

BLM EXPERIMENTATION AND OPTO-ELECTRICAL CHARACTERIZATION IN MICROCHIPS. TOWARDS AN INTEGRATED PLATFORM FOR DRUG SCREENING ON MEMBRANE PROTEINS.

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ABSTRACT

Experimentation on cell membranes and on membrane proteins commonly makes use of planar and simplified membrane models, or bilayer lipid membranes (BLMs). Although these models are extensively employed, the experimentation is tedious and time-consuming, and mostly limited to electrical measurements. We propose here a dedicated microfluidic platform for BLM experimentation using electrical and optical techniques. BLMs are formed in the closed microdevice by exposing a lipid plug to air and buffer to give a bilayer structure. BLMs are highly stable, reproducible and have an excellent sealing resistance. Single protein studies are shown using α -hemolysin as a protein model.

KEYWORDS: microfluidic platform, BLM, membrane proteins, α -hemolysin,

INTRODUCTION

Membrane proteins become preferred targets for drug discovery: they are easily accessible, responsible for the cell communication so that they consist of interesting routes to regulate signaling pathways in case of diseases. As a consequence, novel dedicated platforms are currently highly desired by the pharmaceutical industry for high-throughput screening of drugs on membrane proteins. Good candidates for drug screening assays are microfluidic platforms as they provide a high level of integration with opportunities for parallel investigation and automation. BLMs (bilayer lipid membranes) which are planar models of cell membranes commonly serve as a matrix for membrane protein studies, and they can be integrated in microfluidic platforms. In that context, microsystems bring improved sensitivity for electrical measurements and enhanced bilayer stability, and make simultaneous optical measurements possible [1]. However, so far no fully microfluidic platform for BLM experimentation has been reported, allowing reproducible membrane formation and simultaneous optical and electrical characterization.

EXPERIMENTAL

Microchip fabrication

The microfluidic chip comprises three independent layers as illustrated in figure 1 (left), two glass substrates in which the microfluidic channels are fabricated and a thin substrate placed in-between that contains a micrometer-sized aperture where BLMs are formed. Microfluidic channels are processed in glass using standard wet-etching techniques and fluidic reservoirs powder-blasted in the top glass substrate. The intermediate layer consists of a Teflon membrane. Two fabrication routes have been investigated here to produce such membranes containing micrometer-sized apertures. In a first approach, a FEP commercially available foil is employed and apertures (and fluidic accesses) are made using dry-etching techniques (Bosch process). Alternatively, liquid Teflon AF is spin-coated on a structured PDMS wafer [2].

The three layers are covalently assembled using a novel technique recently developed in our lab [3] that relies on the use of an intermediate layer of glue (NOA 83H).

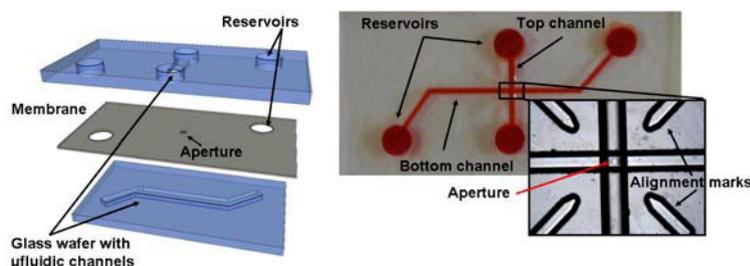


Figure 1: *Integrated microfluidic platform for BLM experimentation.* Left: open view showing the 2 glass layers with the channels ($w= 200 \mu\text{m}$; $h= 50 \mu\text{m}$) and the Teflon layer with the micrometer-sized aperture. Right: Picture of an assembled microchip whose channels are filled with a dye; in inset: enlarged view of the microaperture.

BLM experimentation

The assembled microfluidic chip (fig 1, right) is placed in a dedicated chip-holder (see figure 2) for experimentation, to facilitate the electrical and fluidic connections between the outer world and the chip. BLMs are prepared across the aperture in the Teflon layer by successively flushing a lipid solution, air and buffer in both channels; the exposure of the firstly deposited lipid plug to air and buffer leads to its thinning into a bilayer structure, as discussed in more details in the results section. BLMs are prepared from a mixture of two phospholipids, DPhPC (1,2-diphytanoyl-sn-glycero-3-phosphocholine) and DOPE (dioleoyl-phosphatidylethanolamine) coupled to Rhodamine B in n-decane. The formation of the BLMs is monitored in parallel using a patch-amplifier (Axopatch, Molecular Devices) and microscopy (fluorescence and bright field). In a last series of experiments, a membrane protein (α -hemolysin) is employed; its spontaneous insertion in the membrane confirms the formation of a bilayer structure.

RESULTS AND DISCUSSION

Microchip design and fabrication

We propose here a novel microfluidic platform for BLM experimentation. Although the system is shown here for a single BLM (see fig. 1), the design and fabrication process are easily upgradable for multiplexed measurements on a series of BLMs individually addressed, in a single device. Glass and Teflon are chosen to fabricate the microfluidic networks as well as the intermediate layer because of their excellent insulating properties, chemical inertness and transparency, making both electrical and optical measurements possible in the platform.

The Teflon intermediate layer is produced using two different approaches that both lead to successful and reproducible formation of apertures, down to 10 micron diam. (see figure 3). However, the second approach relying on spinning and molding is more versatile in terms of membrane thickness (10-20 μm) and aperture size (30-100 μm) (figure 3). Leakage free assembly of the microsystem was achieved using an optical adhesive [3] under a microscope. Interestingly, although the bonding proved to be strong, disassembly of the chip and recycling of the glass substrates is possible. For that purpose, the gluing layer is simply dissolved in appropriate solvent, and the glass substrate thoroughly cleaned before being reused.

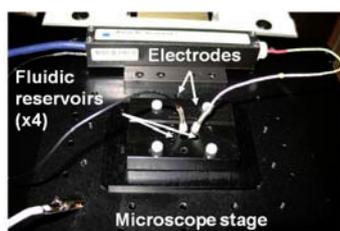


Figure 2: **Home-made chip holder** with external fluidic and electric connections. The holder is connected to a patch-amplifier and placed on the stage of a microscope for simultaneous optical and electrical measurements.

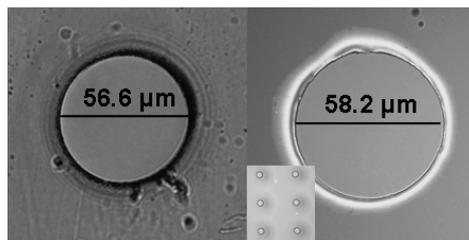


Figure 3: **Fabrication of the microapertures** using DRIE techniques in a FEP Teflon foil (left) or a combination of spinning and molding techniques (right) enabling the production of arrays of microapertures (see inset).

BLM experimentation

As illustrated in figure 4, BLMs are prepared across the aperture in the Teflon substrate by successive flushing of a lipid solution in n-decane, air and buffer in both channels. After deposition of lipids in the micrometer-sized aperture, air or directly buffer is introduced in the channel.

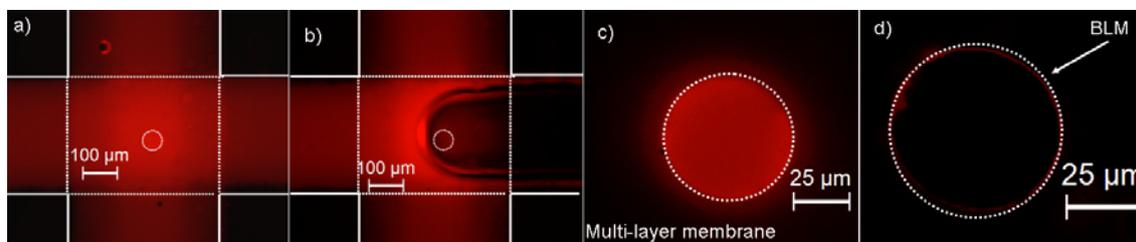


Figure 4: **Monitoring of BLM preparation** in a microchip using fluorescence microscopy. a) Channels are filled with lipid solution of DPhPC:DOPE (1,2-diphytanoyl-sn-glycero-3-phosphocholine, dioleoyl-phosphatidylethanolamine in n-decane). At the aperture a thick lipid plug forms and evolves as a multi-layer membrane upon air-exposure (b, c). (d) A bilayer is obtained after addition of buffer and thinning of the membrane.

Upon exposure to air or another solution, the lipid plug spontaneously thins, as shown in figures 4 and 5, to give a bilayer structure. The resulting bilayer membranes exhibit reproducible characteristics in terms of surface area (capacitance of 10 ± 3.8 pF) and sealing quality (14 ± 4 G Ω). The capacitance values are employed to determine an average surface area of the bilayer of $2.22 \times 10^3 \pm 0.84$ μm^2 (diam. of 53.2 μm). The diameter of the BLMs is measured optically and determined of 47 ± 4 μm .

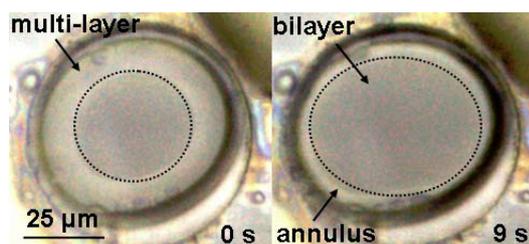


Figure 5: **Thinning of the multilayer** to a bilayer membrane monitored using bright field microscopy. (a) A lipid bilayer starts to form in the center of the aperture (dark area) while a thick lipid annulus remains at its edge; (b) after thinning, the bilayer expands.

Finally, the integrated platform is successfully applied for single protein studies with a commonly used model for membrane proteins, α -hemolysin (see fig. 6). The protein solution is added in the top microfluidic channel, and the protein inserts itself spontaneously in the BLM as shown in figure 6 (right); every jump in the current measured across the membrane corresponds to the insertion of a single protein. This not only confirms the formation of a bilayer structure (as proteins do not insert in multi-layer structures) but also demonstrates the ability of the platform to perform single protein measurements using electrical techniques.



Figure 6: **Single protein studies.** α -hemolysin (left) is used as a model for single proteins studies on-chip. Electrical monitoring of the insertion of individual α -hemolysin molecules (right). Each jump of 50 pA in the current corresponds to the insertion of one protein (applied voltage of 50 mV; buffer conditions: KCl 1 M, HEPES 10 mM).

CONCLUSION

We have developed a novel integrated microfluidic platform for BLM experimentation using both electrical and optical measurements. BLMs are formed in the closed device using a novel methodology, in a reliable and reproducible manner. Early single protein measurements have been successfully performed using the model protein α -hemolysin.

In future we are aiming at the investigation of more relevant membrane proteins, the automation of the membrane formation process and multiplexed studies on a large number of BLMs by simple upgrade of the chip design.

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REFERENCES

- [1] H. Suzuki and S. Takeuchi, Microtechnologies for membrane protein studies, *Anal. Bioanal. Chem.*, vol 391, pp. 2695, (2008).
- [2] M. Mayer, J. K. Kriebel, M.T. Tosteson, and G.M. Whitesides, Microfabricated Teflon membranes for low-noise recordings of ion channels in planar lipid bilayers, *Biophys. J.*, vol 85, pp. 2684, (2003).
- [3] R. Arayanarakool, S. Le Gac, and A. van den Berg, Low-temperature, simple and fast integration technique of microfluidic chips by using a UV-curable adhesive, *Lab Chip*, [10.1039/c004436a](https://doi.org/10.1039/c004436a).

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