Polymersomes are vesicular self-assemblies of amphiphilic diblock copolymers; they consist of a spherical compartment enclosed by a macromolecular bilayer and have great potential as encapsulation and release systems. They offer enhanced mechanical and structural stability as compared to vesicles made from phospholipids or detergents. By tailoring block lengths, block chemistry and functionalization of the copolymers, polymersomes with controlled biological, chemical, and physical properties can be formed. Typically, polymersomes are formed by techniques such as film rehydration, electroformation, phase transfer, and ultrasonication. These techniques rely on the undirected self-assembly of the copolymers, and typically lead to polymersomes with broad size distributions and low encapsulation efficiency. A promising alternative is the directed formation of polymersomes using copolymer-stabilized water/organic solvent/water (W/O/W) double emulsions in microfluidic devices. The assembly of the copolymers is directed by the double-emulsion droplets during evaporation of the organic solvent in which the copolymer is dissolved. A crucial aspect of this technique is the choice of the organic solvent; it must be highly volatile and the diblock copolymer must be highly soluble within it. Moreover, the use of mixed solvents provides additional control over the interaction between the block copolymers in the bilayer. However, many organic solvents either evaporate too quickly causing the copolymers to form precipitates that eventually clog the microfluidic device, or the organic solvent takes too long to be completely evaporated. These problems can be addressed by using mixtures of organic solvents for dissolving the copolymers.

Organic solvents are typically premixed before injection into microfluidic devices for forming the double emulsion templates. As the concentration of the copolymers and the composition of the premixed solvents cannot be tuned inside the device, any copolymer precipitates cannot be easily removed without disruption of the emulsion generation. Thus, the ability to inject additional solvents during the operation of the device would enable in-situ removal of precipitates and eliminate the problem of fouling. Therefore, a microfluidic design that combines the ability to form double emulsions with the ability to inject and mix two organic solvents is desirable. This goal is difficult to achieve using glass capillary microfluidic devices, as the channel design is not easily customized. These limitations can be overcome using lithographic fabrication techniques to produce more sophisticated microfluidic devices. A convenient fabrication technique is soft lithography using poly(dimethylsiloxane) (PDMS), which can be used to fabricate rather sophisticated devices. Unfortunately, PDMS has a low chemical resistance, and swells when it comes in contact with most organic solvents. The resistance of PDMS towards organic solvents can be significantly increased by depositing a glasslike coating using sol–gel chemistry. While this approach has been successfully applied to generate single emulsion drops of organic solvents, its application to sophisticated devices for the fabrication of complex structures such as double emulsions has not been demonstrated. An optimal system for fabricating double-emulsion-templated polymersomes would combine the versatility of stamped microfluidic devices with resistance against organic solvents.

**Fabrication of Polymersomes using Double-Emulsion Templates in Glass-Coated Stamped Microfluidic Devices**

*Julian Thiele, Adam R. Abate, Ho Cheung Shum, Simone Bachtler, Stephan Förster, and David A. Weitz*

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In this paper, we report double-emulsion-templated polymersomes in stamped microfluidic devices. We coat the devices with a sol–gel to produce a durable glasslike layer with tailored surface properties; the coating is evenly distributed throughout the microfluidic device. This increases the resistance of the channel walls against organic solvents, thus enabling the use of organic solvents for dissolving the diblock copolymers. The device geometry allows us to inject two separate streams of organic solvents to form the shell of the double emulsion, and to control the rate at which the solvents are injected. By tuning the ratio of the two organic solvents with different volatilities, the rate at which the solvent mixture is evaporated can be manipulated. The separate injection of the two organic solvents prevents the adsorption of poorly dissolved copolymers on the microchannel walls. Therefore, unlike conventional microfluidic devices using single injection of premixed solvents that fail due to clogging within seconds of operation, our device enables continuous generation of copolymer-stabilized double emulsions with a shell of organic solvents.

In PDMS devices, double emulsions can be formed using a variety of channel geometries including T-junctions and flow-focusing junctions. In this work, we use a flow-focusing cross-junction geometry. Typically double emulsions are produced in an array of two cross-junctions with different wettability. Drops formed in the first junction enter the second junction where they are encapsulated to create double emulsions. However, this device geometry does not allow manipulation of the composition of the shell phase of double emulsions, which is important because mixtures of solvents are often used for dissolving block copolymers in fabricating polymersomes. We therefore introduce a second cross-junction for injecting an additional solvent in our device, as illustrated schematically in Figure 1. For the formation of copolymer-stabilized double emulsions, we dissolve a diblock polymer in the organic solvent stream injected at the first junction, and inject another organic solvent at the second cross-junction, which is miscible with the copolymer-loaded solvent. The device geometry enables the two organic solvents to be injected in separate channels; moreover, it also allows us to control the flow rate of each solvent independently and tune the ratio of the two solvents in which the diblock copolymers are dissolved. To produce double emulsions with a shell of organic solvents, the PDMS devices must resist degradation and swelling due to the organic solvents. We achieve this by coating the PDMS devices using sol–gel chemistry to create a glasslike layer which is both durable and homogeneously distributed even on the rather complex devices, as shown in the scanning electron micrographs of the coated microchannels. A second advantage of the glass coating is the ability to spatially control the wettability of the surface. We achieve this by functionalizing the intrinsically hydrophobic sol–gel with photoreactive silanes. The surface can then be made hydrophilic with high spatial control through the use of a photochemical surface treatment. In this fashion, we make the first and second cross-junctions hydrophobic while the third junction is made hydrophilic; this allows water drops to be dispersed in a continuous phase of organic solvents at the first and second junctions, while the continuous water phase required for the double emulsion is injected at the third, hydrophilic junction.

We demonstrate the concept to form poly(ethylene-glycol)-b-poly(lactic acid), PEG5000-b-PLA5000, polymer vesicles. To form the double-emulsion templates, the diblock copolymer is first dissolved in an organic solvent, chloroform. However, the high density of chloroform causes the double emulsions to sediment, and subsequently wet the bottom of the collection vial, destabilizing the double emulsions. Thus, to lower the density of the organic phase, we add toluene to the copolymer-containing chloroform as a second organic solvent. For the formation of stable polymer vesicles, the osmolarities of the inner and outer phases of the double-emulsion template must be balanced. Otherwise, the size of the polymersomes will change significantly during solvent evaporation due to osmotically driven diffusion of water. We balance the osmolarity by adding glucose to the inner phase and a polyvinyl alcohol (PVA) to the outer phase of our double emulsion. The three phases are fed into the device shown in Figure 2a. Droplets of the innermost phase are emulsified by the copolymer-containing chloroform at the first droplet making junction. Toluene is added at the second droplet making junction. Finally, PVA solution is used for emulsifying the organic solvent phase that contains aqueous inner drops. However, due to the shear-thinning nature of the PVA solution, its viscosity drops significantly when the middle jet with inner droplets flows through the third cross-junction, where the PVA solution is squeezed between the middle jet and the channel wall. Instead of breaking up into double-emulsion
coatings often consist of a nanoporous structure that allows chloroform and toluene to penetrate the sol–gel barrier into the PDMS.\textsuperscript{[30]} Due to a higher swelling ratio in PDMS, chloroform evaporates faster, resulting in a lower chloroform fraction in the solvent mixture. As the solubility of PEG-b-PLA in toluene is significantly lower than in chloroform, the diblock copolymer forms precipitates after the more volatile chloroform starts to evaporate in the microfluidic device. The precipitated copolymers adsorb onto the microchannels and foul the device if the composition of the solvent mixture cannot be maintained; this leads to a buildup of a thick layer of copolymers on the channel walls, as shown in Figure 2b. In this case the hydrophobic PLA-block adheres to the hydrophobic walls, leaving the hydrophilic PEG-block facing the flow within the channels. This results in an inversion of the wettability pattern of the channels and causes the water within the drops to wet the hydrophilic surface. Thus, the drops occasionally merge with other drops,\textsuperscript{[31]} making the drop size ill-controlled, as shown in Figure 2b. As most of the copolymer in the organic solvent mixture precipitates before emulsification, only a small amount of the precipitates stay dissolved in the organic solvent phase, resulting in destabilization of the double-emulsion drops, as shown in Figure 2c. As the double emulsions are not sufficiently stabilized by copolymer molecules, they eventually burst as they flow downstream. With our new device geometry, we separately inject chloroform with PEG-b-PLA in the first cross-junction and toluene in the second cross-junction. Therefore, we can manipulate the composition of the solvent mixture by changing the flow rates of the two organic solvents; thus the loss of chloroform due to evaporation into the PDMS can be compensated for. However, if sufficient diffusive mixing is allowed, precipitation of the copolymer can still take place at the chloroform/toluene interface.\textsuperscript{[32]} We overcome this by using elevated flow rates, and by shortening the microchannel between the second nozzle, where toluene is injected, and the third cross-junction, where the double emulsion is formed. This prevents the copolymer concentration at the chloroform/toluene interface to decrease below its solubility limit. In our experiments, we find flow rates of 1000 $\mu$L h$^{-1}$ for toluene and 500 $\mu$L h$^{-1}$ for chloroform to be optimal; this corresponds to a volumetric ratio of 2:1. Thereby we prevent precipitation of copolymers which otherwise causes failing of the microfluidic device within seconds after injection of copolymer-containing solvents. However, if the volumetric ratio of toluene to chloroform is higher, precipitation of copolymer in the microchannel between the second and third cross-junction is observed. After double emulsions are formed at the third cross-junction, local mixing in the drops leads to a homogeneous distribution of the copolymer in the shell of the double emulsion. Due to its surface activity, PEG-b-PLA adsorbs at the two interfaces of the shell and stabilizes the droplets. The stability can be further increased by adding a homo polymer, PLA$_{4000}$, to the chloroform in the shell.

During solvent evaporation, the PEG-b-PLA-stabilized double emulsions undergo a dewetting transition as the polymersomes are formed. The organic solvent mixture initially wets the entire inner drop and is homogeneously distributed on its surface, as shown in Figure 3a,b; it then dewets from the inner phase, as indicated in Figure 3c. The dewetting is
driven by evaporation of the volatile organic solvents as well as by the relative high surface energy between the inner and outer phase.\[^{[33]}\] The result is a state of partial wetting where the double emulsions adopt an acornlike, asymmetric structure. However, if the volumetric ratio of toluene and chloroform in the initial double emulsions is between 1:1 and 2:1, stable double emulsions are formed, but the drops do not undergo dewetting. If the shell of the double emulsions contains an excess of chloroform, the double emulsions are destabilized due to the density mismatch of the inner drop and surrounding shell. With the optimized volumetric ratio of toluene and chloroform, the diblock copolymer molecules at the two interfaces of the shell self-assemble into a membrane, enclosing the inner phase. Upon dewetting, the bulb of the acornlike dewetted drop which contains the excess diblock copolymer and homo polymer, remains on the surface of the polymersome. After evaporation of the organic solvents, a polymeric aggregate of these polymers remains attached to the surface of the polymersomes, as shown in Figure 3d. Occasionally, the aggregate detaches from the polymersome, as shown in Figure 3e. Since the volume of the inner drop remains unchanged during the dewetting transition, the polymersome size is only determined by the droplet size of the most inner fluid of the double-emulsion template, which can be controlled by tuning the dimension of the nozzle and the flow rate ratio of inner and middle phase.\[^{[19,34]}\] With our microfluidic device we are able to form double-emulsion templates of approximately 100–150 \(\mu\)m in diameter, corresponding with a polymersome diameter of approximately 50–100 \(\mu\)m. However, the principles of polymersome formation should be applicable down to the smallest scale as limited by the feature size of the microfluidic device.

Our new geometry in stamped microfluidic devices allows us to form polymersomes from copolymer-stabilized W/O/W double emulsions. In contrast to the limited flexibility using two cross-junctions for fabricating double emulsions, our modified microfluidic device enables independent injection and mixing of two organic solvents, which form the double-emulsion shell. This is useful for maintaining the ratios of the solvents specific to the diblock copolymers used, and prevents fouling of the channel walls which would cause instantaneous failure of the device. The control over the solvent mixture is important for ensuring continuous operation of the device, and for applying the double-emulsion approach for polymersomes to a wider range of polymers. As the solvent streams do not mix before emulsification, our modified device also enables the preparation of other core–shell structures from rapidly reacting solvent streams. Our approach should also be useful for forming Janus-like particles with freely tunable composition by using two curable monomer streams which can be solidified during emulsification. In addition, the ease of fabrication of stamped PDMS microfluidic devices should facilitate fabrication of highly parallelized devices for larger-scale production of polymersomes.

**Experimental Section**

**Preparation of devices:** The PDMS microfluidic devices are fabricated using soft lithography.\[^{[13]}\] All channels have a fixed height of 100 \(\mu\)m. The PDMS replica is bonded to a glass slide after oxygen plasma treatment. We then coat the PDMS device with a photoactive sol–gel.\[^{[26]}\] The sol–gel is intrinsically hydrophobic, but can be made hydrophilic using photolithographic techniques, though other surface treatments are possible.\[^{[35]}\] We graft patches of hydrophilic poly(acrylic acid) onto the sol–gel using spatially patterned UV light in specific areas. All other parts of the device remain hydrophobic due to the default properties of the sol–gel coating. To form double emulsions with a shell of organic solvents, we pattern the first and second cross-junction to remain hydrophobic and the third cross-junction to be hydrophilic.

**Preparation of double-emulsion templates:** All chemicals are obtained from Sigma-Aldrich Co. unless noted otherwise. PEG\(_{5000}\)-b-PLA\(_{5000}\) and PLA\(_{5000}\) are obtained from Polysciences Inc. Water with a resistivity of 16.8 \(\text{M}\) cm\(^{-1}\) is prepared using a Millipore Milli-Q system. The osmolarities of the inner and continuous phase of the copolymer-stabilized double emulsions are measured with a micro osmometer (Advanced Instruments, Inc., Model 3300). The osmolarities are approximately 104 and 114 mOsm, respectively. We form copolymer-stabilized double emulsions in our modified PDMS microfluidic device by injecting an aqueous solution of glucose (100 m\(\text{M}\)) as the inner phase, chloroform with 120 mg mL\(^{-1}\) diblock copolymer and 40 mg mL\(^{-1}\) homopolymer as the first shell phase, toluene as the second shell phase and an aqueous solution of a polyvinyl alcohol (weight-averaged molecular weight, \(M_w\) 13 000–23 000 g mol\(^{-1}\), 87–89%...
hydrolyzed) at 3% w/w as the continuous phase. A typical set of flow rates of the inner, first shell, second shell, and outer phases is 300, 1000, 500, and 3500 μL.h⁻¹, respectively.

Formation of polymersomes: The copolymer-stabilized double emulsions are collected in a glass vial. We place single samples between a microscopy slide and a cover slip, separated by a silicone isolator, 0.5 mm in thickness. This reduces the rate at which the organic solvents evaporate and allows us to monitor the polymersome formation using optical microscopy. If the double emulsions are left in air, the organic solvents evaporate too quickly destabilizing the double emulsions.

Keywords:
double emulsions · glass-coatings · PDMS microfluidics · polymersomes


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