

Monodisperse semi-permeable microcapsules for continuous observation of cells

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Supplementary information: Formation of microcages with mammalian cells

1. Materials, solution preparation and cell culture

In addition to materials cited in the main text, Dulbecco's modified eagle's medium (DMEM), DMEM powder and Trypsin-EDTA solution were purchased from SIGMA-Aldrich. Fetal bovine serum (FBS) was purchased from Japan Bioserum Co. Ltd.. LIVE/DEAD® Viability/Cytotoxicity kit for Live/Dead assay was purchased from Invitrogen Co.. Mammalian adherent cells used here were 3T3 cells that are mouse fibroblast cells. 3T3 cells were cultured in DMEM supplemented with 10 v/v% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C under a humidified atmosphere of 5% CO₂. Cell viability was examined with LIVE/DEAD® Viability/Cytotoxicity kit following manufacturer's protocol. DMEM (without NaHCO₃) solution was prepared as follows: 2.7 g DMEM powder was added to 200 ml of sterilized water and pH was subsequently adjusted to 7.1 with NaOH. Once a homogeneous solution was obtained, the solution was sterilized by filtration.

2. Methods of producing microcages with mammalian adherent cells

For producing microcages with mammalian adherent cells, we modified the gelation method using GDL and CaCO₃. In the AFFD, corn oil with 2 wt.% lecithin was used as the outer fluid. For the inner fluid, DMEM (without NaHCO₃) with GDL (10 mM) and DMEM (without NaHCO₃) with sodium alginate (2 wt.%) containing insoluble CaCO₃ (75 mg/ml) and 3T3 cells were used, respectively. When we used DMEM instead of DMEM (without NaHCO₃) for producing sodium alginate solution, pH reduction with carbonate ions in DMEM induced the gelation of alginate as calcium ions were released from calcium nano particles. Therefore, we cannot produce monodisperse alginate sol droplets. The inner fluids to produce monodisperse alginate sol droplets with cells were

prepared on the eve of the experiments and mixed in equal portions just before introduction into the inner channel of the AFFD. This mixed inner fluid broke up into monodisperse sodium alginate sol droplets in the AFFD. We obtained monodisperse alginate gel beads after rotating the microtube containing sol droplets for about 10 minutes for the GDL to hydrolyze.

Finally, we extracted the alginate gel beads from the corn oil and transferred them to the cell culture media. We used the same method for the transfer as reported in the main text. However, we substituted the *washing buffer* with DMEM containing CaCl_2 (81 mM), and the TAP buffer with DMEM. As a result of the transfer, we were able to obtain monodisperse alginate gel beads with 3T3 in DMEM. Next, we suspended alginate gel beads in DMEM with PLL (0.01 wt.%) and rotated them for one and a half minutes for producing alginate-PLL membrane on the gel beads. After forming the alginate-PLL membrane, we gently introduced the PLL solution containing the alginate gel beads into DMEM (without NaHCO_3) with sodium alginate (0.5 wt.%) in a microtube and settled the alginate gel beads using centrifugation. Then, we rinsed these gel beads in DMEM by resuspending and centrifuging. Finally, DMEM containing sodium citrate buffer (20 mM) was used to solubilize any alginate that did not react with PLL.

3. Results of alginate gel beads and microcages with mammalian adherent cells

Fig. S1(a) shows monodisperse alginate gel beads containing 3T3 cells produced by our method, demonstrating that our method using GDL and CaCO_3 in the AFFD has the ability to produce monodisperse alginate gel beads encapsulating mammalian adherent cells. In this figure, we fluorescently labeled 3T3 cells by Live/Dead assay; green color shows living cells and red color shows dead cells. Since about 70% of encapsulated 3T3 cells remained viable, we believe that our gelation method is also gentle enough for mammalian cells. Fig S1(b) shows monodisperse microcages containing 3T3 cells labeled by Live/Dead assay. The cell survival percentage was unchanged (~70%), indicating that the PLL membrane formation and alginate gel solubilization processes did not affect the viability of encapsulated cells. We believe that our method facilitates the production of alginate gel beads and microcages capable of encapsulating a wide range of materials, from inorganic materials

such as gold particles, glass beads, etc., to organic materials such as microorganisms and cells (both adherent and non-adherent cells of mammalian and non-mammalian types).

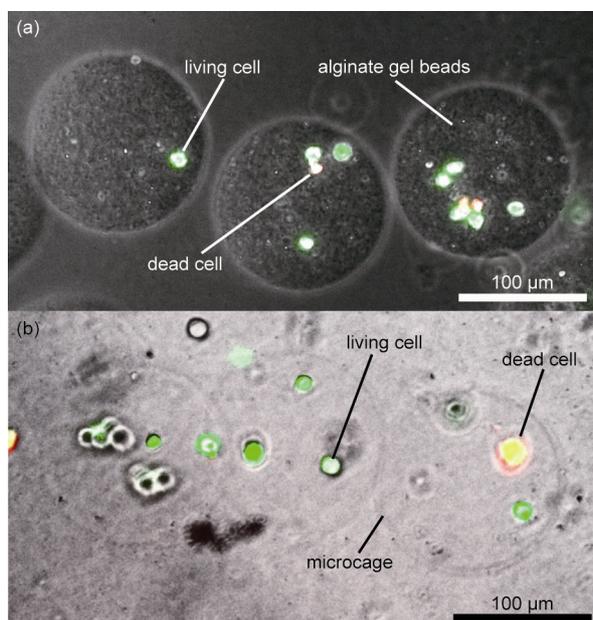


Fig. S1: (a) monodisperse alginate gel beads with 3T3 cells and (b) monodisperse microcages encapsulating 3T3 cells. We stained cells in these photos by Live/Dead assay. Green color shows living cells and red color shows dead cells.