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Label-free on-chip selective extraction of cell aggregate-laden microcapsules from oil into aqueous solution with optical sensor and dielectrophoresis

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Abstract

Microfluidic encapsulation of cells or tissues in biocompatible solid-like hydrogels has wide biomedical applications. However, the microfluidically encapsulated cells/tissues are usually suspended in oil and need to be extracted into aqueous solution for further culture or use. Current extracting techniques are either non-selective for the cell/tissue-laden hydrogel microcapsules or rely on fluorescence labeling of the cells/tissues, which may be undesired for their further culture or use. Here we developed a micro-electro-mechanical system (MEMS) to achieve label-free on-chip selective extraction of cell aggregate-laden hydrogel microcapsules from oil into aqueous solution. The system includes a microfluidic device, an optical sensor, a dielectrophoretic (DEP) actuator, and microcontrollers. The microfluidic device is for encapsulating cell aggregates in hydrogel microcapsules using the flow-focusing function with microchannels for extracting microcapsules. The optical sensor is to detect the cell aggregates, based on the difference of the optical properties between the cell aggregates and surrounding solution before their encapsulation in hydrogel microcapsules. This strategy is used because the difference in optical property

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Supporting Information Available: The following files are available free of charge.

SupportInfo.pdf. The experimental setup; detection after hydrogel microcapsule generation with the optical sensor being located at the downstream of the flow-focusing junction; the time characteristics of the selective extraction system; analysis of the DEP force on the microcapsules for their extraction in the actuator region; purity of label-free extraction of bead-laden hydrogel microcapsules using selective and non-selective extraction methods; typical images showing selective extraction of a cell aggregate-laden in the actuator region; and codes for programmable microcontrollers.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

between the cell aggregate-laden hydrogel microcapsules and empty microcapsules is too small to tell them apart by commonly used optical sensor. The DEP actuator, which is controlled by the sensor and microcontrollers, is for selectively extracting the targeted hydrogel microcapsules by DEP force. The results indicate this system can achieve selective extraction of cell aggregate-laden hydrogel microcapsules with ~100% efficiency without compromising the cell viability, and can improve the purity of the cell aggregate-laden microcapsules by more than 75 times compared with non-selective extraction.

Keywords

MEMS; microfluidics; microencapsulation; hydrogel; actuator

Microencapsulation of cells and tissues in solid-like hydrogels of biocompatible polymers has attracted ever-increasing attention in the field of tissue engineering and regenerative medicine.^{1–8} Microfluidics is a powerful approach for cell/tissue microencapsulation due to its capability of generating microcapsules with well-controlled size and shape.^{2, 9–13} However, the hydrogel microcapsules generated by microfluidics are usually suspended in oil after their generation while cells have to be cultured in aqueous solution, and the cell/tissue-laden hydrogel microcapsules may mix with many unwanted empty hydrogel microcapsules containing no cells/tissues. The latter is particularly true for the microencapsulation of cell aggregates and tissues (e.g., ovarian follicles and pancreatic islets) because their total number is usually small (a few tens to hundreds) from each isolation.^{2, 11, 13–15} Unfortunately, it is difficult to extract cell/tissue-laden hydrogel microcapsules out of empty ones after their generation. This is because the volume ratio of cells to the microcapsules usually is small (less than ~10%), and the difference in the overall electric, optical, and mechanical properties between cell-laden and empty hydrogel microcapsules in the oil phase is too small to be utilized for direct detection of the cell/tissue-laden hydrogel microcapsules.^{16–20}

Nam et al. fabricated a separate device different from the microcapsule generation device for sorting hydrogel microcapsules with different densities (that are dependent on the number of cells in the microcapsules) after off-chip transfer of the microcapsules from oil phase into aqueous phase.²¹ This method can achieve direct separation of cell-laden microcapsules from empty microcapsules in a single aqueous phase. However, the collection of the microcapsules from the generation device, off-chip extraction of the microcapsules from the oil into aqueous phase, and introduction of the microcapsules into the sorting device make the process complex and difficult to conduct. Furthermore, it is easy to lose the cell-laden microcapsules during the lengthy procedure of transferring microcapsules from the generation device into a tube, from the tube to a syringe, and finally from the syringe into the sorting device. This is because the microcapsules have greater density than the carrying aqueous solution (e.g., saline or cell culture medium) and are easy to sink down.

Based on fluorescence activation, several studies successfully achieved the separation of liquid droplets (note: not hydrogel microcapsules) with fluorescent microparticles/cells from droplets without fluorescent objects in oil phase and in the same device as the droplet

generation.^{22–25} The liquid droplets could be squeezed in a narrow channel in order to enhance the detection of the fluorescence signal.²⁵ This strategy unfortunately may not be applicable to the solid-like hydrogel microcapsules because they can be irreversibly damaged under squeezing. Moreover, the method requires labeling the cells with fluorescence, which may not be desired for their further *in vitro* and particularly *in vivo* applications in tissue engineering and regenerative medicine. In addition, the fluorescence-activated detection requires multiple devices such as an illuminator, a photomultiplier, a microscope, and a computer for imaging and fluorescence signal processing. All these may limit the wide applications of the fluorescence-activated detection to selectively extract the cell-laden microcapsules. Moreover, none of these studies has explored the on-chip extraction of cell-laden microcapsules from oil into aqueous solution.

On-chip extraction of cell-laden microcapsules from oil into aqueous phase is important because timely extraction can help maintain high viability of cells inside the microcapsules.^{26–28} We have recently developed a label-free approach to achieve on-chip extraction of hydrogel microcapsules from oil into aqueous solution independent of the stiffness of the microcapsules.²⁷ This is achieved by applying an external dielectrophoresis (DEP) force and utilizing the intrinsic interfacial tension force between the hydrogel microcapsule and oil (the interfacial tension between hydrogel microcapsules and aqueous solution is negligible). However, it lacks the capability of selectively extracting cell-laden microcapsules out of the empty ones.

In this study, we developed an integrated micro-electro-mechanical system (MEMS) that can be used for not only microcapsule generation, but also label-free on-chip selective extraction of cell/tissue-laden microcapsules (minimizing the extraction of empty ones) from oil into aqueous phase. The system includes a microfluidic device, an optical sensor, a DEP actuator, and microcontrollers. The microfluidic device is fabricated for microcapsule generation and extraction. The optical sensor is used to detect cell aggregates in aqueous solution before they are encapsulated, based on the difference in optical properties between the cell aggregate and their surrounding solution. The DEP actuator is utilized to extract the cell aggregate-laden microcapsules from oil into aqueous phase using an externally applied DEP force and the intrinsic interfacial tension force between the hydrogel microcapsule and oil. The microcontrollers in the system couple the optical sensor and DEP actuator, and control the actuator to achieve selective extraction of cell aggregate-laden microcapsules.

EXPERIMENTAL SECTION

Materials

Unless the source is specifically mentioned, all materials were purchased from Sigma (St. Louis, MO, United States).

Modeling of Electric Field and DEP Force

The AC/DC module of the COMSOL Multiphysics (version 5.2) was used to model the electric field in the electrode region and calculate the DEP force that the microcapsules

experience during passing through the electrode region. The governing equation is as follows:

$$\nabla((\sigma + j\omega\epsilon_0\epsilon_r)(-\nabla V)) = 0 \quad (1)$$

where σ is conductivity, ω is angular velocity, ϵ_0 is vacuum permittivity, ϵ_r is relative permittivity, and V is electrical potential. The boundary condition is as follows:

$$\mathbf{n} \cdot \mathbf{J} = 0 \quad (2)$$

where $\mathbf{n}$ is outward normal vector and $\mathbf{J}$ is electrical current. The DEP force $\mathbf{F}$ that the microcapsules experience was calculated using surface integration of the Maxwell stress tensor T as follows:

$$\mathbf{F} = \int_{\partial\Omega} \mathbf{n} T dS \quad (3)$$

where S is surface area and Ω represents the entire surface of the microcapsule. The conductivities of the alginate solution and extraction solution used in this study were set as 1.90 S m^{-1} and 1.80 S m^{-1} , respectively. Their relative permittivity was set as 80. The conductivity σ of the oil emulsion was determined to be as follows:

$$\sigma = \frac{3.6}{1 + e^{8.539 + \frac{E}{50.93}}} \quad (4)$$

where E is the electric field intensity that can be derived from the distribution of voltage in the extraction microchannel. The relative permittivity of the oil emulsion was set as 4.0.²⁷

Ray Optics Simulation

The light path in the sensor region was studied using Ray Optics Simulation that is an open-source web-based software (<https://ricktu288.github.io/ray-optics/>). The refractive index of the aqueous alginate solution, cell aggregate, polystyrene bead, and glass was set as 1.33, 1.37, 1.59, and 1.51, respectively.^{29–30} The thickness of the alginate solution and glass slide was 200 and 170 μm , respectively. Only refraction was considered in this simulation.

Fabrication of Devices

The microfluidic devices were fabricated using standard soft lithography. Firstly, SU-8 2100 photoresist (MicroChem, Westborough, MA, United States) was patterned on a 4-inch silicon wafer. After coating, soft bake, exposure, post exposure bake, development, and rinse and dry, the patterned photoresist on the wafer was obtained. The coating speed of SU-8, the baking time and temperatures, and the exposure time were set according to the data sheet of SU-8 2100 (MicroChem). PDMS (Dow Corning, Auburn, MI, United States) base and its cross-linking agent were mixed at the mass ratio of 10:1, poured on the patterned photoresist

on the wafer, and baked at 72 °C for 5 hours. After the PDMS piece was peeled off from the wafer, it was bonded to a glass slide (170 µm in thickness, Fisher Scientific) to get the microfluidic device. The device was baked at 72 °C for 72 hours before use.

Preparation of Oil Emulsion

A total of 1 g of calcium chloride (CaCl₂) was dissolved in 1 mL of deionized water, and then 1 mL of the CaCl₂ solution and 93.3 µL of Span 80 were added into 5 mL of mineral oil. The oil emulsion was obtained by emulsifying the mixture using the Branson 450 Digital Sonifier with an amplitude of 20% for 1 min.

Preparation of Cell Aggregates

MCF-7 human breast cancer cells were cultured in 75 cm² T-flasks in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and 0.1% (v/v) Plasmocin Prophylactic (InvivoGen, San Diego, CA, United States) at 37 °C in humidified air with 5% CO₂, and the medium was refreshed every two days. The cell aggregates were cultured using hanging drop method as follows: 1), after detached from the flask, the cells were suspended in culture medium at 4000 cells/mL; 2), drops of 25 µL of the medium with cells were placed on the inner surface of the covers of 100-mm petri dishes (Fisher Scientific); 3), the covers with drops were then inverted and put on the petri dishes containing 8 mL of PBS; and 4), the cells were cultured at 37 °C for 3 days to obtain the MCF-7 cell aggregates. The cell aggregates were then transferred and suspended in 2% (w/v) sodium alginate in saline for further use.

Imaging

The movies and time-lapse images were taken using a Zeiss Axio Observer.Z1 microscope with a Zeiss AxioCam MR3 CCD camera.

Statistical Analysis

At least three independent runs were conducted for all the experiments. All data were presented as mean ± standard deviation. Student's two-tailed t test assuming equal variance was conducted using JMP Pro 13 for assessment of statistical significance ($p < 0.05$ or 0.01).

Experimental Setup

The whole experimental setup is illustrated in Figure S1a, with the real images of the microfluidic device and the circuit system being given in Figure S1b and c, respectively. The configuration of the microfluidic channels, inlets, and outlets is shown in Figure 1a. A polydimethylsiloxane (PDMS) slice with microchannel of 300 µm in height was bonded to a thin glass slide (170 µm in thickness, Fisher Scientific, Waltham, MA, United States). The aqueous solution of 2% (w/v) sodium alginate in saline with polystyrene beads (diameter: 105-125 µm, Polysciences, Warminster, PA, United States) or cell aggregates (30 to 170 µm) was introduced into I1 at a flow rate of 200 µL/hr. Oil emulsion was introduced into I2 at a flow rate of 2 mL/hr. The aqueous solution of 1.3% (w/v) carboxymethyl cellulose (medium-viscosity, Sigma) in saline with 10 mM calcium chloride was introduced into I3 as the extraction flow at a flow rate of 2 mL/hr. All solutions were sterilized or filtered using

0.22 μm syringe filters (Corning, NY, United States) before use. The droplets of sodium alginate were generated in the flow-focusing junction, and then gelled into calcium hydrogel microcapsules by Ca^{2+} diffusing from the oil emulsion in the gelling channel. At last, the targeted (i.e., cell aggregate/polystyrene bead-laden) microcapsules were extracted selectively in the extraction channel. The extracted microcapsules were collected from O1, and the rest of the microcapsules were collected from O2. The extraction electrodes E1 and E2 were connected to the circuit of the DEP actuator. When actuated, an electrical potential was applied across the extraction microchannel to generate DEP force for selective extraction of the targeted microcapsules.

RESULTS AND DISCUSSION

Characterization of Optical Sensor

The optical sensor was located at the upstream of the flow-focusing junction, and the 2% (w/v) sodium alginate solution flowing in the microchannel above the sensor is transparent. This makes the beads or cell aggregates visible and allows detection of the beads or cell aggregates with high sensitivity using the optical sensor. The optical sensor consists of an illuminating board (IB, copper tape) with an illuminating hole (IH, diameter: 2 mm), a sensing board (SB, CAD/Art Services, Bandon, OR, United States) with a sensing hole (SH, diameter: 200 μm) and a Vishay (Malvern, PA, United States) Ambient Light Sensor 1206 SMD phototransistor (PT) that was connected to a sensing circuit (Figure 1b-c). A voltage V_s of 8 V was applied to the sensing circuit, and an external resistance R of 33 k Ω was used in series with the phototransistor in the circuit (Figure 1c). The microfluidic device was placed under an LED fiber optic illuminator (AmScope, Irvine, CA, United States), which provided the illumination for the optical sensor from the top of the device. The illuminating board was on the top surface of the microfluidic device, and light could pass through the illuminating hole. The sensing board was located under the microfluidic device, and the sensing hole was aligned with the microchannel, as shown in zoom-in view on the left in Figure 1a. The phototransistor was beneath the sensing hole, and received light from the top of the microfluidic device via the illuminating hole and sensing hole. Compared to the surrounding solution (i.e., the 2% (w/v) sodium alginate solution), the beads or cell aggregates may cause refraction, enhanced absorption, and/or increased scattering of light.²⁹ When a bead or cell aggregate passes through the sensor region, it may block the sensing hole due to the refraction, enhanced absorption, and/or increased scattering of light as illustrated in Figure 1d. This is confirmed by the results shown in Figure S1d-f from the Ray Optics Simulation, for which only refraction is considered. Consequently, the light intensity that the phototransistor receives will decrease, which will decrease the current in the sensing circuit when a bead or cell aggregate passes through the sensor region. This is because the higher the light intensity, the higher the current that can pass through the phototransistor. Consequently, the voltage V_{in} applied on the external resistance R (Figure 1c) will decrease. By inputting the voltage V_{in} into the mbed NXP LPC1768 microcontrollers (Pololu, Las Vegas, NV, United States), the microcontrollers were programmed to detect the voltage drop of V_{in} and control the switch (Pololu basic SPDT relay carrier with 5 VDC relay) and actuator to selectively extract the bead- or cell aggregate-laden microcapsules (Figure 1b).

The performance of the optical sensor was tested first using polystyrene beads (diameter: 105-125 μm). Figure 2a shows the display of the oscilloscope that was used to detect the voltage V_{in} in the sensor when no bead (left) or a bead (right) passes through the sensor region. When no bead passes through the sensor region, the voltage V_{in} varies little. When a bead passes through the sensor region, a peak of voltage drop due to the refraction, enhanced absorption, and/or increased scattering caused by the bead is observable. This voltage V_{in} was input into the controllers that control the actuator by the delay time and active time to selectively extract the bead-laden microcapsules. The efficiency of detecting the polystyrene beads with the optical sensor was 100% ($n = 112$). It is worth noting that the polystyrene beads cannot be detected if the optical sensor is located at the downstream of the flow-focusing junction. As shown in Figure S2 and the corresponding Movie S1, the voltage drops due to the bead-laden hydrogel microcapsules and empty microcapsules are all great and their difference is not distinguishable by the optical sensor. Therefore, we placed the optical sensor at the upstream of the flow-focusing junction to achieve label-free selective extraction of bead or cell aggregate-laden microcapsules in this study.

Characterization of System Controllers

As shown in Figure S1c, two microcontrollers were used in this study: controller 1 is for detecting the voltage drop of V_{in} when a bead or cell aggregate passes through the sensor region, and controller 2 is to control the delay time and active time of the actuator to selectively extract the targeted microcapsules. The controllers are programmable and the codes are shown in Supplemental Information. The Code S1 was for controller 1. When no bead or cell aggregate passes through the sensor region, the voltage V_{in} stays the same and the controller 1 outputs 0 to controller 2. When a bead or cell aggregate passes through the sensor region, the voltage V_{in} decreases, and the controller 1 captures the voltage drop and outputs 1 to controller 2. The Code S2 was for controller 2. When controller 2 receives 0 from controller 1, it outputs 0 to deactivate or keep the switch off; When controller 2 receives 1 from controller 1, after a delay time t_d , it outputs 1 to activate the switch for the active time t_a to extract the targeted microcapsules. As shown in Figure S3a, the delay time t_d set to controller 2 is the average time required for a bead or cell aggregate to travel from the sensor region to the actuator region, and the active time t_a set to controller 2 is the time required for the extraction electrodes E1 and E2 of the DEP actuator to extract the targeted microcapsules from the oil into the aqueous phase. However, we noticed the time for a bead or cell aggregate to travel from the sensor region to the actuator region varies from 4.6 s to 5.4 s (Figure S3b), probably due mainly to the flow instability necessary for generating the hydrogel microcapsules at the flow-focusing junction. Unlike the stable parallel flow in a straight microchannel with no flow instability or perturbation, the aqueous flows in the droplet may rotate or recoil and the flow directions are different at different positions in the droplet at the flow-focusing junction.¹⁰ As a result, the travelling time of the bead or cell aggregate in the flow-focusing junction is dependent on its entry position in the droplets (note: the bead or cell aggregate should flow together with its surrounding fluid in a microfluidic Stokes flow.¹⁰). However, the entry position or time of the bead or cell aggregate in the droplets is stochastic and can occur at any time during the process of droplet formation in the flow-focusing junction. To insure the extraction of all the targeted microcapsules, the active time t_a needs to be set to cover the maximum traveling time

variation. As a result, during the activation of the DEP actuator, one or several empty microcapsules next to the targeted microcapsules may also be extracted from oil to aqueous phase.

Characterization of DEP Actuator

Dielectrophoresis, which is a powerful tool for manipulating or sorting microparticles, was used in the system to extract the hydrogel microcapsules from oil into the aqueous phase.^{27, 31–34} A particle in a medium will experience an electric force (DEP force) in a heterogeneous electric field if the polarization of the particle and the medium is different. If the polarization of the particle is stronger than that of the medium, the DEP force will be positive and towards the stronger electric field region. Conversely, if the polarization of the particle is weaker than that of the medium, it is negative and towards the weaker electric field region. As shown in Figure 1a, the DEP actuator was located at the upstream of the outlets, where 30-gauge Hamilton (Reno, NV, United States) needles were used as electrodes E1 and E2, and placed on the two sides of the extraction channel. A voltage V_A of 200 V was consistently applied on the circuit of the DEP actuator, as shown in Figure 1b. When the switch is on, the voltage is applied on the electrodes E1 and E2 to extract microcapsules from the oil into the aqueous phase. When the switch is off, no voltage is applied on the electrodes and there is no extraction of the microcapsules. Typical images showing controlled extraction of the microcapsules from oil into the aqueous phase when they pass through the actuator region with the actuator system are given in Figure 2b. The corresponding animation is given in Movie S2. Amaranth ($< 0.5\%$ (w/v)) was added into the sodium alginate solution to make the microcapsules more visible in the aqueous solution (which is not needed for detection with the optical sensor). Approximately three or four microcapsules were extracted from oil into the aqueous phase when the active time was set as 0.5 s. In addition, the distance (D) between the microcapsules and the oil-water interface as they move through the actuator region when the DEP actuator is activated, is shown in Figure S4a. The microcapsules start to migrate to the oil-water interface at $X = \sim 300\ \mu\text{m}$ and nearly all hydrogel microcapsules are extracted from oil into the aqueous solution at $X = \sim 100\ \mu\text{m}$ (i.e., D decreases from ~ 75 to $0\ \mu\text{m}$). The velocity of the microcapsules moving in the X direction in the actuator region was measured to be $\sim 6.2\ \text{mm/s}$ on average. Therefore, the migration time for extraction is $\sim 65\ \text{ms}$ (from $X = \sim 300\ \mu\text{m}$ to $\sim 100\ \mu\text{m}$). All the active times (0.2–0.8 s) of the actuator used in this study are longer than the migration time, to ensure enough time for the extraction of the targeted hydrogel microcapsules. Since the size of the microcapsules is not negligible compared to the size of the microchannel, the microcapsules will have a significant influence on the distribution of the electric field in the actuator region. Therefore, the Maxwell stress tensor on the surface of the microcapsule was used to calculate the DEP force applied on the microcapsule using COMSOL Multiphysics version 5.2.²⁷ As shown in Figure S4b, the DEP force that the microcapsules experience in the Y direction in the actuator region is towards the aqueous solution (positive), which drives the microcapsules from the oil into the aqueous solution. Once the microcapsules touch the interface between the oil and the aqueous phase, the interfacial tension force between the hydrogel microcapsules and the oil phase will push the microcapsules into the aqueous phase.³⁵ This extraction mechanism is the same as that reported in our previous study, where microcapsules can be extracted from the oil into the aqueous phase by both the DEP force

and the interfacial tension force between the hydrogel microcapsule and oil, regardless of the stiffness of the microcapsules.²⁷

Selective Extraction of Polystyrene Bead-Laden Microcapsules

Figure 2c and the corresponding Movie S3 show the selective extraction of the bead-laden microcapsules in the actuator region when the delay time and active time were set as 5 s and 0.5 s, respectively. The bead-laden microcapsule and two adjacent empty microcapsules were deflected from the oil emulsion into the aqueous solution, while the rest of the empty microcapsules stayed in oil. Moreover, various active times were used (the delay time was fixed as 5 s, which is the average time for a bead or cell aggregate to travel from the sensing region to the actuating region, as shown in Figure S3b), and the efficiency and purity of the selective extraction were measured to quantify the performance of the selective extraction system. The data are shown in Figure 2d and S5a. The efficiency of the selective extraction is the percentage of the extracted bead-laden microcapsules over the total beads fed through inlet I1 for encapsulation, and the purity is the percentage of the extracted bead-laden microcapsules over the total extracted microcapsules from oil into the aqueous phase. When the delay time was set as 5 s and the active time was set as 0.2, 0.5, and 0.8 s, the efficiency of the selective extraction was 37.9%, 91.0%, and 97.5%, respectively. An increase in the active time from 0.2 s to 0.5 or 0.8 s significantly improves the extraction efficiency. The purity of the selective extraction at the various active times (0.2, 0.5, and 0.8 s) is shown in Figure S5a. The purity for the active time of 0.8 s is significantly lower than that for the active time of 0.5 s. However, it is not significantly different for the active time of 0.2 versus 0.5 s. This is probably because an active time shorter than 0.5 s may be applied too early or too late to select the wrong hydrogel microcapsules for extraction: Movie S4, S5, and S6 show the extraction of the microcapsules when the bead-laden microcapsule comes too early (an empty hydrogel microcapsule is extracted while missing the bead-laden one), just right (a bead-laden hydrogel microcapsule is extracted), and too late (an empty hydrogel microcapsule is extracted while missing the bead-laden one), respectively (delay time: 5 s, active time: 0.2 s). More importantly, with a delay time of 5 s and an active time of 0.5 s, the purity can be improved by more than 75 times from ~0.40% for non-selective extraction to ~30.9%, as shown in Figure S5b. Figure 2e shows typical images of the products of non-selective (i.e., actuator was on without switching off during experiment) versus selective extraction. For the latter, the delay time and active time were set as 5 s and 0.5 s, respectively. For non-selective extraction, the percentage of bead-laden microcapsules in the product is much less than 1% and most of the bead-laden microcapsules were buried in empty microcapsules. It is tedious and difficult to search the bead-laden microcapsules out of the empty ones, which can be overcome with selective extraction.

Selective Extraction of Cell Aggregate-Laden Microcapsules

To test the MEMS for label-free on-chip selective extraction of cell aggregates and tissues, aggregates of MCF-7 cells (diameter: 30-170 μm , similar to ovarian follicles and islets)^{2, 11, 14} were obtained by culturing the cells in hanging drop. Figure 3a shows the display of the oscilloscope used to detect the voltage V_{in} when one cell aggregate passes through the sensor region. When no aggregate passes through the sensor region, the voltage V_{in} varies little (as with that shown in Figure 2a); and when a cell aggregate passes through

the sensor region, a peak of voltage drop may appear due to the refraction, enhanced absorption, and/or increased scattering caused by the cell aggregate. It is worth noting that given the similar size ($\sim 115\ \mu\text{m}$ on average), the voltage drop for a cell aggregate is less than that for a polystyrene bead (Figure 2a and 3a), probably because the cell aggregates are more like the aqueous solution in the microchannel than the polystyrene beads. This is confirmed by the Ray Optics Simulation results shown in Figure S1d-f when only refraction is considered: the refractive index of cell aggregates (1.37) is closer to that of the aqueous alginate solution (1.33) than polystyrene beads (1.59). The efficiency of detecting the cell aggregates with the optical sensor is 100% when they are larger than $100\ \mu\text{m}$ in diameter, as shown in Figure 3b ($n = 252$). This means that the detecting efficiency can reach 100% if the diameter of the cell aggregates is larger than half of the sensing hole ($200\ \mu\text{m}$ in diameter, which is the same as the channel width). For cell aggregates between 82 and $100\ \mu\text{m}$ in diameter (gray region in Figure 3b), they are partially detectable. Cell aggregates smaller than $82\ \mu\text{m}$ in diameter are not detectable by the optical sensor. However, the width of the channel where the optical sensor is located and the diameter of the sensing hole can be decreased for effective detection of the smaller cell aggregates. This is because the capability of the optical sensor in detecting the cell aggregates is dependent on the ratio of the size of the cell aggregates to that of the sensing hole (or the width of the channel that is of the same size as the sensing hole), as shown in Figure 1b and d. Typical images showing selective extraction of a cell aggregate-laden microcapsule near the actuator region are given in Figure S6 (see Movie S7 for the corresponding animation), for which the delay time and active time were set as $5\ \text{s}$ and $0.5\ \text{s}$, respectively. The cell aggregate-laden microcapsule was extracted from oil into the aqueous solution. Figure 3c shows typical images of the products of non-selective versus selective extraction when the delay time and active time were set as $5\ \text{s}$ and $0.5\ \text{s}$, respectively. Again, for non-selective extraction, the percentage of cell aggregate-laden microcapsules in the product is much less than 1% and most of the cell aggregate-laden microcapsules were buried in empty microcapsules. It is tedious and difficult to search the cell aggregate-laden microcapsules out of the empty ones, which can be overcome with selective extraction.

We further studied the impact of the selective extraction procedure on the viability of cells in the cell aggregates, together with the cell viability in control aggregates that were suspended in 2% (w/v) sodium alginate in saline for ~ 2 hours (the typical experimental time). In the selective extraction group, the cell aggregates were suspended in 2% (w/v) sodium alginate in saline, selectively extracted by the selective extraction system, and collected from the system into a 50-mL centrifuge tube with 20 mL of cell culture medium. The Live/Dead (green/red fluorescence) cell viability assay kit (Life Technology) was used to assess the cell viability. Figure 4a shows typical fluorescence images of the MCF-7 cell aggregates in the control (top) and extraction (bottom) groups. The red fluorescence is minimal for both groups, which means minimal dead cells in the cell aggregates. Matlab (R2016b, Natick, MA, United States) was further used to quantify the cell viability in the aggregates via image processing as the ratio of the area of the red fluorescence to the area sum of both the green and red fluorescence, and the data are shown in Figure 4b. The viability of the cells in the control aggregates is $\sim 99.7\%$, while it is $\sim 97.4\%$ in the extracted aggregates. The difference is not statistically significant. This indicates that the on-chip procedure developed in this

study is safe for extracting cell-based aggregates and tissues. This is probably because the conductivity of the microcapsules (1.90 S/m) is much higher than that of the surrounding oil emulsion (< 5 mS/m) due to the high concentration of electrolytes (isotonic saline) inside the microcapsules.²⁷ As a result of the Faraday cage effect, the electric field inside the microcapsules is too weak to cause significant damage to the cells.²⁷

CONCLUSIONS

In summary, a MEMS was developed in this study for the label-free on-chip selective extraction of the bead- or cell aggregate-laden microcapsules from oil into aqueous phase. The optical sensor was located at the upstream of the flow-focusing junction for microencapsulation, which has a high sensitivity to detect the beads or cell aggregates. DEP force was used to move the targeted microcapsules in oil towards the aqueous phase in the DEP actuator. Moreover, two microcontrollers were utilized to detect the voltage drop in the sensor and to control the actuator with the pre-set delay and active times for the selective extraction. The cells in aggregates maintain high viability after the selective extraction due to the protection of the highly conductive hydrogel microcapsules. This MEMS may be valuable for encapsulating cells and tissues with wide applications in the field of tissue engineering and regenerative medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A schematic illustration of microfluidic device and the sensor-actuator system. (a) The microfluidic device coupled with an optical sensor to detect beads or cell aggregates and a dielectrophoretic (DEP) actuator to extract the bead- or cell aggregate-laden microcapsules. The solution of 2% (w/v) sodium alginate in saline with beads or cell aggregates was introduced into the device via I1; the oil emulsion was introduced via I2; the solution of 1.3% (w/v) carboxymethyl cellulose in saline with 10 mM calcium chloride was introduced into I3; the targeted/extracted microcapsules were collected from O1, and the rest of the microcapsules were collected from O2. Electrical potential was applied across the extraction channel via electrodes E1 and E2. (b) The design of the optical sensor together with the circuit and connection of sensor, actuator, and controllers. IB: Illuminating board. IH: Illuminating hole. SB: Sensing board. SH: Sensing hole. PT: Phototransistor. (c) The sensing circuit of the optical sensor for generating the voltage (V_{in}) to be input into the controllers. ($R = 33 \text{ k}\Omega$). (d) Mechanism of the optical sensor illustrated by the absence (left)

and presence (right) of a bead or cell aggregate passing through the microchannel above the sensor/phototransistor.

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Figure 2.

Label-free on-chip selective extraction of polystyrene bead-laden microcapsules from oil into aqueous solution. (a) The signal of V_{in} shown on the display of an oscilloscope in the absence (left) and presence (right) of a bead passing through the microchannel above the sensor/phototransistor. The X axis presents time and the Y axis presents voltage. In the presence of a bead above the sensor, there is a voltage (V_{in}) drop that can be used by the controllers to turn the switch on after a set delay time to activate the actuator for extracting the microcapsules encapsulated with the bead. (b) Typical images showing the extraction of the hydrogel microcapsules from oil into the aqueous phase in the actuator region. The delay and active time of actuator is 5 and 0.5 s, respectively. The numbers (1 to 9) are used to label and distinguish microcapsules. The time interval between adjacent images (i-vi) is 0.17 s. The switch is off for images i, v, and vi while it is on for images ii-iv. Scale bar: 300 μm . (c) Typical images showing the selective extraction of a bead-laden microcapsule (indicated by arrows) in the actuator region. The time interval between adjacent images (i-vi) is 0.17 s. The numbers (1 to 11) are used to label and distinguish different microcapsules. Scale bar: 300 μm . (d) The extraction efficiency for various active times (0.2, 0.5, and 0.8 s). The delay time was set as 5 s. *: $p < 0.05$. (e) Typical images of the products obtained from non-selective (top) versus selective (bottom) extraction. Scale bar: 200 μm .

Figure 3.

Label-free on-chip selective extraction of cell aggregate-laden microcapsules from oil into aqueous solution. (a) The signal of V_{in} shown on the display of an oscilloscope in the absence (left) and presence (right) of a cell aggregate passing through the channel above the sensor/phototransistor. The X axis presents time and the Y axis presents voltage. In the presence of a cell aggregate, a voltage (V_{in}) drop appears, which can be used by the controllers to turn the switch on after a set delay time to activate the actuator for extracting the microcapsules encapsulated with the cell aggregates. (b) The size-dependent capability of the optical sensor located at the upstream of the flow-focusing junction in detecting cell aggregates. For the cell aggregates larger than 100 μm in diameter, the detecting efficiency is 100%. The gray region indicates the overlap of the “Detected” region and “Not detected” region. A total of 252 cell aggregates from 30–170 μm in diameter were studied. (c) Typical images of the products obtained from non-selective (left) versus selective extraction (right). Scale bar: 200 μm .

Figure 4.

Cell viability in the microcapsules after on-chip selective extraction from oil into aqueous solution. (a) Typical bright field and live/dead (green/red) fluorescence images of control (without encapsulation or extraction, top) and selectively extracted (bottom) cell aggregates, showing high cell viability of all the aggregates. Scale bar: 100 μm (b) The quantitative data of cell viability of the control and selectively extracted aggregates.