

**Electronic supplementary information for**

**Formation of monodisperse calcium alginate microbeads by rupture of water-in-oil-in-water droplets with an ultra-thin oil phase layer**

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**Materials**

Polydimethylsiloxane (PDMS; Sylgard 184) was obtained from Dow Corning (Midland, MI, USA). Decane was obtained from Wako Pure Chemical Industries (Osaka, Japan). Sodium alginate was obtained from Kimica Co. (SI-L; Tokyo, Japan). Calcium chloride was obtained from Wako Pure Chemical Industries. Tetraglycerin-condensed ricinoleic acid ester (TGCR; CR-310) was obtained from Sakamoto Yakuhin Kogyo Co. (Osaka, Japan). Sorbitan monooleate (Span 80) and phosphatidylcholine from egg yolk (eggPC) was obtained from Wako Pure Chemical Industries. Sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), and polyoxyethylene (20) sorbitan monooleate (Tween 80) were obtained from Wako Pure Chemical Industries. Dodecyl betaine (DB; Amphitol 20BS) was provided from Kao Co. (Tokyo, Japan) and was purified. New coccine was obtained from Kanto Chemical Co. Inc. (Tokyo, Japan). Sodium chloride and calcium chloride was obtained from Wako Pure Chemical Industries.

**Formation of W/O/W droplets with ultra-thin oil phase layer**

Firstly, the microfluidic device was filled with the external aqueous phase solution. All fluids were injected into the microchannels at constant flow rates controlled by syringe pumps (Pico Plus; Harvard Apparatus, Holliston, MA, USA). Flow injection was started in the order of the external aqueous phase, oil phase and internal aqueous phase. Flow rates of the internal aqueous phase, oil phase, and external aqueous phase were 0.10, 0.10, and 7.02 mL/h for formation of W/O/W droplets without sodium alginate. Flow rates of the internal aqueous phase, oil phase, and external aqueous phase were 0.05, 0.10, and 4.68 mL/h for formation of W/O/W droplets containing sodium alginate.

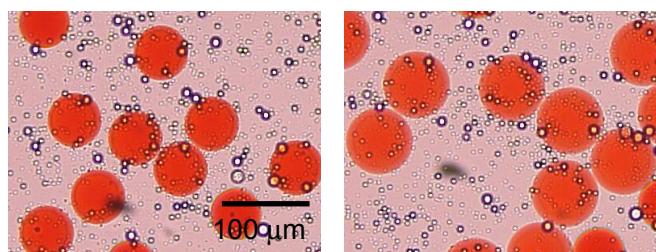


Fig. S1. Volume change of W/O/W droplets by osmotic pressure difference. Microscope images at 0 min (Left) and 9 min (Right) after the W/O/W droplet formation. The osmolarity of the internal and external aqueous phase were 300 and 13.9 mOsm, respectively.



Fig. S2. Encapsulation of solid particles as a model of cells. Fluorescent solid particles were encapsulated into the calcium alginate microbeads. Images were captured using a phase-contrast and fluorescent microscope.