Fabrics of Diverse Chemistries Promote the Formation of Giant Vesicles from Phospholipids and Amphiphilic Block Copolymers

Vaishnavi Girish, Joseph Pazzi, Alexander Li, and Anand Bala Subramaniam

Department of Bioengineering, University of California, Merced, Merced, California 95343, United States

Supporting Information

ABSTRACT: Giant vesicles composed of phospholipids and amphiphilic block copolymers are useful for biomimetic drug delivery, for biophysical experiments, and for creating synthetic cells. Here, we report that large numbers of giant unilamellar vesicles (GUVs) can be formed on a broad range of fabrics composed of entangled cylindrical fibers. We show that fabrics woven from fibers of silk, wool, rayon, nylon, polyester, and fiberglass promote the formation of GUVs and giant polymer vesicles (polymersomes) in aqueous solutions. The result extends significantly previous reports on the formation of GUVs on cellulose paper and cotton fabric. Giant vesicles formed on all the fabrics from lipids with various headgroup charges, chains lengths, and chain saturations. Giant vesicles could be formed from multicomponent lipid mixtures, from extracts of plasma membranes, and from amphiphilic diblock and triblock copolymers, in both low ionic strength and high ionic strength solutions. Intriguingly, statistical characterization using a model lipid, 1,2-dioleoyl-sn-glycero-3-phosphocholine, revealed that the majority of the fabrics yielded similar average counts of vesicles. Additionally, the vesicle populations obtained from the different fabrics had similar distributions of sizes. Fabrics are ubiquitous in society in consumer, technical, and biomedical applications. The discovery herein that biomimetic GUVs grow on fabrics opens promising new avenues in vesicle-based smart materials design.

INTRODUCTION

When dispersed at low concentrations in aqueous solutions, amphiphiles with cylindrical packing shapes, such as certain phospholipids, amphiphilic block copolymers, and fatty acids, self-assemble into complex suspensions of nanotubes, nanosheets, and vesicles of various sizes and lamellarieties. Of these structures, giant unilamellar vesicles (GUVs), vesicles larger than 1 μm and composed of a single bilayer, are of interest because they mimic the dimensions and compartmentalization properties of biological cell membranes. GUVs can encapsulate and protect hydrophilic cargo, and their membranes can host hydrophobic molecules and amphipathic proteins. Thus, GUVs are useful for biomimetic targeting and delivery of cargo. GUVs are also useful for biophysical experiments and for creating synthetic cells.

Obtaining a large fraction of GUVs in aqueous solutions requires directed assembly. Procedures for directing the assembly of GUVs fall into two general categories, solvent-based methods and solvent-free methods. For solvent-based methods, an aqueous solution is first emulsified in a continuous phase of oil or volatile organic solvent containing dissolved lipids. The amphipathic lipids adsorb as a monolayer on the interfaces of the aqueous droplets. Next, the lipid monolayer-stabilized droplets are transferred into a second aqueous continuous phase. As the droplets cross the interface, they entrain a layer of solvent or oil along with a second monolayer of lipids. Slow dissolution of the solvent results in thinning of the solvent film, allowing the two monolayer leaflets to form bilayers. With appropriate solvent combinations, dewetting of the solvent film to form attached solvent droplets also allows the formation of bilayer membranes. Manual agitation followed by centrifugation or microfluidic flows can accomplish emulsification and transfer of droplets. Related procedures employ microfluidic jetting to disrupt solvent-stabilized black lipid membranes to form GUVs. Non-negligible solubility of most organic solvents in the aqueous phase (e.g., toluene solubility in water: 103 parts per million, 5.7 mM) and in the hydrophobic core of the lipid bilayer, however, suggests that residual solvent remains in the membrane and in the aqueous phases at equilibrium. This residual solvent effects properties such as the bending rigidity and the phase transition temperatures of the membranes. Organic solvents are toxic, making the GUVs unsuitable for use in biomedical applications without additional solvent-removal steps. For solvent-free methods, the process begins by spreading a film of lipids dissolved in a volatile organic solvent on a solid surface. Traces of solvent that remain in the film after ambient evaporation are driven-off in vacuo. When placed in an aqueous solution, the dry solvent-free film assembles into lamellar stacks of lipid bilayers. Various methods are known to direct the vesiculation of the lamellar stacks to form GUVs. In the gentle hydration method, up to 20 mol % of charged lipids is added to the lipid mixture and hydrodynamic flows are minimized for several hours to several days. In the electroformation method, an oscillating electric field is applied...
orthogonal to the plane of the lamellar stacks. Recently, we reported that GUVs formed spontaneously on cellulose fibers when dry lipid-coated cellulose filter papers and cotton fabric are hydrated in aqueous solutions (PAPYRUS, paper-abbetted amphiphile hydration in aqueous solutions). GuVs formed from a wide variety of glycerophospholipids and sphingolipids, including those with charged headgroups and those with saturated and unsaturated alkyl chains. GuVs can be formed in water, sugar solutions, and physiologically relevant ionic buffers, such as phosphate buffered saline (PBS). Cellulose, even at elevated temperatures, is insoluble in aqueous solutions and in organic solvents typically used to dissolve lipids, such as chloroform, methanol, and toluene. The insobility of cellulose allows the formation of GuVs with single and multicomponent membranes containing lipids with high chain-melting temperatures that are free from contamination from cellulose. Cellulose paper also promotes the formation of giant polymer vesicles (polymersomes) and fatty acid vesicles from amphiphilic block copolymers and fatty acids. Compared to gentle hydration that requires hours to days to form GuVs, the formation of GuVs on cellulose paper was rapid (typical time <90 min).

Cellulose paper is the first fibrous porous substrate reported to direct the formation of GuVs. Thus, it is unknown if the chemical nature of cellulose or the geometric characteristics of the fibrous substrate is dominant for directing the formation of GuVs. Here, we conduct systematic experiments with fabrics composed of pure fibers of cotton, silk, wool, rayon, polyester, nylon, and fiberglass. The fabrics have geometric properties similar to those of cellulose paper: they are composed of an entangled mesh of cylindrical fibers, while being chemically distinct.

Silk and wool are natural protein-based fibers obtained from animals. Cocoons of the silkworm Bombyx mori are the primary source of commercial silk. Silk fibers are composed of the insoluble protein fibroin. The fleece of sheep is the primary source of commercial wool fibers. Wool fibers are composed of the insoluble protein keratin. Cotton is a natural polysaccharide fiber obtained from the bolls that form around the seeds of the cotton plant (Gossypium sp.). Cotton fibers are composed of the insoluble polysaccharide cellulose in the cellulose I crystal structure. Rayon is a semisynthetic polysaccharide fiber that is regenerated from natural cellulose feedstock. Wet-spinning of a cellulosic solution through a polysaccharide fiber obtained from the bolls that form around the seeds of the cotton plant (Gossypium sp.). Cotton fibers are composed of the insoluble polysaccharide cellulose in the cellulose I crystal structure. Rayon is a semisynthetic polysaccharide fiber that is regenerated from natural cellulose feedstock. Wet-spinning of a cellulosic solution through a spinneret followed by chemical regeneration of the cellulosic polymers results in long fibers of rayon of controlled size and crystallinity. Regenerated cellulose is in the cellulose II crystal structure. Nylon 6,6 is a synthetic fiber made by melt-spinnning polyamides that result from the polycondensation of hexamethylenediamine and adipic acid. Polyester fibers are melt-spun poly(ethylene terephthalate). Raw materials for these synthetic fibers originate from petroleum by-products. Fiberglass is solidified extruded molten glass.

We find that GuVs formed on all the fabrics. Furthermore, similar to cellulose fibers, the process of vesicle formation on the fabrics was general to a wide variety of lipids and other lamellar-phase-forming amphiphiles, such as the triblock copolymer polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO 4 PPO 7 PEO 4, Pluronic L121) and the diblock copolymer poly(butadiene-b-ethyleneoxide) PBD 46 PEO 30. These results reveal that the chemistry of cellulose is not the dominant characteristic that promotes the formation of GuVs on paper and cotton fabric. Despite the disparate chemical structure of these fibers, statistical comparison using GuVs grown from the model phospholipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) showed that properties such as the distribution of diameters and the average number of GuVs per unit mass of lipid obtained from the different fabrics were remarkably similar. Differences were statistically significant only for populations of GuVs obtained from rayon and wool. This result further suggests that the chemical properties of the fiber play a secondary role in the formation of GuVs on paper and fabrics. A geometric characteristic of fibrous porous media—we propose the curvature of the fibers—could be dominant in promoting the formation of giant vesicles.

All fabrics have a long history of use as consumer textiles and thus are more accessible than previously reported substrates used to form GuVs, such as glass, indium tin oxide-coated slides, and various hydrogels. As consumer products, the fabrics are relatively cheap and can be purchased commercially from a wide variety of sources, such as through brick-and-mortar or online vendors, through distributors, and through fabric stores worldwide. For biophysical experiments and model membrane studies that require relatively small numbers of GuVs of high purity, selection of a specific fabric type to fabricate GuVs can be made on the basis of costs, reusability, and depending on the scale of fabrication and the impact on the environment. Moreover, cotton, rayon, silk, nylon, and polyester fabrics are biocompatible and are used in biomedical applications. Since giant vesicles can encapsulate hydrophilic cargo in their lumens and dissolve hydrophobic molecules in their membranes, our novel demonstration that these fabrics promote the formation of giant vesicles opens hitherto unconsidered avenues in smart textile and biomaterials design.

**EXPERIMENTAL SECTION**

**Materials.** We purchased sucrose (BioXtra grade, purity ≥ 99.5%) glucose (BioXtra grade, purity ≥ 99.5%), casein from bovine milk (BioReagent grade), and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEO 4 PPO 7 PEO 4, Pluronic L121) from Sigma-Aldrich. We purchased 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phospho(1'-rac-glycerol) (DOPG), cholesterol (ovine wool), 1-palmitoyl-2-(dipymetheneboron difluoride) undecanoyl-sn-glycero-3-phosphocholine (TopFluoPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rhod-PE), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and Escherichia coli polar lipid extract from Avanti Polar Lipids, and poly(butadiene-b-ethylene oxide) (PBD 46 PEO 30) (P9095-BdEO, lot# P9757) from Polymer Source Inc (Montreal, Canada). We purchased the fabrics from online vendors and from a local branch of a national fabric store Jo-Ann, LLC. The fabrics we purchased were silk (Silk Dupioni, bright white, 100% silk), rayon (Sportswear Modal Fabric, white, 100% rayon), nylon (Sport Nylon Fabric, white, 100% nylon), and polyester (Satin Taffeta, white, 100% polyester). We purchased wool fabric (100% Merino Wool Interlock—Washable) from Nature’s Fabrics (naturesfabrics.com) and plain weaved fiberglass fabric (3 oz Fabric Style120 E-Glass) from Fibre Glass Developments Corp. We obtained 18.2 MΩ ultrapure water from an ELGA Purelab Ultra water purification system (Woodridge, IL).

**Preparation of Substrates.** We used a previously published procedure to clean the fabrics. We cut each piece of fabric into 10 × 10 cm². Working in a chemical fume hood, we placed the fabric in 100 mL of neat chloroform in a 100 mL glass media bottle. We
used a magnetic stirrer and Teflon stir bar to agitate the fabric in chloroform for 30 min. We repeated the process twice with fresh chloroform each time. After the final chloroform wash, we removed the fabric and allowed the solvent to evaporate from the fabric. We then placed the fabric in a 1000 mL glass media bottle and alternated between soaking and rinsing the fabric in fresh batches of 1000 mL of ultrapure water over the course of 3 h. Ambient drying and storage in a clean Petri dish completed the process.

**Scanning Electron Microscopy.** To prepare the fabrics for imaging, we cut a small 5 mm × 5 mm piece of the cleaned fabrics and mounted them on aluminum scanning electron microscopy (SEM) stubs using double-sided copper tape. We used a field emission scanning electron microscope (GeminiSEM 500, Zeiss, Germany) to obtain images of the surfaces of the fabrics. The fabrics were exposed to a beam accelerating voltage of 1 kV, and the secondary electrons that were scattered from the surface were collected using an Everhart-Thornley detector. We collected images at a pixel resolution of 1333 nm/pixel for the low-magnification images and a pixel resolution of 147 nm/pixel for the high-magnification images.

**Deposition of Lipids and Growth of DOPC GUVs for Quantitative Characterization of Yields and Size Distributions.** We standardized growth conditions to allow comparison between the different substrates. We prepared a solution of 99.5:0.5 mol % DOPC/TopFluor-PC in neat chloroform at a concentration of 2 mg/mL. We cut each piece of fabric into 9.5 mm diameter circular disks with a pair of scissors and deposited appropriate volumes of the lipid solution to obtain 3 μg of lipid per 1 mg of the substrate. For wool, we used 1.5 μg of lipid per 1 mg of substrate. After the solvent had evaporated ambiently, we placed the lipid-coated fabric in a vacuum chamber for 1 h to drive off residual solvent. The substrates were then placed into 2.0 mL Eppendorf tubes. We added 500 μL of a 100 mM solution of sucrose to hydrate the lipid-coated substrates and incubated the substrates for 60 min.

**Growth of GUVs with Varying Membrane Compositions on the Fabrics.** All amphiphile-coated substrates were placed under vacuum for a minimum of 1 h to drive off residual solvent and then incubated in an aqueous solution for 60 min before imaging. Growth of giant vesicles from the various amphiphiles required the use of varying growth temperatures and nominal surface concentrations. For the amphiphilic triblock Pluronic L121 polymersomes, we deposited 14 μg of 99.5:0.5 mol % Pluronic L121/TopFluor-PC per 1 mg of the substrate. Growth was performed at room temperature. For the

---

**Table 1. Properties of the Fabrics**

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Structural Formula</th>
<th>Fiber diameter, μm (mean ± sd)</th>
<th>Contact angle</th>
<th>Fabric Weave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silk62</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>10.6 ± 1.7</td>
<td>72° ± 70</td>
<td>Plain</td>
</tr>
<tr>
<td>Wool63</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>18.5 ± 4.8</td>
<td>78° ± 59</td>
<td>Jersey</td>
</tr>
<tr>
<td>Cotton64</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>15.8 ± 3.7</td>
<td>34° ± 60</td>
<td>Plain</td>
</tr>
<tr>
<td>Semisynthetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rayon65</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>12.0 ± 1.5</td>
<td>30° ± 50</td>
<td>Plain</td>
</tr>
<tr>
<td>Synthetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nylon66</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>22.9 ± 1.3</td>
<td>68° ± 60</td>
<td>Plain</td>
</tr>
<tr>
<td>Polyester67</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>12.8 ± 1.4</td>
<td>42° ± 60</td>
<td>Twill</td>
</tr>
<tr>
<td>Inorganic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiberglass (alumino-borosilicate)</td>
<td><img src="attachment" alt="Structural Diagram" /></td>
<td>4.7 ± 0.4</td>
<td>0° ± 71</td>
<td>Satin</td>
</tr>
</tbody>
</table>

*Structural formula diagrams were obtained from the refs 62–68. Water contact angles were obtained from refs 60, 69 and 70. The fiber diameters were measured from SEM images.*
amphiphilic diblock PBD₄₆PEO₃₀ polymersomes, we deposited 7 μg of 99.5:0.5 mol % PBD₄₆PEO₃₀/TopFluor-PC per 1 mg of the substrate. Growth was performed at 80 °C. For 89.5:10:0.5 mol % DOPC/DOPG/TopFluor-PC, we deposited 3 μg of the lipid mixture per 1 mg of the substrate. Growth was at room temperature. For 89.5:10:0.5 mol % DOPC/DOTAP/TopFluor-PC, we deposited 7 μg of the lipid mixture per 1 mg of the substrate. Growth was at room temperature. For 99.5:0.5 mol % DOPC/TopFluor-PC, we deposited 3 μg of the lipid mixture per 1 mg of the substrate. Growth was at room temperature. For 99.5:0.5 mol % DPPC, we deposited 3 μg of the lipid mixture per 1 mg of the substrate. Growth was at 65 °C. For 35.5:36:28:0.5:0.5 mol % DOPC/DPPC/cholesterol/TopFluor-PC/Rhod-PE, we deposited 8 μg of the lipid mixture per 1 mg of the substrate. Growth was at 65 °C. For the E. coli membrane extract we deposited 3 μg of the lipid mixture per 1 mg of the substrate. Growth was at 37 °C. To obtain GUVs in standard PBS, we first incubated the lipid-coated fabrics in ultrapure water for 10 min. We then added a concentrated stock of the buffer salts (Amresco phosphate buffered saline (PBS) 20X concentrate, 2.74 M sodium chloride, 0.54 M potassium chloride, 0.2 M sodium phosphate, pH 7.5) to obtain standard PBS and allowed the vesicles to grow for an additional 50 min before imaging.

**Harvesting of the GUVs.** We placed a 100 μL droplet of a 100 mM solution of sucrose on a clean glass coverslip. We removed the substrates from the Eppendorf tubes using forceps and quickly immersed the wet substrate into the droplet on the coverslip. We harvested the GUVs by gently aspirating the solution into a 1000 μL pipette tip while moving the tip systematically over the surface of the fabric. We enlarged the opening of the pipette tip by cutting off the end of the tip. The larger opening minimizes damage to the GUVs by reducing fluid shear forces during aspiration.

**Confocal Imaging.** We fabricated square imaging chambers (width × length × height = 6 mm × 6 mm × 1 mm) from poly(dimethylsiloxane) bonded onto glass slides. We passivated the surface with casein to prevent rupture of GUVs onto the bare glass. We chose aliquot sizes that resulted in approximately 100 000 GUVs per all the fabrics, except for rayon, where we used 2 μL aliquots. Isomolar solutions of glucose (100 mM) were added to bring the total volume in the chamber to 60 μL. The sucrose-filled vesicles, which had a higher density, sedimented to the bottom of the chamber. After 3 h, we used an upright confocal laser scanning microscope (LSM 880 with Airyscan+FAST, Axio Imager.Z2m, Zeiss, Germany) to capture single-plane confocal images of the entire area of the chamber using an automated tile scan routine (850.19 μm × 850.19 μm, 2140 pixels × 2140 pixels per image, 64 images). We used a 10X EC PlanNeofluar objective with a numerical aperture, NA = 0.3 to image. The TopFluor dye was excited using a 488 nm argon laser set at 4% power. For images of GUVs growing on the fibers of the fabric, we collected confocal z-stacks with an axial-spacing of 0.67 μm using a 20X W Plan-Apochromat water immersion objective with an NA = 1.0.

**Image Analysis.** We used a custom routine written in MATLAB (Mathworks Inc., Natick, MA) to analyze the images. The routine used an intensity threshold followed by watershed segmentation to identify fluorescent objects. The native regions routine tabulated the equivalent diameters and mean fluorescence intensities of the objects. GUVs were distinguished from other lipid structures on the basis of their mean intensities and their size (>1 μm). To obtain the counts of GUVs normalized per unit mass of lipid, we use the following: GUVs per mass lipid = \( \frac{V_{\text{aliquot}}V_{\text{harvested}}}{M_{\text{aliquot}}M_{\text{harvested}}} \). In this equation, GUVs number is the number of GUVs counted in the chamber, \( V_{\text{aliquot}} \) is the aliquot volume, \( V_{\text{harvested}} \) is the volume of the harvested solution, and \( M_{\text{aliquot}} \) is the mass of lipid that was deposited on the fabrics. Two-sample t-tests were performed in MATLAB to test for statistical significance.

### RESULTS AND DISCUSSION

#### Properties of the Fabrics.

We tested fabrics woven from pure fibers of silk, wool, rayon, nylon, polyester, or fiberglass. Table 1 shows the structural formulas, the average diameter of the fibers, the weave pattern, and the single-fiber water contact angle of the fabrics. The structural formulas and water contact angle of these fibers have been characterized in the literature. Fiberglass, cotton, and rayon have smaller water contact angles compared with silk, wool, rayon, nylon, and polyester. All fibers have water contact angles of <90°, which indicates that the surfaces of the fibers are hydrophilic. Figure 1 shows scanning electron microscope images of the fabrics. Images on the left column are lower magnification images that show the microstructure of the fabrics. Images on the right column are higher magnification images that show the surfaces of the fibers. Silk, rayon, nylon, polyester, and fiberglass. Scale bars (a)–(g): left column 100 μm, right column 10 μm.
fiberglass fibers had smooth surfaces. Wool had a scaly surface. Furthermore, the rayon, nylon, polyester, and fiberglass fibers appeared very uniform, reflective of their synthetic nature. The fiber sizes, measured from SEM images, ranged from an average diameter of 4.7 ± 0.4 μm for fiberglass to 22.9 ± 1.3 μm for nylon. The radii of curvature of the fibers on the different fabrics were thus within a relatively narrow range of 0.1–0.4 μm\textsuperscript{−1}.

**All Fabrics Promoted Vesiculation of GUVs on Their Fibers.** Figure 2 shows confocal microscope images of the

![Figure 2. Confocal microscope images of the surface of the hydrated fabrics after 1 h. (a) Silk, (b) wool, (c) rayon, (d) polyester, (e) nylon, and (f) fiberglass. GUVs are the bright green circles with dark interiors. Although not fluorescently labeled, the fibers are visible due to the fluorescent lipid coating. (a–f) Scale bars: 25 μm.](Image)

lipid-coated fabrics 1 h after incubation in an aqueous buffer. All fabrics tested had GUVs growing from the surfaces of their fibers. Most of the GUVs had tethers to the lipid layer coating the fibers.\textsuperscript{48} These results show that the spontaneous formation of GUVs is not limited to cellulose and is general to fibers of differing surface chemistries.

Silk and rayon had spherical GUVs that appeared qualitatively similar to those seen previously on cellulose paper and cotton fabric\textsuperscript{49} (Figure S1a). The GUVs were highly abundant on these fabrics. GUVs coated the fibers in stacked layers that filled the pores between the fibers of the fabric. Wool had noticeably fewer GUVs than the other fabrics. Nylon, polyester, and fiberglass fabrics had an intermediate number of GUVs, with some regions having a higher abundance of GUVs and others having a lower abundance of GUVs. We observed that GUVs formed only from the lipid layer that coated the fibers. Lipid deposits that spanned the gaps between the fibers gave rise to multilamellar lipid structures (Figure S2). These lipid deposits were more prevalent on wool, nylon, polyester, and fiberglass than on rayon, silk, or cotton. Thus, along with GUVs, other lamellar structures were present on the wool, nylon, polyester, and fiberglass fabrics.

All fabrics promoted the growth of GUVs from a wide variety of lamellar-phase-forming amphiphiles. GUVs could be obtained in both low ionic strength solutions, such as ultrapure water and sugar solutions, and in high ionic strength solutions, such as standard phosphate buffered saline (standard PBS: 137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium phosphate, pH 7.4–7.6). GUVs composed of lipids with long saturated alkyl chains that require growth at elevated temperatures could also be obtained since all fibers are thermally stable to 100 °C (the boiling point of water) or higher.\textsuperscript{51,52} Figure 3 shows representative confocal images of a

![Figure 3. Confocal microscope images of giant polymersomes and giant lipid vesicles on the surface of silk fibers after 1 h in aqueous buffer. (a) Giant polymersomes of the amphiphilic triblock copolymer Pluronic L121 in a solution of sucrose. (b) Giant polymersomes of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in a solution of sucrose. (c) Giant vesicles with negatively charged membranes (89.5:10:0.5 mol % DOPC/DOPG/TopFluor-PC) in a solution of sucrose. (d) Giant vesicles with positively charged membranes (89.5:10:0.5 mol % DOPC/DOTAP/TopFluor-PC) in standard PBS. (e) Giant vesicles with zwitterionic membranes composed of two unsaturated alkyl chains (DOPC, chain-melting temperature −20 °C) in standard PBS. (f) Giant vesicles with zwitterionic membranes composed of saturated alkyl chains (DPPC, chain-melting temperature −45 °C) in standard PBS. (g) Giant vesicles with membranes exhibiting liquid–liquid phase coexistence composed of 35.5:36:28:0.5:0.5 mol % DOPC/DPPC/cholesterol/TopFluor-PC/Rhod-PE in standard PBS. (h) Giant vesicles composed of membrane extracts from *E. coli* in standard PBS. (a, b, d, e) Scale bars 15 μm. (c, f, g, h) Scale bars 25 μm.](Image)

giant polymer and lipid vesicles of various membrane compositions growing both in low ionic strength solutions (100 mM sucrose, Figure 3a–c) and high ionic strength solutions (standard PBS, Figure 3d–f) on silk fibers. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46} Figure 3a,b shows giant polymersomes composed of the amphiphilic triblock copolymer Pluronic L121 and polymersomes composed of the amphiphilic diblock copolymer PBD\textsubscript{a}PEO\textsubscript{b} in standard PBS. For growth in PBS, samples were incubated in ultrapure water for 10 min before a concentrated stock of the buffer salts was added to obtain standard PBS. We allowed the vesicles to continue to grow in standard PBS for a further 50 min before imaging. Gradients in salt concentration between the continuous phase and the lumens of the GUVs were rapidly equilibrated through the nanotube tethers that connected the GUVs to the external aqueous phase.\textsuperscript{46}
GUVs were grown at 65 °C to ensure that the membranes were fully mixed. Upon cooling to room temperature, the membranes phase-separated into liquid ordered and liquid disordered domains, as evidenced by the partitioning of Rhod-PE (false-colored red) and TopFluor-PC (false-colored green) into distinct compartments in the membrane. Rhod-PE partitions strongly into the liquid disordered phase, whereas TopFluor-PC partitions equally between the liquid disordered phase and liquid ordered phase.11,47 Figure 3f shows GUVs obtained from the polar fraction of membrane extracts of the bacteria E. coli. The membranes, composed of phosphatidylethanolamine/phosphatidylglycerol/cardiolipin 67.0:23.2:9.8 wt/wt % (manufacturer’s specifications), are highly negatively charged.

Quantification of Effective Yield of GUVs from the Fabrics. Having shown that vesicle growth on the fabrics is general to a wide range of solution ionic strengths, temperatures, and lamellae-forming amphiphile types, we next used the model lipid DOPC to conduct statistical experiments to quantify the distribution of sizes and effective yields of GUVs harvested from the fabrics. We performed three independent experiments for each type of fabric. We harvested the lipid structures from the fabrics and imaged aliquots from the suspensions after allowing the structures to sediment for 3 h in a custom imaging chamber. A fraction of GUVs and other structures remain trapped in the fabric after the harvesting process. We were careful to reproduce similar harvesting conditions for all fabrics to allow a comparison of the effective yields. We analyzed our images using a custom MATLAB routine. Each experiment had large sample sizes ranging from \( n = O(10^5) \) to \( O(10^3) \) GUVs. GUVs were identified on the basis of their fluorescence intensity48,49 and their size (>1 μm). Control experiments using coupled sedimentation and dye leakage confirmed that membrane fluorescence intensity distinguishes GUVs from other structures (Supporting Information, Figures S3 and S4). Furthermore, measurements of lipid mobility using fluorescence recovery after photobleaching (FRAP) yielded a diffusion coefficient, \( D = 7.9 \pm 1.7 \text{ μm}^2/\text{s} \) for the lipids in the membranes of vesicles (Supporting Information, Figure S5). This value of the diffusion coefficient is consistent with those reported in the literature for GUVs composed of DOPC.72–74

Figure 4a–f shows a typical field of view obtained from a 4 μL aliquot of the harvested solution diluted in 56 μL of isomolar glucose buffer. Structures harvested from silk and rayon, similar to those from cotton fabric (Figure S1b) and cellulose paper,49 appeared predominantly to be GUVs. Consistent with our direct observations of the structures on the fabrics, a larger fraction of lipid aggregates and lipid nanotubes were present in the samples harvested from wool, nylon, polyester, and fiberglass. On the basis of typical numbers reported in the literature (ten to hundreds of vesicles),10–11 the number of GUVs in a characteristic field of view obtained from all fabrics was sufficient to perform biophysical experiments.

Figure 4g shows a bar and scatter plot of the number of GUVs obtained from the fabrics arranged in a descending order of counts per microgram of lipid from left to right. The height of the bar is the average of the three experiments for each type of fabric. The points are the counts from the three independent experiments. To allow comparison between the fabrics, we normalized the data to the mass of lipid deposited on the fabrics. Rayon appeared to produce the highest average number of GUVs per unit mass of deposited lipid at \( 2.6 \times 10^5 \) GUVs per μg of lipid and wool had the lowest average number of GUVs at \( 6.2 \times 10^4 \) GUVs per μg of lipid. Two-sample t-tests between rayon and all fabrics showed that the higher yield from rayon was statistically significant (\( p < 0.05 \)), except when compared to nylon, where \( p = 0.1373 \). Two-sample t-tests showed no statistical difference between yields obtained from cotton, silk, nylon, polyester, and fiberglass with each other. The lower yield of wool was statistically significant (\( p < 0.05 \)) when compared with the other fabrics, except when compared with polyester (\( p = 0.2032 \)). A matrix of the pairwise \( p \)-values between the fabrics is shown in Table S1. These results show that when measured at large sample sizes, yields from chemically and structurally distinct fabrics, such as silk, cotton, fiberglass, nylon, and polyester, were statistically indistinguishable, whereas the higher yield of GUVs from rayon and lower yield of GUVs from wool were statistically distinguishable. Note that even the lowest yielding fabric, wool, produced a larger number of GUVs than electroformation on stainless steel electrodes.75 Furthermore, unlike electroformation, directing the assembly of GUVs on fabric does not require access to electrical power, electronic equipment, or specialized chambers.

Similar to cotton fabric,49 the process of GUV growth did not alter the fibers of the fabrics. All fabrics supported at least five cycles of GUV growth and harvest with no measurable changes in the characteristics of the GUVs (data not shown).

Distribution of Sizes of the GUVs Obtained from the Fabrics. We next analyzed the sizes of the DOPC GUVs that we obtained from the different fabrics. Figure 5a shows a histogram of the diameters of the GUVs harvested from silk. The bin width is 1 μm, and the sample size, \( n = 113 \) 461. The distribution of diameters was unimodal with a prominent right tail. Smaller GUVs were more abundant than larger GUVs. Populations of GUVs harvested from the other fabrics also showed similar unimodal right-skewed distribution of diameters (Figure S6). These results further add to the growing body...
of GUVs harvested from the fabrics. The height of the bar is the average of the three experiments for each type of fabric. The points are the median diameters of the population from the three experiments on each type of fabric. The populations of GUVs harvested from silk had a median diameter of 2.7 μm. Two-sample t-tests revealed that the median diameter of GUVs obtained from silk was statistically distinguishable from that of all other fabrics, except when compared with GUVs obtained from rayon, which had a median diameter of 2.7 μm (p = 0.0520). A matrix of the pairwise p-values comparing the median diameters of the population from each fabric type is shown in Table S2. Overall, it is striking that the distribution of diameters was surprisingly similar despite the different chemistries of the fibers. We note that this result would be expected if the geometry of the fibers controlled the sizes of the GUVs; the diameter of the fibers in the fabrics ranged between ~5 μm for fiberglass and ~22 μm for nylon.

## CONCLUSIONS

We have shown that the formation of GUVs and polymersomes from lamellar films of phospholipids and amphiphilic block copolymers is general to a surprisingly wide variety of fabrics composed of cylindrical fibers of different surface chemistries. Fabrics are widely used in various consumer and technical applications including in biomedical applications. Thus, our demonstration that vesicles can form on a range of fabrics opens new avenues for designing smart functionalized materials. Interestingly, despite the large differences in the chemistry and microstructure of the fabrics, the effective properties of the populations of GUVs such as the median diameters and the number of GUVs obtained per unit mass of lipid were remarkably similar across different types of fabrics. Our results thus suggest that a unified understanding of the mechanism of formation of GUVs on paper, fabrics, and other fibrous porous media possibly lies in a geometric characteristic of the fibers. We speculate that the relatively small variation in curvatures between the fibers of the different fabrics (0.1–0.4 μm⁻¹) might explain the similar size ranges of the GUVs obtained from the fabrics. Effects of curvature on the micrometer scale have been shown to be important in membrane processes such as in the localization of membrane domains and in the localization of bacterial membrane proteins. Further studies on the contribution of curvature to the interaction energy between the lamellae in multibilayer stacks will likely yield additional mechanistic insights.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.9b01621.

Technique for selecting GUVs based on the analysis of membrane fluorescence intensity; coupled sedimentation and fluorescence leakage assay to determine unilamellarity; GUVs on cotton fabric and GUVs harvested from cotton fabric; multimembrane vesicles and other structures that formed from lipid deposits that span fibers; representative data from the analysis of membrane fluorescence intensity; schematic and results of the coupled sedimentation and dye leakage assay; representative still images and FRAP recovery curve from GUVs harvested from silk; representative histo-
gram of sizes of giant vesicles obtained from different fabrics; p-values for the effective counts of GUVs from the fabrics; p-values for the median diameter of GUVs from the fabrics (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
E-mail: asubramaniam@ucmerced.edu.

**ORCID**
Anand Bala Subramaniam: 0000-0002-1998-9299

**Author Contributions**
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Funding**
This work was funded by NSF Award CBET-1512686 and NSF-CREST; Center for Cellular and Biomolecular Machines at the University of California, Merced (NSF-HRD-1547848). The data in this work was collected, in part, with a confocal microscope acquired through the National Science Foundation MRL Award Number DMR-1625733 and a scanning electron microscope acquired through NASA Grant NNX15AQ01A.

**Notes**
The authors declare no competing financial interest.

**REFERENCES**

Ramamurthi, K. S.; Lecuyer, S.; Stone, H. A.; Losick, R.