 μl of the solution was injected as close to the BLM as possible, whilst nystatin-containing vesicles were fused with the BLM following the method developed by Woodbury [4].

4. Results and discussion

Figure 3 shows a sequence of optical reflection images that illustrate the spontaneous thinning of a BLM across the aperture in a silicon nitride membrane. Interference fringes due to the thick initial lipid film can be clearly observed (top left of Figure 3). As the lipid film thins, these fringes recede and the area of the BLM appears black (bottom right of Figure 3). The thinning of the bilayer was also monitored electrically by measuring the change in capacitance, which was seen to increase as the bilayer thinned. The bilayers were extremely stable with minimal sensitivity to moderate mechanical vibrations and with a lifetime of several hours.

Figure 4 shows a current-time trace obtained shortly after the injection of the solution containing gramicidin monomers. Single-channel events can be clearly observed. Similarly, Figure 5 shows an electrical recording acquired during the fusion of nystatin loaded vesicles. Fusion of an individual vesicle with the BLM results in a current spike that decays with time, as the nystatin oligomers disassociate in the BLM into individual nystatin monomers, which do not function as ion-channels.

5. Conclusions

The use of microfabricated silicon devices for electrically and optically monitoring artificial BLM systems has been demonstrated. Stable BLMs were successfully produced and single-channel recordings were obtained from channels formed from re-constituted membrane proteins.

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References

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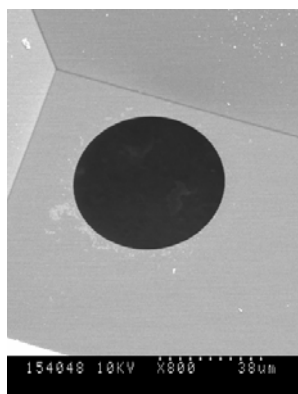


Figure 1. An SEM of the aperture in a silicon-nitride membrane.

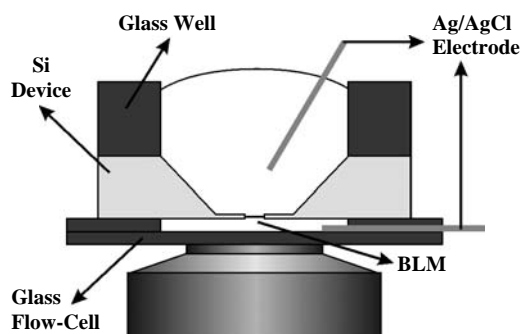


Figure 2. Experimental apparatus for simultaneous electrical and optical recording of BLMs.

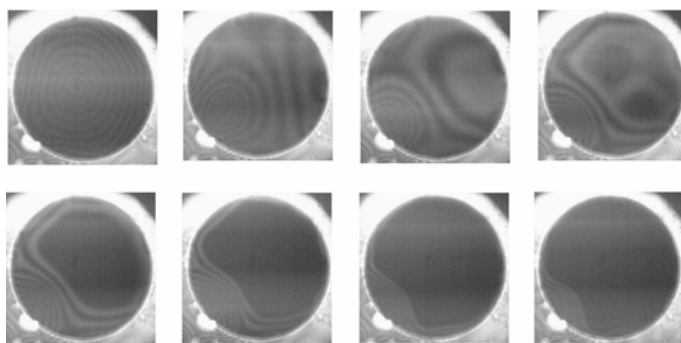


Figure 3. A time sequence of reflectance images (approximately 20s long) illustrating the spontaneous thinning of a BLM.

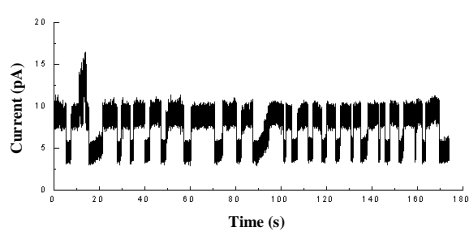


Figure 4. A current-time trace that shows ion conduction through single gramicidin dimers, that form temporarily when two gramicidin monomers come together by diffusion.

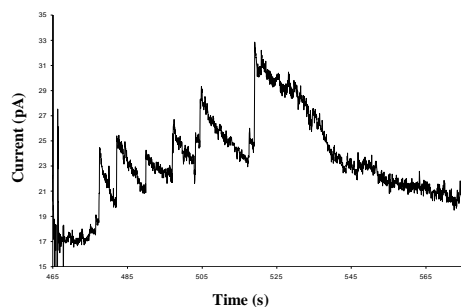


Figure 5. A current-time trace showing the fusion of individual nystatin-containing vesicles with the BLM.