Polymer Vesicles

DOI: 10.1002/ange.201006023

Multicompartment Polymersomes from Double Emulsions**

*Ho Cheung Shum, Yuan-jin Zhao, Shin-Hyun Kim, and David A. Weitz**

Polymersomes are vesicles which consist of compartments surrounded by membrane walls that are composed of lamellae of block copolymers.[1] They are important for numerous applications in encapsulation and delivery of active ingredients such as food additives, drugs, fragrances, and enzymes.[2] Polymersomes are typically prepared by precipitating block copolymers from their solvents through addition of a poor solvent for the copolymers,[3] or by rehydrating a dried film of the copolymers.[4] The unfavorable interactions between blocks in the copolymer and the poor solvent induce formation of aggregate structures ranging from micelles, wormlike micelles, and vesicles. However, the resultant polymersomes are highly polydisperse and have poor encapsulation efficiency. Recently, a new approach has been developed to fabricate monodisperse polymersomes by using double emulsions as templates.[5] Water-in-oil-in-water (W/O/W) double emulsions with a core–shell structure are first prepared in capillary microfluidic devices.[6] Diblock copolymers, dissolved in the oil shell phase, assemble into the walls of the polymersomes upon removal of the oil by evaporation[5a,7] after adhesion of the diblock copolymer-adsorbed interfaces.[8] This approach leads to polymersomes with high size uniformity and excellent encapsulation efficiency and also enables precise tuning of the polymersome structures.

Advances in techniques for fabricating polymersomes have led to controlled spherical polymersomes with a single compartment. However, non-spherical capsules with multiple compartments also have great potential for encapsulation and delivery applications.[9] By storing incompatible actives or functional components separately, polymersomes with multiple compartments can achieve encapsulation of multiple actives in single capsules and reduce the risk of cross-contamination. Moreover, multiple reactants can be encapsulated separately to allow reaction upon triggering. By tuning the number of compartments containing reactant, the stoichiometric ratio of the reactants for each reaction can be manipulated. These multicompartment polymersomes will create new opportunities to deliver not only multiple functional components, but also multiple reactants for reactions on demand. In addition, with the versatility of synthetic polymer chemistry to tune properties such as polymer length, biocompatibility, functionality, and degradation rates, non-spherical polymersomes with multiple compartments can be tailored for specific delivery targets. However, polymersomes that have been reported to date are almost exclusively spherical in shape, and have only one compartment. Since most conventional polymersome fabrication processes rely on self-assembly of the block copolymer lamellae, little control over the size and structure of the resultant polymersomes is achieved. With the conventional emulsion-based methods, non-spherical droplets are also not favored because interfacial tension between the two immiscible phases favors spherical droplets, which have the smallest surface area for a given volume. Recent advances in microfluidic technologies[10] enable high degree of control in droplet generation, and ease in tuning the device geometry. This offers a new opportunity to fabricate double emulsion with controlled morphology,[11] which serve as templates for fabricating the non-spherical multicompartment polymersomes. However, such investigations have not, as yet, been carried out.

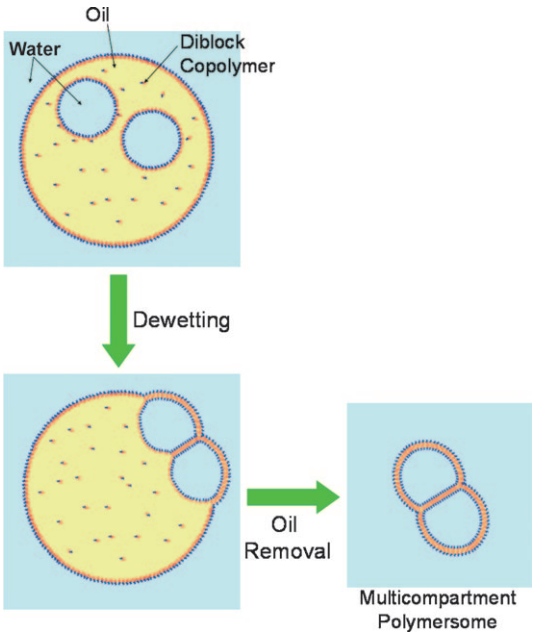
Here, we demonstrate the generation of non-spherical polymersomes with multiple compartments. We use glass capillary microfluidics to prepare W/O/W double emulsions with different number of inner aqueous drops. These emulsions are initially stabilized by the amphiphilic diblock copolymers in the oil shells, which consist of a mixture of a volatile good solvent and a less volatile poor solvent for the copolymers. As the good solvent evaporates, the copolymers at the W/O and the O/W interfaces are attracted towards each other to form the membranes. As a result, neighboring inner droplets adhere to one another and this leads to formation of multicompartment polymersomes, as illustrated in Scheme 1. We also use a modified glass capillary device for generating double emulsions with two distinct inner phases containing different encapsulants. This process leads to the fabrication of non-spherical polymersomes with multiple compartments for separate encapsulation of multiple actives.

A glass capillary microfluidic device is used to generate double emulsions with controlled morphology (see Figure S1 in the Supporting Information).[6b] Due to the high degree of control afforded by microfluidics, the number of inner droplets in a W/O/W double emulsion system can be controlled by varying the flow rates of the three phases independently.[9,12] An example of the process is shown in Figure 1 a. The thickness of the double emulsion shells can be adjusted by changing the flow rates. However, as long as the flow rates are not altered enough to change the number of

[*] Dr. H. C. Shum,[+] Y. J. Zhao, Dr. S. H. Kim, Prof. D. A. Weitz
School of Engineering and Applied Sciences, Department of Physics and Kavli Institute for Bionano Science and Technology, Harvard University, Cambridge, MA 02138 (USA)
E-mail: weitz@seas.harvard.edu
Homepage: http://www.seas.harvard.edu/projects/weitzlab/
Y. J. Zhao
State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096 (China)
[+] Current address: Department of Mechanical Engineering
University of Hong Kong
Hong Kong (China)

[**] This work was supported by Amore-Pacific Co., the NSF (DMR-1006546), the Harvard MRSEC (DMR-0820484), and BASF.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201006023.



Scheme 1. Formation of multicompartment polymersomes from double emulsion drops with multiple inner droplets.

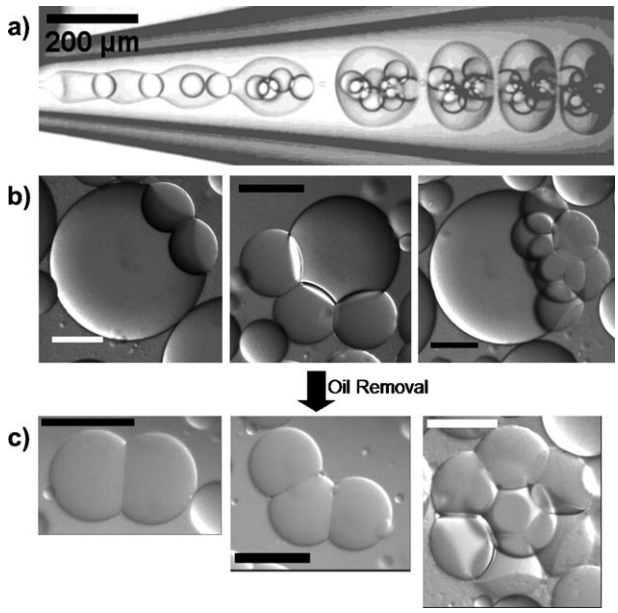


Figure 1. a) Generation of double emulsion drops with multiple inner droplets in a glass capillary microfluidic device. b) Optical microscopy images showing dewetted double emulsion drops with two (left), three (middle), and eight inner droplets (right). Scale bars are 50 µm. c) Optical microscopy images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

inner droplets of the double emulsion templates, change in shell thickness does not affect the morphology of the final polymersomes since all solvents in the shells is removed in subsequent steps. To prepare the double emulsion templates, multiple inner droplets are dispersed in drops of a mixture of chloroform and hexanes (36:64 v/v) with 10 mg mL^{-1} poly(ethylene glycol)-*b*-poly(lactic acid), (PEG(5000)-*b*-PLA(5000)). The drops-in-drops are suspended and stabilized in a poly(vinyl alcohol) (PVA) solution. A homopolymer, PEG, is added to the inner droplet phase to match the osmolalities 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.[8] We demonstrate such a process with double emulsion drops with two, three, and eight 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-outer 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 a hydrophobic environment that enables encapsulation of hydrophobic compounds in the membrane, as shown in Figure S2. Polymer vesicles with two, three, and eight compartments are formed after removing the solvent drops either by evaporation, or due to shear in microfluidic flow.[8] The dewetted drops and the resultant polymersomes are shown in Figure 1 b and c. This double emulsion-templated approach can also be applied to systems with different solvent mixtures, and diblock copolymers with different block lengths (see Figure S3).

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 Figure 2. The polydisperisty in terms of the number of compartments is low when the number of compartments is small (see Figure S4). 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 arrange-

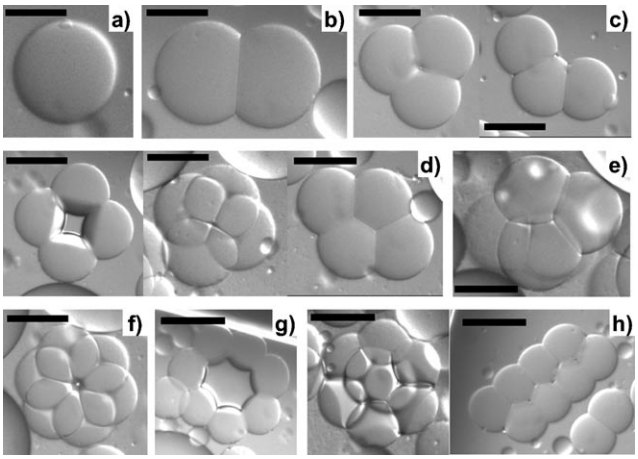


Figure 2. Optical microscope images of PEG(5000)-*b*-PLA(5000) polymersomes with a) one, b) two, c) three, d) four, e) five, f) six, g) seven, and h) eight compartments. The orientation of the compartments is not unique for polymersomes with the same number of compartments, as shown in (c), (d), and (h). Scale bars are 30 μm.

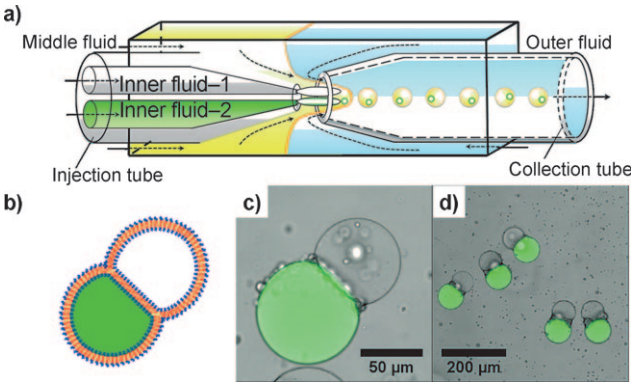


Figure 3. a) Capillary microfluidic device for preparing double emulsions with two distinct inner phases. b) Polymersome with two compartments for encapsulating two different actives. c,d) Overlays of optical microscope images and fluorescence microscope images of c) a PEG(5000)-*b*-PLA(5000) polymersome with FITC-Dextran in one compartment and PEG in the other compartment, and d) a monodisperse population of PEG(5000)-*b*-PLA(5000) polymersomes encapsulating FITC-Dextran and PEG separately in their two compartments.

ments, as shown in Figures 2c, d, and h. The total membrane area of the polymersomes is set by the total interfacial area of all the inner droplets. The shape of the polymersomes 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.[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 Figure S2 a–c). 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 have designed a microcapillary device using a round capillary with two separate microchannels[14] for injection of the two distinct inner phases of the double emulsions, as shown in Figure 3a. Similar techniques have previously been demonstrated in two-dimensional microfluidic devices.[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 osmolalities of the two phases are matched to avoid net diffusion of water across the droplets. The double emulsion collected undergoes dewetting to form multicompartment polymersomes whose structure is illustrated in Figure 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 Figure 3c and d. This highlights the effectiveness of our approach for separately encapsulating different active ingredients and the potential of multicompartment polymersomes as a novel encapsulating system in drug and vaccine delivery.[16] Moreover, these polymersomes are ideal for encapsulating reactants for triggered reactions, since they allow tuning of the amount of reactants according to the stochiometric 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 multicompartment 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 multicompartmental polymersomes as controlled reaction vessels that enable triggered reactions with controlled stoichometry of the reactants. Moreover, our approach is general and should also enable fabrication of controlled liposomes with multiple compartments.

Received: September 26, 2010
Revised: November 15, 2010
Published online: January 7, 2011

Keywords: emulsions · membranes · microfluidics · polymers · vesicles

[1] B. M. Discher, Y. Y. Won, D. S. Ege, J. C. M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, *Science* **1999**, *284*, 1143 – 1146.

[2] S. F. M. van Dongen, W. P. R. Verdurmen, R. J. R. W. Peters, R. J. M. Nolte, R. Brock, J. C. M. van Hest, *Angew. Chem.* **2010**, *122*, 7371 – 7374; *Angew. Chem. Int. Ed.* **2010**, *49*, 7213 – 7216.

[3] a) H. W. Shen, A. Eisenberg, *Macromolecules* **2000**, *33*, 2561 – 2572; b) H. W. Shen, A. Eisenberg, *J. Phys. Chem. B* **1999**, *103*, 9473 – 9487.

[4] a) M. Antonietti, S. Forster, *Adv. Mater.* **2003**, *15*, 1323 – 1333; b) H. Kukula, H. Schlaad, M. Antonietti, S. Forster, *J. Am. Chem. Soc.* **2002**, *124*, 1658 – 1663.

[5] a) H. C. Shum, J. W. Kim, D. A. Weitz, *J. Am. Chem. Soc.* **2008**, *130*, 9543 – 9549; b) H. C. Shum, D. Lee, I. Yoon, T. Kodger, D. A. Weitz, *Langmuir* **2008**, *24*, 7651 – 7653.

[6] a) R. K. Shah, H. C. Shum, A. C. Rowat, D. Lee, J. J. Agresti, A. S. Utada, L. Y. Chu, J. W. Kim, A. Fernandez-Nieves, C. J. Martinez, D. A. Weitz, *Mater. Today* **2008**, *11*, 18 – 27; b) A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone, D. A. Weitz, *Science* **2005**, *308*, 537 – 541.

[7] a) R. C. Hayward, A. S. Utada, N. Dan, D. A. Weitz, *Langmuir* **2006**, *22*, 4457 – 4461; b) E. Lorenceau, A. S. Utada, D. R. Link, G. Cristobal, M. Joanicot, D. A. Weitz, *Langmuir* **2005**, *21*, 9183 – 9186.

[8] H. C. Shum, E. Santanach-Carreras, J. W. Kim, A. Erhlicher, J. Bibette, D. A. Weitz, unpublished results, **2010**.

[9] D. Lee, D. A. Weitz, *Small* **2009**, *5*, 1932 – 1935.

[10] S. Okushima, T. Nisisako, T. Torii, T. Higuchi, *Langmuir* **2004**, *20*, 9905 – 9908.

[11] H. C. Shum, A. R. Abate, D. Lee, A. R. Studart, B. G. Wang, C. H. Chen, J. Thiele, R. K. Shah, A. Krummel, D. A. Weitz, *Macromol. Rapid Commun.* **2010**, *31*, 108 – 118.

[12] K. L. Lao, J. H. Wang, G. B. Lee, *Microfluid. Nanofluid.* **2009**, *7*, 709 – 719.

[13] a) M. P. Aronson, H. M. Princen, *Nature* **1980**, *286*, 370 – 372; b) H. M. Princen, M. P. Aronson, J. C. Moser, *J. Colloid Interface Sci.* **1980**, *75*, 246 – 270.

[14] B. J. Sun, H. C. Shum, C. Holtze, D. A. Weitz, *ACS Appl. Mater.* **2010**, DOI: 10.1021/am100860b..

[15] P. Panizza, W. Engl, C. Hany, R. Backov, *Colloids Surf. A* **2008**, *312*, 24 – 31.

[16] a) Y. Barenholz, E. Kedar, Y. Louria-Hyaon, A. Joseph, E. Raz, K. Takabayshi, WO Patent WO/2003/000,232, **2003**; b) L. S. Mendonça, J. N. Moreira, M. C. P. de Lima, S. Simões, *Biotechnol. Bioeng.* **2010**, *107*, 884 – 893; c) C. Hatzifoti, A. Bacon, H. Marriott, P. Laing, A. W. Heath, *PLoS One* **2008**, *3*, e2368.

