Polymer Cushions Functionalized with Lipid Molecules

Dong-Chan Lee, † Bong-Jun Chang, † Luping Yu, *,† Shelli L. Frey, † Ka Yee C. Lee,*,† Sirisha Patchipulusu,‡ and Connie Hall*,‡

Department of Chemistry and The James Frank Institute, The University of Chicago, 5735 South Ellis Avenue, Chicago, Illinois 60637, and Department of Biomedical Engineering, Illinois Institute of Technology, 10 West 32nd Street, Chicago, Illinois 60616

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This Letter reports a novel approach to the fabrication of a biomimicking surface by modification of an end-functionalizable smooth polymer cushion constructed via chemoselective ligation with a phospholipidlike molecule containing oxyamine groups. The mobility of a phospholipid bilayer formed by vesicle fusion on the phospholipid-like molecule terminated polymer film was characterized by fluorescence recovery after bleaching. Platelet adhesion, as one measure of biocompatibility of the film was also studied and compared to other surfaces such as polyethylene or poly(dimethylsiloxane). The results show that the end-functionalized smooth polymer cushion has potential as a biocompatible platform to reconstitute membrane proteins.

Supported lipid bilayers have been extensively used for reconstituting membrane proteins to mimic cell surfaces as well as to function as biosensors. The simplest approach deposits a single phospholipid bilayer onto a rigid solid support where an ultrathin ($\sim 1-2$ nm) water layer is trapped between the hydrophilic support and the lower leaflet of the bilayer. This trapped water layer renders the bilayer mobile despite its proximity to the solid substrate. However, frictional coupling between the substrate and the lower leaflet restricts its mobility compared to the upper leaflet. 1-4 Furthermore, it has been demonstrated that transmembrane proteins with large extracellular domains are often immobilized due to interactions with the solid substrate,5 thereby making the solid-supported bilayer a somewhat suboptimal model system for mimicking cell membranes. Due to these limitations, there have been numerous attempts to reduce substrate effects by using adsorbed polymer cushions,6 though physical adsorption to the polymer alone does not provide necessary long-term stability. Several groups have overcome this problem by developing tethered polymersupported planar lipid bilayers, where the phospholipid bilayer is mechanically stabilized by controlled covalent tethering at the polymer-substrate and polymer-bilayer interfaces.⁶⁻⁹ While these polymer-anchored systems support fluid lipid bilayers, their design makes it difficult to control polymer film characteristics, such as deposition thickness and density of functional groups. There have been other attempts to reduce substrate effects by utilizing lipid multilayers. For instance, the second lipid bilayer adsorbed on top of a supported bilayer is known to be more mobile and is therefore likely to serve as a better cell membrane mimic. While it is easy to obtain full coverage for the first lipid bilayer on a solid substrate, it is difficult to form a contiguous second bilayer by methods other than Langmuir-Blodgett deposition. These studies provide a motivation for designing a functionalized substrate to combine the advantages of polymer cushions and multilayer lipid systems. An ideal candidate for polymer cushion should possess flexibility, stability, and end-functionalizability to immobilize bioactive compounds.

In this Letter, we report a new polymer cushion system whose thickness can be controlled with nanometer resolution and the surface easily functionalized with phospholipid-like molecules. The resulting phospholipidterminated polymer cushions can serve as a support for stable, yet mobile lipid bilayers and may provide an improved platform to study transmembrane proteins. These lipid-functionalized surfaces also exhibit the potential for improved biocompatibility compared to common surface coatings.

The polymer cushions were prepared by using a new layer-by-layer polymer deposition approach, developed based on the chemoselective ligation between oxyamine and aldehyde groups. 10,11 This layer-by-layer methodology allows straightforward fabrication of robust and functionalizable polymer nanofilms. In addition, the polarity of the side groups of the polymers can affect the swellability of the cushion, which may be useful for drug encapsulation. Two polymers functionalized with aldehyde and oxyamine side chains (polymers A and B in Chart 1) were used to prepare the multilayer polymer cushion. To immobilize polymers onto solid supports, a surface modification reagent (1)12 was synthesized and covalently coupled to glass substrates.

After deprotection, the oxyamine groups were chemoselectively coupled to aldehyde-functionalized polymer A to produce a surface-attached single polymer layer. The

[†] The University of Chicago.

[‡] Illinois Institute of Technology.

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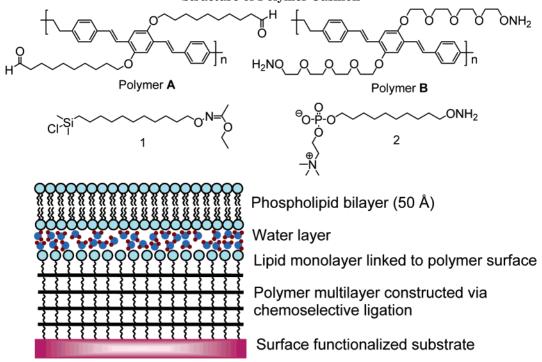
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⁽¹²⁾ Supporting Information.

Chart 1. Structures of Functional Polymers and Molecules for Chemoselective Ligation and a Schematic Structure of Polymer Cushion



surface of the resulting layer contains aldehyde groups that can react with polymer ${\bf B}$ to form the second layer. The process can be repeated by alternately dipping the substrate into two different polymer solutions. The surfaces of these polymer layers became very smooth after seven layers were deposited. The detailed synthesis and characterizations of polymers (${\bf A}$ and ${\bf B}$) and the characteristics of the multilayer are described in the previous report. 11

Surface morphologies are investigated by atomic force microscopy (AFM). All AFM images shown were taken in air and imaged with contact mode AFM using minimal contact force so as not to damage the surface under investigation. Figure 1A shows the AFM image of a cleaned glass substrate to demonstrate the roughness of the

starting surface. A measure of the roughness is given as the root-mean-squared (rms) value of the height in the direction perpendicular to the interface within a given area. For the glass substrate shown here, the rms is almost 2 nm. A larger scan of the same surface was also characterized and showed that the surface roughness is homogeneous across the glass substrate.

In contrast, upon deposition and covalent attachment of seven alternating layers of polymers onto a similar glass substrate, the roughness intrinsic to the glass is smoothened out (see Figure 1B imaged at the same scale). Section analysis of this sample shows a much reduced rms roughness value of 0.7 nm. Apart from demonstrating the ability of the polymer deposition to "heal" the roughness of the glass substrate and give rise to a smoother surface,

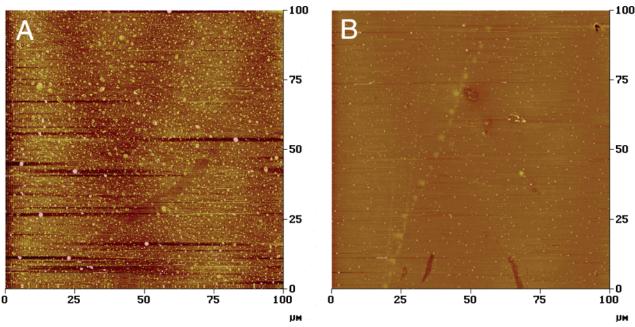


Figure 1. AFM images of (A) glass substrate and (B) seven polymer layers.

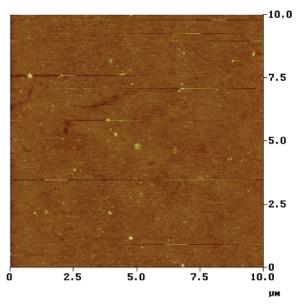


Figure 2. AFM image of phospholipid-terminated surface.

topographic imaging of the surface also proves that polymer coverage on the glass substrate is very uniform.

When the attachment of phospholipid-like molecules to form a monolayer on the polymer cushion is done at a concentration below its critical micelle concentration (cmc), very smooth phospholipid-terminated surfaces (rms of 0.36 nm) (Figure 2) with roughness comparable to that of the seven-layer polymer surface can be achieved. It should also be noted that unlike a single layer of phospholipid that is physically adsorbed to a hydrophobic substrate, this phospholipid layer is stable for days even with its headgroup facing the air due to the covalent attachment.

Since the surface of the polymer cushion terminates with a zwitterionic, and therefore hydrophilic, phospholipid headgroup, it presents a novel platform to ameliorate the substrate effect of reduced lipid mobility for the lower bilayer leaflet of adsorbed bilayers. Phospholipid bilayers of egg-phosphatidylcoline (egg-PC), labeled with 0.5 mol % Texas Red-DHPE, can be deposited by vesicle fusion on such modified phospholipid-terminated polymer surfaces. 12 Coverage of the adsorbed bilayer as well as the lateral mobility of the lipid molecules on this phospholipidterminated polymer substrate can be monitored by directly visualizing the lipid film using fluorescence microscopy. Egg-PC bilayers adsorb evenly on the polymer substrate as shown by an even field of fluorescence (Figure 3A). A nonuniform or unformed bilayer would appear as a fluorescent field with dark regions distributed throughout. The brighter portion in the center of the image is caused by slightly uneven illumination in our setup and is not inherent to the sample. This can be corrected during image analysis if raw images are subtracted using a reference image.

The bilayer lipids are laterally mobile, as demonstrated by fluorescence recovery after photobleaching (FRAP) experiments (Figure 3A). Estimates of the diffusion coefficient were obtained by observing the time scale of the recovery of the bleached spot (Figure 3B), resulting in diffusion measurements more qualitative in nature. 13 Trend comparisons between samples can provide pertinent information about the mobility of the lipids in a bilayer on the construct versus mica, a commonly used solid support. The immobile lipid fraction is similar for both types of substrates, approximately 35%. The diffusion

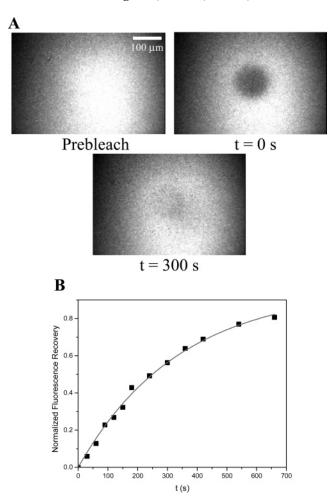


Figure 3. (A) Fluorescence micrographs of a FRAP experiment on fluorescently labeled EggPC bilayer deposited on a phospholipid terminated polymer cushion substrate at room temperature. (B) Normalized fluorescence recovery curve extracted from the image sequence. Experimental data are indicated by squares. A best fit of the exponential recovery model (solid line) yields the translational diffusion coefficient as described in the main text.

coefficient found for an egg-PC bilayer on a mica substrate is $\sim 3 \,\mu\text{m}^2/\text{s}$ while that for lipid on the modified substrate is $\sim 10 \,\mu\text{m}^2/\text{s}$. FRAP measurements provide evidence of a more mobile lipid bilayer when supported on the phospholipid terminated polymer-cushioned substrate, with an enhanced lipid diffusion coefficient compared to that for a control bilayer on a typical mica substrate.

These polymer-lipid systems also mimic the natural cell membrane and could be used as a biocompatible surface. Thus, platelet adhesion studies as one measure of potential biocompatibility were performed with these polymer-lipid films. Platelet adhesion to lipid-terminated surfaces and the two precursor layers has been compared to adhesion on borosilicate glass, poly(dimethylsiloxane) (PDMS), and polyethylene (PE). PE and PDMS are commonly used in blood contacting applications and are

⁽¹³⁾ The FRAP analysis originally developed by Axelrod¹⁴ and modified by Soumpasis 15 assumes laser photobleaching (typically subsecond) which is fast with respect to recovery time. This ensures that the bleaching and the lipid diffusion process leading to the recovery of the fluorescence are temporally separated. However, photobleaching with a mercury lamp needs longer illumination times leading to blurring at the edges of the photobleached spot because substantial diffusion occurs while bleaching takes place.
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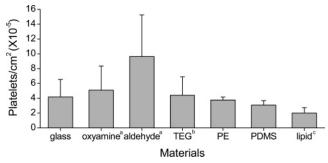


Figure 4. Number of adherent platelets per unit surface area. Static adhesion from platelet-rich plasma after 2 h; see text for the labels. (a) Polymer multilayers with top layer containing the corresponding functional groups. (b, c) TEG- and lipid-terminated polymer multilayers, respectively.

generally considered fairly biocompatible. Washed platelets were suspended in autologous plasma at 5×10^7 platelets per mL and surfaces were exposed to the mixture for 1 h at 37 °C. Platelet attachment was quantified using a lactate dehydrogenase assay. 12 The precursor layers (oxyamine, aldehyde, and tetraethylene glycol) (5.09, 9.63, and 4.39 \times 10^5 platelets/cm²) and glass (4.19 \times 10^5 platelets/cm²) demonstrated greater adhesion than PE, PDMS, or lipid surfaces. However, lipid surfaces presented significantly lower platelet attachment (2 \times 10^5 platelets/cm²), 47% and 35% less attachment than PE or PDMS, respectively (Figure 4).

In conclusion, we have developed a new polymer cushion system that offers precise control in film thickness, film smoothness, and surface functional groups. An oxyamine compound with phospholipid head was synthesized and immobilized to the polymer cushion surface. The resulting polymer lipid surfaces exhibit high hydrophilicity and were able to support lipid bilayers. The unique feature of these support bilayers is that they exhibit high lateral mobility and show potential as a platform to reconstitute membrane proteins. The polymer lipid surfaces were shown to enhance biocompatibility compared to standard coatings. Our results also demonstrate that the polymer cushion can provide an effective platform to present any desired bioactive molecules via chemoselective ligation for the enhancement of biocompatibility.

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Supporting Information Available: Details of the synthesis of materials, assembly procedure, and experimental procedure for diffusion coefficient measurements and the blood compatibility. This material is available free of charge via the Internet at http://pubs.acs.org.

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