Characterizing the chemical complexity of patterned biomimetic membranes

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ABSTRACT

Biomembranes are complex, heterogeneous, dynamic systems playing essential roles in numerous processes such as cell signaling and membrane trafficking. Model membranes provide simpler platforms for studying biomembrane dynamics under well-controlled environments. Here we present a modified polymer lift-off approach to introduce chemical complexity into biomimetic membranes by constructing domains of one lipid composition (here, didodecylphosphatidylcholine) that are surrounded by a different lipid composition (e.g., dipentadecylphosphatidylcholine), which we refer to as patterned backfilled samples. Fluorescence microscopy and correlation spectroscopy were used to characterize this patterning approach. We observe two types of domain populations: one with diffuse boundaries and a minor fraction with sharp edges. Lipids within the diffuse domains in patterned backfilled samples undergo anomalous diffusion, which results from nonideally mixed clusters of gel phase lipid within the fluid domains. No lateral diffusion was observed within the minor population of domains with well-defined borders. These results suggest that, while membrane patterning by a variety of approaches is useful for biophysical and biosensor applications, a thorough and systematic characterization of the resulting biomimetic membrane, and its unpatterned counterpart, is essential.

1. Introduction

Microdomains (such as "lipid rafts" [1]) in biological membranes have been proposed to participate in numerous functions, such as signal transduction [2,3] and membrane trafficking [4]. Given the inherent complexity of cell membranes, model systems (i.e., giant unilamellar vesicles [5], supported planar lipid bilayers and monolayers [6–8] and black lipid membranes [9]) have been developed to provide a simpler platform for step-by-step studies in controlled environments. Domains of different lipid phases can form spontaneously when two or more lipids are mixed together [8,10–12], where segregation of lipids into different phases occurs mainly due to the inherent properties of the participating lipid molecules. Due to this spontaneous segregation, these model systems do not allow us to probe the dynamics of microdomains in a spatially and temporally well-controlled manner. As a result, newly developed micropatterning approaches such as microcontact printing [13–15], agarose stamping [16], UV photolysis [17–20] and a polymer-based dry lift-off approach [21,22] may allow formation of spatially organized microdomains of one lipid composition within the context of another lipid composition, thereby allowing us to extend beyond the phase diagram. Such spatially organized model systems

could then act as building blocks for integrated biosensors to facilitate studies for a variety of cellular processes [22].

Planar supported membranes consist of a continuous bilayer of lipids that have been deposited onto a solid or a polymer-based support [23] as a result of vesicle fusion [12] or Langmuir–Blodgett/Langmuir–Schaefer deposition [6]. Due to the presence of thin film of water between the bilayer and substrate, supported bilayers retain many of the physical characteristics of naturally occurring cell membranes and are amenable to a variety of measurement and surface modification techniques, such as micropatterning, due to their planar geometry. To date, arrays of single composition lipid domains on solid supports has been accomplished using microcontact printing [13], UV photolysis [17,19,20], agarose stamping [16] and a polymer-based dry lift-off approach [21,22,24]. We focused in this work on the latter patterning method, in which a parylene film is patterned via microlithography, the biomolecule (here, lipids) deposited, and the parylene film removed to reveal arrays of biomolecule on the substrate. To date, parylene lift-off has been used to deposit domains of haptenated bilayers for antibody recognition [24] and for cell stimulation [22,25]; however, introducing further chemical complexity within the bilayer itself by surrounding the domains with another lipid composition had not yet been done.

In this work, we describe a combination of polymer lift-off and backfilling to construct chemically more complex supported bilayers. For our initial studies, we used lipids that are immiscible: didodecylphosphatidylcholine (di12:0 PC) which is fluid at room temperature and

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dipentadecylphosphatidylcholine (di15:0 PC) which is in the gel phase. We find that the backfilled interdomain regions of gel phase spatially restrict the fluid domains, and two types of domains exist on patterned backfilled samples, which we characterize with fluorescence microscopy and fluorescence correlation spectroscopy (FCS). We find that the two types of domains vary in properties (e.g., melting behavior, diffusion), which we pin down to their respective sources. We determined that the backfilling step introduces gel phase lipids inside fluid patterns that cluster and act as obstacles leading to anomalous diffusion of fluid lipids. Further, these results suggest that if backfilling is used to introduce complexity into patterned biomimetic membranes, it will be critical to optimize lipid concentrations on unpatterned bilayers first to determine concentrations that lead to minimal intercalation of the backfilling lipid. These results lead us to suggest that a thorough, quantitative characterization of patterned bilayers, regardless of the patterning method used, is essential for any study in which the membrane properties must be known and well-defined.

2. Materials and methods

2.1. Materials

1,2-didodecyl-*sn*-glycerol-3-phosphocholine (di12:0 PC) and 1,2-dipentadecyl-*sn*-glycerol-3-phosphocholine (di15:0 PC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The fluorescent lipid analogs, 1,1′-dihexadecyl-3,3,3′,3′-tetramethylindocarbocyanine (diI-C_{16}) and 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (bodipy PC), were purchased from Invitrogen (Carlsbad, CA, USA). Lipids and fluorescent analogs were used without additional purification.

2.2. Preparation of supported planar bilayers

The day prior to an experiment, small unilamellar vesicles (SUVs) were formed as described previously [26,27]. Lipids at a desired composition, e.g., di12:0 PC±0.5 mol% bodipy PC or di15:0 PC±0.5 mol% diI-C_{16}, were added to a test tube, previously cleaned in ethanolic potassium hydroxide, and dried under nitrogen. Dried lipids were then resuspended at a 2 mM concentration in 50 mM Tris, pH 7.4, 100 mM NaCl (TBS), probe sonicated until clarified and subjected to ultracentrifugation (Airfuge, 30 psi, 1 h). The top quarter of the supernatant was collected and stored overnight at room temperature and used within 24 h. On the day of an experiment, the SUV solution (75 μL) was applied to a sandwich made of a detergent-cleaned 3"×1" glass slide and a 22 mm×22 mm glass coverslip that had been cleaned in oxygen plasma immediately prior to application of the SUV suspension. SUVs spontaneously fuse to form uniform lipid bilayers, and after a 30 min incubation in a humidified chamber, samples were rinsed with TBS to remove unfused vesicles. Bilayer samples were sealed with VALAP (Vaseline:lanolin:paraffin [2:1:1, wt/wt]) and measurements carried out immediately. Samples formed in this manner will be referred to as unpatterned bilayers. For some control experiments, unpatterned di12:0 PC±0.5 mol% bodipy PC bilayers were incubated with 75 μL of either di15:0 PC±0.5 mol% diI-C_{16} SUVs or TBS for 30 min. These bilayers were then extensively rinsed with TBS, sealed with VALAP and used for imaging and FCS measurements.

2.3. Preparation of patterned surfaces via polymer lift-off

Parylene C (Parylene Deposition System 2010, Specialty Coating Systems; Indianapolis, IN, USA) was vapor deposited onto a detergent-cleaned glass substrate (22 mm×22 mm). After parylene deposition (Fig. 1), a 100 nm film of aluminum metal (Thermionics VE-90 thermal evaporator; Port Townsend, WA, USA) was evaporated onto the parylene coated surface, followed by application of a 1.2 μm-thick film of Shipley 1827 positive photoresist. The substrate was baked for 90 s
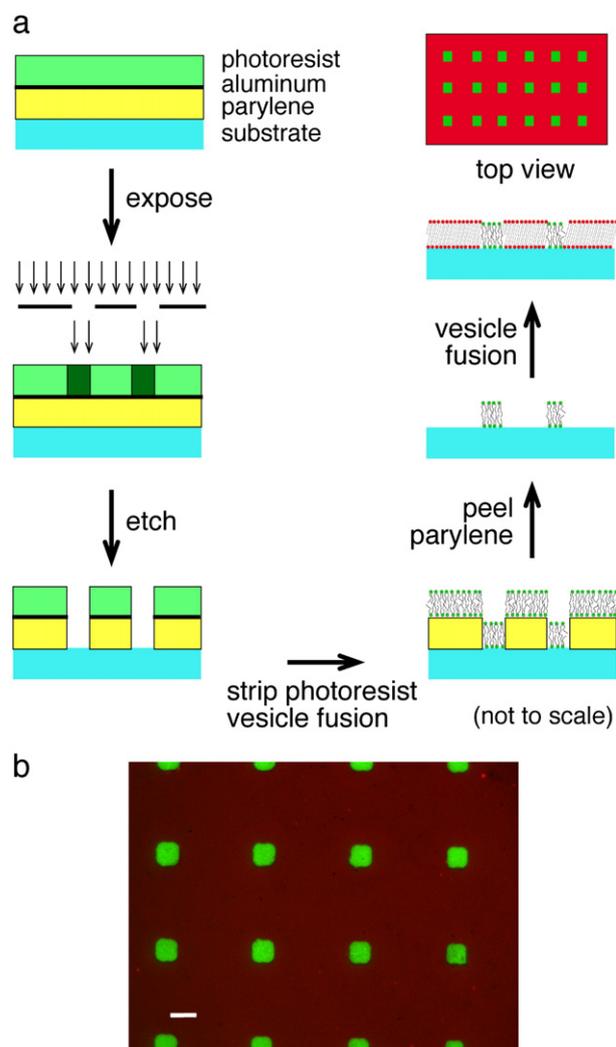
Fig. 1. Schematic illustration of lipid micropatterning via a modified polymer lift-off approach and a representative image. (a) Parylene, aluminum and photoresist were deposited on a glass substrate. Following exposure to ultraviolet light through a photomask, the photoresist and aluminum layers were developed and removed, and parylene was dry-etched in an oxygen-rich plasma. Substrates were cleaned and incubated with SUVs (e.g., bodipy PC-labeled di12:0 PC) to form domain arrays after removal of parylene. The exposed substrate was then coated with a bilayer of another lipid composition (e.g., di15:0 PC labeled with diI-C_{16}) that had formed via vesicle fusion. (b) A representative image of patterned di12:0 PC containing 0.5 mol% bodipy PC (green) and backfilled di15:0 PC with 0.5 mol% diI-C_{16} (red). Images were captured sequentially with appropriate emission filters to minimize bleedthrough between the two fluorescence signals. Bar, 10 μm.

at 105 °C. The pattern from a quartz photomask was transferred to the photoresist via UV exposure in a contact aligner. The photoresist and the underlying aluminum were developed in CD26 base. The pattern was then dry-etched in a reactive ion etcher (Plasma Therm 720 RIE Reactive Ion Etch System; St. Petersburg, FL, USA) under the following conditions: 5 sccm argon, 90 sccm oxygen and 10 sccm carbon tetrafluoride at 400 W and 50 mTorr. As a result of dry-etching, exposed parylene was etched to the bare glass surface (Fig. 1), as assessed by X-ray photoelectron spectroscopy (not shown). Samples were rinsed with acetone and isopropyl alcohol, the aluminum was dissolved in CD26, and finally the sample was rinsed with deionized water and dried under nitrogen.

2.4. Patterned bilayer formation

The patterned substrate was first cleaned in an oxygen plasma. SUVs (75 μL) of fluid lipids (e.g., di12:0 PC labeled with bodipy PC)