Dewetting-induced membrane formation by adhesion of amphiphile-laden interfaces

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Experimental Section

Materials: Glass microcapillaries were purchased from World Precision Instruments, Inc. and Atlantic International Technologies, Inc.). Materials we used to prepare the outer phase of the double emulsions 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.). Middle phase oils included poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA, Polysciences, Inc.) with block lengths of (PEG: 5000 g·mol^{-1}/ PLA: 5000 g·mol^{-1}) and (3000 g·mol^{-1}/3000 g·mol^{-1}), poly(ethylene glycol)-b-poly(caprolactum) (PEG-b-PCL) with block lengths of (PEG: 5000 g·mol^{-1}/ PCL: 9000 g·mol^{-1}), and 25-[N-[(7-nitro-2-1,3-benzoxadiazol-4-yl)methyl]amino]-27-norcholesterol (NBD-norcholesterol, Avanti Polar Lipids, Inc.) dissolved in mixtures of chloroform (Mallinckrodt Chemicals, Inc.), hexanes (EMD Chemicals, Inc.) and silicone oil (Dow-corning, Inc.) with viscosities of 0.65 cSt, 1 cSt, and 1.5 cSt. Innermost phase consisted of 8-hydroxypyrene-1.3,6-trisulfonic acid trisodium salt (HPTS), polyethylene glycol (Mw: 6000 g·mol^{-1}, Sigma-Aldrich Co.) and water (18.2 MΩ·cm^{-1}, Millipore Milli-Q system). Double emulsions and polymersomes prepared were collected into a vial containing water with sodium chloride (Sigma-Aldrich Co.) with osmolality matched to that of the innermost phase. In addition to polymersomes, we also prepared phospholipid vesicles for fluorescence recovery after photobleaching (FRAP) experiments via electroformation. We deposited a small aliquot (~10 μL) of 1 mg/mL 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids, Inc.) and 0.07 mg/mL NBD-norcholesterol in chloroform uniformly on an indium tin oxide (ITO) coated glass slide,

which was then vacuum dried for 2 hr. The dried phospholipids layer was enclosed with a silicone isolator (Invitrogen, Inc.) and another ITO coated glass slide. The resultant chamber was filled with 100 mM sucrose solution. An alternating current with a peak voltage of 2 V at 10 Hz was applied across the chamber for about 5-10 hours. The vesicles were finally transferred into a 100 mM glucose solution for FRAP experiments.

Microfludics: Monodisperse W/O/W double emulsions were prepared using glass capillary-based microfluidic devices.[1,2] The round capillaries (World Precision Instruments, Inc., Sarasota, Florida), with inner and outer diameters of 0.58 mm and 1.0 mm, respectively, were tapered to desired diameters with a micropipette puller (P-97, Sutter Instrument, Inc.) and a microforge (Narishige International USA, Inc., East Meadow, New York, USA). Two tapered capillaries were aligned inside square glass capillaries (Altantic International Technology, Inc., Rockaway, New Jersey, USA) with an inner dimension of 1.05 mm. A transparent epoxy resin (5 Minute Epoxy, Devcon, Danvers, Massachusetts, USA) was applied to seal the capillaries where necessary. The outer radii, R_o, of the double emulsions ranged from 60 to 200 μm, while the inner radii, R_i, ranged from 50 to 120 μm. These values were controlled by the size of the capillaries and the flow rates of the different phases.[2] Fluorescent dyes and PEG were pre-dissolved in the inner phase for subsequent encapsulation in double emulsion drops. Positive syringe pumps (PHD 2000 series, Harvard Apparatus, Holliston, Massachusetts, USA) were used to deliver the different phases at desired flow rates. A typical set of flow rates for the outer, middle and inner phases was 4000, 2000 and 150 μL/h respectively and the

droplet-generation frequency was about 500 Hz. Samples were collected into a vial filled with sodium chloride solution for sedimentation of polymersomes .

Sample characterization: The microfluidic process was monitored using an inverted optical microscope (DM-IRB, Leica) fitted with a fast camera (Phantom V9, Vision Research, Inc.). Bright-field and fluorescence images were obtained with 10× objectives at room temperature using an automated inverted microscope with fluorescence (DMIRBE, Leica Microsystems, Inc.) equipped with a digital camera (QICAM 12-bit, QImaging, Inc.). Suspensions of polymersomes were freeze-dried using a FTS Dura-Stop tray drier (Dura-Stop, FTS Systems Inc.) coupled with a condenser module (Dura-Dry-MP, FTS Systems, Inc.). Scanning electron microscopic (SEM) images of dried capsules coated with a thin layer of platinum and palladium were taken using a Zeiss Supra 55VP field emission scanning electron microscope (FESEM, Carl Zeiss SMT, Inc.) at an acceleration voltage of 4.5 kV. Freeze-fracture cryo-SEM was performed on a Zeiss Supra 40V field emission scanning electron microscope at an acceleration voltage of 1.6 kV. A small aliquot of sample was placed on a sample stub and plunged into a slush of liquid and solid nitrogen. The sample was transferred in a EM VCT100 vacuum cryo transfer system (Leica Microsystems, Inc.). The frozen sample was fractured with a sharp blade before imaging. The size of the aggregates in the solvent was determined using dynamic light scattering (ALV 5000, 532nm laser, 90° scattering angle).

To characterize diffusion within membranes of the vesicles, a small volume (~50 μL) of vesicles was placed in an elastomeric isolation chamber and covered with a glass coverslide. The vesicles were allowed to settle in the sample chamber and the membranes

were studied using fluorescence recovery after photobleaching (FRAP), which was performed using a confocal microscope (TCS SP5, Leica Microsystems, Inc.). An argon (Ar) laser at a wavelength of 488 nm was used to photobleach the dyes and the fluorescence intensity as a function of time was measured.

Supplementary figures

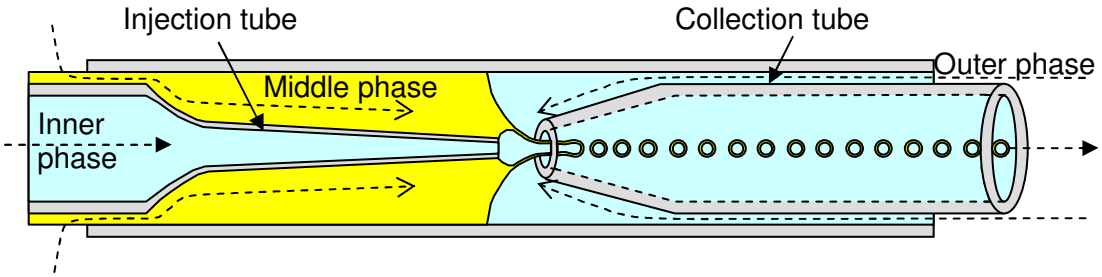

Figure S1: Schematic of a glass capillary microfluidic device for generating double emulsions

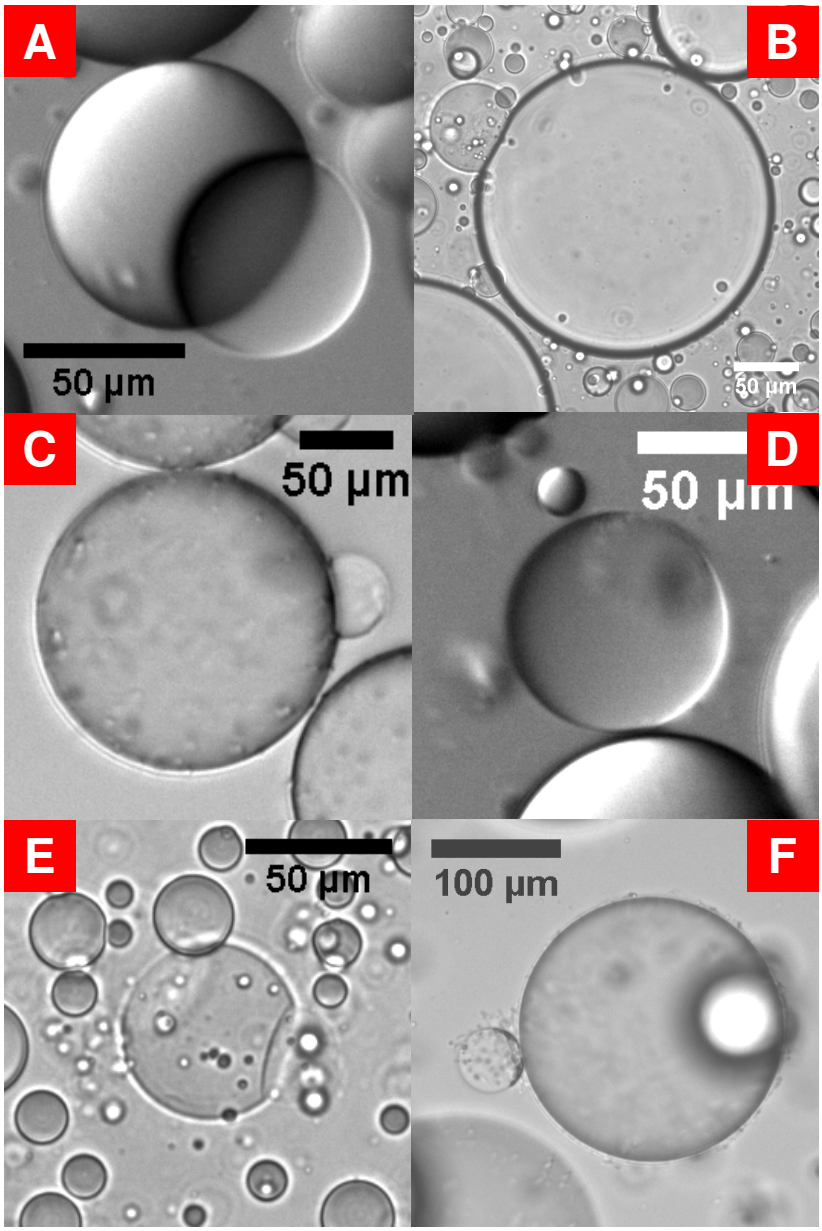

Figure S2: Optical microscope images of dewetted drops with shell phases of A) a mixture of chloroform and hexanes (36:64 v/v) with 10 mg/mL PEG(5000)-b-PCL(9000), B) a mixture of chloroform and hexanes (32:68 v/v) with 10 mg/mL PEG(3000)-b-PLA(3000), and C) a mixture of chloroform and poly(dimethyl siloxane) oil with a viscosity of 1 cSt (46:54 v/v) with 10 mg/mL PEG(5000)-b-PLA(5000). Optical microscope images of D) a PEG(5000)-b-PCL(9000) polymersome, E) a PEG(3000)-b-PLA(3000), and F) a PEG(5000)-b-PLA(5000) polymersome after dewetting from their corresponding shell phases of D) a mixture of chloroform and hexane (36:64 v/v) with 10 mg/mL PEG(5000)-b-PCL(9000), E) a mixture of chloroform and hexanes (32:68 v/v) with 10 mg/mL PEG(3000)-b-PLA(3000), and F) a mixture of chloroform and poly(dimethyl siloxane) oil (41:59 v/v) with a viscosity of 0.65 cSt with 10 mg/mL PEG(5000)-b-PLA(5000) respectively.

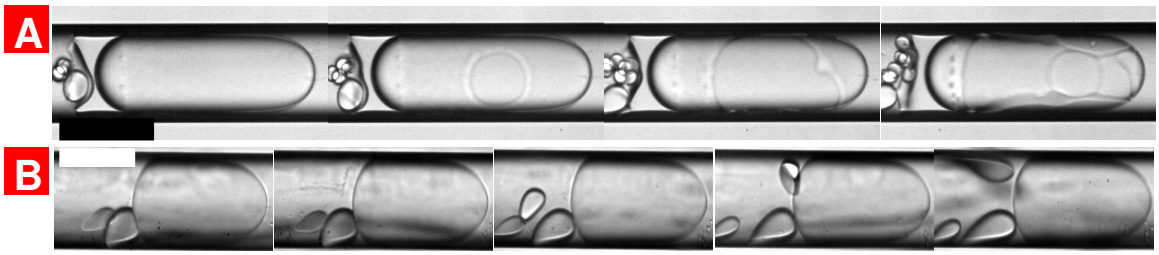

Figure S3: A) Series of optical microscope images showing the dewetting of a double emulsion drop in a straight microchannel. The time interval between successive images is 10 ms and the scale bar is 100 μm. B) Series of optical microscope images showing the detachment of the small oil drop from the polymersome in a straight microchannel. The time interval between successive images is 150 ms and the scale bar is 80 μm.

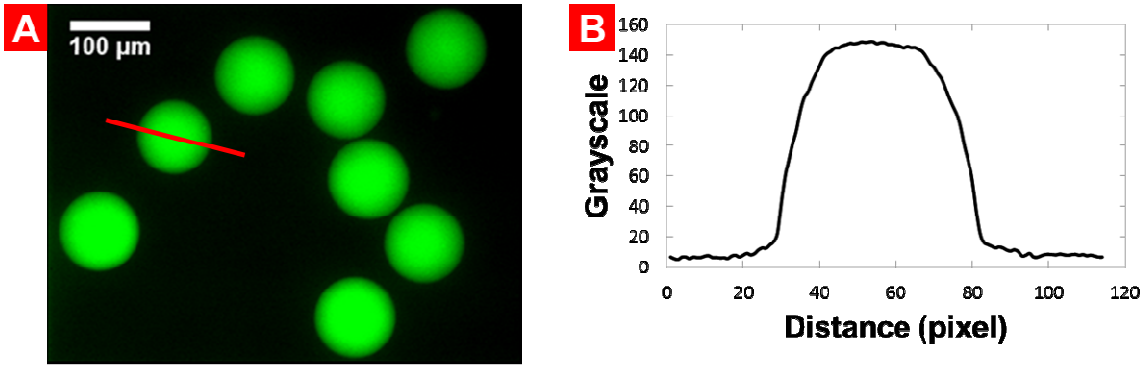

Figure S4: A) Confocal laser scanning microscope image of PEG(5000)-b-PLA(5000) polymersomes encapsulating a fluorescent dye (HPTS). The red line indicates the position of the line profile displayed in B); B) Line profile of the fluorescence intensity across a polymersome. The profile indicates that the fluorescence intensity drops sharply at the edge of the polymersome, suggesting that almost all fluorescent dyes are encapsulated inside the polymersome.

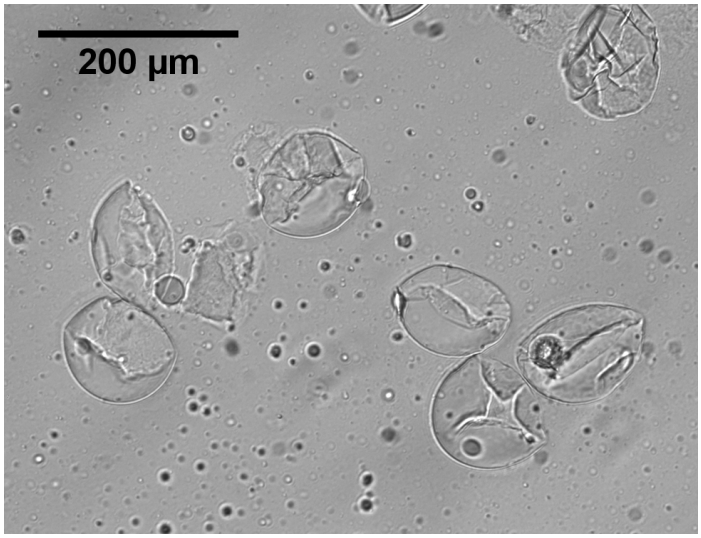

Fig. S5: Optical microscope image of crushed polymersomes with 125 mM sucrose as the original internal phase.

Reference

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