Supplementary Materials (ESI)

Centrifuge-based cell encapsulation in hydrogel microbeads using sub-microliter sample solution

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1. Detailed design of the acrylic holder

The acrylic holder to fix the pulled glass capillary was made by machining 2-mm-thick acrylic plates as shown in Figure S1.

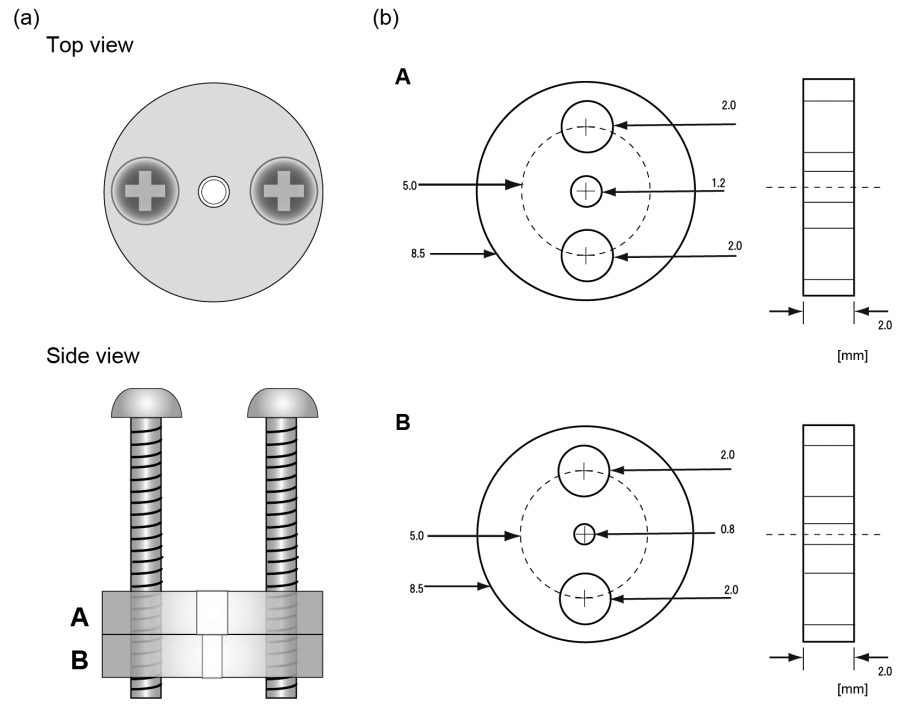

Figure S1. The design of the acrylic holder. **(a)** Schematic illustration of the acrylic holder. **(b)** Design of the acrylic holder.

2. Stability of the Ca-alginate hydrogel microbeads in culture medium

To confirm the stability of fabricated Ca-alginate hydrogel microbeads during cell culture, we evaluated the diameter change in the hydrogel microbeads immersed in culture medium for 0-4 days. We used a pulled glass capillary with ~100 μm tip diameter for the CDSD, 4% sodium alginate solution for hydrogel microbeads, 150 mM $CaCl_2$ solution for gelation and applied centrifugal gravity at ~2000 G. After the bead fabrication, we moved the microbeads into culture medium (DMEM) supplemented with 10% FBS and 1% AB. The diameter of the microbeads was measured by using an inverted phase-contrast microscope (IX-72, Olympus). As shown in Figure S2, we obtained the Ca-alginate hydrogel microbeads having 114 ± 5.2 μm (mean ± s.d., *n*=30) in diameter just after the bead fabrication. After one and four days, the diameter of the hydrogel microbeads increased by the swell of the hydrogel in the culture medium at day 1 (141 ± 3.5 μm), but the microbeads maintained their diameter for 4 days (145 ± 3.8 μm). This result shows that the Ca-alginate microbeads suspended in culture medium are stable for several days.

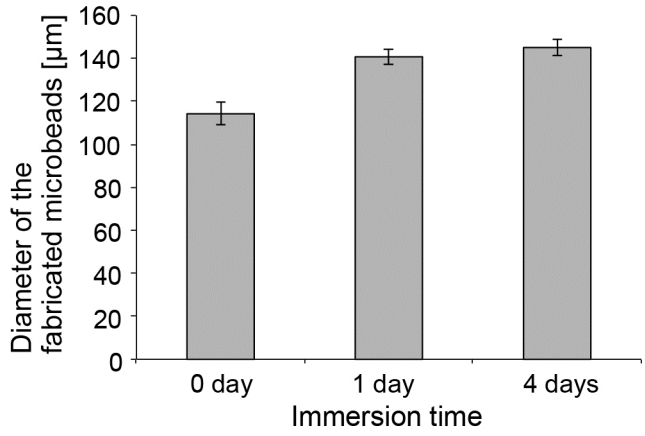

Figure S2. Dhange in the averaged diameter of the fabricated microbeads immersed in culture medium. Error bars indicate standard deviation (*n*=30).

3. Detailed design of the acrylic holder

The initial volume of Na-alginate solution, $V_{initial}$, and remained volume of sample solution after centrifuge, V_{after}, were estimated by microscopic images. Typical examples of the images before and after centrifuge are shown in Figure S3(a) and (b), respectively. For both cases, we roughly calculated the volume of the Na-alginate plug as cylinders or half spheres depending on their shapes. Note that we ignored any other tiny fluorescent signals on the inner wall of the glass capillary after the centrifuge because their volumes were sufficiently small to be ignored. The efficiencies of the ejection by centrifugal gravity force, $E_{ejection}$, was 99.6 ± 0.2%, 96.0 ± 4.0% and 98.6 ± 3.3% (mean ± s.d., n=9) for 0.5 ± 0.1 μL, 1.4 ± 0.1 μL and 2.3 ± 0.1 μL, respectively. Thus, the expected volume of sample loss in this experiment was in the scale of several tens of nanoliters.

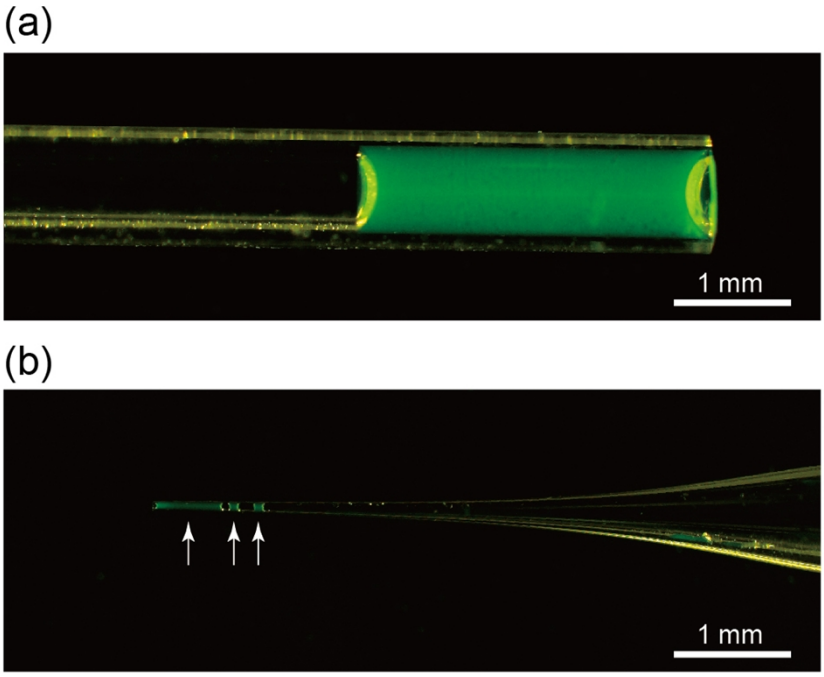

Figure S3. The images of Na-alginate solution before and after centrifuge. **(a)** ~1.4 μL solution of Na-alginate containing calcein loaded at the end of glass capillary. **(b)** Remaining Na-alginate solution (indicated by the arrows) at the tip of the glass capillary after centrifuge at 2000 G for 20 sec.