Supporting Information

for

Stepwise synthesis of giant unilamellar vesicles on a microfluidic assembly line

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Contents

Materials and Methods

Preparation of lipid/oil mixtures

1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids, Alabaster, AL) was dissolved in chloroform (10 mg/ml), dried under gentle nitrogen flow (45 min, 37°C), then dried under vacuum (2 h, room temperature). Dried lipids were dissolved in dodecane or hexadecane with shaking overnight (1 mM, 37°C, 100 rpm).

Device microfabrication and operation

The device was fabricated in polydimethylsiloxane (PDMS) using soft lithography and bonded to glass. Briefly, PDMS pre-polymer was mixed with curing agent (10:1 w/w, SYLGARD 184, Dow Corning, Midland, MI), degassed under vacuum and poured onto the 40-μm-high SU-8 device mold (Stanford Microfluidics Foundry, Stanford, CA). The polymer was cured (1.5 h, 70 °C), removed from the mold, and diced into individual devices. Reservoirs were created with a biopsy punch (0.75 mm, Harris Uni-Core). Punched devices and borosilicate glass slides were hydrolyzed (5:1:1 water:HCl:H_2O_2, 30 min), dried, and placed into contact to bond the PDMS device to the slide. All channels were 40 μm deep. Prior to the Y-junction, the droplet forming channel was 60 μm wide, the extracellular aqueous channel was 100 μm wide and the phase transfer channel is 200 μm wide (technical device drawings are provided).

Fluids were driven through the circuit with syringe pumps (Harvard Apparatus, Holliston, MA) through micro-bore tygon tubing (0.03 in OD × 0.01 in ID, Saint-Gobain). Water droplets in oil were formed at a T-junction with a flow rate ratio > 3 (oil:water).

Droplet flow was delivered to the central channel where a stable two-phase oil/water interface was generated with a co-flowing extracellular aqueous flow (AQ_{ex}). Using dodecane as the carrier oil phase, cytoplasmic droplet aqueous flow (AQ_{cy}) was 0.1 μL/min, oil was 2 μL/min, and AQ_{ex} was 2 μL/min.

Vesicle characterization and imaging

Droplets containing Cascade Blue−labeled dextran (1% w/w, 10 kDa, Invitrogen, Carlsbad, CA) and fluorescein dye (10 μM, 332 Da) in phosphate buffered saline were formed in a DOPC/dodecane suspension (1 mM). Output vesicles were collected and tested for permeability and lamellarity using *S. aureus* α-hemolysin toxin (50 μg/mL, Sigma-Aldrich, St. Louis, MO). Two-color confocal fluorescence imaging microscopy time courses of both the dextran size exclusion marker ($\lambda_{ex}/\lambda_{em}$ = 400 nm/420 nm) and the small molecule fluorescein dye (490 nm/520 nm) were acquired (FV1000, Olympus, Center Valley, PA), and intensity and sizing data were quantitated (ImageJ, NIH, Bethesda, MD). Fluorescence data following hemolysin treatment were normalized to the pre-treatment intensity. DIC imaging was conducted using an oil immersion objective (40X 1.3 NA, Olympus).

Encapsulation yields were measured by dividing the fluorescence intensity of a product vesicle after phase transfer by the fluorescence intensity of the droplet just prior to phase transfer. Encapsulation yield was calculated for each vesicle assembly event. Fluorescein photobleaching and vesicle integrity were assessed by monitoring the time evolution of fluorescein fluorescence in vesicles without α-hemolysin treatment and in droplets to deconvolve photobleaching and small molecule leakage. Fluorescence was normalized to the time = 0 intensity. Exposure time fore both vesicle integrity and photobleaching experiments was 3.5 s.

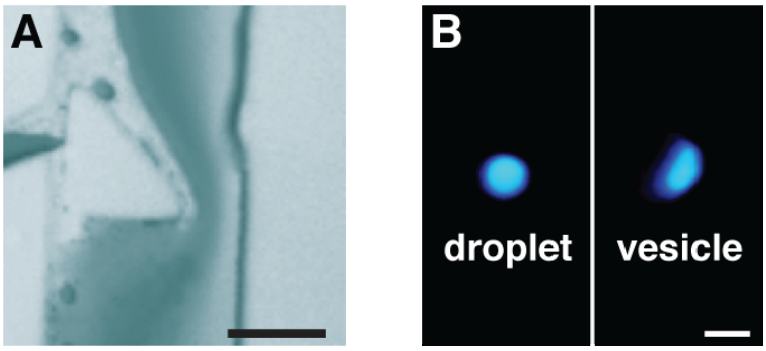

Figure S1. Example raw image data for vesicle size programmability and encapsulation yield, and selective permeabilization assay schematic. **(A)** Droplet intermediates are shown in the lipid/oil phase above the triangular post, and product vesicles are in the extracellular aqueous phase below (scale bar = 100 μm). **(B)** A droplet containing fluorescein dye (*left*) transits the interface to become a vesicle (*right*). Integrated fluorescence intensity data collected before and after phase transfer give the encapsulation yield (scale bar = 50 μm).

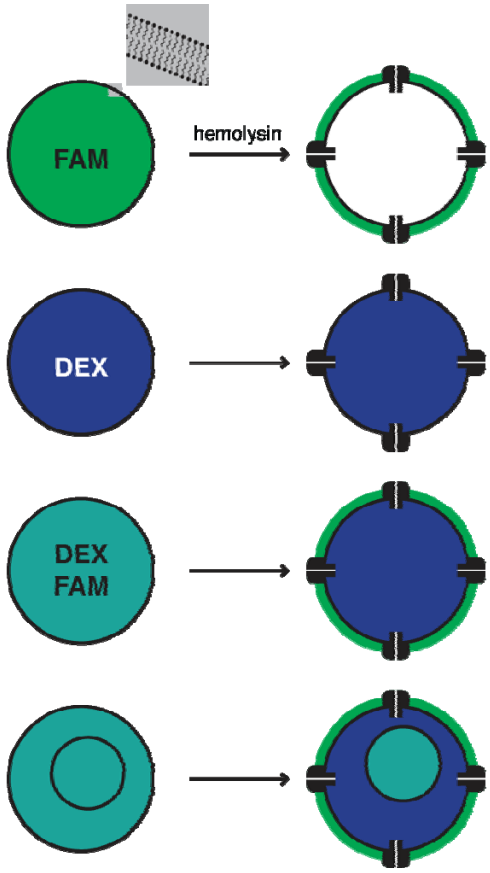

Figure S2. Functional incorporation and assembly of the homoheptameric bacterial pore alpha hemolysin results in selective permeabilization of unilamellar vesicles to small molecule reporter fluorescein (FAM, green), which diffuses through permeabilized bilayers. Macromolecular cargo (> 500 Da), reported by 10-kDa blue-fluorescent dextran (DEX, blue), remains trapped in permeabilized vesicles. Permeabilized unilamellar vesicles loaded with both reporters (cyan) will selectively retain dextran while completely discharging fluorescein. Exogenous hemolysin only permeabilizes the outermost bilayer of multi-lamellar vesicles, revealing the presence of internal lamellae.

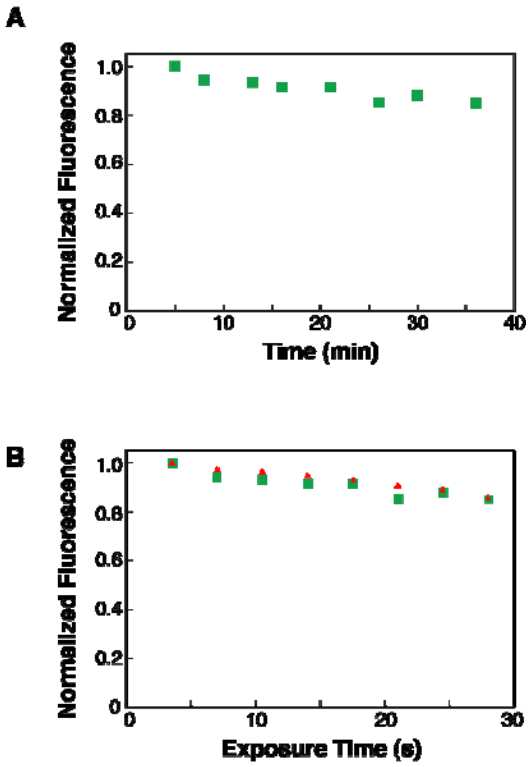

Figure S3. Vesicle integrity in the absence of α-hemolysin toxin and fluorescein photobleaching. **(A)** Vesicles exhibited modest decrease in fluorescein fluorescence when monitored continuously over a 40-min experiment in the absence of α-hemolysin. **(B)** To account for photobleaching, fluorescein fluorescence in droplets stabilized by DOPC in dodecane was observed with identical exposure time and wavelength. Fluorescence for droplets (triangles) and vesicles (squares) decreased identically with exposure time, confirming that leakage across the DOPC bilayer was negligible over the hemolysin assay time course.

Vesicle Diameter (μm)	CV
25	0.056
44	0.127
54	0.105
58	0.125
61	0.069

Table T1. Coefficients of variance (CV) for DOPC vesicles prepared on the microfluidic assembly line.

Movie Information

Movie M1. Parallel flow of DOPC-stabilized water-in-oil droplets and extracellular aqueous phase in the microfluidic assembly line. Both the droplet and the extracellular aqueous phase are stained with bromophenol blue. Movies were taken with a MacroFIRE color CCD camera (Optronics, Goleta, CA). For detailed experimental conditions, see **Materials and Methods**.

Movie M2. DOPC-stabilized droplets transiting the oil/water interface. PBS was used as both AQ_{cy} and AQ_{ex}. Movies were taken with a Phantom Miro eX4 high-speed camera (1,000 fps, Vision Research, Wayne, NJ).