Multicompartment polymersome gel for encapsulation

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Abtract

We introduce an approach that combines the concepts of emulsion-templating and dewetting for fabricating polymersomes with a large number of compartments. The resultant polymersome gel behaves as a gel-like solid, but is a true vesicle suspended in an aqueous environment. Due to the thin membranes that separate the compartments, the polymersome gels have a high volume fraction of internal phase for encapsulation of hydrophilic actives; they also provide large surface area of diblock copolymer membrane for encapsulation of lipophilic actives. Multiple actives can also be encapsulated in the gel without cross-contamination. Our technique represents a simple and versatile bulk approach for fabricating polymersome gels; it does not require the use of any specialized equipment or subsequent polymerization steps to solidify the gel. The resultant polymersome gel is promising as an encapsulating structure as well as scaffold for tissue engineering.

Polymer vesicles or polymersomes are capsules with shells consisting of bilayers of amphiphilic block copolymers.[1,2] They are of great interest for encapsulation of active ingredients in a wide range of areas, including food, beverage, cosmetics, as well as the pharmaceutical industry. Typically, these vesicles are spherical and have only one compartment. However, polymersomes with multiple compartments have great potential as

they allow encapsulation of multiple actives without cross-contamination. Moreover, since the compartments can be packed tightly together and are separated by a thin polymer bilayers, polymersomes with large number of compartments can achieve a very high internal phase volume fraction; thus, such polymersomes have properties similar to high-internal-phase emulsions (HIPE), and can serve as templates for synthesis of highly porous polymers, known as polyHIPE[3], with potential as low-weight materials[4], scaffolds in tissue engineering[5,6], sensor materials[7] as well as supports for solid phase synthesis[8] and for hydrogen storage[9]. Conventionally, HIPEs are used for these applications, but their stability is often affected by droplet coalescence and phase inversion.[10] As a result, the amount of surfactant needed for stabilization of HIPEs can often reach up to 30 wt % of the external phase; this makes the properties of the HIPE highly dependent on the properties of the surfactants that are difficult and costly to remove.[11] In addition, the structure of the voids in conventional polyHIPEs are usually spherical; the low packing density of spherical droplets can limit the volume fraction of the polyHIPEs. Polymersomes with compartments separated by thin walls of copolymers potentially achieve higher volume fraction of internal phase, and the copolymers walls also prevent cross-contamination of actives in different compartments. Despite these potential advantages, approaches to fabricate these multi-compartment polymersomes have been limited.

In this work, we present a new approach to fabricate polymersomes with a large number of compartments for encapsulation of multiple actives. We use a template consisting of a water-in-oil (W/O) emulsion; the continuous phase of this emulsion consists of amphiphilic block copolymers dissolved in a mixture of a volatile good solvent and a less volatile poor solvent for the copolymers. The W/O emulsion template is then added to water as a second continuous phase. By tuning the ratio of the two solvents, the adhesion between the neighboring inner droplets can be adjusted. To achieve high volume fraction of the internal phase, the compartments of the vesicles must adhere to each other strongly to squeeze

out the solvent in the thin film separating neighboring compartments.[12] As the inner droplets stick to each other, a gel-like polymersome with multiple compartments is formed (**Fig. 1a**). We demonstrate that the approach is highly versatile and allows encapsulation of multiple hydrophilic actives in the internal phase, and hydrophobic actives in the bilayer membrane separating the compartments.

Experimental Section

We prepared a W/O emulsion by shaking or vortexing in a mixer (Vortex-Genie 2, VWR Scientific Industries, Inc.) for 1 minute. The aqueous phase was made up of water and in some cases, with fluorescein isothiocyanate-dextran (FITC-Dextran, Mw: ~10,000 g·mol-1, Sigma-Aldrich Co.) or Rhodamine B isothiocyanate-dextran (RhoB-Dextran, Mw: ~10,000 g·mol-1, Sigma-Aldrich Co.) as model encapsulants and polyethylene glycol (Mw: 6000 g·mol-1, Sigma-Aldrich Co.) for increasing the density of the droplet phase. Middle phase oils included poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA, Polysciences, Inc.) with block lengths of (PEG: 5000 g·mol-1/ PLA: 5000 g·mol-1) and (3000 g·mol-1/3000 g·mol-1) dissolved in a mixture of chloroform (Mallinckrodt Chemicals, Inc.) and hexanes (EMD Chemicals, Inc.). The oil phase consisted of ~10 mg/mL poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA, Polysciences, Inc.) with block lengths of (PEG: 5000 g·mol-1/ PLA: 5000 g·mol-1) dissolved in a mixture of 36 vol% chloroform (Mallinckrodt Chemicals, Inc.) and 64 vol% hexanes (EMD Chemicals, Inc.). Fluorescent tracers, 25-[N-[(7-nitro-2-1,3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol (NBD-norcholesterol, Avanti Polar Lipids, Inc.) or 8-Acetoxymethyl-2,6-diethyl -1,3,5,7-tetramethyl pyrromethene fluoroborate (Pyrromethene 605, Exciton, Inc.), were sometimes added for visualization the membranes of the vesicle gel. We then poured the W/O emulsion poured into a glass vial with 5-10 mL of water. The W/O emulsion, which stayed atop the layer of water, was left at room temperature for 15-30 hours for evaporation of the mixture of chloroform and hexane. Photographs of the resultant vesicle

gels were taken with a digital camera (PowerShot SD1100 IS, Canon, Inc.) and a confocal microscope (TCS SP5, Leica, Inc.) with an argon laser at a wavelength of 488 nm and a Helium Neon laser at a wavelength of 543 nm. Vesicle gels were also dried at room temperature for a day for imaging with scanning electron microscopy. Images of the dried samples coated with a thin layer of platinum and palladium were taken using a Zeiss Supra 55VP field emission scanning electron microscope (FESEM, Carl Zeiss SMT, Inc.) at an acceleration voltage of 4.5 kV.

We prepare a W/O emulsion by homogenizing a mixture of an active-containing aqueous phase and an oil phase, which consists of diblock copolymers of poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA) dissolved in a mixture of 36vol% chloroform and 64vol% hexane. This composition of the oil phase is chosen to facilitate formation of bilayer polymersome membranes, which has been applied to fabricate polymersomes with single[13] and multiple compartments[14] in microfluidic devices. A homopolymer, PEG, is added to the active-containing aqueous phase to increase the osmolarity of the innermost phase to match that of the continuous phase so that diffusion of water across the oil phase and the final polymersome membrane is minimized. This also increases the density of the innermost phase to facilitate the sedimentation of the final polymersomes insolution; no additional effects are observed due to the addition of PEG. In the resultant W/O emulsion, the amphiphilic diblock copolymers adsorb at the W/O interfaces, stabilizing the droplets against coalescence with one another; a photograph of this stable W/O emulsion is shown in **Fig. 1b**. The emulsion is subsequently added into another aqueous phase, which will form the eventual continuous phase of the polymersomes, as shown in **Fig. 1c**. The W/O emulsion and the aqueous phase are left at room temperature for the organic solvents to evaporate. Since chloroform, which is a good solvent for PEG-b-PLA, evaporates faster than hexane, a poor solvent for PEG-b-PLA, in the solvent mixture, solubility of PEG-b-PLA is reduced as the solvents evaporate. As a

result, the PEG-b-PLA-laden interfaces become attracted to each other, leading to formation of an adhesive emulsion gel. Similarly, the interfaces of the emulsion gel also stick to the interface between the solvent and the additional aqueous phase into which the emulsion has been added. This results in a dewetting transition, where the organic solvents are expelled from the diblock copolymer-laden interfaces. After the solvent removal, the interfaces solidify to form membranes in gel phase. These dewetting-induced membranes have been shown to be bilayered.[13] Solvent evaporation continues to take place until all solvents are removed from the membranes. While the more volatile chloroform evaporates first, hexanes are poor solvent for PEG-*b*-PLA and therefore, the resultant membranes are not expected to entrap hexanes. Since 10wt% PEG has been added to the droplet phase of the W/O emulsion, making it heavier than the continuous phase, the emulsion gel separates from the oil phase, sinking to the bottom aqueous phase; in this fashion, a multicompartment vesicle gel is formed (**Fig. 1d**). With this approach, the final vesicle gel behaves as a dry foam where the solid membrane layer is free of organic solvent, and where no further drainage of solvents occurs. Remarkably, the entire structure is comprised of amphiphilic block copolymers, which act as a surfactant. Nevertheless, the vesicle gel is mechanically stable and lasts for weeks, until the constituent PEG-b-PLA ultimately hydrolyzes in solution. This approach for forming multicompartmental vesicle gels is also applicable to other amphiphilic block copolymers,[13] which result in vesicle gels with different membrane properties.

The multicompartment vesicle gel differs from conventional vesicle gels that are typically concentrated suspensions of many individual vesicles. In our multicompartment vesicle gels, neighboring compartments are separated by a single membrane of diblock copolymers. As a result, the multicompartment vesicle gel does not break up into individual vesicles upon dilution. Due to the stickiness of the emulsion droplets during formation of the vesicle gel, the compartments are packed closely against each other, acquiring a foam-like structure that is characterized by closely packed polyhedra such as dodecahedra and truncated octahedra, as

shown by the scanning electron microscope (SEM) 2-dimensional image of a dried vesicle gel in **Fig. 2a**. Due to the structural change from spherical droplets to foam-like structure, the total interfacial areas increase while the volume of the droplet phase remains constant. A transition from a spherical droplet to a dodecahedron results in about 9.8% increase in total surface area, while a transition to a truncated octahedron leads to a 9.9% increase. Therefore, excess diblock copolymers are added to the oil phase of the emulsion to stabilize the additional interface during the transition. The foam-like structure and the thin membranes separating the compartments produces the high volume fraction of internal phase exhibited by our polymersome gel. Unlike typical HIPE systems, the polymersome gel does not require compression by creaming or sedimentation to achieve high internal phase. The adhesiveness of the W/O emulsion droplets induced by the precipitation of the diblock copolymers adsorbed at the W/O interfaces causes the expulsion of the organic solvents, spontaneously increasing the volume fraction of the internal phase. The membranes of the polymersome gels can also be used for encapsulating lipophilic actives; we demonstrate this by encapsulating a fluorescent NBD-cholesterol, as shown by the fluorescently labeled foam-like edges in **Fig. 2b**. The vesicle gels enable the encapsulation of both hydrophilic and lipophilic active ingredients within the same structure. We demonstrate this by encapsulating hydrophilic FITC-Dextran, and a lipophilic dye in **Fig. 2c**.

Our vesicle gel also enables simple encapsulation of multiple actives within the same encapsulating structures. For the different actives to be encapsulated without cross-contamination, the fluid phases containing different actives have to be separated during fabrication of the encapsulating structures. This is accomplished by designing devices with complicated geometries to separately inject the different phases.[14,15] However, the complexity involved increases with the number of active-containing fluids that need to be encapsulated, limiting the use of multicompartment structures for encapsulation applications. With our approach for encapsulating multiple actives in multicompartment vesicle gels, multiple active-

containing fluid phases can be emulsified separately to form multiple W/O emulsions. These emulsions are subsequently mixed gently and added to the same aqueous phase. Since the actives are contained inside droplets stabilized by diblock copolymers, cross-contamination can be avoided. We demonstrate this by encapsulating FITC-Dextran and RhoB-Dextran separately in the polymersome gel; after addition of the two W/O emulsions to the same continuous phase, there is no cross-contamination between the pink and the yellow droplets, as shown in **Fig. 3a**. Upon evaporation of solvents, the droplets become adhesive and dewet to form a multicompatment vesicle gel that encapsulates FITC-Dextran and RhoB-Dextran separately, as shown by the separation of the pink and the yellow regions in the polymersome gel in **Fig. 3b**. By preparing different types of W/O emulsions containing different hydrophilic actives, the process can be easily adapted to encapsulate multiple actives in the polymersome gels. This highlights the versatility of this approach for encapsulating multiple active ingredients. Our approach also does not require the use of any specialized equipment or subsequent polymerization steps to solidify the gel; the resultant gel is a true vesicle suspended in an aqueous environment. The gel has a high porosity and tunable pore sizes for cell seeding, In addition, nutrients can diffuse across the thin membranes separating the compartments throughout the whole structure; thus,it has great potential as a scaffold for tissue engineering by allowing cell attachment and growth while remaining encapsulated. Due to the biodegradability of PEG-b-PLA, the gel can also be absorbed by the surrounding tissues without the need for subsequent surgical removal. Nevertheless, using the current technique, it remains difficult to separately encapsulate multiple lipophilic actives, which get mixed within the organic solvents before evaporation. A novel strategy for segregating the different lipophilic actives in separate regions within the membrane is needed.

In summary, we have developed a new approach for fabricating multicompartment polymersome gels by dewetting of a W/O emulsion into an aqueous continuous phase. The resultant polymersome gels have a foam-like structure with high volume fraction of the

internal phase for encapsulation. We demonstrate that while the compartments of the polymersome are ideal for hydrophilic actives, lipophilic actives can also be encapsulated in the membrane of the vesicle gel. Our approach is highly versatile and allows encapsulation of multiple active ingredients without cross-contamination. By applying our approach to block copolymers with appropriate properties and functionalities, the polymersome gel can be customized for specific applications. This would produce more complex polymersome membranes, for example, with tunable mechanical properties, permeabilities, binding affinities to biomolecules and selectivities towards different chemical species.

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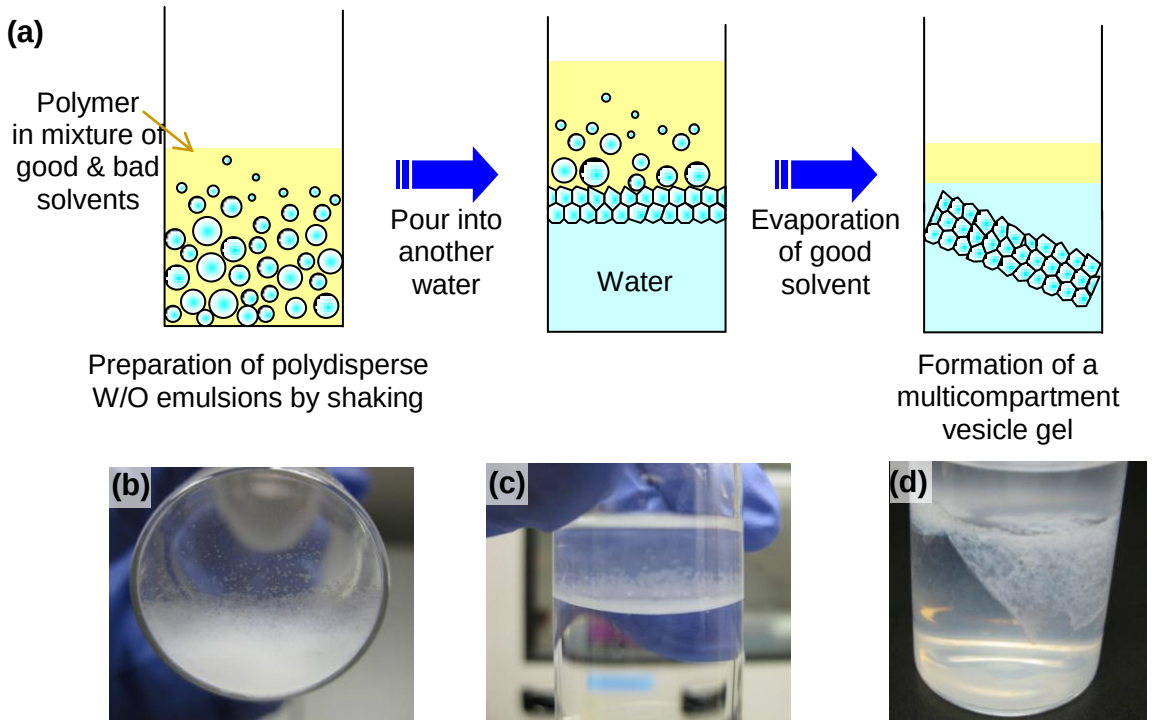

Figure 1: a) Schematic of the fabrication of multicompartment vesicle gel; b-d) photographs of b) a water-in-oil (W/O) emulsion with water droplets dispersed in a mixture of chloroform and hexane (36:64 v/v) with 10 mg/mL PEG(5000)-b-PLA(5000) dissolved in it, and c) the same W/O emulsion floating on a layer of water. d) The multicompartment vesicle gel forms after evaporation of some of the volatile solvent.

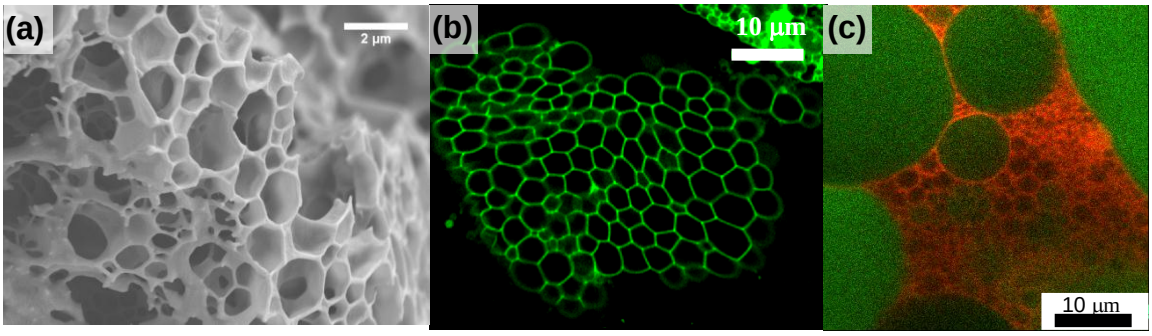

Figure 2: a) Scanning electron microscope (SEM) image of a dried vesicle gel; b-c) confocal laser scanning images of b) multicompartment vesicle gels with NBD-cholesterol in the membrane walls separating the compartments, and c) multicompartment vesicle gels with FITC-Dextran inside the compartments and trace amount of Pyrromethene 605 in the membrane walls.

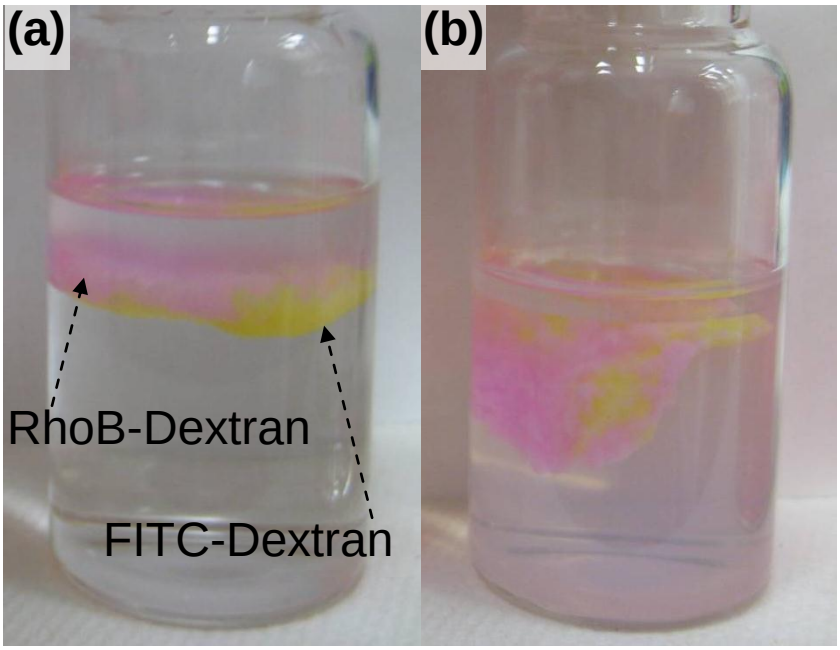

Figure 3: Photographs of a) a water-in-oil (W/O) emulsion with droplets of water with FITC-Dextran and water with RhoB-Dextran dispersed in a mixture of chloroform and hexane (36:64 v/v) with 10 mg/mL PEG(5000)-b-PLA(5000) floating on a layer of water, and b) the resulting multicompartment vesicle gel encapsulating FITC-Dextran and RhoB-Dextran separately.

Multicompartment polymersome gels are fabricated by dewetting of a block copolymer-stabilized water-in-oil (W/O) emulsion into an aqueous continuous phase. The resultant polymersome gels have a foam-like structure with high volume fraction of the internal phase for encapsulation of hydrophilic actives and large membrane surface area for encapsulation of lipophilic actives.

Polymeric Materials

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Multicompartment polymersome gel for encapsulation

ToC figure ((55 mm broad, 40 mm high))