One-Step, Chemisorbed Immobilization of Highly Stable, Polydiacetylenic Phospholipid Vesicles onto Gold Films

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This communication reports the structural formation and characterization of polymerized phospholipid vesicles chemisorbed onto conductive gold films. Vesicles, which are microscopic spheroidal capsules composed of self-assembled surfactants, have been intensively and extensively investigated in several scientific disciplines and exploited for numerous technological applications.[1] Structurally, vesicles retain many of the properties inherent in natural biological membranes. Functionally, vesicles have been designed to mimic the highly selective, sensitive, and efficient modes of separation, detection, locomotion, synthesis, catalysis, and signal and energy transduction found in natural systems.[2] However, fine control for these processes requires a novel architecture in which to receive and transceive signals for performing a desired task to a given extent and at a specified rate. Stable vesicles immobilized onto solid supports appear to be the ideal construct to capitalize on signal transduction induced from chemical, optical, electrical, magnetic, or thermal transitions.

A number of techniques such as steric entrapment,[3] hydrophobic binding,[4] attractive surface-polarizable binding,[5] and avidin−biotin affinity binding[6] have been used to immobilize vesicles, and recently polymerized micelles,[7] onto or within solid supports. For most of these techniques, immobilized vesicles have proven extremely valuable for chromatographic purification of a variety of drugs,[8] peptides,[9] proteins,[6c] and antibodies,[10] and more recently, they have been incorporated in the design of an optochemical sensor.[11] However, in all of these reports, the stability and the structural integrity of vesicles have not been fully addressed. In fact, vesicles in contact with solid surfaces are known to unravel and to form a lipid monolayer or bilayer(s),[12] also known as supported membranes. The formation of supported membranes relative to that of vesicle surface binding is predicted to depend on a competition between the adhesive energy and the vesicle bending energy.[13] Given enough time, however, conventional vesicles will aggregate, fuse, and eventually precipitate, which re-emphasizes the issue of vesicle instability.[1]

Our efforts are focused on stabilizing both the vesicle structure and the vesicle to surface attachment using short linkers on a technologically advantageous flat conducting thin film. Polymerizing lipid surfactants within the vesicle wall enhances its structural stability[14] and stable vesicle to gold surface attachment is achieved by incorporating lipids containing disulfide functionality on the polar headgroup segment (Figure 1). It is important to note that disulfide relative to sulfhydryl functionality not only forms stronger bonds on gold surfaces,[15] but being in its oxidized state prevents vesicle aggregation. To our knowledge, this is the first report of construction and visual characterization of a highly monodisperse, unilamellar population of polymerized vesicles onto flat Au films using in situ AFM. This construct should have a substantial technological impact and advance the fields of bioelectronics, biosensors, bioseparations, biosynthesis, and bioenergetics.[16]

Extruded vesicles composed of 1,2-bis(tricosa-10,12-diynoyl)-*sn*-3-glycerophosphocholine ($DC_{8,9}PC$) as the major component and doped with 1,2-bis(tricosa-10,12-diynoyl)-*sn*-3-glycerophospho-2-[(2-hydroxyethyl) disulfanyl)] ethanol ($DC_{8,9}PDSEtOH$) at 1 mol % were size characterized by laser light scattering[17] and transmission electron microscopy to be of a unilamellar, highly monodisperse dispersion. Vesicles upon polymerization remained intact and retained their size and shape. Vesicle stability, which is dictated by the degree of polymerization,[18] is also assessed by visibly observing no precipitates over a period of weeks. Glass substrates coated with gold films were allowed to incubate at room temperature in a suspension of disulfide-functionalized, polymerized vesicles, and the films were characterized by in situ AFM.

In situ AFM images of bare, unmodified gold (0.3 nm surface roughness, not shown) and exposure of bare gold films to a suspension of polymerized $DC_{89}PC$ vesicles without $DC_{8,9}PDSEtOH$ films (Figure 2) display a relatively smooth surface with no identifiable structures. The low surface area roughness of 2.2 nm for the latter film does not support the formation of a lipid monolayer or bilayer, since the bilayer thickness of hydrated $DC_{8,9}PC$ is 6.9 nm.[19] It is unlikely that $DC_{8,9}PC$ or some supramolecular structures thereof are adsorbed onto the surface, since application of a large force using the AFM tip did not dislodge this unknown matter. Undesired surface adsorption or surface defects produced during gold deposition may be a possible phenomenon leading to these artifacts. From these observations, we may conclude that polymerized vesicles composed solely of $DC_{8,9}PC$ are not influenced by the gold surface, at least for the duration and condition of the experiment.

Alternatively, Figure 3 shows a pronounced difference between the in situ AFM image of polymerized $DC_{8,9}PC$ vesicles containing $DC_{8,9}PDSEtOH$ immobilized to a conductive gold surface

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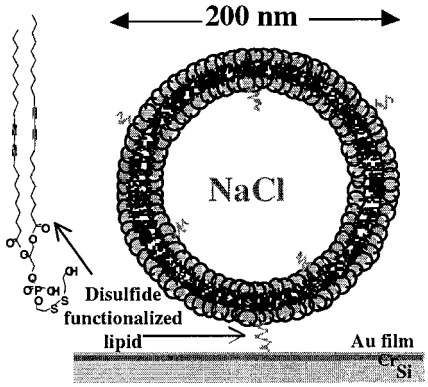

Figure 1. A cartoon depiction (not drawn to scale) of a unilamellar, polymerized vesicle with a diameter of 200 nm immobilized via a thiolate linkage to a gold film bonded to a chromium layer bonded to glass.

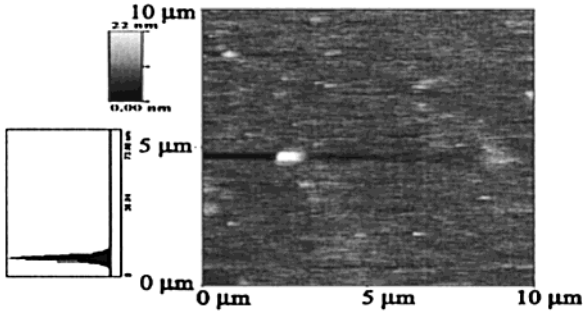

Figure 2. An AFM image of a gold film after exposure to polymerized vesicles without $DC_{8,9}$PDSEtOH. A surface roughness of 2.2 nm was determined for this sample. A histogram providing the topological height distribution is shown (lower left).

relative to that without dopant. A surface area roughness of 83 nm provides strong evidence that large curved, supramolecular structures such as vesicles are coating the surface. Qualitatively, we observe a large degree of vesicle surface deposition, whereby complete and rapid coverage occurs within 1 h. Over 3 days of buffer incubation, similar surface morphology is obtained with these vesicle-immobilized films suggesting that our structures remain intact. The critical contact pressure for rupturing immobilized nonpolymerized dipalmitoyl phosphatidylcholine in situ was determined to be $\sim$0.9 N/m^2.[20] Since we estimate an imaging contact pressure of 0.75 N/m^2 and since polymerization imparts greater structural stability to the vesicles, the image quality of Figure 3 is presumed satisfactory. In contrast, we have observed multilayered lipid films formed with nonpolymerized $DC_{8,9}$PC vesicles doped with $DC_{8,9}$PDSEtOH (not shown). These films were much smoother than their polymerized counterparts, having a surface roughness of 3.8 nm and a depth height determined by scribing of 19.1 nm.

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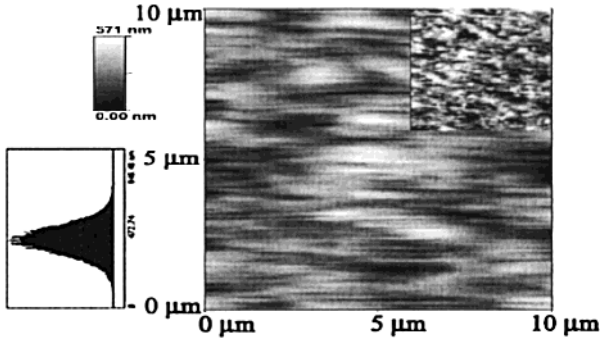

Figure 3. An AFM image of the surface morphology of polymerized vesicles doped with $DC_{8,9}$PDSEtOH that are chemisorbed onto a gold film. This sample contains an abundance of vesicles with a surface roughness of 83 nm. A horizontally shadowed image (inset) presents finer visual detail of the surface scan. A histogram providing the topological height distribution is shown (lower left).

Scribing surfaces with AFM is not uncommon[21] and is used here to rupture these surface-immobilized vesicles (see Figure S1 in the Supporting Information). An AFM tip force of 30 nN was applied to scribe the surface and to have direct contact with the gold film. Albeit this rupture force is in excess to that predicted to break the thiolate gold bond ($\sim$44 kcal/mol[15]),[21c] this demonstrates that our polymerized vesicles are stably linked onto the gold surface. A depth profile of Figure 3 reveals a height (181 nm) congruent to that of the vesicle diameter, indicating that the structural integrity of vesicles is not compromised. This result also reinforces the fact of only a monolayer deposition of vesicles. Vesicle stacking of multilayers is perceivably possible through weak noncovalent interactions but is not observed with our system. Excessive rinsing of the chemisorbed vesicle layer, however, does not lead to vesicle dislodgment (see Figure 3), providing additional evidence to the high degree of vesicle to surface stability.

We have developed the structural foundation upon which to fabricate a stable vesicle-based, submicron electrode. Our methodology is simple, rapid, versatile, and relatively inexpensive. Our vesicles are highly monodispersed, unilamellar, and physically robust, and form a stable link onto Au films. Presently, we are investigating alternative lipid chemistries, vesicle surface order, and the electrochemical surface stability of our chemisorbed vesicles.

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Supporting Information Available: A detailed description of experimental procedures, an AFM image, and references (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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