

TOWARDS SIMULTANEOUS ELECTRICAL AND OPTICAL INVESTIGATION OF BLMS USING A NOVEL MICROFLUIDIC DEVICE

Iris van Uitert¹, Yanina Cesa², Hans de Boer¹, Johan Bomer¹,
Martin Bennink², Séverine Le Gac¹, and Albert van den Berg¹

¹*BIOS, the Lab-on-a-Chip Group, MESA⁺ Institute for Nanotechnology, THE NETHERLANDS* and ²*Biophysical Engineering, MESA⁺ Institute for Nanotechnology, THE NETHERLANDS*

ABSTRACT

We firstly describe the influence of the phospholipid (PL) composition of bilayer lipid membrane on their electrical properties: (i) the more unsaturations in the tail, the earlier the BLM breakdown and (ii) the bulkier the head group, the less stable the membrane. Secondly, we design and fabricate novel devices that couple such electrical characterization to optical investigation and that enable the preparation of asymmetrical membranes: a “macro” device including a drilled PMMA plate as well as microfluidic device consisting of a glass-teflon foil-glass sandwich.

KEYWORDS: BLMs, membrane composition, asymmetry, electrical properties.

INTRODUCTION

Understanding the properties of cellular membranes may provide a means for more controlled and reproducible cellular experiments. The cell membrane consists of a bilayer formed from various PLs whose distribution is asymmetrical: the inner leaflet of the membrane is composed of negatively charged PLs whereas the outer leaflet mostly includes neutral PLs. Furthermore, the inner leaflet is more fluid than the outer one since PL hydrocarbon tails present less unsaturations in the outer leaflet than in the inner one [1]. It has already been demonstrated that both the heterogeneous composition and the asymmetrical character of the membrane influence its properties [2, 3]. A good model for studying membranes consists of bilayer lipid membranes (BLMs). These planar mimics of cell membranes are conventionally prepared on a substrate including a small aperture, and current work on BLMs is mostly restricted to symmetrical membranes. In this work we aim at getting a better understanding of the electrical properties of cell membranes with both symmetrical and asymmetrical BLMs prepared using the painting and the Montal-Mueller [4] technique, respectively. We particularly investigate the influence of the membrane composition (nature of the PLs) on its capacitance and breakthrough voltage.

EXPERIMENTAL

BLMs are prepared in appropriate device using the painting technique, restricting thereby BLM studies to symmetrical BLMs. We use here various PLs (Avanti Polar Lipids Inc.), alone or in mixtures: (i) 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DOPC), a neutral PL found in the outer leaflet of the membrane, (ii) brain phosphatidylserine (PS), a negatively charged PL and (iii) bovine liver phosphatidylinosi-

tol (PI), another negatively charged PL that contains a bulky head and a highly unsaturated tail (Fig. 1a). For all membrane compositions we measure the *capacitance* as well as the *breakdown voltage* (voltage leading to membrane rupture).

RESULTS AND DISCUSSION

BLMs made from PC, PS and PI introduced in various weight ratios (Fig. 1b-d) are prepared and characterized. The membrane breakdown voltage (BV) is influenced by the BLM composition and the PL density which is directly linked to (i) the number of unsaturations, (ii) the charge and (iii) the bulkiness of the head groups of PLs. The stability of homo BLMs is as follows: PC>PS>PI. PC has no unsaturation and is neutral while PI is highly unsaturated and has a bulky charged head. Hetero BLMs are characterized by a higher BV. Increasing x_{PI} in PS:PI and PC:PI BLMs leads to a decrease in membrane stability (Fig. 2c, d) accounted for by the introduction of more unsaturations, bulky heads, and more charges (PC:PI BLMs). For PC:PS BLMs, two trends are seen: a first stabilization of the membrane followed by a destabilization (Fig. 2b). The stabilization can be explained by charge interactions between the head groups of the PLs while the destabilization is due to both the increased number of unsaturations and charge repulsion between the negative heads.

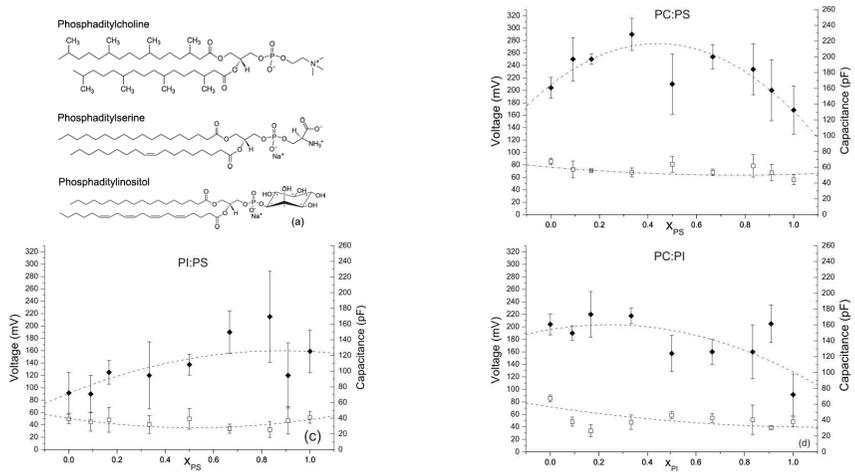


Figure 1: (a) Structure of PC, PS and PI. (b-d) Electrical characterization of BLMs made from PC:PS (b), PI:PS (c) and PC:PI (d) membranes: breakdown voltage (\blacklozenge) and capacitance (\square) as a function of the PL weight fraction, $x_{PL_i} = m_{PL_i} / \sum_l m_{PL_l}$.

In parallel we have developed a novel device that enables us to prepare asymmetrical BLMs and to couple their electrical characterization to optical studies (Fig 2). Membranes are prepared on a PMMA substrate that includes an aperture ($< 350 \mu\text{m}$) (Fig. 2a). This PMMA plate is placed either in a vertical holder for the preparation of asymmetrical BLMs or in a horizontal holder that fits on a microscope stage for simultaneous electrical and optical investigation (Fig. 2c). Figure 2d represents a PC membrane prepared using the painting technique in our novel device.

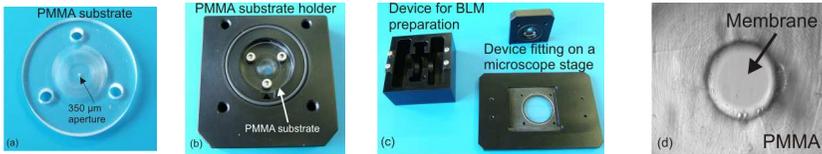


Figure 2: (a-c) Novel device for preparing asymmetrical membranes and the simultaneous electrical and optical investigation of BLMs. (d) Picture of a PC membrane prepared in our novel device.

We have also designed and fabricated a fully integrated microfluidic device for the preparation of horizontal asymmetrical membranes and their dual (electrical and optical) characterization. The system is made from glass and includes a Teflon foil with a small aperture for the BLM preparation (Fig. 3).

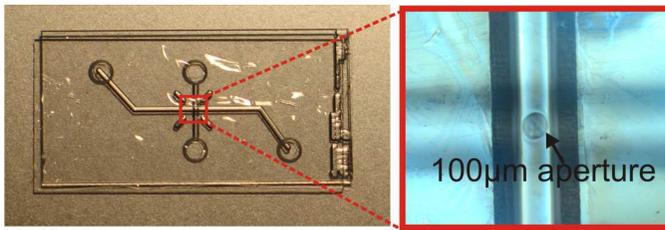


Figure 3: Fully integrated microfluidic device for the preparation of horizontal asymmetrical BLMs and their dual characterization. Left: microsystem; Right: enlarged view on the aperture fabricated in a Teflon foil for preparing BLM.

CONCLUSIONS

We describe here the influence of the chemical composition of BLMs prepared from mixtures of PC, PI and PS on their electrical properties (breakdown voltage) using a conventional device. We are currently investigating the preparation of similar BLMs in novel devices, macro and microfluidics, enabling coupled electrical and optical characterization as well as the preparation of asymmetrical BLMs.

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