CENTRIFUGE-BASED SINGLE CELL ENCAPSULATION IN HYDROGEL MICROBEADS FROM ULTRA LOW VOLUME OF SAMPLES
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ABSTRACT
This paper describes a simple, rapid and inexpensive cell encapsulation method using ultra low volume (sub-microliter) of cell-suspended pre-gel solution. This method requires only a glass capillary, acrylic holder and tabletop centrifuge, and 20 seconds to complete single cell encapsulation that ensures high viability of encapsulated cells and high efficiency of cell encapsulation. Our cell encapsulation method would be a promising tool for encapsulating rare and precious samples in biological analyses, and would conveniently be acceptable to non-specialists of microfluidics working in biological research and point-of-care medicine.

KEYWORDS: Centrifuge, Calcium alginate, Hydrogel, Single cell, Encapsulation

INTRODUCTION
Cell microencapsulation has recently been a common technology for drug screening, single cell analysis and medical implantation [1, 2]. One of the next targets is use of rare and precious cells such as primary cells from individual human, looking ahead to clinical treatment and point-of-care medicine. However, available amount of these cells is usually quite low: order of 10⁴ cells or less, and sometimes several tens of cells in several microliter solution. Thus, current microencapsulation techniques using microfluidic devices, which usually require several hundred microliter of solutions, cannot meet this demand because of the dead volume of samples in the device. In this paper, for encapsulating small amount of samples, we apply a centrifuge-based droplet shooting device (CDSD) that we previously developed [3] to single cell encapsulation (Fig. 1). Important feature of our approach is that, in principle, all of loaded sample can turn into hydrogel microbeads because of no dead space in our device. Taking advantage of this feature, we focus on minimizing the amount of samples for single cell encapsulation.

CELL ENCAPSULATION IN CALCIUM ALGINATE HYDROGEL MICROBEADS USING CDSD
We used sodium alginate (Na-alginate)/CaCl₂ system to fabricate cell-encapsulating Ca-alginate microbeads (Fig. 1)

Figure 1: Conceptual illustration of our centrifuge-based cell encapsulation by using ultra low volume of samples. Small amount of cell suspension (~0.5 µL) is introduced pulled glass capillary and turned into microbeads by using cross-linking of alginate polymers with Ca²⁺ ions. By optimizing the cell density of the suspension, single cell encapsulated in single cell beads can be obtained efficiently from the ultra low volume of samples.
using a CDSD [3] composed of a pulled glass capillary (length: 10-15 mm, tip diameter: ~50 µm) and a lab-made acrylic holder for fixing the glass capillary in a microtube (Fig. 2). After introducing Jurkat-suspended Na-alginate solution into the capillary, we applied centrifugal gravity force to our device to generate Ca-alginate microbeads encapsulating Jurkat cells. The fabricated microbeads were reasonably sphere-shaped (average diameter: 68.4 µm ($n = 141$)), and Jurkat cells were successfully encapsulated at high viability (>95%).

**CELL ENCAPSULATION USING ULTRA LOW VOLUME OF SAMPLES**

We next examined the capability of the bead formation from ultra low volume of samples, 0.5 µL. After centrifugation for 20 s, we successfully obtained ~900 microbeads from 0.5 µL Na-alginate solution (Fig. 3 (A)) and found that some of the microbeads encapsulated single Jurkat cells inside (Fig. 3 (B)). Efficiency of the microbead formation of our device was estimated to more than 50%, meaning that the loss of the Na-alginate solution was less than 0.25 µL.

We evaluated the efficiency of single-cell encapsulation in single microbeads at three different cell concentrations (2-180 cells/µL) in the 0.5 µL sample solution. We obtained high cell encapsulation efficiency (>80%) for all the cell

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**Figure 2: Setup for the fabrication of cell-encapsulating hydrogel microbeads by centrifugal gravity.** All the instruments are inexpensive and available for non-specialists of microfabrication in most labs.

**Figure 3: Microscopic images of all of the formed Ca-alginate microbeads from 0.5 µL Na-alginate solution.** (A) A whole view of the formed microbeads that were sandwiched between a cover glass and a culture dish. (B) A magnified image of the Ca-alginate microbeads. Some of the beads encapsulated a single cell (labeled with green fluorescent dye) indicated by arrows.
concentrations. Yield of cell-containing microbeads increased as the increase of cell concentration. In the cell-encapsulating microbeads, percentage of single cell encapsulation were as high as ~80% at cell concentration of 144-180 x 10^3 cells/mL. Taken together, we achieved to encapsulate ~80% of the initial cells into microbeads, and ~80% of cell-encapsulating microbeads have single cells inside. We believe that this method would be a useful and convenient tool for encapsulating rare and precious samples in biological analyses.

CONCLUSION
In conclusion, we proposed a simple, rapid and inexpensive cell-encapsulating method using 0.5 µL sample solution using a centrifuge-based droplet shooting device. The viability of encapsulated cells were ~95%, and efficiency of cell encapsulation were ~80% at cell concentration of 144-180 x 10^3 cells/mL. By combining our method with cell sorting methods such as standard cell sorter apparatuses or microfluidic sorters, we believe that this method would be a useful tool for encapsulating rare and precious samples in biological analyses.

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