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# Construction and Characterization of Soft-Supported Lipid Bilayer Membranes for Biosensors Application

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***Abstract*—A model cell membrane can be ingeniously used to mimic biological processes with wide applications in the biomedical field. For instance, a model cell membrane can be used as a functionalized element integrated to a sensor for identification of specific proteins or other molecules in a biofluid. A special characteristic of the model membrane described in this work is the fluidic or surface mobility feature given by a thin film of grafted polymer that serves as a cushion layer to support lipid bilayers. Lipid bilayers were deposited on a polyethylene glycol (PEG) thin film over a polished glass substrate using the Langmuir-Blodgett technique. Topographical characterization of the bilayers was accomplished using atomic force microscopy (AFM). Results revealed that the grafted polymeric PEG cushion layer confers mobility to the model membrane.**

## I. Introduction

SOFT-supported lipid bilayer membranes can be used to mimic cell membranes. In this fashion, many of the processes that take place in the cell membrane can be reproduced *in vitro* and used in bioengineered design of biosensors to improve their performance. Lipid bilayers can be used to study immobilized glycolipids, membrane receptors and membrane proteins to generate information about cell membranes and tissue surfaces. They can also be used for the stimulation of cell proliferation and tissue growth in a controlled manner, or for the suppression of cellular apoptosis[1].

An important characteristic of cell membranes is that while being a barrier, they maintain surface mobility and lateral fluidity. This characteristic allows other molecules such as proteins, glycoproteins, and carbohydrates to interact and integrate biological functions of lipid bilayers.

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For the effects of this work, soft-supported model lipid bilayer membranes have been constructed. They have been separated from the solid substrate by a thin water and chemically grafted PEG film (~30 nm), which allow the maintenance of the structural and thermodynamic properties of free lipid bilayers [2]. Ideally, the interactive forces between membranes and solids have to be only either weakly attractive or repulsive to avoid dewetting of the soft layered films following bilayer deposition [1]. A useful strategy to avoid de-wetting is to chemically graft a polymer cushion to the surface.

In this work, a polyethylene glycol (PEG) cushion layer was created as a soft support for a lipid bilayer membrane [3]. PEG is a linear or branched near neutral polyether soluble in water and most organic solvents. Its chemical formula is: $HO\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2OH$. PEG was chosen for this study because its biocompatible properties. PEG is nontoxic, it has shown immunogenicity and nonantigenicity and it does not harm active proteins or cells even if it interacts with real cell membrane [4]. In addition, PEG coated surfaces become hydrophilic and protein rejecting [3]. This property is desirable for the effects of biosensor design and for making the detection process more specific. The inert character of PEG is based on its molecular conformation in aqueous solution, where PEG exposes slightly negative hydrophilic groups which attract water and shows very high surface mobility (steric exclusion). It is worth mentioning that in order to maintain its biological and biocompatible properties, the PEG has to be chemically grafted to the surface.

An important application of the soft hydrated polymer cushion is to provide a lubricating surface, which enables the self-healing of defects in the supported membranes. This is essential to reduce nonspecific bindings [1].

## II. Experimental Procedure

### A. Construction of the Soft Support Layer

Polyethylene glycol (PEG, 400 Da, P3265-500G, Sigma,) was grafted to polished glass surfaces (rectangular hemacytometer cover glasses, 12-519-10, Fisher Sci.). Using the methodology explored by Alcantar et al. (2000) [3]. The polished glass was first activated by submerging it in a 10 % w sodium hydroxide (NaOH, 1310-73-2, Acros Organics) solution and sonicating (Model FS30, Fisher Sci.) it for 5 minutes. The activation process consisted on the production of silanol groups (Si-OH) on the glass surface

that later reacted with the end alcohol group of the PEG molecules (Fig. 1A). The glass was retrieved from the NaOH solution and was vigorously rinsed with milli-Q water and dried with ultrapure nitrogen. Note that any traces of water can interfere with the PEG reaction and any traces of NaOH can interfere with the lipid deposition. The cleaned and activated surfaces were submerged in liquid PEG for one hour at 100°C maintaining constantly stirring (Fig. 1A and 1B). The reaction and conditions have been verified and determined in a previous published work [3]. Once the PEG grafting reaction ended, the surfaces were retrieved from the hot PEG solution, were gently rinsed with water avoiding direct contact with the water flow, and dried with ultrapure nitrogen.

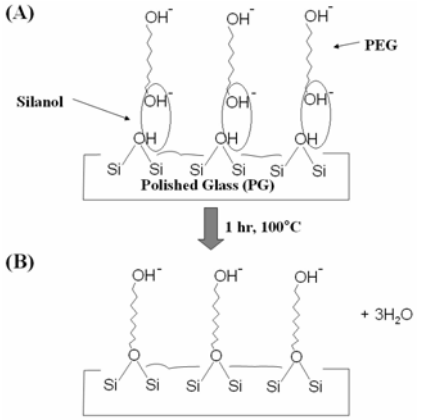


Fig. 1. (A) Schematic of the grafting reaction, where silanol groups on the surface lose the hydrogen, forming water with the end hydroxyl group of the PEG molecules. (B) Representation of the PEG surface grafted.

### B. *Lipid Bilayer Deposition*

The lipid used for the study was 1, 2 - Distearoyl - sn - Glycero - 3 - Phosphoethanolamine (DSPE, 850715X, Avanti Polar Lipids Inc.). The lipid bilayers were deposited onto the grafted PEG layer using the Langmuir-Blodgett deposition technique (LB trough, Model 611D, Nima Technology). The final lipid bilayers were kept inside of a laminar flow cabinet (Class 100) to reduce contamination with environmental particles. The deposition was done at a set barrier speed of 25 $cm^2$/min. The initial uncompressed surface area was 300 $cm^2$. The area, deposition pressure, and lipid concentration used for the depositions were chosen based on previous experiments [5], where 100 μL of DSPE dissolved in Chloroform (HPLC grade, 67-66-3, Acros Chemicals.) at a concentration of 15 μg/μL was gently spread on the air/water interface using a gas tight Hamilton syringe (giving 5 minutes to allow the solvent to evaporate). During the deposition (Fig. 2A and 2B), the surface pressure was kept constant at 30 mN/m. The deposition took place at constant pressure and using a slow barrier velocity of 50 $cm^2$/min.

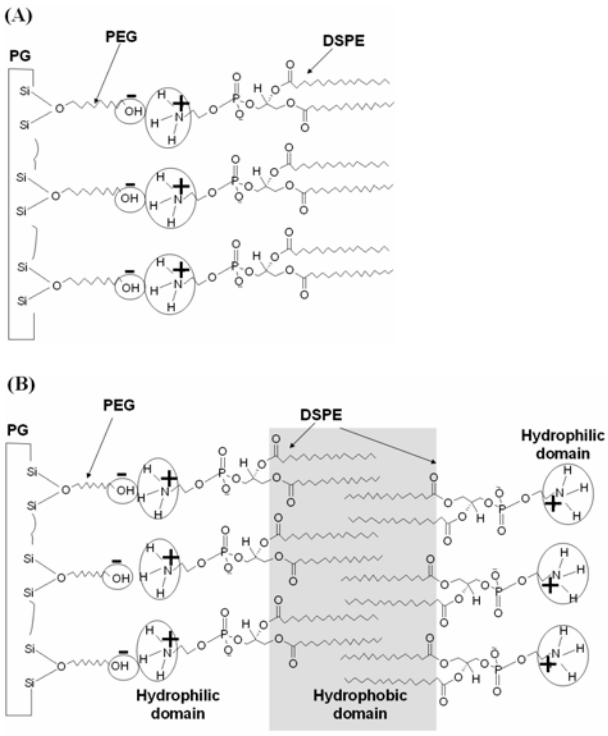


Fig. 2. (A) Schematic of first lipid monolayer deposited on the cushioned layer. (B) Representation of the soft-supported lipid bilayer. Hydrophilic and hydrophobic domains are shown.

### C. *Soft-supported lipid bilayer characterization*

The surface topology of the soft-supported membranes was analyzed with atomic force microscopy (AFM) (MFP-3D, Asylum Research, Santa Barbara, CA). The surfaces were scanned using non-contact tapping mode under deionized water to preserve the integrity of the lipid bilayer using silicon cantilevers with typical spring constant < 1 N/m. Scan sizes were 10x10 $\mu m^2$.

## III. Results and Discusion

Figure 3A shows the entire deposition process from the moment of monolayer compression until the deposition of the whole lipid bilayer. One revealing event that confirmed the lipids-PEG physical interaction was observed after the first DSPE lipid layer was deposited and during the second layer transference. That is, the part of the lipids deposited on the back side of the glass slide returned to the air/water interface as reflected in the area of the deposition (Fig 3B). This corroborates that as the substrate was withdrawn from the air/water interface, the lipids were adhering on the PEG layer rather than on the glass side (only one side on the polished slide remained with PEG).

For the second layer deposition, the substrate was then submerged under water again. During this process the interaction between the PEG and the lipids layer can be explained since the lipids interact and adhere stronger to the

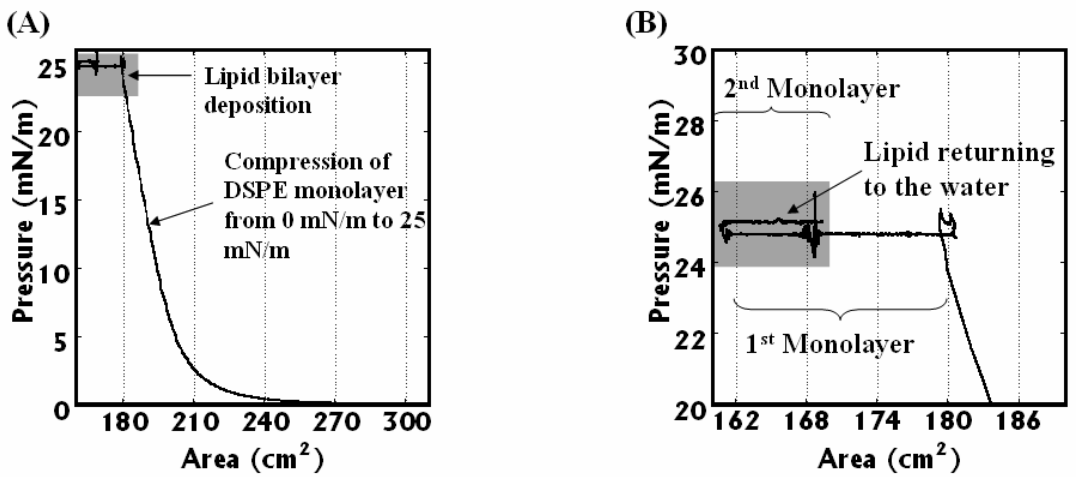


**Fig. 3. (A)** Representation of the lipid bilayer deposition process: with maximum (initial) area of 300 $cm^2$ and minimum area of 160 $cm^2$. The lipids were compressed in a monolayer, from 300 $cm^2$ to 180 $cm^2$. The first layer was deposited from 180 $cm^2$ to 160 $cm^2$, and the second layer was deposited from 160 $cm^2$ to 170 $cm^2$. **(B)** Detailed view of the shaded area from (A). The two layers during deposition are monitored: observe that during the deposition of the second layer the area (shaded rectangle) is increasing, meaning that some lipids return to the water.

PEG cushion layer than to the bare glass. Thus, the behavior observed in Fig. 3B is expected during the deposition process. After the LB deposition, the bilayers were analyzed with AFM. A control glass surface without undergoing the PEG reaction was also analyzed by AFM the LB data showed that the transfer of lipids to the glass was poor and resulted in no bilayer formation.

From the AFM analysis (Fig. 4), it can be observed that the surface roughness decreased with the grafted PEG layer onto the polished glass. This was also corroborated in a previous publication [1]. The PEG cushion layer for the lipids is provides a lubricating surface that enables the self-healing of defects in the supported lipid bilayer membrane. This self-healing mechanism plays an important role in terms of lipid bilayer stability. This effect can be confirmed with the fact that the bilayer shown in Fig. 4 maintained surface mobility even a week after being deposited.

## IV. Conclusions

Polyethylene glycol (PEG) was used as a stabilizing cushion support for the lipid bilayers. The physical interactions, which are being investigated, allow the lipids to adhere strongly enough to the polymeric layer and maintain a stable membrane structure. Future work includes the measurement of interaction forces between the PEG and the lipid bilayer using the surface force apparatus (SFA) and the insertion of other molecules such as proteins, cholesterol, and glycoproteins to this lipid bilayer to construct a functionalized membrane that can be integrated to a sensor device. Additionally, the PEG layer offers a biocompatible and nontoxic surface which is suitable for biosensors that require the contact with *in vivo* or *in vitro* biofluids.

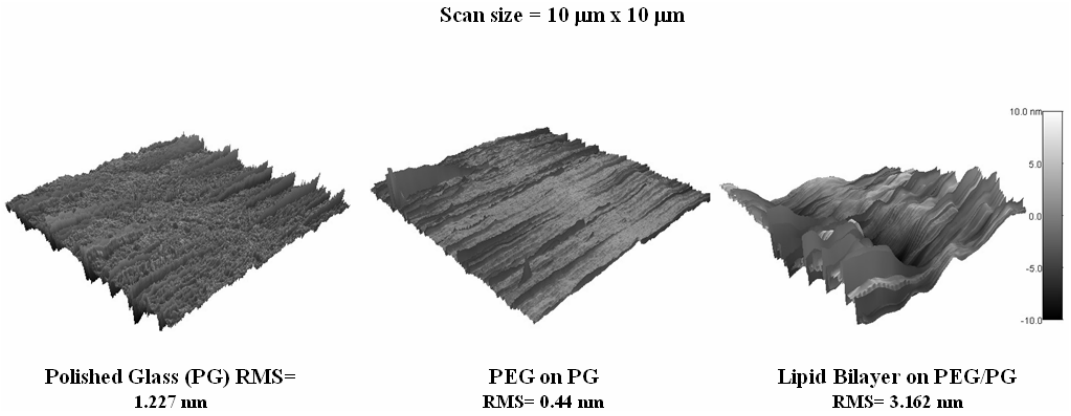


**Fig. 4.** AFM pictures characterizing the process of soft-supported lipid bilayer construction at each stage, observe the change in roughness (RMS) corresponding to each stage.

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