MICRO-PATTERNING OF IONIC RESERVOIRS WITHIN A DOUBLE BILAYER LIPID MEMBRANE TO FABRICATE A 2D ARRAY OF ION-CHANNEL SWITCH BASED ELECTROCHEMICAL BIOSENSORS

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Submitted to:
NANOTECH 2004 CONFERENCE AT BOSTON, MA (March 7-11, 2004).
Micro-Patterning of Ionic Reservoirs within a Double Bilayer Lipid Membrane to Fabricate a 2D Array of Ion-Channel Switch Based Electrochemical Biosensors

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ABSTRACT

We present a simple approach for the design of ionic reservoir arrays within a double phospholipid bilayer to ultimately develop a 2D array of ion-channel switch based electrochemical biosensors. As a first step, a primary bilayer lipid membrane is deposited onto an array of electrodes patterned onto a substrate surface. Subsequently, an array of microvoids is created within the bilayer by a wet photolithographic patterning of phospholipid bilayers using a deep UV light source and a quartz/chrome photomask. To ensure registry, the photomask used to pattern bilayers is designed to match up the microvoids within the primary bilayer with the array of electrodes on the substrate surface. The deposition of a secondary bilayer lipid membrane onto the primary bilayer that spans across the patterned microvoids leads to the formation of the array of ionic reservoirs within the double phospholipid bilayer. This is accomplished using giant unilamellar vesicles and by exploiting membrane electrostatics. The use of ion-channels incorporated into the secondary bilayer that covers the individual ionic reservoirs allows the construction of a 2D array of ion-channel switch based electrochemical biosensors that are able to recognize different target-agents simultaneously.
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ABSTRACT

We present a new approach for the design of ionic reservoir arrays within a double phospholipid bilayer to ultimately develop a 2D array of ion-channel switch based electrochemical biosensors. As a first step, a primary bilayer lipid membrane (BLM) is deposited onto an array of electrodes patterned onto a substrate surface. Subsequently, an array of microvoids is created within the bilayer by a wet photolithographic patterning of phospholipid bilayers using a deep UV light source and a quartz/chrome photomask. The deposition of a secondary BLM onto the primary bilayer that spans across the patterned microvoids leads to the formation of an array of ionic reservoirs within the double phospholipid bilayer. This is accomplished using giant unilamellar vesicles and by exploiting membrane electrostatics. The eventual use of ligand-gated ion-channels incorporated into the secondary bilayer that covers the individual ionic reservoirs will lead to a 2D array of ion-channel switch based electrochemical biosensors.

Keywords: bilayer lipid membrane, ionic reservoir, ion channel, electrochemical biosensor

1 INTRODUCTION

Ion-channel based biological systems have not been extensively employed in current sensor technology because of the serious problems in reproducing a native-like microenvironment necessary for proper functioning. In fact, there are several problems in achieving this.

1. One needs to create a lipid bilayer, on a solid surface, that is sufficiently flexible and defect free such that ion-channel activity can actually be measured.
2. The lipid membrane must be in a physical state where biological molecules function as if in a natural system.
3. One needs to entrap an ionic medium between the membrane and the supporting electrode in order to facilitate the ion exchange through the ion-channels incorporated into the membrane.

A previous approach based on Gramicidin-A pairs, by Cornell et al. [1], exhibits limitations related to a choice of ion-channel switching mechanism that is different from any of those present in cell membranes. In addition, the preparation of the ionic reservoir structure between the membrane and the electrode evidences high experimental difficulties.

In contrast, surface micro-patterning methods in the solid state have been particularly useful for patterning supported phospholipid bilayers. These patterned phospholipid bilayers have played an important role to understand, emulate, pattern, and exploit many functions of cell membranes for fundamental biophysical research and many biomedical and sensing technologies [2-8].

The micro-patterning of supported membranes has been achieved by a variety of methods that fall into two broad categories:

1. Use of pre-patterned substrate surfaces that present chemical and/or electrostatic barriers to membrane formation [9-14].
2. Application of soft-lithographic methods of patterned deposition (stamping) or removal (blotting) using polymeric stamps [15, 16].

However, the methods requiring substrate pre-patterning depend on the prior deposition of exogenous materials on the substrate surface and form single patterns. While methods based on polymer stamps circumvent these issues, they require optimization of the physical contact, associated contact pressure, and deformability of the polymer stamps. Moreover, difficulties associated with achieving contact uniformity for large-area patterning remain.

In this approach we describe a simple light-directed method for patterning supported phospholipid membranes over large substrate areas in a non-contact manner and without requiring prior substrate patterning. The approach is generally analogous to solid-state lithographic methods developed for dry-state DNA and peptide arrays [17], but is applicable for the aqueous phase and fluid bilayer lipid membranes (BLM). It simply relies on the spatially-directed illumination of phospholipid bilayers submerged in an aqueous ambient to UV light. The pattern of exposure to light through a mask, or by other spatially addressable means, determines the relief pattern generated within the bilayer. Ultimately, an experimental procedure based on this approach has been developed to prepare ionic reservoirs within a double lipid bilayer with independent electrodes connected to individual ionic reservoirs.
2 APPROACH

The general procedure that we proposed to pattern supported membranes onto a solid substrate is schematically shown in Figure 1.

![Figure 1](image1.png)

Figure 1. Direct patterning of voids within bilayer membranes using deep UV photolithography.

The process begins with the preparation of a continuous supported bilayer lipid membrane (sBLM) on a hydrophilic silica surface. To enable fluorescence measurements, vesicles are doped with appropriate concentrations of labeled lipids (e.g., 1 mol% 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt abbreviated as Texas Red-DHPE). Next, a lithographically produced quartz mask (e.g., an array of square opaque (chrome) elements over a UV transparent quartz mask) is then brought in soft contact with the sBLM. Deep UV light in the 184–257 nm range, produced by low or medium pressure Hg lamp housed in a used quartz envelope (10-20 mW cm\(^{-2}\)), is then directed through the mask at the bilayer samples submerged in phosphate buffered saline (PBS) for ~120 min. Upon separation of the mask from the sample under the buffer, high-fidelity patterns of membrane bilayers comprising intact lipid patches in UV-protected areas and lipid-free regions in the UV-exposed areas are obtained. Using this approach, one can create both patterns of voids and isolated membrane islands.

The previous general procedure to pattern supported membranes onto a solid substrate has to be modified in order to prepare ionic reservoirs within a double lipid bilayer with independent electrodes connected to individual ionic reservoirs (Figure 2).

![Figure 2](image2.png)

Figure 2. Schematic representation of an ionic reservoir formation within a double lipid bilayer constructed. A) Self assembly of alkanethiol (RSH) molecules on Au; B) Vesicle spreading on hydrophobized RSH/Au to produce a primary hybrid bilayer; C) Photochemical patterning of the hybrid bilayer; D) Deposition of the second bilayer that spans across the void in the primary bilayer.

First, a prior deposition of a metal onto the substrate with the same spatial distribution as the micro-patterned voids, using a customized mask with holes following the same distribution as in the previously mentioned quartz mask, is carried out. Next, the gold surface is hydrophobized by the self-assembly of alkanethiol molecules (Figure 2a) to allow the spreading of a vesicle to produce a primary hybrid bilayer (Figure 2b). The photochemical patterning of the hybrid bilayer leads to the formation of an array of voids within the bilayer (Figure 2c). Finally, the deposition of a second lipid bilayer that spans across the voids in the primary bilayer leads to the formation of a 2D array of ionic reservoirs (Figure 2d).
The eventual use of ligand-gated ion-channels incorporated into the secondary lipid bilayer that covers the individual ionic reservoirs will lead to a 2D array of ion-channel switch based electrochemical biosensors (Figure 3).

![Figure 3. Electrochemical biosensors array based on ion-channels incorporated into the secondary lipid bilayer that covers the ionic reservoirs connected to individual electrodes.](image)

In this kind of electrochemical biosensors the signaling occurs by electrochemical detection of changes in conductance when ion-channels open or close because of binding of the target-agent. Taking into account that each electrode is independently connected, one can get individual electric signals from each ionic reservoir.

3 CONCLUSIONS

In summary, the wet membrane photolithography approach presented here is simple; inexpensive, and an enabling procedure to create optically-defined patterns of fluid bilayer lipid membranes submerged in the aqueous phase. This approach allows the preparation of ionic reservoirs within a double bilayer with independent electrodes connected to individual ionic reservoirs for the development of a 2D array of ion-channel switch based electrochemical biosensors.

REFERENCES