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Rapid one-step purification of single-cells encapsulated in alginate microcapsules from oil to aqueous phase using a hydrophobic filter paper: Implications for single-cell experiments

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By virtue of the biocompatibility and physical properties of hydrogel, picoliter-sized hydrogel microcapsules have been considered to be a biometric signature containing several features similar to that of encapsulated single cells, including phenotype, viability, and intracellular content. To maximize the experimental potential of encapsulating cells in hydrogel microcapsules, a method that enables efficient hydrogel microcapsule purification from oil is necessary. Current methods based on centrifugation for the conventional stepwise rinsing of oil, are slow and laborious and decrease the monodispersity and yield of the recovered hydrogel microcapsules. To remedy these shortcomings we have developed a simple one-step method to purify alginate microcapsules, containing a single live cell, from oil to aqueous phase. This method employs oil impregnation using a commercially available hydrophobic filter paper without multistep centrifugal purification and complicated microchannel networks. The oil-suspended alginate microcapsules encapsulating single cells from mammalian cancer cell lines (MCF–7, HepG2, and U937) and microorganisms (*Chlorella vulgaris*) were successfully exchanged to cell culture media by quick (~10 min) depletion of the surrounding oil phase without coalescence of neighboring microcapsules. Cell proliferation and high integrity of the microcapsules were also demonstrated by long-term incubation of microcapsules containing a single live cell. We expect that this method for the simple and rapid purification of encapsulated single-cell microcapsules will attain widespread adoption, assisting cell biologists and clinicians in the development of single-cell experiments.

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1 Introduction

Observation and investigation of the hallmark traits of individual living cell populations while excluding the effects of adjacent cells presents a useful experimental paradigm in cell biology [1]. To date, biological questions regarding cellular heterogeneities remain unresolved, even though various microfluidic techniques have been applied to these questions [2,3]. Particularly, monodisperse droplets generated by microfluidics in a rapid and high-throughput manner are capable of confining single cells within a well-controlled microenvironment to perform single-cell analysis based on cellular fluorescence detection [4,5], time-resolved analysis [6–8], and drug screening [9]. However, a microdroplet-based single-cell analysis has the drawback of volume loss from long-term incubation, which requires the constant introduction of solvent [10].

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Abbreviations: PBS, phosphate-buffered saline ; **PDMS**, poly(dimethylsiloxane)

A preferable system is highly monodispersed hydrogel microcapsules obtained via microfluidic formation of a water-in-oil emulsion. Monodispersed hydrogel microcapsules have been widely used as tiny incubators designed to permit facile diffusion of oxygen and nutrients to and from the encapsulated single cells through the nano-sized pores in a microcapsule [11]. In this regime, various applications of cell and/or microbe encapsulation have been utilized for long-term culture [12], in situ analysis of cellular content [13], transplantation [14], and cellular function analysis [15]. In comparison to conventional liquid droplets dispersed in immiscible carrier oil, hydrogel microcapsules are attractive because: (i) the cells are conserved from liquid evaporation; (ii) the encapsulated living cells display a high viability and cultivability; and if necessary, (iii) it is possible to easily recover the trapped individual cell from the microcapsule by dissolving it into a biocompatible buffer. To maximize the merits of hydrogel microcapsules, it is vital to purify hydrogel microcapsules from oil directly into a cell culture media.

The most common method for the depletion of immiscible fluid from hydrogel microcapsules is stepwise rinsing of oil after microcapsule collection [16]. Conventionally, the microcapsules are first dissolved in an organic solvent, such as chloroform, isopropanol or hexane, to remove the surrounding oil phase [17]. However, the viability of the cells would be threatened due to the toxicity of the solvent used during the extraction of the cell-encapsulating microcapsules from the oil phase. Many researchers prefer the alternative of repeated rounds of off-chip washing with distilled water and low-speed centrifugation, but the collected microcapsules can aggregate, resulting in a decrease in the monodispersity and recovery yield of the microcapsules [18]. Additionally a time-consuming off-chip manipulation of cell-laden hydrogel microcapsules reduce cell viability. Microfluidic modules for on-chip continuous extraction have also been demonstrated. However, they require surface patterning the poly(dimethylsiloxane) (PDMS) channel [19], or the complex microchannel network with multiple inlets and outlets [20, 21], which is not accessible or attractive to end-users.

The aim of this study is to demonstrate a simple one-step purification approach for the rapid extraction of single-cell-encapsulated hydrogel microcapsules from oil phase to aqueous phase. We recently introduced a microfluidic control module for on-chip polymerization and recovery of the size-controlled hydrogel microcapsules by changing flow rates [22]. Since the hydrogel microcapsule collecting chambers are configured to be compatible with commercially available hydrophobic filter paper with a small pore size (~0.45 μm), the filter paper can be impregnated with the oil phase containing hydrogel microcapsules. Consequently, quick depletion of the surrounding oil promoted by the hydrophobic filter paper is suitable for one-step purification of microcapsules, shortening extraction time (~10 min for removal of the oil phase) and simplifying the laborious process of washing out the residual oil phase. After the external gentle washing for culture medium exchange, the cell-laden hydrogel microcapsules are resuspended with no unwanted aggregation of neighboring microcapsules and show a high monodispersity. To confirm that the proposed method could be utilized to rapidly extract single-cell-encapsulated hydrogel microcapsules, we encapsulated single cells from cell lines and microorganisms in monodispersed alginate hydrogel microcapsules and demonstrated the extraction of the resulting microcapsules from a carrier oil. In addition, we measured cell proliferation to see if the encapsulated cells can actually survive in the purified microcapsules.

2 Materials and methods

2.1 Material preparation

Sodium alginate (1% w/w; A0682–100G, Sigma) in phosphate-buffered saline (PBS; Gibco) was prepared and filtered with a 0.22 μm syringe filter (Millex-GV, Millipore) to remove any clumps of alginate. Oleic acid (Sigma) with Abil EM 90 surfactant (4% w/w) was injected as a continuous phase. The calcified oleic acid was prepared by dissolving 0.6 g of calcium chloride (C7902–500G, Sigma) in 25 mL of oleic acid via ultrasonication.

2.2 Preparation of mammalian cells and algal cells

Cells were cultured in the appropriate growth media and incubated at 37°C in 5% CO_2 in a humidified water-jacketed incubator. MCF–7 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen, CA), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell layers cultured on Petri dishes were rinsed with PBS and then detached using trypsin-EDTA solution (0.25% trypsin and 1 mM EDTA·4Na; Gibco). U937 cells were cultured in RPMI 1640 medium (Invitrogen). Prior to encapsulation, appropriate aliquots of the cells were diluted to 1.0×10^7 cells/mL. *Chlorella vulgaris* (KMMCC–274) was obtained from the algal culture collection at the Korean Marine Microalgae Culture Center (Busan, Korea). All cultures were incubated under constant shaking with an agitation speed of 120 rpm and continuous illumination with a 3000–lux intensity lamp.

2.3 Design and fabrication of a PDMS-based microfluidic module

A microfluidic device for microcapsule generation was fabricated using a conventional PDMS (Sylgard 184; Dow

Corning, Midland, MI) molding process. The PDMS replica mold was obtained by SU–8 photoresist (Microchem Corp., St. Newton, MA) patterning on a silicon wafer via two-step soft lithography. The device consisted of two microchannels for alginate precursor and oleic acid (40 and 60 µm wide, respectively), the microbridge (15 µm wide and 300 µm long), the main channel (100 µm wide), and a diverging microchannel near the outlet (200 µm wide). The first layer of photolithography defined the cross-junction (35 µm in depth), while the second layer defined the microbridge structures and the main channel (100 µm in depth), which facilitated recovery of the droplet shape from a plug to a sphere. The PDMS microchannel was then irreversibly sealed by plasma activation on a glass slide for 1 min. Then the microfluidic devices were sterilized in an autoclave at 120°C for 15 min and dried at 65°C for 1 day to allow recovery of the hydrophobic nature of the microchannel. To reduce the adherence of cells to the channel walls, 1% (w/v) Pluronic F127 in distilled water was coated onto the surface of the microchannel before cell introduction.

2.4 Centrifugal purification of hydrogel microcapsules

Centrifugal purification of hydrogel microcapsules from oil phase to aqueous phase was performed as follows. First, the microcapsules prepared in the microfluidic module were collected at the microtube. After gently adding PBS to the microtube, the microtube was centrifuged in 500 rpm for 5 min, and then washed with PBS to remove oil, surfactant and other residues. The washing process was repeated three times.

2.5 Purification of hydrogel microcapsules using a hydrophobic filter paper

The sodium alginate precursor microdroplets each containing a single cell were dispersed in oleic acid using a flow-focusing method at a cross-junction in the microfluidic module (Fig. 1A). The calcified oleic acid was injected into the additional channel leading to polymerization of the monomer droplets into microcapsules via the microbridge structures. Then the polymerized microcapsules were collected at the microfluidic outlet and were transferred into an Eppendorf microtube with a pipette (Fig. 1B). Fig. 1C shows a schematic representation of the simple one-step purification of hydrogel microcapsules from oil phase to aqueous phase using commercially available 47 mm diameter hydrophobic filter paper (Millipore Co., MA) made from polyvinylidene fluoride with a 0.45 µm pore size. First, the polymerized microcapsules generated in the microfluidic module were transferred to the hydrophobic filter paper with a pipette. The oil phase immediately begins to permeate through the filter paper because of its hydrophobicity. After 10 min, we turned the oil impregnated paper over and placed it on a PBS drop in a Petri dish. As the liquid drop was spread out on the surface of Petri dish, the hydrogel microcapsules were submerged in the aqueous solution immediately. After

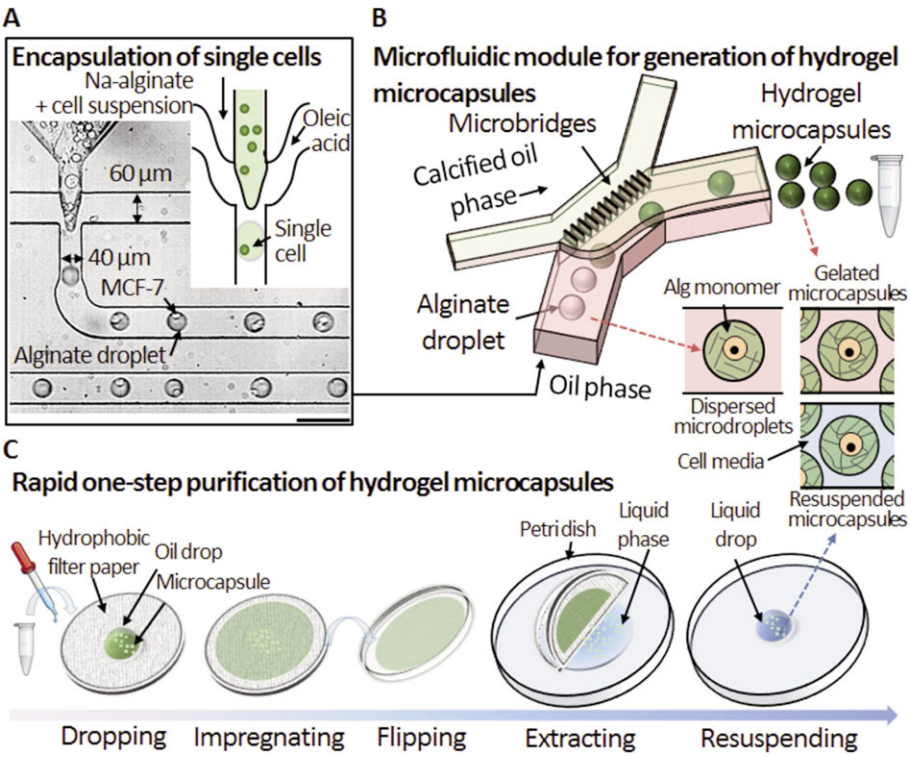

Figure 1. Schematics of the process for the extraction of alginate hydrogel microcapsules from oil phase using hydrophobic filter paper. (**A**) Schematic diagram and bright-field micrograph showing MCF-7 cell encapsulation into a sodium alginate precursor droplet at the cross junction. Scale bar: 50 µm. (**B**) Schematic diagram of operating principles for the rapid preparation of single-cell-encapsulated hydrogel microcapsules. (**C**) Schematic of the one-step purification of the hydrogel microcapsules using a hydrophobic filter paper.

removing the filter paper, the oil phase was completely depleted, leading to an extraction of the microcapsules into the liquid phase. The microcapsule-suspending PBS liquid drop was finally placed on the Petri dish, which facilitates the storage of microcapsules for a long period.

2.6 Experimental setup

All cell-containing fluids were introduced using a syringe pump (KDS200; KD Scientific Inc., Holliston, MA). The movements of droplets, including single cells, were recorded with a computer-controlled high-speed camera (Hotshot 512sc; NAC Image Technology, Simi Valley, CA) mounted onto an inverted optical microscope (TS100; Nikon Co., Tokyo, Japan).

3 Results and discussion

3.1 Extraction of alginate microcapsules

Figure 2A shows photographs depicting the continuous process of extracting hydrogel microcapsules from oil phase to liquid phase. The 100 µL drop of oily microcapsule suspension was transferred to the hydrophobic filter paper using a pipette tip (step I). To visualize microcapsules, orange food dye was mixed with the alginate precursor solution. After 10 min, the oil was completely impregnated through the filter paper due to it's inherent hydrophobicity, but the hydrogel microcapsules could not passed through the filter paper due to the small pore size (step II). We could observe the microcapsules attached to the oil impregnated paper microscopically (see inset of step II). Step III shows the arrangement of the paper which was completely immersed in the oil phase. A 200 µL PBS drop was placed on a Petri dish and then the paper was positioned onto the PBS drop with a pair of tweezers. Subsequently we observed that the orange dye diffused away from the hydrogel microcapsules, resulting in a color change in the PBS solution. The microcapsules were washed and finally resuspended in PBS (step IV).

To validate monodispersity, hydrogel microcapsules prepared using the conventional centrifugal method were compared to those obtained with the simple one-step method with regard to morphology. We were able to obtain alginate microcapsules in PBS using a benchtop centrifuge, however, we observed that the resulting microcapsules showed low monodispersity (Fig. 2B). Neighboring microcapsules aggregated with each other due to the high centrifugal force. This unwanted clumping and aggregation of microcapsules reduce both monodispersity and single-cell encapsulation efficiency. On the contrary, through the quick depletion of the oil phase using a hydrophobic filter paper, we successfully

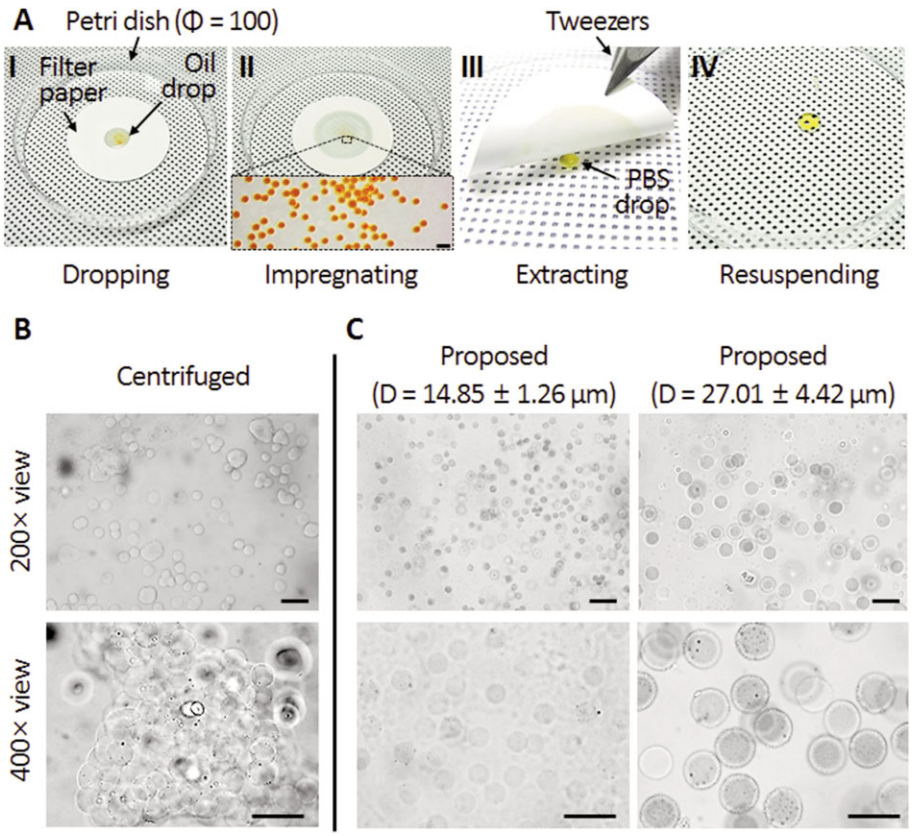

Figure 2. Simple one-step preparation of liquid-suspended alginate microcapsules using hydrophobic filter paper. (**A**) Photographs depicting the continuous process of extracting hydrogel microcapsules from oil phase to liquid phase. (I–II) The oil-suspended microcapsules were transferred and impregnated through hydrophobic filter paper. Bright-field micrograph of the yellow-dyed microcapsules attached to the oil-impregnated filter paper (see inset). (III–IV) After placement of the oil impregnated paper on a 200 µL PBS drop in a Petri dish, the microcapsules were washed and finallly resuspended in a drop of PBS. (**B**,**C**) The fabricated alginate microcapsules were isolated and stained by methylene blue. The morphology of the microcapsules purified via the conventional (**B**) and single step method (**C**) was compared. Scale bar: 50 µm.

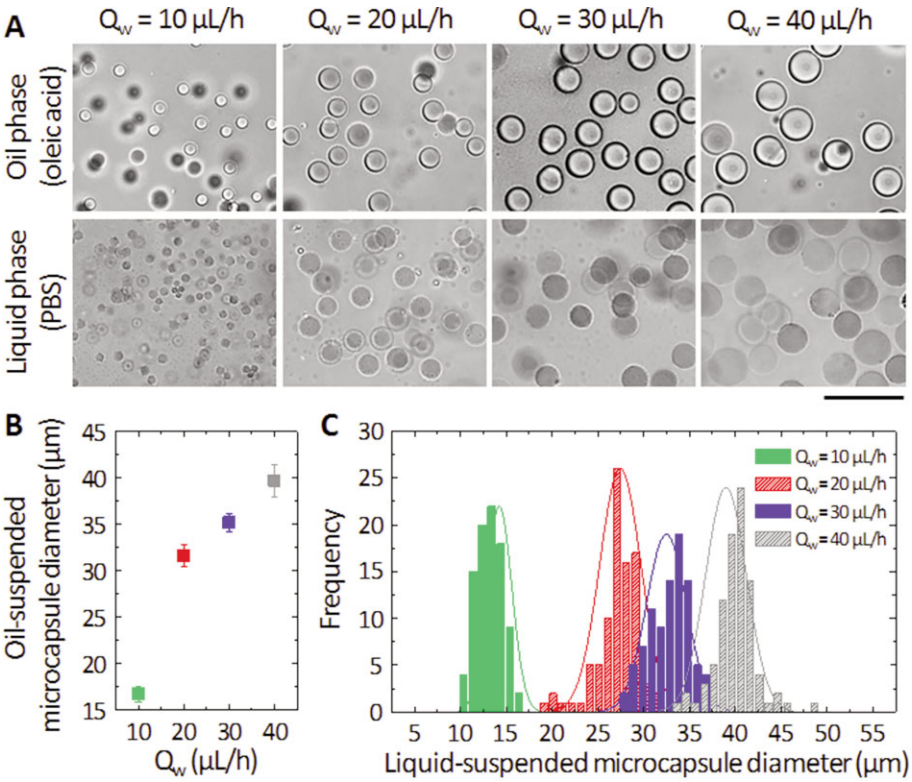

Figure 3. Prepared alginate microcapsules, size control, and size distribution. (**A**) Microscopic image of the microcapsules in oil and liquid phase when the alginate flow rate (Q_w) in the microfluidic module was varied. The oil flow rate was set at 50 µL/h. Scale bar: 100 µm. (**B**) Plot of the diameter of oil-suspended microcapsules with respect to Q_w. Data are represented as mean ± SD of three experimental replicates. (**C**) Distribution of the diameter of microcapsules purified using a hydrophobic filter paper according to the variance of Q_w (n = 90).

prepared spherical alginate microcapsules with no aggregation (Fig. 2C).

By combining the microfluidic module with integrated microbridge structures, and the hydrophobic-paper-based oil extraction, we were able to produce monodisperse alginate microcpasules and to precisely control their size. Figure 3A shows the microcapsules in oil and liquid phase obtained when the alginate flow rate (Q_w) was varied as indicated. The water/oil interface of the oil-suspended hydrogel microcapsule appeared clearly, whereas that of the liquid-suspended microcapsule did not. The diameters of the microcapsules were 16.73 ± 0.85, 31.64 ± 2.09, 35.18 ± 0.98, and 39.68 ± 1.76 µm, when the Q_w was 10, 20, 30, and 40 µL/h, respectively, based on three replicates (Fig. 3B). Figure 3C illustrates the variability in microcapsule size with various Q_w when generating alginate microcapsules after oil extraction (n = 90). As the flow rate ratio (Q_w/Q_o) at a steady oil flow rate (Q_o) of 50 µL/h increased, the microcapsule diameter increased. These liquid-suspended microcapsules were monodisperse since the coefficient of variation (CV) was less than 5%. This monodispersity results from the sufficient spacing between two neighboring monomer droplets via microbridge structures, which guarantees the stable formation of alginate microcapsules without the aggregation of surrounding microcapsules. Thus we confirmed that the microcapsule sizes were precisely controllable by adjusting the input flow rate.

3.2 Preparation of liquid-suspended alginate microcapsules containing a single live cell

Shrinkage and evaporation of the oil-suspended microcapsule were also examined. Evaporation of the encapsulated liquid solution due to the surrounding oil phase has previously limited the time for which microcapsules can be stored. The oil- and liquid-suspended microcapsules encapsulating a single MCF–7 cell were compared with regard to on-stage incubation (Fig. 4A). The diameter of the prepared microcapsules were approximately 32 µm. The time-lapse bright-field microscopic images showed that the spherical shape of the oil-suspended microcapsules was distorted considerably and they eventually collapsed on a glass surface. When the incubation time varied from 0 to 30 min, the diameter of the oil- and liquid-suspended microcapsules changed from 30.53 ± 3.76 to 13.57 ± 4.26 µm, and 31.64 ± 3.39 to 31.42 ± 3.44 µm, respectively. We observed that as incubation time increased, the diameter of oil-suspended microcapsules decreased (Fig. 4B). However, the diameter of the microcapsules suspended in culture media was stably maintained irrespective of the incubation time; indicating that the liquid-suspended microcapsules were successfully conserved from liquid evaporation during 30 min incubation ($p = 2.78 \times 10^{-9}$, statiscal anlaysis was conducted using the Student's t test, $^{*}p < 0.05$, n = 10). Therefore we confirmed that the prepared microcapsules were robust

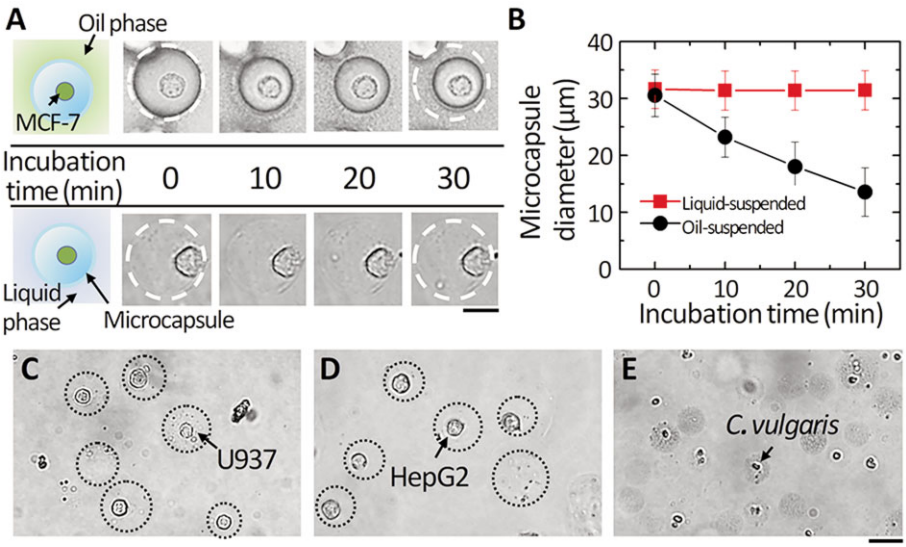

Figure 4. Liquid-suspended alginate microcapsules encapsulating various kinds of cells and their integrity. (**A**) Bright-field images of the oil- (top) and liquid-suspended (bottom) microcapsules encapsulating single MCF–7 cells as the aqueous phase evaporates for 30 min. White dotted circles indicate the initial diameter of microcapsule. Scale bar: 20 μm. (**B**) Variation in the mean diameters of the oil- and liquid-suspended microcapsules as a function of time. The diameter of oil-suspended microcapsules were significantly different from the diameter of liquid-suspended microcapsules at an incubation time of 30 min (statiscal anlaysis was conducted using the Student's *t*–test, $^{*}p < 0.05$, $n = 10$). Data are represented as mean ± SD. Bright-field images of alginate microcapsules containing a single (**C**) U937, (**D**) HepG2, and (**E**) *C. vulgaris* cell. Black arrows indicate encapsulated cells in hydrogel microcapsules. Scale bar: 30 μm.

and their quality could be maintained in the cell culture medium for several hours.

For practical purposes, this microfluidic approach was used to encapsulate various kinds of cells into monodispersed microcapsules. Microcapsules were obtained using cell lines, including U937 (Fig. 4C) and HepG2 (Fig. 4D), and microalgae of *Chlorella vulgaris* (Fig. 4E), and then resuspended in the culture media. Due to the smaller diameter of microalgae, we prepared smaller diameter microcapsules (~20 μm) to encapsulate single alga. Irrespective of the cell type, the shape and monodispersity of the microcapsule were maintained since multiple alginate microcapsules did not coalesce. The advantage of our microcapsule preparation method is its high applicability, which stems from the following features: (i) the purification method stands on the basis of commericially available apparatus and thus can be established more easily than other conventional purification methods; and (ii) the purification procedures can be performed on a clean bench within several minutes while maintaining cell viability. Therefore, we anticipate that the simplicity of cell-laden microcapsule purification will gain widespread adoption from cell biologists and clinicians to facilitate their therapeutic strategies.

3.3 Long-term cell culture in microcapsules

To verify the viability and cultivability of the encapsulated cells, we have demonstrated the ability of the purification method for the culture of living single cells. A sample of microcapsules each containing a single MCF–7 was incubated at 37°C for 6 days. At culture day 0, the single-cell-laden spherical microcapsules were dispersed in culture media. After six days of incubation, the MCF–7 cell within each microcapsule was found to have proliferated and aggregated and the morphology of the cells were clearly observed by bright-field microscopy (Fig. 5). Despite the acidity and low gas permeability of oleic acid, the viability of MCF–7 was maintained, this was because the oleic acid was immediately changed into mineral oil at the microfluidic collection chamber by manual pipetting. We also conducted the long-term culture of *C. vulgaris* within alginate microcapsules over 19 days (Supporting information, Fig. S1). Supporting information, Fig. S1B, shows that distribution of the number of *C. vulgaris* cells encapsulated per microcapsule, when the initial cell density was ~5.0×10^7 cells/mL, follows a Poisson distribution, as has been reported previously [13]. After 19 days, the cell had divided into a number of daughter cells, resulting in the increase of cells (>2 cells) per microcapsule. Several studies have already indicated that cell proliferation in microcapsules purified by the conventional centrifugal extraction method was slower than that in microcapsules purified by extraction without centrifugation [20, 21]. Therefore, the high cell viability demonstrated by our simple and non-centrifugal purification provides a feasible tool for long-term culturing of single cells.

This simple purification technique could be widely used to encapsulate cells in alginate microcapsules. Alginate has an additional advantage in that the encapsulat-

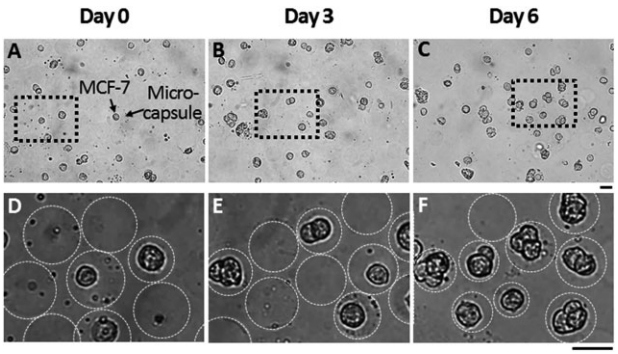

Figure 5. Morphology comparison of single MCF–7 cells within alginate microcapsules after long-term cell culture. Bright-field images of alginate microcapsules (**A**,**D**) at culture day 0, (**B**,**E**) at culture day 3, and (**C**,**F**) at culture day 6. A MCF–7 cell within the microcapsule was proliferated and aggregated and the morphology observed by magnified bright-field microscopy. Scale bars: 20 μm.

ed cells can be easily retrieved by dissolving the microcapsule with calcium chelating agents. When a solution of sodium citrate (100 mM) was introduced to the sample, the alginate microcapsules were rapidly dissolved and the cells were recovered within 10 s. The one-step method has more universal applications than other techniques, as it is not restricted to alginate precursors and can be simply modified to generate hydrogel microcapsules using other polymerizing chemistries. For instance, the UV-crosslinkable poly(ethylene glycol) and temperature-polymerized agarose microcapsules in the oil phase could be purified into the aqueous solution. This versatility can be valuable for an encapsulating experiment that requires a precise control of cell number and suitability of the surrounding scaffolds.

Furthermore, the hydrophobic filter paper can be easily integrated with the microdevice outlet; a strip of hydrophobic filter paper could be integrated into a droplet-based microfluidic platform that takes advantage of the ability to generate, polymerize, and extract alginate microcapsules in a single chip, resulting in preparation of monodisperse cell-laden microcapsules in a simple and high-throughput manner. The paper is integrated near to the microfluidic outlet chamber and absorbs the surrounding carrier oil by capillary force, enabling oil to be drained away along the paper without any active pumping. In this regime, the maintenance of constant temperature, humidity, and CO_2 allows the preservation of cell viability. This integrity accelerates the overall extraction process and will contribute to the mass production of microcapsules.

4 Concluding remarks

In this study, we developed a simple technique to prepare monodisperse alginate hydrogel microcapsules encapsulating single cells. First, continuously generated microdroplets containing single mammalian cells were polymerized immediately by the calcified oleic acid. Rapid oil impregnation, using commercially available hydrophobic filter paper, enabled the hydrogel microcapsules to be rapidly resuspended from a carrier oil into an aqueous solution, maintaining their monodispersity without unwanted aggregation of neighboring microcapsules. The microcapsule size was controlled precisely by adjusting the flow rates, making the microcapsules appropriate for the encapsulation of a single cell. These microcapsules have the capability to encapsulate various types of cells and microorganisms. Cell proliferation and the high integrity of microcapsules were also demonstrated by the long-term incubation of single-cell-encapsulating microcapsules. The one-step method of microcapsule purification is a versatile tool with the potential to enable biofunctional assays and single-cell studies. The accessibility of this simple and user-friendly method will lower the barrier to entry for cell biologists wanting to develop their own single-cell experiments.

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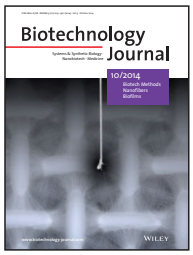

This regular issue of BTJ includes the new section "Biotech Methods", and features articles on nanofibers and biofilms. The cover illustrates the technique of electrospinning, which is applied for the production of artificial filaments that can be organized to mats, which has many applications such as wound coverning or for stabilization of bioprinting/3D tissue units. It is described that silica [bio-silica] converts those mats to morphogenetically active materials. Image by Werner Mülller. See the article by Müller et al.
http://dx.doi.org/10.1002/biot.201400277

Biotechnology Journal – list of articles published in the October 2014 issue.

Editorial: "Biotech Methods" – bringing methods to the forefront of biotechnology
Judy Peng
http://dx.doi.org/10.1002/biot.201400492

Commentary
Biosilica-loaded poly(ε-caprolactone) nanofibers: A step closer to bioprinted materials with tunable properties
Georg M. Guebitz and Enrique Herrero Acero
http://dx.doi.org/10.1002/biot.201400383

Biotech Method
Rapid one-step purification of single-cells encapsulated in alginate microcapsules from oil to aqueous phase using a hydrophobic filter paper: Implications for single-cell experiments
Do-Hyun Lee, Miran Jang and Je-Kyun Park
http://dx.doi.org/10.1002/biot.201400319

Biotech Method
Size-selective DNA separation: Recovery spectra help determine the sodium chloride (NaCl) and polyethylene glycol (PEG) concentrations required
Zhangyong He, Hong Xu, Min Xiong and Hongchen Gu
http://dx.doi.org/10.1002/biot.201400234

Biotech Method
Phenylboronate chromatography selectively separates glycoproteins through the manipulation of electrostatic, charge transfer, and *cis*-diol interactions
Rimenys J. Carvalho, James Woo, M. Raquel Aires-Barros, Steven M. Cramer and Ana M. Azevedo
http://dx.doi.org/10.1002/biot.201400170

Biotech Method
Fluorescent dye ProteoStat to detect and discriminate intracellular amyloid-like aggregates in *Escherichia coli*
Susanna Navarro and Salvador Ventura
http://dx.doi.org/10.1002/biot.201400291

Biotech Method
Phosphorylation of silk fibroins improves the cytocompatibility of silk fibroin derived materials: A platform for the production of tuneable materials
Vadim Volkov, Andreia Vasconcelos, Marisa P. Sárria, Andreia C. Gomes, Artur Cavaco-Paulo
http://dx.doi.org/10.1002/biot.201400302

Research Article
A functional high-content miRNA screen identifies miR-30 family to boost recombinant protein production in CHO cells
Simon Fischer , Theresa Buck, Andreas Wagner, Carolin Ehrhart, Julia Giancaterino, Samuel Mang, Matthias Schad, Sven Mathias, Armaz Aschrafi, René Handrick and Kerstin Otte
http://dx.doi.org/10.1002/biot.201400306

Research Articles
Feeding strategies enhance high cell density cultivation and protein expression in milliliter scale bioreactors
Georg Faust, Nils H. Janzen, Christoph Bendig, Lin Römer, Klaus Kaufmann and Dirk Weuster-Botz
http://dx.doi.org/10.1002/biot.201400346

Research Article
Bioactive nanoparticles stimulate bone tissue formation in bioprinted three-dimensional scaffold and human mesenchymal stem cells
Guifang Gao, Arndt F. Schilling, Tomo Yonezawa, Jiang Wang, Guohao Dai and Xiaofeng Cui
http://dx.doi.org/10.1002/biot.201400305

Research Article
Biosilica-loaded poly(e-caprolactone) nanofibers mats provide a morphogenetically active surface scaffold for the growth and mineralization of the osteoclast-related SaOS-2 cells
Werner E.G. Müller, Emad Tolba, Heinz C. Schröder, Bärbel Diehl-Seifert, Thorben Link and Xiaohong Wang
http://dx.doi.org/10.1002/biot.201400277

Research Article
High-level conversion of L-lysine into 5-aminovalerate that can be used for nylon 6,5 synthesis
Si Jae Park, Young Hoon Oh, Won Noh, Hye Young Kim, Jae Ho Shin, Eun Gyo Lee, Seungwoon Lee, Yokimiko David, Mary Grace Baylon, Bong Keun Song, Jonggeon Jegal, Sang Yup Lee and Seung Hwan Lee
http://dx.doi.org/10.1002/biot.201400156

Research Article
Synergistic effect of Aspergillus *niger* and *Trichoderma reesei* enzyme sets on the saccharification of wheat straw and sugarcane bagasse
Joost van den Brink, Gabriela Piccolo Maitan-Alfenas, Gen Zou, Chengshu Wang, Zhihua Zhou, Valéria Monteze Guimarães and Ronald P. de Vries
http://dx.doi.org/10.1002/biot.201400317

Research Article
Solid support membrane-aerated catalytic biofilm reactor for the continuous synthesis of (*S*)-styrene oxide at gram scale
Babu Halan, Thomas Letzel, Andreas Schmid and Katja Buehler
http://dx.doi.org/10.1002/biot.201400341

Research Article
Metabolic modeling of spatial heterogeneity of biofilms in microbial fuel cells reveals substrate limitations in electrical current generation
Nadeera Jayasinghe, Ashley Franks, Kelly P. Nevin and Radhakrishnan Mahadevan
http://dx.doi.org/10.1002/biot.201400068