

Supplementary Information

Multicompartment microfibers: fabrication and selective dissolution of composite droplet-in-fiber structures

*Eujin Um, Janine K. Nunes, Tamara Pico, Howard A. Stone**

Experimental

Channel fabrication: The microfluidic device for making multicompartment microfibers was fabricated from polydimethylsiloxane (PDMS) by soft lithography. The mold of the microchannel, including a sequence of flow-focusing channels, was fabricated by standard photolithography from photoresist (SU-8 2010 and SU-8 2025, MicroChem) on a silicon wafer. The mold of the top layer of the channel was fabricated on the silicon wafer using two-step lithography of 30 μm and 90 μm in height, and the bottom layer was fabricated to be 80 μm in height. Channel dimensions are shown in Fig. S2. A degassed 10:1 mixture of PDMS prepolymer and curing agent (Sylgard 184, Dow Corning) was poured on the channel mold and released after 2 hours of curing at 65°C. The closed channel structure with openings for inlets and outlets was formed by bonding top and bottom layers of PDMS with plasma treatment. We heated the devices at 95°C for a day to recover hydrophobicity of the PDMS channels, and stored them in vacuum overnight, before localized grafting of the hydrophilic polymer, poly(acrylic acid) (PAA).¹ We masked the channel with black electrical tape leaving only the region for oil droplet generation transparent. We filled the channel with 10% w/v benzophenone (photoinitiator) in acetone for 70-90 seconds, and flushed with air. Then the channel was filled with 10 wt% acrylic acid in DI water and exposed under a 335 nm – 610 nm Bandpass color filter to UV light for 90-150 seconds. In this time range, we increased the time of treatment with benzophenone and acrylic acid if the oil solution wet the PAA grafted region when forming a droplet, and decreased the time if the alginate stream started to adhere to the channel wall upon contact with calcium chloride solution. The channel was washed with ethanol, pH 10 water, and DI water respectively, before fiber fabrication.

Chemical sample preparation: (i) For the sheath flow of the fiber formation, we used 1.5-2% (w/v) of calcium chloride solution in DI water. (ii) For the alginate fiber, we used 0.5-1 wt%

sodium alginate (from brown algae) with 0.1-0.25 wt% poly(vinyl alcohol) (PVA; Molecular weight, 31,000-50,000) dissolved in DI water or PBS. PVA is a known biocompatible polymer.² Also, after collecting the fibers, we rinsed them with DI water or PBS, and stored them in DI water to allow any water-soluble components in the fiber to be removed. Then we used UV-Vis spectroscopy to determine the presence of PVA, which shows an absorbance maximum absorbance around 216 nm, but we observed that there were no absorbance peaks in the wavelength range 210-240 nm from the sample solution over 24 hours. For cell encapsulation, the alginate-PVA solution was filtered through 0.22 μm pore-sized filter prior to mixing with cells. The mammalian cells, NIH/3T3 mouse fibroblast cells (ATCC), were cultured in Dulbecco's modified Eagle medium (DMEM; ATCC) supplemented with 10% (v/v) calf bovine serum and 1% (v/v) penicillin. The cells were prepared to be $1\text{-}2 \times 10^5$ cells mL^{-1} in the final alginate solution. For encapsulating mammalian cells in the alginate fiber, we used a solution of 0.5 wt% alginate with 0.1 wt% PVA dissolved in PBS. The bacterial cells were *Escherichia coli* (*E. Coli*, S17-1 λ pir) that express green fluorescent protein (GFP) from a plasmid, induced by isopropyl β -D-1-thiogalactopyranoside (IPTG). More details on the cells, including plasmid construction, can be found in the work of Drescher et al.³ The *E. coli* cells were prepared in the alginate solution to be $4\text{-}5 \times 10^6$ cells mL^{-1} . For encapsulating bacterial cells, we used a solution of 1 wt% alginate with 0.25 wt% PVA dissolved in PBS. (iii) For the oil droplet compartment, we used mineral oil with 2 wt% Span 80 in general. For solidifying inner alginate droplets to prevent them from escaping the oil compartment, we mixed 5% (v/v) of calcium chloride-saturated undecanol with the oil solution. The concentrations of the cargos to be carried in the oil compartment were 0.3 mg mL^{-1} for Nile red, 0.8% (v/v) for magnetic particles in oil-phase (EMG 909, FerroTec), and 10 and 20% (v/v) for eugenol. To demonstrate the antibacterial effect of the oil compartment to the bacterial cells encapsulated in the fiber, eugenol was first mixed with undecanol and added to the final concentration of 10 and 20% (v/v) in the oil compartment of 2 wt% Span 80 in mineral oil. The final concentration of undecanol in the oil solution was 5% (v/v). In the case of using just eugenol for the oil compartment, we added 2 wt% Span 80 to eugenol to form stable droplets. (iv) The concentration of alginate-PVA solution in the inner alginate compartment was kept the same as the alginate fiber. As for the cargos, 0.5% (v/v) 200 nm orange fluorescent particles (FluoSpheres, Invitrogen), 1% (w/v) FITC conjugated BSA, or 0.04% (v/v) of 190 nm Dragon Green fluorescent particles (Uniform Dyed Microspheres, Bangs

Laboratories, Inc.) were mixed with the alginate solution. For water-based magnetic particles, 2% (v/v) of 1 μm -magnetic polystyrene particles were mixed in the alginate solution. Chemicals not indicated with vendors were purchased from Sigma-Aldrich.

Fiber collection and treatment: Multicompartment fibers were collected as suspensions in DI water or wound between two rotating needles controlled by a motor (Barnant Motor Mixer, Model No. 700-5412). Fibers encapsulating the fibroblast cells were collected in DMEM supplemented with 10% calf bovine serum and 1% penicillin, and stored in the 5% CO_2 -humidified incubator at 37 °C. To test the cell viability inside the multicompartment fibers, 2 μM of calcein AM and 4 μM of ethidium-homodimer-1 (EthD-1) from LIVE/DEAD Viability/Cytotoxicity kit for mammalian cells (Molecular Probes) were applied in the DMEM 1 hour prior to the observation under a fluorescent microscope. The cell viability was analyzed with the green (calcein AM) and red (EthD-1) fluorescence images using image analysis software, ImageJ (NIH). *E. coli*-encapsulated fibers were either collected in LB media containing 1mM IPTG and 100 $\mu\text{g mL}^{-1}$ Kanamycin (kanamycin sulfate; Fisher). For selective dissolution of the cargos from the oil compartment of the fibers, ethanol (Reagent alcohol, BDH) was added to the fiber suspensions. The de-cross-linking agent, 160 mM sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, Sigma-Aldrich) in DI water was used as calcium chelator to degrade the alginate fiber or inner alginate particles.

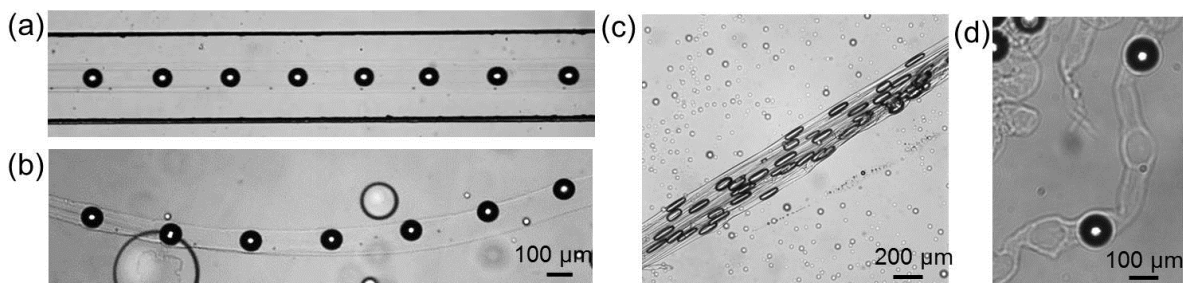

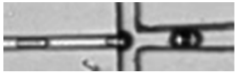

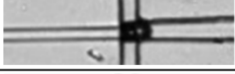

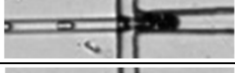

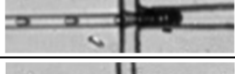




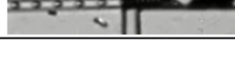


Figure S1. Bright-field images of the oil droplets-in-fiber structure (a) generated inside a main channel, (b) freely suspended in an aqueous solution, and (c) spun using a motor. The fibers are stretched and therefore the shapes of inner oil compartments are elongated as well. (d) The resulting fibers after some of the oil droplets have escaped.

Table S1. The flow rate conditions of the inner alginate solution and the number of the inner alginate particles per oil drop at each flow rate of the oil phase. The average length of the inner alginate droplet was measured inside the channel of 30 μm in height and 30 μm in width. The flow rates of calcium chloride solution and the alginate solution for fibers are kept at 6 mL hr⁻¹ and 0.6 mL hr⁻¹, respectively. When more than four alginate particles are inside the oil droplet, the actual number of the particles per droplet was difficult to distinguish by image analysis (see column 6 below), so we captured high-speed movies (column 7) at the junction of double emulsion droplet formation, and counted the number of alginate droplets encapsulated as the oil phase breaks into droplets. This number is listed in column 5.

Oil phase		Inner alginate phase		Number of alginate particles per oil drop	Formation of double emulsions at the focusing junction	
$Q_{\text{oil phase}}$ (μL hr ⁻¹)	Average droplet diameter (μm)	$Q_{\text{inner alginate}}$ (μL hr ⁻¹)	Average droplet length in the channel (μm)			
5	114	0.5	91	2		
20	121	5	38	1		
40	133	16	47	4		
60	135	20	38	6		
70	142	23	34	10		
90	148	29	31	13		
110	148	42	29	started wetting		

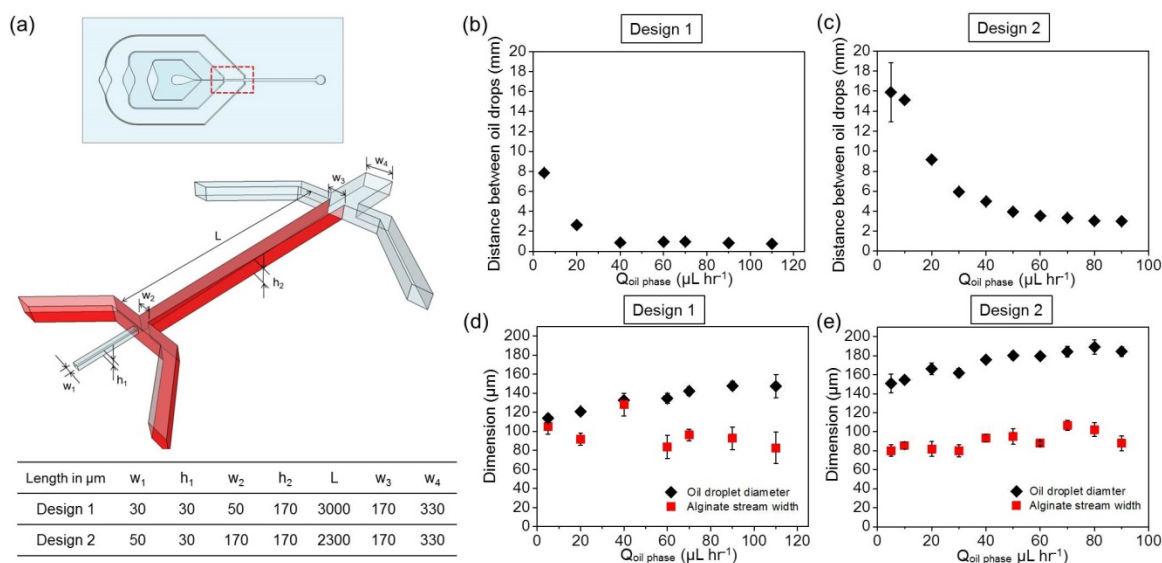


Figure S2. (a) The channel geometry of two different dimensions to fabricate the multicompartment fibers, Design 1 and Design 2. (b-e) The dimensions of the multicompartment fibers as a function of the oil phase flow rate are shown, while the flow rates of calcium chloride solution and the alginate solution for fibers are fixed to be 6 mL hr^{-1} and 0.6 mL hr^{-1} , respectively. The overall distance between oil droplets in the fiber in Design 1(b) is shorter than in Design 2(c). The diameter of oil droplets and the width of alginate stream (measured in the region with no droplets) in Design 1 (d) and Design 2 (e) are also shown. Overall, the increase in the widths w_1 and w_2 (Design 2) compared to the Design 1 resulted in the larger diameters of oil droplets and the longer distance between the droplet compartments in the fiber.

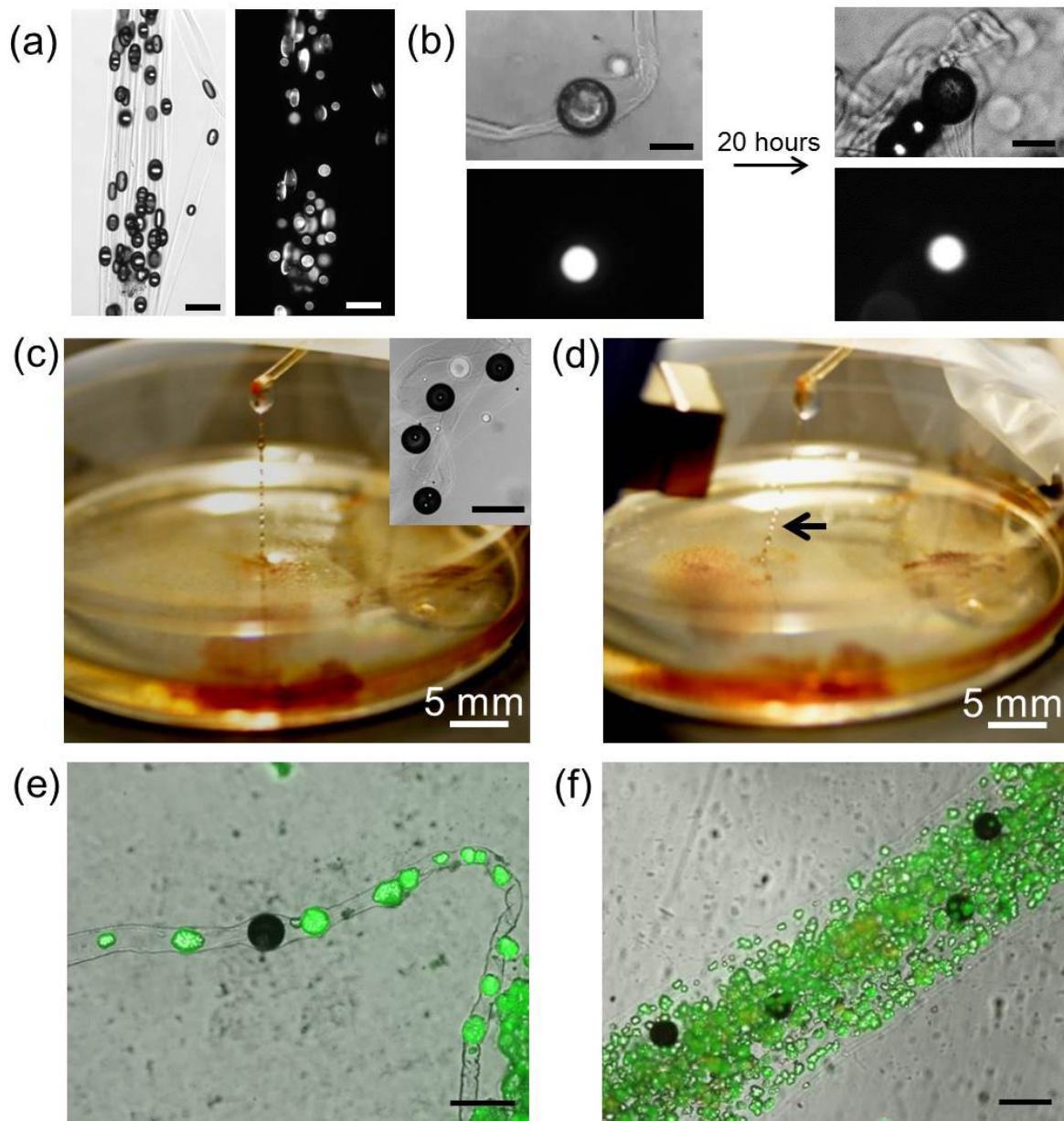


Figure S3. (a) Bright-field and fluorescence images of alginate-in-oil-in-alginate fibers where fluorescent nanoparticles are encapsulated in the inner alginate particles of the fiber. The fibers are spun using a motor. Scale bars = 200 μm . (b) Encapsulation of FITC conjugated BSA in the inner alginate particles in the alginate-in-oil-in-alginate fiber composite structure stored in DI water for 20 hours. Scale bars = 100 μm . (c) Alginate-in-oil-in-alginate fiber, where the oil compartment contains a ferrofluid, flowing out of the outlet tubing of the microfluidic device. Inset bright field image shows the structure of the magnetic multicompartiment fiber; scale bar = 200 μm . (d) The fiber is deflected in the direction of a magnet, as indicated by the arrow. (e) Fibroblast cells after 6 days of growing inside the fiber with ferrofluid-oil droplets, forming the cell spheroids. (f) The cell-encapsulated fibers, spun on the needles using a motor, form a thin cell-sheet of alginate hydrogel embedded with ferrofluid-oil droplets. Scale bars = 200 μm .

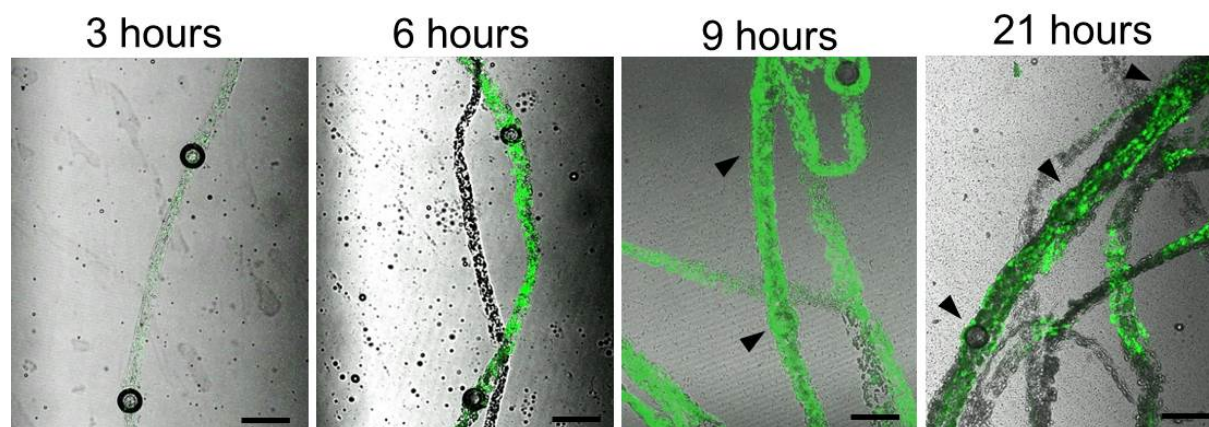


Figure S4. Growth of GFP-expressing *E. Coli* around the oil compartment of 2 wt% Span 80 in mineral oil in the alginate fiber. Black arrows indicate where the oil droplets are inside the fiber. Scale bars = 200 μm .

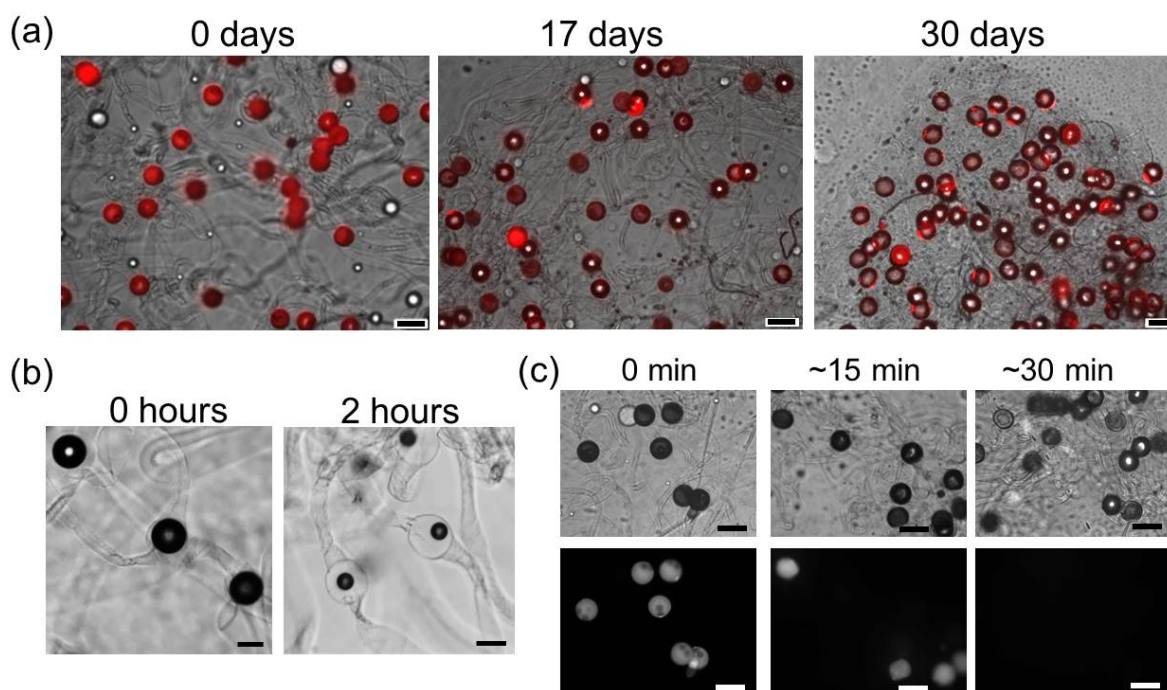


Figure S5. (a) Overlaid bright-field and fluorescence images of alginate-in-Nile Red oil-in-alginate fibers suspended in water showing the long-term stability of the multicomponent fibers. Over a one-month time frame, the average diameter of the oil droplets which are composed of mineral oil with 2 wt% Span 80 and 0.3 mg mL^{-1} Nile Red, decreased slightly from $144.1 \pm 5.2 \text{ }\mu\text{m}$ to $141.5 \pm 9.2 \text{ }\mu\text{m}$ during one month. Scale bars = 200 μm . (b) The oil compartment can dissolve and shrink in volume in water in case of using more soluble oil, such as eugenol, as the oil compartment instead of mineral oil. Scale bars = 100 μm . (c) Bright-field and fluorescence images of alginate-in-Nile Red oil-in-alginate fibers in a water/ethanol solution, where the presence of ethanol causes the contents of the oil compartment to escape the fiber as shown by the loss of dye from the oil compartments. Scale bars = 200 μm .

Supplementary Information Movie S1. Formation of multicompartiment fibers in microfluidic device

References

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