One-Step Synthesis of a Fluorescent Phospholipid−Hydrogel Conjugate for Driving Self-Assembly of Supported Lipid Membranes

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ABSTRACT: We have synthesized a phospholipid−hydrogel conjugate that appears to drive the self-assembly of lipid membranes. A one-step radical polymerization synthesis of the conjugate was devised using lipid anchors that contain a fluorescent marker and a reactive vinyl group. The anchors preferentially located at oil−water interfaces during hydrogel formation and through their vinyl groups became covalently attached at the hydrogel surface. X-ray photoelectron spectroscopy and laser scanning confocal microscopy confirmed surface localization of the anchors. Upon mixing the conjugates with liposomes, lipid membranes readily self-assembled around the lipid-modified hydrogel surfaces. Control experiments with anchorless hydrogels indicate that anchors influenced the surface density and morphology of the lipid membranes. The one-step synthesis allows for simple control of surface anchor density and thus potential control of lipid membrane properties; the fluorescent markers facilitate anchor quantitation and further membrane interaction studies. Potential applications include high throughput drug screening, diagnostics, cell models, and drug delivery.

Introduction

To investigate the biochemical function of membrane proteins, proteins are usually reconstituted into liposomes.[1] Fragile liposomes can be stabilized by external polymeric coating.[2−6] Alternatively, the stability and properties of phospholipid bilayers can be regulated by internal supports. An internal hydrophilic support of molecular dimensions may not be sufficiently large to accommodate membrane proteins with large intracellular components. In contrast, a macroscopic hydrogel-filled core should provide more internal flexibility for a supported bilayer and make the overall construct more similar to a biological cell.

Previously, Jin, Pennefather, and Lee[7] established a process to make poly(vinyl alcohol) beads, esterified the surface of the beads with fatty acid anchors, and showed that, upon the addition of phospholipids, a lipid membrane barrier formed on the surfaces of the polymer beads. This construct, called a Lipobead, provided both hydrophobic anchors and a large internal hydrophilic space. Although a complex multistep process was required to prepare the supported lipid membrane and no quantitation of anchor surface density was provided, this Lipobead construct demonstrated the potential for creating self-assembling cell surrogates.

The overall objective of our work was to make a hydrogel-supported phospholipid bilayer that can be used as an artificial biological cell. Such a cell surrogate would have potential applications in high throughput drug screening, diagnostic assays, cell models, and drug delivery. We hypothesized that lipid anchors covalently attached to the hydrogel would facilitate, with fast kinetics, the self-assembly of a phospholipid bilayer on the surface of that hydrogel (Figure 1) by lowering the energy barrier for fusion of membrane vesicles with the hydrogel surface. In such a cell surrogate, the hydrogel core provides a large volume of hydrophilic space to house the intracellular segments of membrane proteins and to store drugs, diagnostic agents, or intracellular signaling molecules. Although there are purely mechanical methods for encapsulating hydrogels with lipid bilayers,[8,9] the lipid anchors will serve to seed the coating process as well as providing a scaffold to support and stabilize the membrane coating.

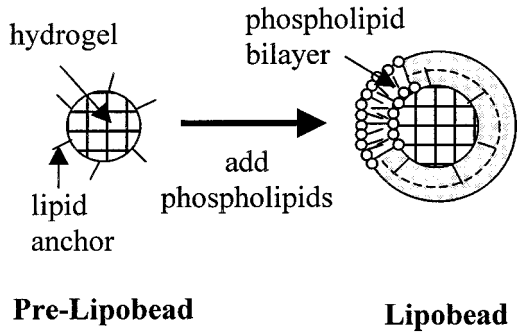

Figure 1. Schematic illustration of the self-assembly of a lipid membrane around a hydrogel−phospholipid conjugate.

Here we describe development of a robust and rapid way for generating supported lipid bilayers using a hydrogel support whose surface has been modified with a phospholipid at that surface during hydrogel polymerization. We report a novel one-step process for the synthesis of a hydrogel−phospholipid conjugate that can serve as a bilayer support, demonstrate the presence of lipid anchors on the hydrogel surface, and show that a phospholipid membrane self-assembles on the hydrogel surface where the anchors are present. Fluorescently labeled lipid anchors were used to facilitate detection and quantitation and to allow for further membrane studies such as energy-transfer experiments using additional fluorescent probes. For ease of discussion, cross-linked hydrogel beads with covalently attached lipid anchors on the surface will be called pre-Lipobeads, and

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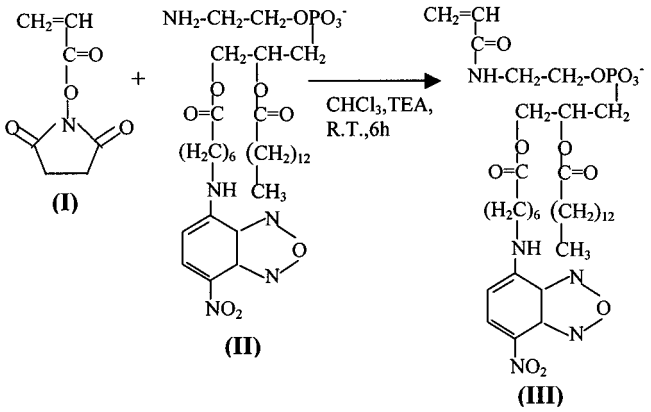
Scheme 1. Lipid Anchor Synthesis



pre-Lipobeads with a complete lipid membrane coating will be called Lipobeads (Figure 1). Analogous planar constructs will be called pre-Liposheets and Liposheets, respectively.

Experimental Section

Materials. 1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1-myristoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]caproyl]-*sn*-glycero-3-phosphoethanolamine (PE-NBD), 1-palmitoyl-2-[6-[(7-nitro-2−1,3-benzoxadiazol-4-yl)-amino]caproyl]-*sn*-glycero-3-phosphocholine (PC-NBD), and a mini-extruder and its accessories were obtained from Avanti Polar Lipids. Acrylic acid succinimide ester, triethylamine, *N*-isopropylacrylamide (NIPAAm), dimethylacrylamide (DMAA), *N*,*N*′-ethylenebis(acrylamide) (E-BIS), *N*,*N*′-methylenebis-(acrylamide) (M-BIS), sorbitan monostearate, cholesterol, and egg phosphatidylcholine (ePC) were obtained from Sigma-Aldrich Canada. NIPAAm was recrystallized from heptane: toluene (50:50 v/v) prior to use. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED) and ammonium persulfate were obtained from Baker Chemical Co. and Caledon Laboratories Ltd., respectively. HPLC grade chloroform was obtained from Fisher Scientific. FluoroSpheres (F-8853, 2 μm) were obtained from Molecular Probe. All other chemical used were reagent grade.

Thin-layer chromatography (TLC) plates (5 cm by 10 cm, 60 Å particle size, KSF silica gel) were obtained from Whatman. Silica and amino-modified solid-phase extraction cartridges (3 mL), ninhydrin, and molybdenum TLC sprays were obtained from Supelco.

Lipid Anchor Synthesis. Acrylic acid succinimide ester (2 mmol, **I**), unlabeled DMPE (0.5 mmol), and fluorescent PE-NBD (0.05 mmol, **II**) were dissolved in 10 mL of chloroform. Triethylamine catalyst (80 μL) was added, and the reaction proceeded at room temperature for 6 h under continuous stirring (Scheme 1). The fluorescent lipid anchor product (**III**) and its nonfluorescent analogue were isolated from the reaction mixture by solid-phase extraction (SPE). A 5 mL aliquot of the reaction mixture was poured into a hexane-washed SPE silica cartridge and allowed to drain by gravity. Liquid collection was started 2 min thereafter. 12 mL of chloroform: acetic acid (100:1 v/v) eluted the fatty acids; 5 mL of methanol: chloroform (2:1 v/v) eluted a yellow solution. This yellow fraction was dried with a stream of compressed air, redissolved in chloroform, and poured into a prewashed amino cartridge. The desired product was eluted from the amino cartridge with 5 mL of chloroform:2-propanol (2:1 v/v). The overall molar yield of the purified product relative to the initial PE and PE-NBD was about 25%. Product purity was confirmed by thin-layer chromatography using a 65:25:4 (v/v) mixture of chloroform: methanol:water as the developing solvent. Fourier transform infrared (Galaxy series FTIR 5000) and nuclear magnetic resonance (Varian 500 NMR) spectroscopies were used to analyze the purified product.

One-Step Pre-Liposheet Polymerization. A solution of monomer (either NIPAAm or DMAA, 350 mg) and cross-linker (M-BIS, 20 mg) in water (5 mL) was immersed in an ice bath and purged with nitrogen for 15 min. Then 400 μL of TEMED was added, and the mixture poured into molds of desired size. 20−40 μL of a lipid anchor/decane solution of predetermined concentration was added to the monomer solution. The lower density lipid anchor/decane solution remained on top of the aqueous monomer solution. Ammonium persulfate solution (100 mg/mL in water, 20 μL per 100 μL of monomer solution) was added to initiate polymerization. Care was taken to keep the monomer solution below the lower critical solution temperature (LCST) of the polymer so that a clear, homogeneous gel was produced. Polymerization was allowed to proceed overnight at room temperature in a covered reactor. The hydrogel product was washed with hexane to remove unreacted anchors and excess decane, then further washed, and soaked in water to remove unreacted monomer and to achieve equilibrium hydration.

One-Step Pre-Lipobead Polymerization. To a round-bottom reaction flask equipped with a Teflon stirrer, 1.9 mL of DMAA, 0.15 g of E-BIS, 14 mL of water, 50 mg of ammonium persulfate, 50 mL of hexane, and 29 mL of carbon tetrachloride were added. With the use of hexane or carbon tetrachloride, the density of the organic phase was adjusted to be slightly higher than water. The reaction flask was immersed in a 40 °C water bath and purged with nitrogen gas for 15 min. With continuous stirring at 200−300 rpm, the lipid anchors (5−20 mg) and 70 mg of sorbitan monosterate were added to stabilize the monomer droplets. After 10 min of emulsification, 170 μL of TEMED was added to the mixture to initiate polymerization. Within 2 h, polymer beads were formed. The beads were washed sequentially with 2-propanol, methanol, water, ethanol, chloroform, and ethyl acetate and dried in a vacuum oven for about 2 days. The overall yield of polymer beads based on the initial mass of monomer was about 90%.

Characterization of the Phospholipid−Hydrogel Conjugate. Low-resolution X-ray photoelectron spectroscopy (XPS) was used to confirm the presence of lipid anchors on the lyophilized pre-Liposheet. Sample spectra was generated by a Leybold MAX 200 spectrometer equipped with a 15 kV/25 mA Al Kα X-ray and had a base pressure maintained at 3×10^{-9} mbar during data acquisition, and the data obtained were normalized to unit transmission. By integrating peak areas (ESCATOOLS, Surface/Interface Inc., Mountain View, CA) and applying appropriate sensitivity factors provided by the manufacturer, the relative atomic percent of the sample was determined. Laser scanning confocal microscopy (LSCM) was used to observe the distribution of the fluorescent anchors on the hydrated hydrogel. Images were obtained using a model 5.10 Carl Zeiss Axiovert 100M laser scanning confocal microscope equipped with a C-Apochromat 63×/1.2 water immersion lens, a 10×/0.5 Fluar lens, an argon laser using the 458 nm line, a beam splitter HFT 458, and an emission filter LP505. The pinhole size of 135 μm used along with the 63× lens corresponded to an optical section of 1 μm.

Liposome Preparation. The following lipid solutions in chloroform were prepared: ePC, 10 mg/mL; DMPE, 5 mg/mL; cholesterol, 5 mg/mL; and PC-NBD, 1 mg/mL. A thin lipid film (consisted of 200 μL of ePC, 200 μL of DMPE, and 20 μL of cholesterol) was formed in the bottom of a flask as the chloroform evaporated in a Rotavapor. The film was dried overnight in a vacuum oven and was rehydrated with 2 mL of pH 7.0 Hepes buffer. The buffer contained 0.14 M NaCl, 0.5 mM sodium azide, 0.01 M *N*-[2-hydroxyethyl]piperazine-(*N*′-[2-ethanesulfonic acid]), and its sodium salt and droplets of diluted sodium hydroxide solution to adjust the pH to 7.0. The lipid suspension was maintained at temperature above 40 °C and was extruded through a 100 nm polycarbonate membrane 11 times to obtain unilamellar vesicles. The size of the vesicles was determined by dynamic light scattering (Brookehaven 90Plus particle sizer, 90 °C as the angle of incidence, refractive index of 1.5). Fluorescent vesicles were prepared using the same procedure but by replacing 10 μL of ePC with 100 μL of PC-NBD to give vesicles with 3 wt % fluorescent constituents.

Results and Discussion

Lipid Anchor Synthesis. TLC, FTIR, and NMR confirmed that lipid anchors were synthesized. A comparison of the FTIR spectra of the purified lipid anchor product, **III**, and unmodified DMPE, **II**, shows the presence of two peaks in the product that are not present in the unmodified DMPE: a strong IR peak at 1660 cm^{-1} that indicates the presence of an amide bond in the product and a weak peak at 1380 cm^{-1} that may be indicative of a vinyl carbon−carbon bond (C=C). These functional groups are consistent with the chemical structure of the desired product (**III**) shown in Scheme 1.

Spotted TLC plates were sprayed first with ninhydrin which stains primary amines spots pink and then with molybdenum blue which stains phosphate-constaining spots blue. Unmodified DMPE, containing both a primary amine and a phosphate group, stained positive for both sprays and was consistent with its chemical structure. The stain position corresponded to a relative mobility of 0.40 ± 0.04. The purified lipid anchor product showed a blue stain at a new position $R_f = 0.53$ ± 0.03 and no red stain. The staining pattern is consistent with the absence of primary amine and the presence of a phosphate group in the lipid anchor. This spot is yellow in visible light and absorbs UV light, suggesting the presence of the NBD fluorescent group and a UV-absorbing acrylamide structure. Taken together, the TLC staining pattern, the yellow color of this spot, and the FTIR amide and C=C peaks strongly indicate that this spot corresponds to the desired lipid anchor product, acrylo-PE-NBD (**III**), and the presence of only one stained spot is indicative of the purity of the product. A comparison of the NMR ($CDCl_3$) spectra of the anchor and unmodified DMPE shows the disappearance of the proton shift at 8.3 ppm, and the appearance of new proton shifts at 5.5 to 6.4 and 12.1 ppm. The change in spectrum is consistent with the synthesis scheme that the terminal, primary amine (at 8.3 ppm, singlet, two protons) on phosphoethanolamine is modified to an amino group (at 12.1 ppm, singlet, one proton). A group of new proton shifts between 5.5 and 6.4 ppm is also consistent with the addition of a vinyl group (CH_2=CH−) to the anchor molecule (one proton at 5.75 ppm, a second proton at 6.20 ppm, and a third one at 6.25 ppm).

Characterization of the Phosphoipid−Hydrogel Conjugate by XPS. Acrylo-PE-NBD (**III**) was designed to be covalently attached to a hydrogel as well as to intercalate into a lipid bilayer and therefore facilitate the assembly of a bilayer on the hydrogel surface and act as a tether for the supported bilayer. The lipid anchor is a modified phospholipid molecule and possesses four desirable features: (1) a vinyl group on the hydrophilic end to participate in a one-step copolymerization with any vinyl monomer, (2) a lipid chain on the other end for tethering a bilayer to the hydrogel surface, (3) surface active properties to allow surfactant function and proper orientation at oil−water interfaces during polymerization, and (4) a fluorescent group to facilitate quantitation. During the synthesis of pre-Liposheets and pre-Lipobeads, **III** preferentially situates at oil/water interfaces, thus leading to the positioning of anchors at the surface of the phospholipid−hydrogel conjugates or pre-Lipobeads. The reactive vinyl group of the anchor is at the hydrophilic end of the molecule and should therefore be available to copolymerize with NIPAAm or DMAA in the aqueous phase.

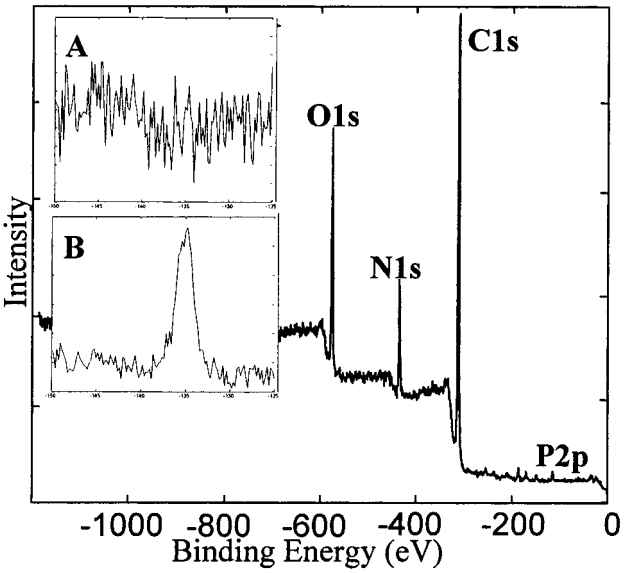


Figure 2. X-ray photoelectron spectrograph of a poly(dimethylacrylamide) (pDMAA) hydrogel modified with lipid anchor. The angle of incidence was 90°. Inset A: expanded view of the phosphorus peak area (−150 to −125 eV) in an unmodified pDMAA hydrogel. Inset B: expanded view of the phosphorus peak in a pDMAA pre-Liposheet.

XPS confirmed the incorporation of lipid anchors at the surface of pre-Liposheets. Inset A of Figure 2 shows a low-resolution XPS scan (−150 to −125 eV region) of a poly(dimethylacrylamide) (pDMAA) gel that did not contain the lipid anchor. The XPS spectrum did not reveal any phosphorus, and the C:O:N:P atomic ratio of 5.3:1.2:1:0 correlated well with the theoretical monomeric ratio 5:1:1:0 for pDMAA. In contrast, the XPS of a pDMAA pre-Liposheet, a flat pDMAA gel with lipid anchors present during polymerization, showed a C:O:N:P atomic ratio of 7.7:1.6:1:0.05 (Figure 2). The presence of phosphorus (Figure 2, inset B) and the enrichment of carbon and oxygen were consistent with the presence of lipid anchors on the hydrogel surface. The phosphate and ester groups in the lipid anchors likely contributed to the increased amount of oxygen atoms, whereas the fatty chains on the anchor increase the carbon atom composition. Since the surface of the lyophilized pre-Liposheet was rough and porous, angle-resolved XPS revealed no clear trend in surface composition at different incident angles. Moreover, XPS could not be used to determine the absolute lipid anchors density, as the scattered X-ray intensity and the mean free path of emitted electrons were not accurately known.

Characterization of the Phosphoipid−Hydrogel Conjugate: Confocal Microscopy. LSCM was used to visualize the fluorescent lipid anchors on the pre-Liposheets (Figure 3) and pre-Lipobeads (Figure 4). To facilitate the visualization of the bulk hydrogel, FluoroSpheres were incorporated in the hydrogel. The pinhole of the confocal microscope was set to visualize 1 μm thick optical *z*-sections of a pre-Liposheet (Figure 3). These pictures showed no fluorescence when the plane of focus was above the hydrogel surface, but intense fluorescence at the hydrogel surface, indicating the surface localization of fluorescent lipid anchors, and only spherical fluorescent objects below the surface, indicating the presence of FluoroSpheres in the bulk of

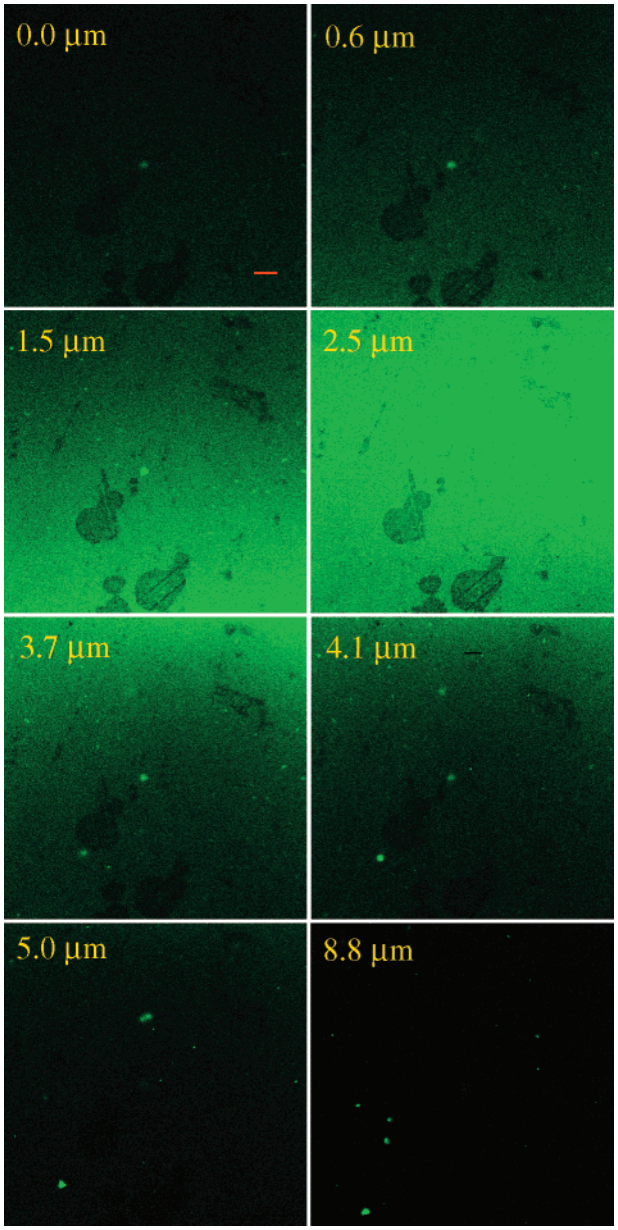


Figure 3. *Z*-section scanning laser confocal images of a poly(*N*-isopropylacrylamide) pre-Liposheet. Numbers in each image indicate the distance in the *z*-direction from a reference point. The red bar indicates a length scale of 10 μm, and each picture reflects the fluorescent intensity collected from a 1 μm optical section. FluoroSpheres, the round spherical objects, were incorporated to facilitate bulk hydrogel visualization.

the hydrogel. It is notable that surface FluoroSpheres could be detected before the diffuse fluorescence of the anchors, indicating that the region occupied by the anchors is much less than 2 μm thick. When FluoroSphere-containing hydrogels without any lipid anchors were imaged, FluoroSpheres were visible in the bulk of the hydrogel, but no fluorescence could be seen at the hydrogel surface (images not shown). Clearly, lipid anchors were positioned at the surface of the pre-Liposheets.

Similarly, the optical *z*-section of a pre-Lipobead (Figure 4A) also showed that the fluorescent anchors were localized at the bead surface. Some angular positions showed more intense fluorescence than others. The cause for the apparent nonuniformity in anchor distribution is not immediately obvious. One possible reason may be related to the lateral phase separation of phospholipids when the temperature of the system is below the gel-to-fluid transition temperature of the phospholipids. As a result, the small fraction of total lipid that is fluorescently labeled may form clusters at the surface prior to complete polymerization of the conjugate. No fluorescence was detected from the perimeter of a plain pDMAA hydrogel bead (image not shown). The core of the hydrogel appeared to have some light scattering properties (Figure 4B), and similar light scattering structures were observed using nonconfocal fluorescent microscopy (Figure 4C). In a control experiment, preformed hydrogel beads without anchors were suspended in a hexane/carbon tetrachloride solution containing dissolved fluorescent anchors. Some fluorescent anchor molecules did localize at the solvent−hydrogel interface. The beads were then subjected to the same washing protocol as the pre-Lipobeads. No fluorescence could be detected on the beads' perimeter after washing, indicating that the washing procedure was adequate to remove any anchors that were not covalently attached to the hydrogel, and therefore anchors on the pre-Lipobeads were most likely covalently attached.

Lipid Layer Self-Assembly around Pre-Lipobeads. Fluorescently labeled liposomes (62% ePC, 32% DMPE, 3% PC-NBD, 3% cholesterol) with a mean diameter of 140 nm were added to pre-Lipobeads to form Lipobeads. The *z*-section confocal image of a Lipobead (Figure 5) shows that the surface fluorescence had become smooth and uniform in appearance, suggesting that a lipid membrane covered the entire surface. No fluorescent lipid penetration into the core of the gel bead could be detected.

Since both the lipid anchors and the liposomes were fluorescently labeled, the fluorescent ring seen in Figure 5 may be attributed to either the anchors or PC-NBD from liposomes. To distinguish between these two possibilities, fluorescent liposomes were added to pre-Lipobeads of pDMAA made with nonfluorescent anchors. Any surface fluorescence on the bead would then indicate the presence of the added lipids and thus the self-assembly of a lipid membrane around the anchored hydrogel. Unbound liposomes were washed away with water, and the beads were recovered and imaged by LSCM (Figure 6). An intense ring of fluorescence is visible around the beads, indicating the fusion of fluorescent liposomes with the nonfluorescent pre-Lipobeads (Figure 6A1,B1). These figures provide evidence that self-assembly of a lipid membrane around pre-Lipobeads occur spontaneously upon the addition of liposomes.

To understand whether surface anchors are needed for lipid membrane formation, additional experiments were performed in which fluorescent liposomes were added to preformed pDMAA beads without anchors. The beads were either hydrated (Figure 6A2,B2) or dried (Figure 6A3,B3) at the time of addition of the liposome suspension. A fluorescent ring is visible around hydrogel beads that had been dry when mixed with liposomes (Figure 6A3); however, the fluorescence intensity is much lower than that on the Lipobead, indicating that less lipid has been attracted to the surface. Only minimal fluorescence is visible on the gel surface of hydrated pDMAA beads that had been hydrated prior to mixture with liposomes. A closer examination with a 63× lens shows ripples and patches in the fluorescent

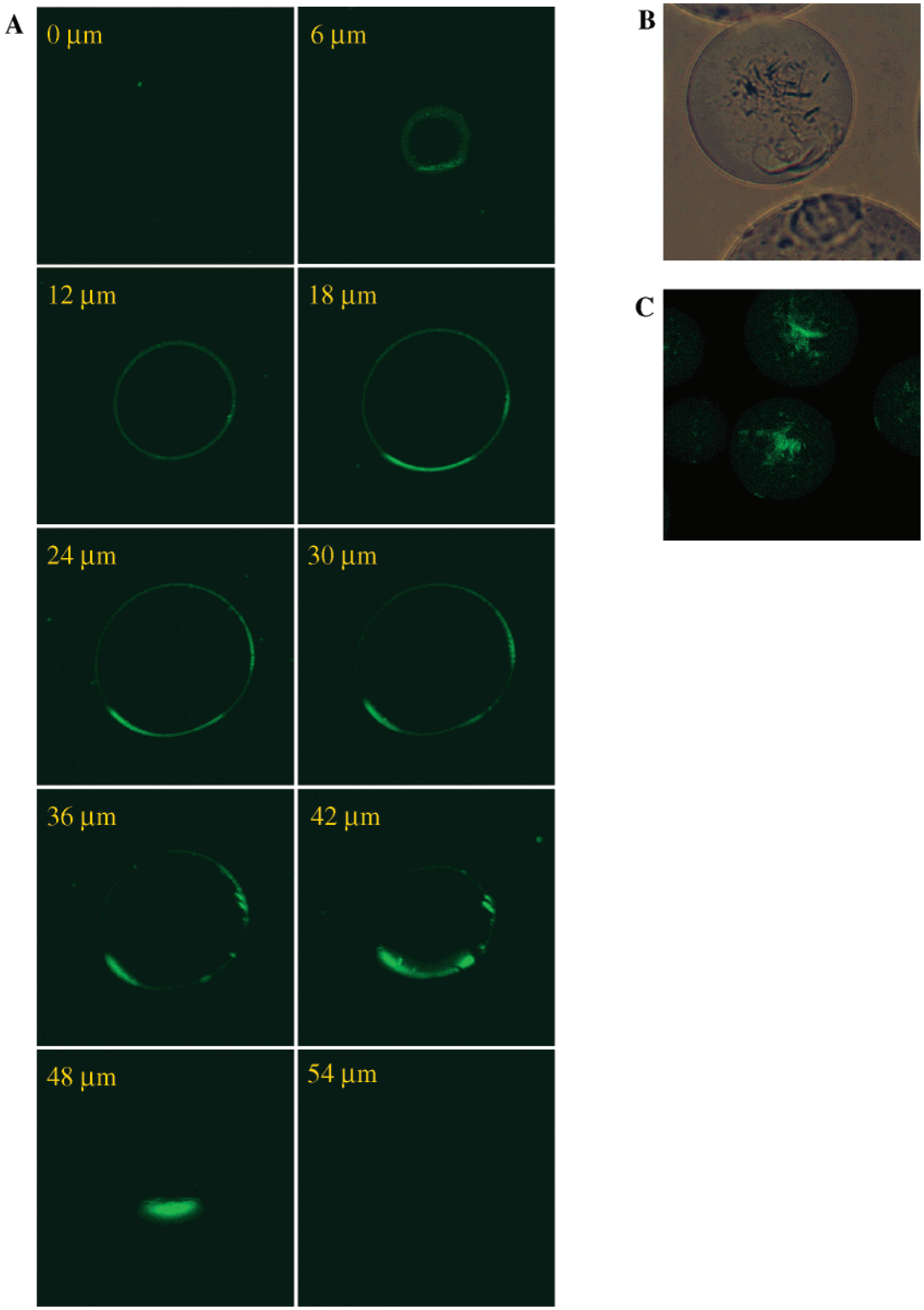


Figure 4. Images of poly(dimethylacrylamide) (pDMAA) pre-Lipobeads and pDMAA hydrogel beads. (A) *Z*-section scanning laser confocal images of a pDMAA pre-Lipobeads. Numbers in each image indicate the distance in the *z*-direction from a reference point. All images were collected with a 63× water immersion lens and a 1 μm optical section. (B) pDMAA hydrogel beads under a 10× light microscope. (C) pDMAA hydrogel beads under a 10× nonconfocal fluorescent microscope.

ring around pDMAA hydrogel beads without anchors that had been mixed with liposomes after previous hydration (Figure 6B2) or drying (Figure 6B3). It is clear that the absence of anchors results in differences in surface morphology and lipid distribution. The mechanism underlying these surface differences is unknown; however, since the amount of surface lipid is dependent on the prehydration status of anchorless beads, it is possible that nonspecific adsorption of liposomes to gel surfaces may be a significant mechanism of surface lipid deposition in the absence of anchors.

The role of anchors in the formation of lipid layers around hydrogels and the nature of such layers is a question that remains to be answered. Kiser et al.[9] reported that, with the input of energy, lipid layer formed upon the hydration of anchorless poly(methacryl-

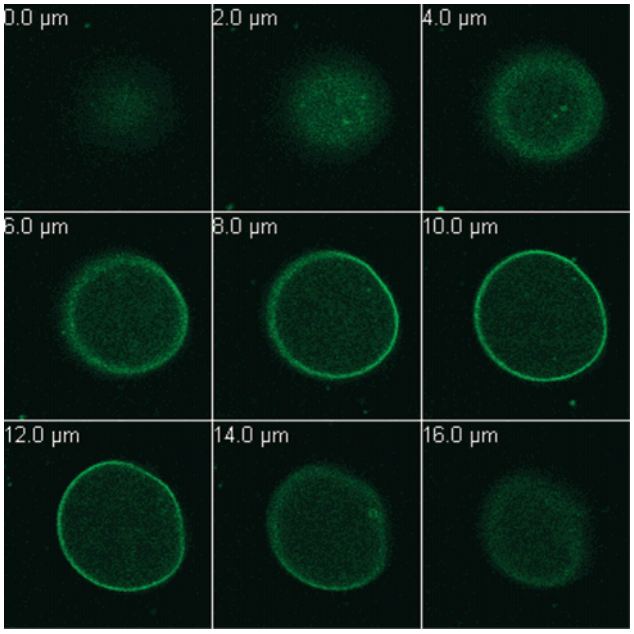


Figure 5. *Z*-section scanning laser confocal images of a poly(dimethylacrylamide) Lipobead (after the addition of fluorescent liposomes). The top left number on each image indicates the distance the lens focus had moved in the *z*-position. All pictures were taken with a 63× water immersion lens and a 1 μm optical section.

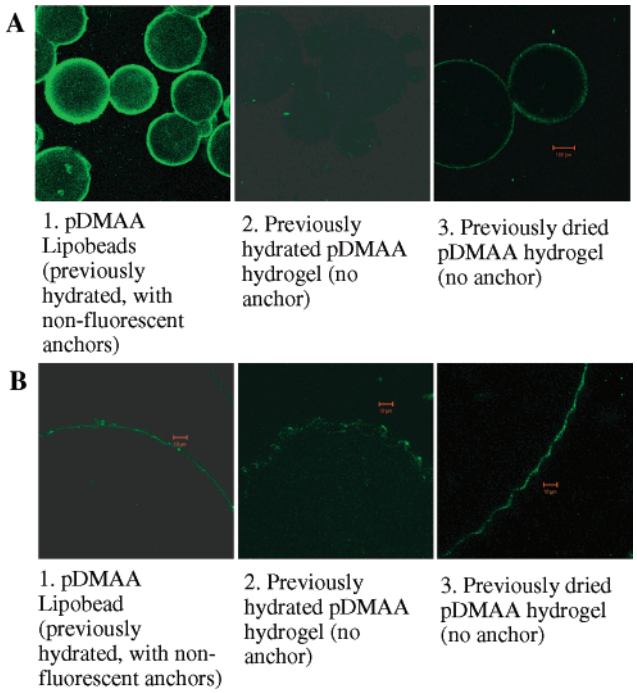


Figure 6. Scanning laser confocal images of poly(dimethylacrylamide) (pDMAA) Lipobeads and pDMAA hydrogels after the addition of fluorescent lipid vesicles. The red bar indicates a length scale of 100 μm and 10 μm in the 10× and 63× images, respectively. (A) Images collected with a 10× lens and 10 μm optical section. (B) Images collected with a 63× lens and 1 μm optical section.

ic acid) microgels with a lipid suspension and that the layer was complete enough to function as a diffusional barrier until it was intentionally ruptured. Others have shown that lipid anchors on gold[10−12] or dextran[13] substrates promote the self-assembly of phospholipid layers. Our results indicate that lipid layers can self-assemble on hydrogel surfaces in the absence of anchors, but the presence of anchors enhances this process, resulting in a more complete coating, a smoother membrane surface morphology, and higher surface lipid density. Further studies, including capacitance measurements and mass transfer experiments, are planned to further investigate the role of anchors in determining lipid layer properties. Anchors may also play a role in accelerating the kinetics of the lipid membrane assembly process by lowering the activation energy barrier for liposome fusion with the hydrogel surfaces.

To overcome the problem of liposome fragility, hydrophilic polymer coatings on liposome external surfaces have been developed that reduce the attractive interactions between bilayers and thus minimize liposome fusion and prolong blood circulation times.[2] The coating can also be used to regulate liposome properties and are typically copolymers composed of an aliphatic segment such as octadecylacrylamide and a hydrophilic segment such as poly(ethylene glycol) (PEG)[2] or poly(*N*-isopropylacrylamide) (PNIPAAm).[3] The aliphatic part of the copolymer inserts into the phospholipid bilayer, while the hydrophilic part remains on the surface and acts to exclude nearby vesicles. For example, Ringsdorf et al.[3] and Polozova et al.[4,5] synthesized a copolymer of NIPAAm and pyrene-labeled octadecylacrylamide and used the copolymer as the external coating to achieve temperature responsive release of drugs from liposomes. They suggested that the mechanism of drug release was the result of temperature-induced polymer conformational changes which, when conveyed by the lipid anchors to the bilayer, disrupted bilayer integrity. Instead of having a lipid-anchored polymeric support on the external leaflet of phospholipid membranes, the crosslinked hydrogel of a Lipobead provides support and maintains membrane stability by insertion of the anchors into the internal membrane leaflet.

Other studies that involve internal membrane support may not have provided enough space for the proper positioning of transmembrane proteins with large intracellular components. Cornell et al.[14] studied bilayers coupled to gold surfaces via lipid anchors and showed that they can be used as biosensors following the incorporation of modified gramicidins. Half-membrane-spanning phytanyl lipids were covalently attached to the gold surface via disulfide linkages, and hydrophilic poly(ethylene oxide) spacers provided a 4 nm thick aqueous cushion between the bilayer and the support substrate.

Soft hydrogel supports on flat surfaces have also be developed. Sackmann et al.[15] investigated dextran-supported phospholipid bilayers for various applications. Theato et al.[16,17] and Seitz et al.[18] synthesized a triblock copolymer in which one aliphatic end acted as anchor for a bilayer, the other end formed a disulfide linkage with gold surface, and a remaining central poly(dimethylacrylamide) block acted as a hydrophilic cushion for the bilayer. Changes in contact angle qualitatively confirmed the attachment of aliphatic anchors. Their surface plasmon spectroscopy data suggested that liposomes fused with the modified surface rapidly, but whether the anchors facilitated the fusion process was untested.

An interesting supported bilayer structure developed by Wagner et al.[19] involved a bifunctional PEG with lipid anchor on one end and a triethoxysilane group on the other end for attachment to silicate substrate; a central PEG segment provided a molecular scale hy-

drophilic cushion for the bilayer. The mobility of reconstituted membrane proteins (cytochrome b_5 and annexin V), as determined by fluorescence recovery after photobleaching (FRAP), were found to be higher than in bilayers supported directly on a solid substrate. In contrast to those previous studies involving bilayers supported on solid or flat surfaces, we are able to coat soft surfaces that are either flat or curved.

This newly developed pre-Lipobead synthesis scheme is easier and faster than the procedure described by Jin et al.[7] Furthermore, it allows for identification and quantitation of the fluorescent anchors. The fluorescent anchors can also be used with other fluorescent probes to conduct energy transfer studies in different environments. Our novel construct eliminates the need for a solid substrate, and instead of a molecular layer of hydrophilic support, the macroscopic hydrogel in our construct would provide a large aqueous environment as well as mechanical stability to the inner leaflet of the lipid bilayer. Many experiments for examining bilayer properties and protein−bilayer interactions are now possible.

Using our synthetic scheme, any vinyl monomer may be used with the specially synthesized anchor to make the phospholipid−hydrogel conjugate. Moreover, the chemical composition and the quantity of the anchor on the conjugate may also be varied. This artificial cell surrogate allows one to probe how the bilayer may be influenced by the nature of the support substrate and anchor density. By reconstituting specific membrane protein on the surface of the pre-Lipobead, one can look at the function of the protein (e.g., transport function and ligand binding) in a high-throughput format. Encapsulation of different materials by the pre-Lipobead can be achieved by hydrating the bead in a solution containing that material prior to the addition of liposomes and formation of the Lipobead. Ultimately, artificial cells or artificial cell organelles with specific desired properties may be constructed.

Conclusions

Using a specially synthesized lipid anchor and a radical polymerization technique, a one-step synthesis of a fluorescent phospholipid−hydrogel conjugate was developed. The conjugate may be made into bead or slab form. Using XPS and LSCM, lipid anchors were shown to be located only on the surface of the hydrogel. LSCM also showed that, upon the addition of pre-Lipobeads into a suspension of liposomes, lipid membranes were formed around the pre-Lipobeads. The hydrogel support provides mechanical stability and facilitates ease of handling. We are currently examining the influence of anchor density on lipid membrane properties and exploring the use of Lipobeads to characterize reconstituted membrane proteins.

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