Colloido-polymersomes: capsules consisting of a composite layer of particles and polymer

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ABSTRACT

We present an approach to fabricate capsules consisting of a composite layer of particles and block copolymers . The capsules are fabricated by directed assembly of block copolymers at the interfaces of a double emulsion template and electrostatic adsorption of particles onto the copolymers. The double emulsion drops are prepared using capillary-based microfluidics. The solvent phase of the double emulsion is then evaporated to yield polymersomes. The particles are locked together on the surface of the polymersomes. The resultant structures, which we call "colloido-polymersomes", are hollow shells of particle-coated thin polymer films. Our approach provides a simple strategy for fabricating vesicles with a complex structure for encapsulation-related applications; by tuning the characteristics of the particles attached, the final vesicles can be used for attaching biomolecules such as proteins and DNA, and thus are useful for biomedical applications such as biosensing.

Keywords: vesicles, polymersomes, colloidosomes, colloids, microfluidics

1 INTRODUCTION

Amphilic molecules such as phospholipids and surfactants are known to form aggregates in aqueous solution to minimize the exposure of the hydrophobic segments of the molecules to water; vesicles are one such aggregate structures. Vesicles, which are compartments of aqueous cores surrounded by a membrane, have demonstrated excellent potential for encapsulation of actives ranging from biomolecules, drugs, flavors, growth factors, to cells for applications in biomedical engineering, pharmaceuticals, cosmetics as well as fundamental biological studies. Natural membranes such as cell membranes are examples of vesicles. As a result, vesicles are not only of interest as biochemical reactors but also as model structures for fundamental understanding of natural membranes. The constituent amphiphiles of natural membranes include phospholipids, skin lipids and other naturally-occuring lipids. Recently, amphiphilic block copolymers have been used to form vesicles that have a more robust membrane when compared with phospholipid vesicles.[1] Advances in synthetic chemical techniques enable the tuning of the properties of block copolymers to achieve different functionalities, facilitating the manipulation of the applicability of the resultant vesicles. Therefore, block copolymer vesicles, or polymersomes are of increasing interest as an encapsulating structure. Another approach to strengthen the mechanical properties of vesicles while maintaining the tunability of their functionalities is by replacing the small amphiphilic molecules that form the membrane layer with a layer of particles, resulting in an alternative type of vesicles known as colloidosomes.[2, 3] By choosing particles of different sizes and nature, properties of the colloidosomes such as membrane permeabilities and rigidity can be manipulated. While colloidosomes are mechanically more robust than polymersomes, polymersomes achieve better encapsulation of active ingredients with a small molecular size. Therefore, it is advantageous to combine the benefits of both types of vesicles; a robust approach to form such novel vesicles is needed.[4]

Traditionally, polymersomes are prepared by hydrating dried films of amphiphilic block copolymers; the thin films become gradually swollen and fold into vesicular structures. This procedure can be assisted by the application of an alternating electric field in a process called electroformation. Alternatively, polymersomes are also formed by mixing a amphiphile-rich solvent phase with a miscible anti-solvent phase, inducing the amphiphiles to precipitate out following the reduction in their solubility upon mixing of the solvent and the anti-solvent.[5, 6] While these techniques are simple and robust, little control can be achieved due to the self-assembly nature of the processes. Colloidosomes are typically prepared by emulsion-templated methods, where colloidal suspensions are used as one of the emulsion phases. The colloidal particles are attracted to the interfaces of the emulsions to minimize the total energy of the system, forming particle-coated droplets.[2] Similar emulsion-templated approach has recently been applied to prepare polymersomes; in this case, water-in-oil-in-water (W-O-W) double emulsions are used as templates.[7-9] The amphiphilic block copolymers are first dissolved in a solvent phase that forms the shell layer of the double emulsions. The solvent is then removed through processes such as evaporation so that the amphiphiles attached to the inner-middle and middle-outer interfaces aggregate to form a bilayer membrane. By generating the double emulsion templates using microfluidic techniques, high uniformity in sizes as well as

enhanced encapsulation efficiency has been achieved. The level of compartmentalization enabled by the technology creates new opportunities to make novel core-shell structures with sophisticated shell compositions.

In this work, we have combined the double-emulsion-templated approach for fabricating polymersomes and electrostatic attachment of charged colloidal particles onto charged block copolymers to prepare particle-armored polymersomes, which we call colloido-polymersomes. A schematic of the process is shown in Fig. 1.

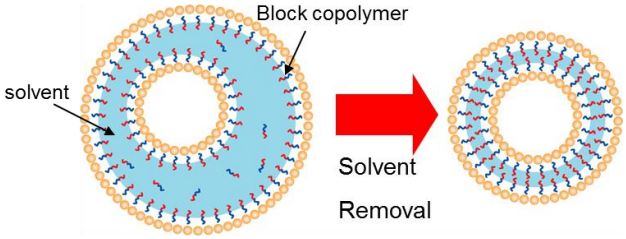

Figure 1: Schematic illustrating the approach for forming colloido-polymersomes from double emulsion templates.

2 EXPERIMENTAL SECTION

Materials. Materials used to prepare the outer phase were water (18.2 MΩ•cm-1, Millipore Milli-Q system), and poly(vinyl alcohol) (PVA; Molecular weight Mw: 13,000-23,000 g•mol-1, 87-89% hydrolyzed, Sigma-Aldrich Co.). The middle aqueous phase was cationic diblock copolymer poly(butadiene-b-N-methyl-4-vinyl-pyridinium iodide) (PBd(120000)-b-P4VPQI(28200), Polymer Source Inc.), neutral diblock copolymer poly(ethylene-glycol)-b-polylactic acid (PEG(5000)-b-PLA(5000), Polysciences Inc.) or anionic diblock copolymer poly(styrene-b-acrylic acid) (PS(16000)-b-PAA(4300), Polymer Source Inc.) dissolved in mixtures of toluene, chloroforme and hexanes. The inner oil phase is made up of water or Trizma buffer solution (pH 7.2, Sigma Aldrich, Inc.) with fluorescent plain silica particles (diameter: 500 nm, Interfacial Dynamics Inc.) or crosslinked latex particles (diameter: 500nm, Interfacial Dynamics Inc.).

Microfluidics. The capillary microfluidic devices consist of coaxial assemblies of round and square glass capillaries on glass slides.[10, 11] For the microfluidic device for fabricating single-core double emulsions, the round glass capillary tubes (World Precision Instruments) with outer and inner diameters of 1.0mm and 580μm, respectively, were tapered to the desired orifice using a capillary puller (Sutter Instrument, P-97) and a microforge (Narishige, MF-830). the diameters of typical orifices of the capillaries for the inner phases and for collecting the final emulsions were 10-30μm and 50-200μm, respectively. The tip of the capillary for injection of the inner phase was coated with a hydrophobic reagent (Trimethoxy(octadecyl)silane, Sigma-Aldrich Co.). The tapered capillaries for the inner phase and for collecting the final emulsions were then fitted into a 1.05mm inner diameter square capillary (AIT Glass) with a distance of 50-150μm. A transparent epoxy resin was used to seal the tubes where required. To generate the double emulsion templates, all the fluids were pumped into the capillary microfluidic device using syringe pumps (Harvard PHD 2000 series), as shown in Fig. 2. A typical set of flow rates for the outer, middle, and inner phases were 800, 1800, and 5000 μL/hr, respectively. The generated emulsions were collected in glass vials filled with water or Trizma buffer solution with nanoparticles. The collected samples were allowed to evaporate under vacuum for 1-2 days.

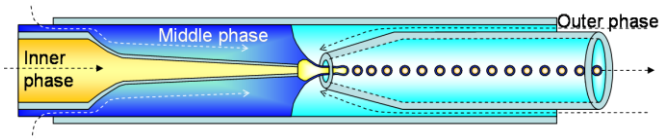

Figure 2: Schematic of the glass-capillary microfluidic devices for generating the double emulsion templates. The inner and outer phases contain the charged colloidal particles for attaching to the surface of the diblock copolymers, which is dissolved in a solvent and injected as the middle phase.

Characterization. The double emulsion generation processes in our capillary microfluidic device were monitored using an inverted optical microscope (DM-IRB, Leica) fitted with a fast camera (Phantom V9, Vision Research). Bright-field images and fluorescence images were obtained with 10× objectives at room temperature using an inverted microscope with fluorescence (DMIRBE, Leica Microsystems, Inc.) equipped with a digital camera (QICAM 12-bit, QImaging, Inc.).

3 RESULTS & DISCUSSION

To prepare the colloido-polymersomes, we generate monodisperse double emulsions in glass-capillary microfluidic devices. We first encapsulate particles that we desire to decorate the inner wall of the polymersomes with inside the inner droplets of the double emulsions. Due to electrostatic attraction, the negatively charged plain silica particles are adsorbed onto the surface of the positively charged PBd(120000)-b-P4VPQI(28200) inside the inner droplets, as shown in Fig. 3. When the focal plane is near the bottom of the inner droplets, fluorescently labelled particles can be distinctly observed on the surface of the inner droplets(Fig. 3A). As we gradually move the focal plane towards the top plane of the droplets, a ring of distinct particles can be seen at the inner-middle interfaces while blurry green halos due to particles in planes that are out of focus are observed near the center of the droplets (Fig. 3B-E). On the top plane of the inner droplets, clear fluorescent particles are present but at a lower number density than in the bottom plane since the silica particles are heavier than the surrounding medium (Fig. 3F). These indicate that the particles are indeed adsorbed on the diblock copolymers at

the inner-middle interfaces. To confirm this, we also repeated the same experiments with diblock copolymers that are neutral (PEG(5000)-b-PLA(5000)) and that have the same charge (PS(16000)-b-PAA(4300)) such that electrostatic attraction is not expected. With these diblock copolymers, no distinct particles are observed at the inner-middle interfaces; instead the fluorescent silica particles remain evenly dispersed in the inner droplets, as shown by uniform fluorescence in the inner droplets of the double emulsion drops in Fig. 4. This agrees with our idea that the armor of particles on the surface of the block copolymer membranes are adsorbed electrostatically.

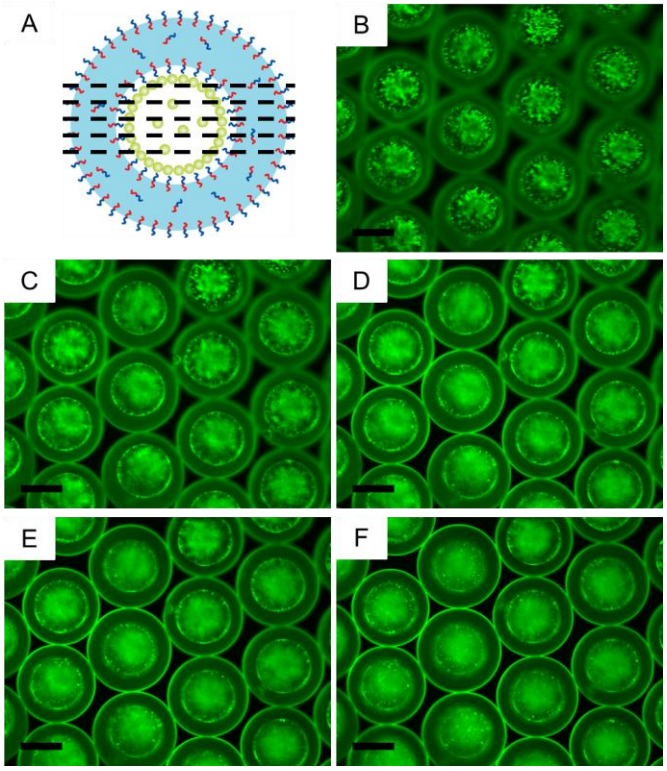

Figure 3: (A) Schematic of the double emulsion template for forming the colloido-polymersomes. The dotted lines indicate the different planes along which the double emulsion templates are imaged in Fig. 3B-F. (B-F) Fluorescent microscope images of the double emulsion templates with fluorescent plain silica particles (500nm) in inner droplets stabilized by a cationic diblock copolymers imaged at different heights. Scale bars are 80 μm.

After forming these particle-laden inner droplets, the double emulsion drops are converted into polymersomes through solvent evaporation, as demonstrated previously[7, 12]. While the inner walls of the membranes are adorned with particles initially added to the innermost phase, the outer walls are decorated with particles added subsequently to the solutions in which the double emulsions are collected. After complete solvent removal, the colloidal particles remain attached to the surface of the polymersomes, as demonstrated by the texture shown on the surface of the colloido-polymersome in Fig. 5A and by

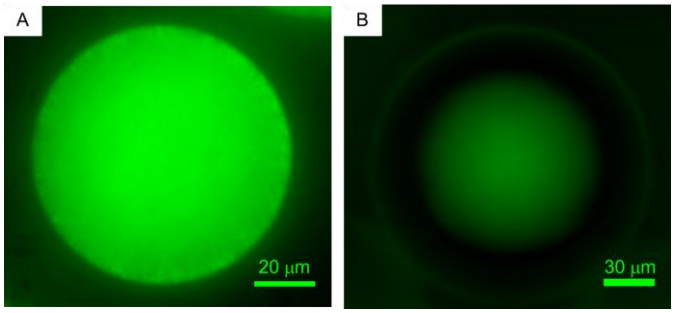

Figure 4: Fluorescent microscope images of the double emulsion templates with fluorescent plain silica particles (500nm) in the inner droplet stabilized by a (A) neutral and (B) anionic diblock copolymers.

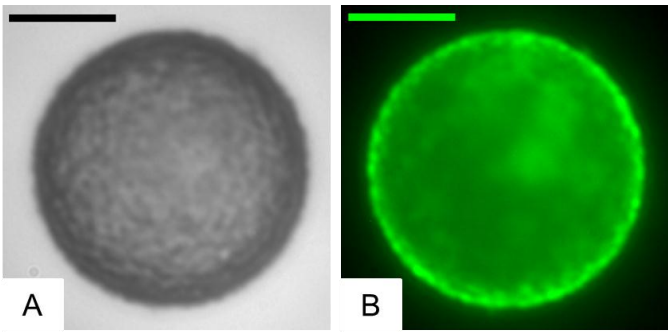

Figure 5: (A) Optical microscope image and (B) fluorescent microscope image of a colloido-polymersomes. All scale bars are 10 μm.

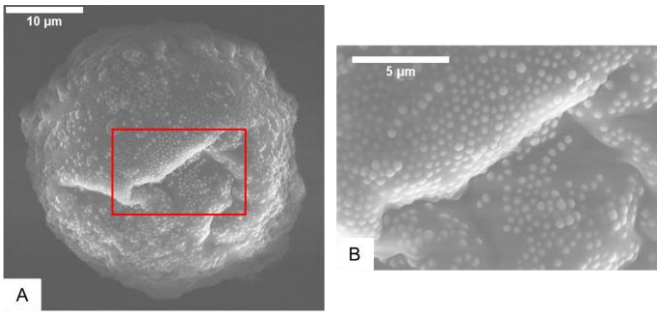

Figure 6: Scanning electron microscope (SEM) images of (A) a crumpled colloido-polymersome; (B) is a magnified image of the area in the red square shown in A.

the fluorescent shell in Fig. 5B. The resultant colloido-polymersomes exhibit excellent mechanical rigidity; upon drying, their shells are only buckled but remains unruptured by stresses induced by drying, as shown in the scanning electron microscope image of the dried colloido-polymersomes in Fig. 6. The enhanced rigidity can be attributed to the particle layer embedded inside the polymer matrix. Due to the compartmentalization enabled by the microfluidic technology, this approach allows different particles to be added to the two sides of the membranes, offering versatility to engineer the properties and functionality of the two sides of the membranes. For instance, the inner wall can be coated with particles with

specific binding to the active ingredients to be delivered for enhanced encapsulation efficiency, whereas the outside wall can be armored with particles with specific ligands to attach to the target organs. This illustrates how the colloido-polymersomes present a novel way to achieve targeted delivery. In addition, the colloido-polymersomes can also be used as a microscopic bioreactors to mimic natural cells.

4 CONCLUSION

We have introduced a microfluidic approach to prepare colloidal-particle-armored polymersomes or colloido-polymersomes through double-emulsion-templating and electrostatic attraction of particles to charged diblock copolymers. The resultant particle-coated polymer membrane shows excellent mechanical rigidity and enables encapsulation of small active ingredients. Apart from the increased mechanical stability, the colloidal particle layers allows modification of the properties and functionality of the two sides of the polymer membranes through attachment of different functional particles. Our approach represents a first demonstration of this concept by combining microfluidic compartmentalization and electrostatic attraction; this should also be easily adapted to the attachment of biomolecules, such as DNA, proteins and cells, to membranes of vesicles for applications including targeted delivery and mimicking of natural cell aggregates[13].

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