Multicompartment Polymersomes from Double Emulsions

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to the inner droplet phase to match the osmolarities of the inner and outer phases thus preventing net diffusion of water across the shell phase. In the middle phase of the double emulsions, the amphiphilic diblock copolymers, PEG(5000)-b-PLA(5000) adsorbs at the O/W and W/O interfaces. The composition of the middle phase is chosen to facilitate dewetting of the double emulsion induced by adhesion of copolymer monolayers at the interfaces.\cite{18} We demonstrate such a process with double emulsion drops with 2, 3 and 8 inner droplets. As chloroform in the middle shell phase evaporates, the diblock copolymers at the interfaces become less soluble in the solvent; as a result, the interfaces become adhesive, leading to dewetting. The inner droplets stick to each other, and the inner-middle interfaces also adhere with the middle-outter interface, expelling the solvent in the shell layer to form drops of solvent attached to the sticky inner droplets, as illustrated in Scheme 1. The region between the two polymer-laden interfaces provides an hydrophobic environment that enables encapsulation of hydrophobic compounds in the membrane, as shown in Fig. S2 in Supporting Information. Polymer vesicles with 2, 3 and 8 compartments are formed after removing the solvent drops either by evaporation, or due to shear in microfluidic flow,\cite{19} the dewetted drops and the resultant polymersomes are shown in Fig. 1B & C. This double emulsion-templated approach can also be applied to systems with different solvent mixtures, and diblock copolymers with different block lengths. (See Fig. S3 in Supporting Information)

With our approach, the number of compartments of the final polymersomes is fixed by the number of inner droplets in the double emulsion templates, which can be tuned by varying flow rates of the three phases. In the absence of osmotically driven transport of water across the shells of the double emulsion, the sizes of the compartments in the final polymersomes are also controlled by the sizes of the inner droplets (See Supporting Information). Using this approach, we have fabricated polymersomes with number of compartments ranging from one to eight, as shown in Fig. 2. The polydispersity in terms of the number of compartments is low for small number of compartments. (See Fig. S4 in Supporting Information.) Due to the nature of the vesicle formation approach, the spatial configuration of the compartments is not unique. As soon as sufficient chloroform is removed from the solvent phase, the reduced solubility of the diblock copolymers provides a driving force for the copolymers to aggregate; therefore, the copolymer-laden interfaces attract each other. This suggests that the process is kinetically controlled and does not allow rearrangement of the inner droplets in the step of double emulsion-to-polymersome transition. As a result, for polymersomes with the same number of compartments, the spatial arrangement of the compartments is not unique. The inner droplets may have different relative orientations in different double emulsion drops when the interfaces become adhesive, thus compartments in polymersomes with the same number of compartments can have different spatial arrangements, as shown in Fig. 2C, D & H. The total membrane area of the polymersome is set by the total interfacial area of all the inner droplets. The shape of the polymersome is controlled by the contact angle between the inner droplets during dewetting, which in turn is determined by the strength of the adhesion between the copolymer monolayer as predicted by the Young-Dupré equation.\cite{13} There is no theoretical limit to the number of compartments that this approach enables; we demonstrate this by fabricating polymersomes with tens of compartments (See Fig. S2A-C in Supporting Information). Our approach provides a robust and versatile way to fabricate polymersomes with controlled number of compartments.

The ability to fabricate vesicles with multiple compartments creates new opportunities for encapsulating multiple actives within the same vesicular structures. This requires the capability to create double emulsions with not only multiple inner droplets, but also inner droplets containing different contents. To accomplish this, we design a microcapillary device using a round capillary with two separate microchannels\cite{14} for injection of the two distinct inner phases of the double emulsions, as shown schematically in Fig. 3A. Similar techniques have previously been demonstrated in two-dimensional microfluidic devices.\cite{10,15} Using our modified devices, we have generated double emulsions with two inner phases containing different model encapsulants, one with a fluorescein isothiocyanate-dextran (FITC-Dextran) solution, and the other with a PEG solution. The osmolarities of the two phases are matched to avoid net diffusion of water across the droplets. The double emulsion collected undergoes dewetting to form multicompartiment polymersomes whose structure is illustrated in Fig. 3B. With fluorescence and optical microscopy techniques, we observe encapsulation of the fluorescent FITC-Dextran solution and the non-fluorescent PEG solution in separate compartments of the resultant polymersomes without cross-contamination, as shown in Fig. 3C & D. This highlights the effectiveness of our approach for separately encapsulating different active ingredients and the potential of multicompartiment polymersomes as a novel encapsulating system in drug and vaccine delivery.\cite{16} Moreover, these polymersomes are ideal for encapsulating reactants for triggered reactions, since they allow tuning of the amount of reactants according to the stoichiometric ratio of the desired reactions by adjusting the number of compartments that contains the different reactants.

In summary, we have shown that polymersomes with multiple compartments can be fabricated by using double emulsion with different morphology as templates. With capillary microfluidic devices, the number of inner droplets in the double emulsion can be controlled by adjusting the flow rates of the phases. The transition from double emulsion to polymersomes is induced by the reduction in solubility of the diblock copolymers in the shells of the double emulsions, which leads to the adhesion of the copolymer-laden interfaces. Our approach provides a unique way to fabricate multicompartiment vesicles that could be utilized for encapsulation of multiple actives. To that end, we have demonstrated the encapsulation of multiple model encapsulants separately using a modified capillary microfluidic device. This creates new opportunities to use these multicompartimental polymersomes as controlled reaction vessels that enable triggered reactions with controlled stoichiometry of the reactants. Moreover, our approach is general and should also enable fabrication of controlled liposomes with multiple compartments.

Scheme 1. Schematic for the formation of multicompartiment polymersomes from double emulsion drops with multiple inner droplets.
Figure 1. A) Generation of double emulsion drops with multiple inner droplets in a glass capillary microfluidic device; B) Optical microscopy images showing dewetted double emulsion drops with (left) two, (middle) three, and (right) eight inner droplets; C) Optical microscopy images of PEG(5000)-b-PLA(5000) polymersomes with (left) two, (middle) three, and (right) eight, after complete removal of the oil phase of the double emulsions. Scale bars are 50 μm.

Figure 2. Optical microscope images of PEG(5000)-b-PLA(5000) polymersomes with A) one, B) two, C) three, D) four, E) five, F) six, G) seven and H) eight compartments. The orientation of the compartments is not unique for polymersomes with the same number of compartments, as shown in C, D and H. Scale bars are 30 μm.

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Vesicles with multiple compartments: A microfluidic technique is used to generate polymersomes with multiple compartments using double emulsion with different morphology as templates. Our approach provides a unique way to fabricate multicompartment vesicles that could be utilized for encapsulation of multiple actives. This also creates new opportunities to use these polymersomes as controlled reaction vessels that enable triggered reaction with controlled stoichiometry of the reactants.