Vesicle budding from polymersomes templated by microfluidically prepared double emulsions

Julian Thiele, Venkatachalam Chokkalingam, Shaohua Ma, Daniela A. Wilson, Wilhelm T. S. Huck*

[*] Dr. J. Thiele, Dr. V. Chokkalingam, Dr. D. Wilson, Prof. Dr. W. T. S. Huck, Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen (The Netherlands); S. Ma, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW (UK)

Supplemental Information

This supplemental information contains details on the synthesis of PS-PEG. In addition, we provide information on the fabrication of durable PDMS-based microfluidic devices with controlled wettability to form copolymer-stabilized double emulsions. If you have additional questions, feel free to send an email to j.thiele@science.ru.nl.

General information

All reagents and chemicals were used as received unless otherwise indicated. Styrene was distilled prior to use to remove the inhibitor. Anisole and N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA) were distilled prior to use. Ultrapure MilliQ water was obtained from a Labconco Water Pro PS purification system with a resistivity of 18.2 MΩ. The supplemental part includes high-speed video files of double emulsion formation with 8 % (w/w) PS-PEG as well as of a double emulsion-to-polymersome transition and the formation of vesicle buds. Abbreviations IP, MP and OP in the high-speed video files refer to the flow rates of inner, middle and outer phase, respectively, denoted in µL h^{-1} (fps = frames per second, exposure = shutter speed, XXx = objective magnification).

Instrumentation

NMR spectra were recorded on a Varian Inova 400 spectrometer with $CDCl_3$ as a solvent and TMS as internal standard. The molecular weight of the diblock copolymer was measured on a

Shimadzu Prominence GPC system equipped with a PL gel (5 µm) mixed D column (Polymer Laboratories) and differential refractive index and UV (254 nm) detectors. THF was used as an eluent with a flow rate of 1 mL min^{-1}. Polystyrene standards in the range of 580 to 377,400 Da were used for calibration. Scanning electron microscopy (SEM) was performed on a JEOL 2300 microscope operated at an acceleration voltage of 3 kV. For each experiment, 15 µl of polymersome solution were air-dried on a silicon wafer, placed on conductive tape and attached to an aluminum sample block (dry SEM sample preparation). The sample was then sputtered with an Au/Pd alloy to give a thickness of approximately 10 nm before further characterization. Bright-field microscopy was performed using an IX71 microscope (Olympus) equipped with objectives ranging from 2x (air) to 100x (UPLFLN 100X, N.A. 1.30) and a Phantom high speed MIRO camera (Vision Research Inc., UK) to allow for imaging single droplet formation inside the microfluidic device. Fluorescence and confocal microscopy was carried out on an IX81 confocal microscope (Olympus), equipped with an Andor iXon3 camera, Andor 400-series solid state lasers, a Yokogawa CSU-X1 spinning disk and an Andor FRAPPA photo-bleach module.

Synthesis of diblock copolymer PS_{134}-*b*-PEG_{45}

The amphiphilic diblock copolymer containing poly(ethylene glycol) as a hydrophilic block and poly(styrene) as a hydrophobic block was synthesized by atom-transfer living radical polymerization (ATRP). The poly(ethylene glycol) macro-initiator with a degree of polymerization DP = 45 and the copolymer PS-*b*-PEG were synthesized according to a method reported previously.[1] The synthesis was optimized to yield a poly(styrene) block of approximately 134 repeating units. The diblock copolymer was characterized by ^{1}H NMR and GPC to evaluate the molecular weight and size distribution. GPC traces of the polymer used for the formation of PS-PEG polymersomes are shown in Figure S1. The low polydispersity index of the sample in the range from 1.07 to 1.1 is typical for a controlled living polymerization.

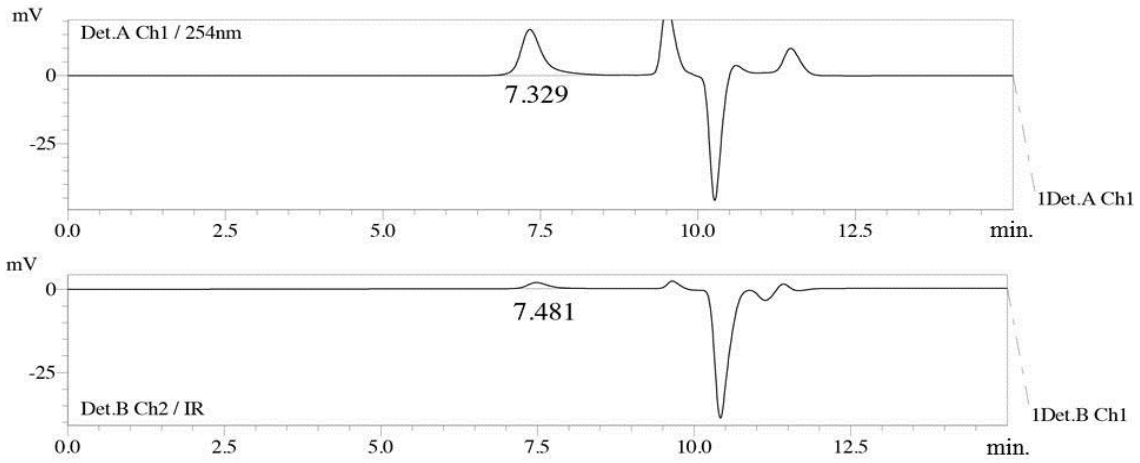

Figure S1. GPC traces of the amphiphilic diblock copolymer PS_{134}-*b*-PEG_{45} used for forming giant polymersomes.

Fabrication of microfluidic devices

Microfluidic devices were fabricated using soft lithography in PDMS.[2] The channel width at the first and the second cross-junction was 60 µm and 120 µm, respectively. All channels had a fixed height of 80 µm. A PDMS replica of this channel design was bonded to a glass slide using oxygen plasma treatment. To form copolymer-stabilized double emulsions, PDMS-based microfluidic devices need to resist degradation due to organic solvents and require controlled wettability to form water-in-organic solvents-in-water (W/O/W) double emulsions.[3,4] The controlled surface modification of our microfluidic devices was carried out following a procedure for sol-gel glass coatings and graft photo-polymerization of hydrophilic polymers by Abate et al.[5] which was optimized for our needs. In brief, 11.0 g Irgacure 2959 photoinitiator (Ciba), 0.01 g hydroquinone and 49.4 µL dibutyltin dilaurate in 20.0 mL of dry chloroform were stirred under nitrogen until homogenous. 3-(triethoxysily)propyl isocyanate (12.1 mL) was slowly added over 30 min. under stirring. The reaction mixture was heated to 50 °C and stirred for additional 3 h to allow for complete conversion, obtaining the desired silane-coupled photoinitiator. The solvent was removed under vacuum, yielding a highly viscous, yellow, transparent liquid.

A photo-reactive sol-gel mixture was prepared by combining 1 mL tetraethylorthosilicate (TEOS), 1 mL methyltriethoxysilane (MTES), 0.5 g of the above prepared photoinitiator-silane, 0.5 mL (heptadecafluoro-1,1,2,2-tetrahydrodecyl)triethoxysilane, 2 mL trifluoroethanol and 1 mL aqueous HCl (set to pH = 1). The mixture was reacted in an oil bath at 70 to 80 °C for 0.5 h under reflux, yielding a slightly yellow, homogeneous solution. The solution was ready to be applied for coating microfluidic devices with a hydrophobic, solvent resistant sol-gel and can be stored at 2 to 8 °C in the dark up to one month. The microchannels were made solvent-resistant by filling the device with the above solution, followed by incubation at 120 °C for 1 min. The excess solution was flushed out of the device with 20 mL air. To form a solvent-resistant sol-gel, the microfluidic device was incubated at 120 °C for another 2 h.

To spatially pattern the wettability of our coated microfluidic devices and allow for formation of W/O/W double emulsions, the second cross-junction and the meander-shaped outlet channel were made hydrophilic by UV-induced photo-polymerization of acrylic acid. A monomer solution composed of acrylic acid (0.2 mL), $NaIO_4$ (0.8 mL, 5 mM in water), ethanol (1 mL), acetone (0.5 mL) and benzophenone (0.05 g) was purged with nitrogen for 0.5 h to remove dissolved oxygen. To spatially pattern the device wettability, we used the flow-confinement method.[6,7] Inert oil, HFE 7500 (3M), was used to block all sol-gel-coated microchannels, but the second cross-junction and the meander-shaped outlet channel. The inert oil was injected at 2000 µL h^{-1}, and the hydrophilic monomer solution was injected at 1000 µL h^{-1}, as sketched in Figure S2. The oil

phase and the monomer solution formed a stable interface at the second cross-junction and the two phases exited the device through the later outer-phase inlet channel. The monomer was grafted onto the sol-gel-coated microchannels by exposing the monomer solution to a focused UV beam in an Olympus IX81 microscope, equipped with a 100 W mercury lamp (365 nm, USH-1030L, USHIO Inc.), for 30 s, thus rendering the exposed microchannels hydrophilic.

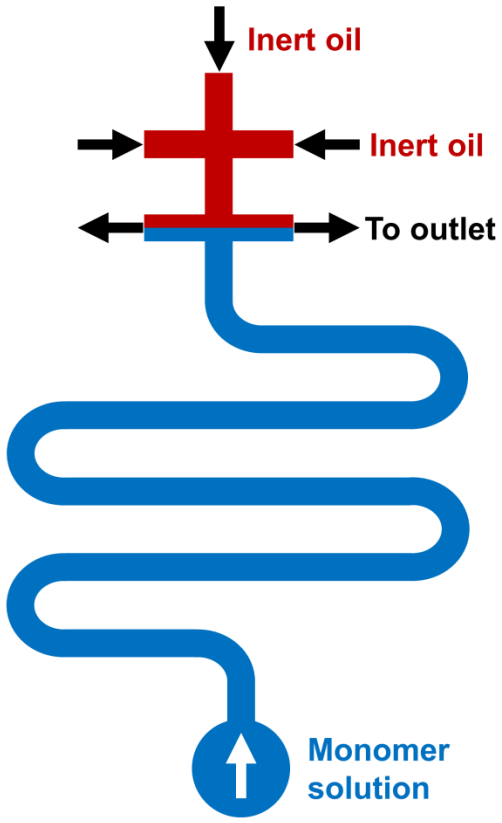

Figure S2. Schematic representation of the surface modification of microfluidic devices using the flow-confinement method.[6,7]

Formation of PS-PEG-stabilized double emulsions

All solutions were injected using gastight syringes (Hamilton 1000 series) mounted onto syringe pumps (Cetoni® neMESYS, 14.5 gear) connected to the microfluidic device via PTFE tubing (Novodirect GmbH, inner diameter = 0.53 mm, outer diameter = 1.03 mm). We formed copolymer-stabilized double emulsions by injecting an aqueous solution of glucose (50 mM) with sodium dodecyl sulfate (0.1 % w/w) as the inner and outer phase and chloroform / hexane (80 : 20 v/v) with 0.5 % to 16 % (w/w) PS-PEG as the double emulsion's shell phase into the microfluidic device. A typical set of flow rates of the inner, middle and outer phase was 150, 330,

and 750 μL h^{-1}, respectively. The outlet tubing of the microfluidic device was fed into a microscope chamber slide separated by a silicone isolator (SecureSeal™, diameter = 20 mm, height = 0.5 mm), where copolymer-stabilized double emulsions were collected for further characterization. This reduced the rate at which the organic solvents evaporated and allowed us to monitor the formation of giant polymersome templates as well as vesicle budding using optical microscopy. If the double emulsion was simply left in air, the organic solvents would evaporate too quickly and destabilize the emulsion upon double emulsion-to-polymersome transition.

References

(1) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. *Adv. Mater.* **2009**, *21*, 2787-2791.

(2) Xia, Y.; Whitesides, G. M. *Annu. Rev. Mater. Sci.* **1998**, *28*, 153-184.

(3) Abate, A. R.; Lee, D.; Do, T.; Holtze, C.; Weitz, D. A. *Lab Chip* **2008**, *8*, 516-518.

(4) Schneider, M. H.; Willaime, H.; Tran, Y.; Rezgui, F.; Tabeling, P. *Anal. Chem.* **2010**, *82*, 8848-8855.

(5) Abate, A. R.; Krummel, A. T.; Lee, D.; Marquez, M; Holtze, C.; Weitz, D. A. *Lab Chip*, **2008**, *8*, 2157-2160.

(6) Bauer, W. A. C.; Fischlechner, M.; Abell, C.; Huck, W. T. S. *Lab Chip* **2010**, *10*, 1814-1819.

(7) Abate, A. R.; Thiele, J.; Weinhart, M.; Weitz, D. A. *Lab Chip* **2010**, *10*, 1774-1776.