

Supplementary Material of Manuscript

**Rapid exchange of oil-phase in microencapsulation chip
to enhance cell viability**

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The effect of flow rate on the size and throughput of alginate bead

The size and throughput of the droplets were controlled by the flow rates of oleic acid and alginate solutions. First, the flow rates of alginate solutions were changed from 40 $\mu\text{L/h}$ to 100 $\mu\text{L/h}$, whereas the flow rate of oleic acid was fixed at 0.3 mL/h (Fig. S-1a). In this test, the shearing rate was almost identical, and the supply flow for droplet generation was changed. The bead diameter was increased from 100 μm to 140 μm with a small standard deviation ($\sigma=4.47\%$). When the flow rate of the alginate solution was less than 40 $\mu\text{L/h}$, the backflow of alginate solution occurs as the result of the high pressure of oleic acid. On the other hand, when the flow rate was in excess of 100 $\mu\text{L/h}$, a thread of alginate solution was extended over the bottleneck and the droplets were generated at the downstream channel via Rayleigh-Plateau instability. It was noted that the production rate of the alginate beads was almost proportional to the flow rate of the alginate solution ($\sigma=1.69\%$).

Second, the flow rate of oleic acid was changed from 0.3 mL/h to 0.9 mL/h, whereas the flow rate of alginate was fixed at 160 $\mu\text{L/h}$ (Fig. S-1b). In this case, the shear force was proportional to the flow rate of oleic acid and the supply of alginate solution is constant. When the flow rate of oleic acid was increased from 0.3 mL/h to 0.9 mL/h, the diameter of the droplets was reduced, and the throughput was inversely proportional. As the shear force of oleic acid was increased, the frequency of bead generation became larger, and finally the diameter of the droplets was decreased.

Cytotoxicity test of oleic acid and mineral oil

In order to test the toxicity of oleic acid and mineral oil, P19 EC cells were added into e-tube contained mineral oil, oleic acid and oleic acid contained 1% CaCl_2 , and then they were cultured at 37 °C for 10 min. Cells were washed and collected by a centrifuge.

Here, trypan blue was used to divide live and dead cells. Cells are selective in the compounds that pass through the membrane; trypan blue is not absorbed in a live cell, but it traverses the membrane in a dead cell. Therefore, only dead cells will show a blue color. All toxicity assays were repeated three times. P19 EC cells in the mineral oil were lived a mean 90.4 % ($\sigma=$ 5 6.33%), but cell viability were s a mean 0.01 % ($\sigma= 0.02\%$) in the oleic acid and cells were lived a mean 0.11 % ($\sigma= 0.2\%$) in the oleic acid contained 1% CaCl_2 . From the results of experiment, oleic acid has deadly the toxicity for the cell viability. On the contrary, the mineral oil is not harmful for it.

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List of Figures

Figure S-1. Characteristics of alginate beads; (a) the bead sizes and throughput with the variation in the alginate acid flow rates (oil flow rates: 0.3 mL/h) (b) the bead sizes and
5 throughput with the variation of flow rates of Oil (alginate flow rate: 160 μ L/h).

Figure S-2. Cell toxicity of oleic acid, oleic acid contained 1% CaCl_2 and mineral oil.