Supporting Information

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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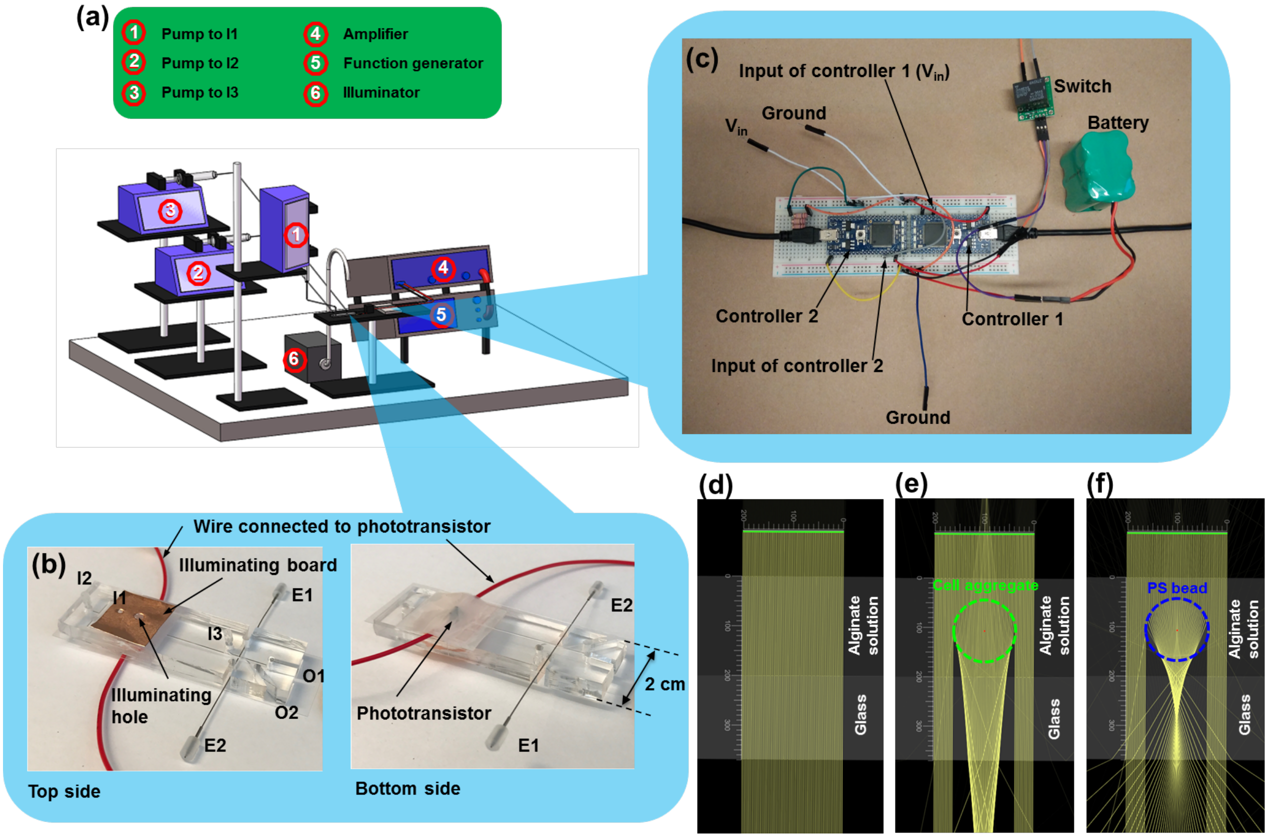

Figure S1. Experimental setup. (a) A 3D sketch of the whole experimental setup. (b) Real images of the microfluidic device (top side: left; bottom side: right). I1, I2, and I3 are inlets 1, 2, and 3, respectively. O1 and O2 are outlets 1 and 2, respectively. E1 and E2 are electrodes 1 and 2, respectively. (c) A real picture showing controller 1, controller 2, switch, battery, and connections. The output of controller 1 is 0 (when no bead or cell aggregate passes through the sensor region) or 1 (when a bead or cell aggregate passes through the sensor region), which is the input of the controller 2. (d-f) The light path determined by Ray Optics Simulation with: (d) no cell aggregate or polystyrene bead, (e) a cell aggregate, and (f) a polystyrene bead in the sensor region. The light is from top to bottom. Only refraction was considered in this simulation of light path. The phototransistor was located at the bottom of the glass slide. Unit: µm.

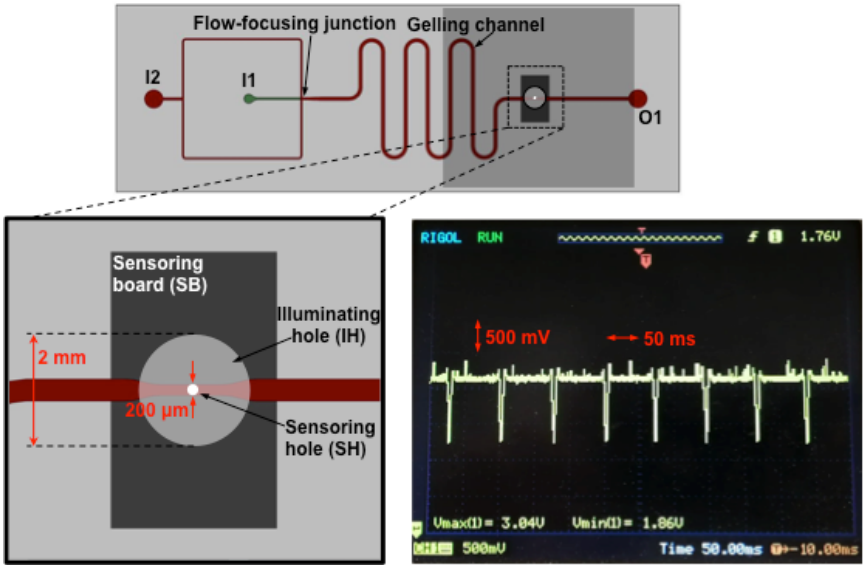

Figure S2. Detection after hydrogel microcapsule generation with the optical sensor being located at the downstream of the flow-focusing junction (FFJ): a schematic illustration of the microchannel design (top) and the position of the sensoring hole (bottom left), and a typical display of the voltage drop on oscilloscope due to empty and cell-aggregate-laden hydrogel microcapsules (bottom right). The corresponding animation showing the encapsulation of a cell aggregate at the FFJ is given as Movie S1. The aqueous solution of 2% (w/v) sodium alginate in saline with cell aggregates (diameter: 112 to 151 µm) was introduced into I1 at a flow rate of 200 µL/hr, and the oil emulsion was introduced into I2 at a flow rate of 8 ml/hr. The channel width where the sensoring hole (200 µm in diameter) is located is 200 µm. The diameter of the hydrogel microcapsules is ~195 µm. The voltage drops due to empty and cell-aggregate-laden hydrogel microcapsules are similar and indistinguishable by the optical sensor. For the display of the oscilloscope, the X axis presents time and the Y axis presents voltage.

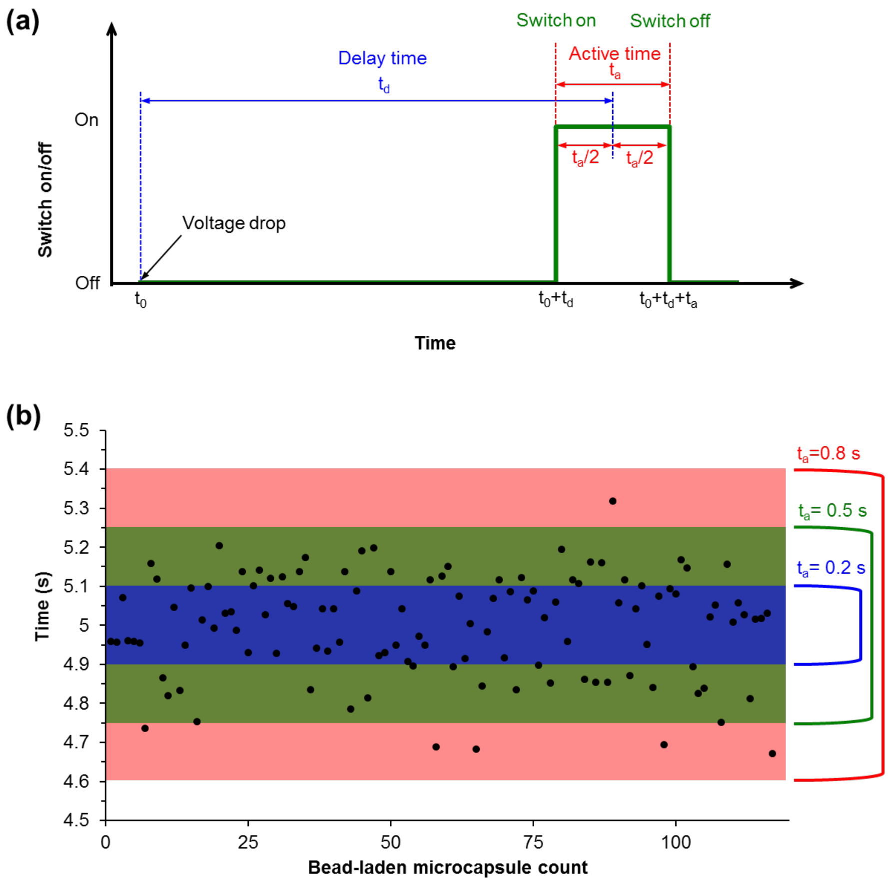

Figure S3. Time characteristics of the selective extraction system. (a) At t_0, a bead or cell aggregate passes through the sensor region, and the controllers capture the voltage drop. After a delay time $t_d - \frac{t_a}{2}$, the controllers activate the actuator by turning on the switch for the active time t_a to extract the targeted (i.e., bead- or cell-aggregate-laden) microcapsules. The average time required for a bead or cell aggregate to travel from the sensor region to the actuator region is t_d, and t_a is the time required for the actuator to extract the targeted microcapsules from oil into the aqueous phase. Due to the flow instability necessary for generating the microcapsules at the flow-focusing junction, the time needed for a bead or cell aggregate to travel from the sensor region to the actuator region may vary slightly and the maximum value should be used to ensure the extraction of all bead- or cell-aggregate-laden microcapsules. (b) The variation in time for the polystyrene beads (or cell aggregates) to travel from the sensor to the actuator region via the flow-focusing junction where they are encapsulated in alginate hydrogel microcapsules. The red, green, and blue regions show the time windows of the active time of the DEP actuator being 0.8 s, 0.5 s, and 0.2 s, respectively.

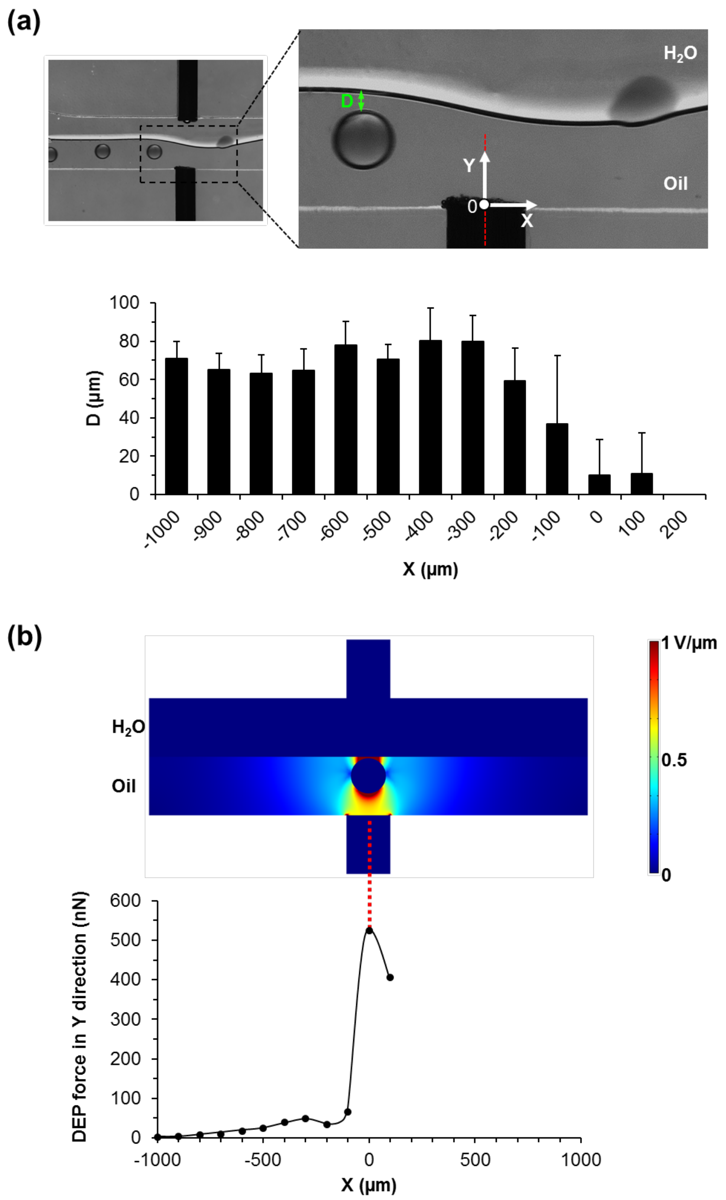

Figure S4. Analysis of the DEP force on the microcapsules for their extraction in the actuator region. (a) The distance (D) between the top surface of the hydrogel microcapsules and the oil-water interface in the actuator region when the DEP actuator is activated. The data were obtained with a total of 111 microcapsules. The microcapsules start to migrate to the oil-water interface at X = ~-300 µm and nearly all microcapsules are extracted into the aqueous phase at X = ~100 µm. The speed of the microcapsules moving in the X direction in the actuator region is measured to be ~6.2 mm/s on average, and it takes ~65 ms for the microcapsules to be extracted from oil into the aqueous phase. (b) The distribution of electric field (E) in the actuator region obtained from modeling using COMSOL Multiphysics (version 5.2), and the calculated Y-direction DEP force that the microcapsules experience when they pass through the actuator region. The positions of the microcapsules in Y direction at various X positions were set as that shown in (a). The DEP force in the Y direction is towards the aqueous (H_2O) solution, and moves the microcapsules in the oil emulsion towards the aqueous solution.

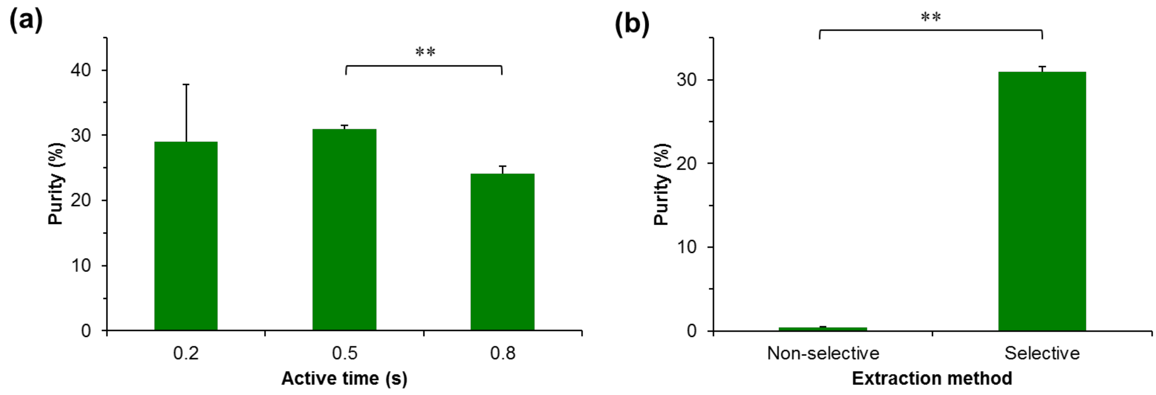

Figure S5. Purity of label-free extraction of bead-laden hydrogel microcapsules using selective and non-selective extraction methods. (a) The purity of selective extraction at various active times (0.2, 0.5, and 0.8 s) of the actuator. The delay time was set as 5 s. (b) The purity of non-selective versus and selective extraction (delay time: 5 s and active time: 0.5 s). **: $p < 0.01$

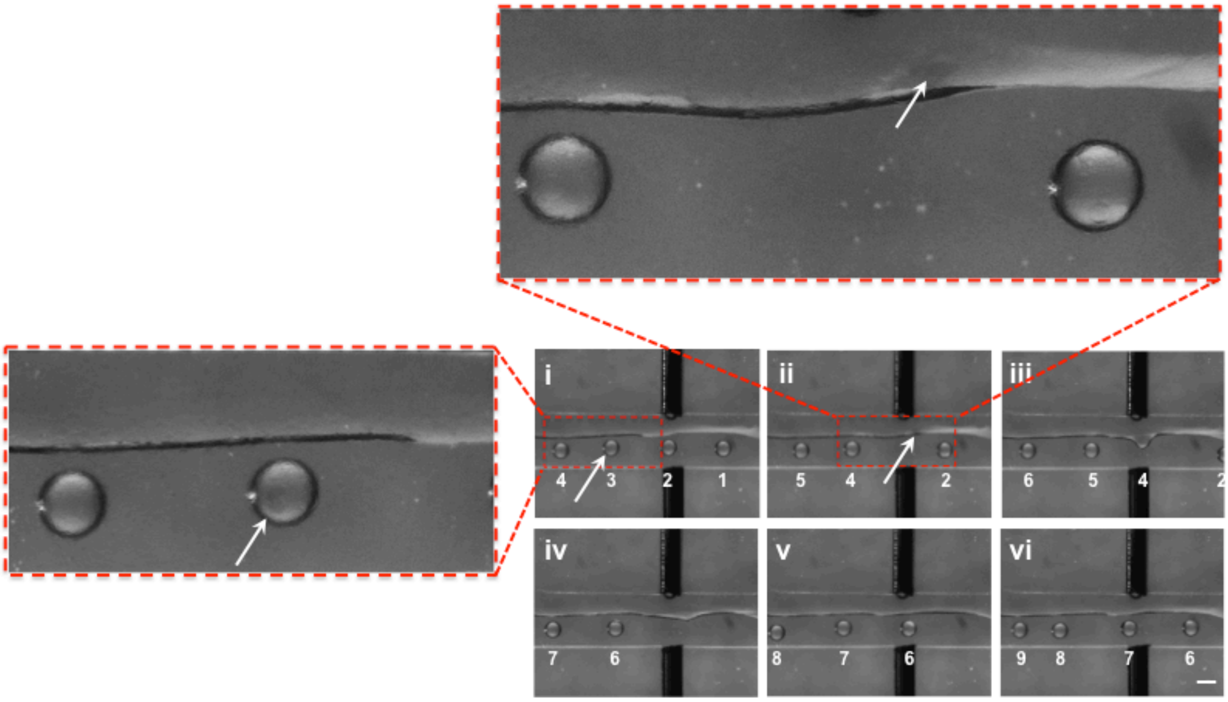

Figure S6. Typical images showing selective extraction of a cell-aggregate-laden microcapsule (indicated by arrows) in the actuator region. The delay time and active time were set as 5 s and 0.5 s, respectively. The numbers (1 to 9) are used to label and distinguish microcapsules. The cell-laden microcapsule (3) together with empty ones (4 and 5) was extracted in this specific case. The time interval between adjacent images is 0.17 s. Scale bar: 300 μm.

Code S1


```
#include "mbed.h"

AnalogIn ain(p15);
AnalogOut aout(p18);
DigitalOut dout(LED1);

Serial pc(USBTX, USBRX);

int main(void)
{
    float n[17] = {0};
    dout = 0;
    aout = 0;
    int number = 0;
    while (1)
    {
        for (int i = 0; i < 16; i++)
            n[i] = n[i+1];
        n[16] = ain;
        if (n[0] + n[1] + n[2] + n[3] > n[16] + n[15] + n[14] + n[13] + 0.05)
        {
            dout = 1;
            aout = 1;
            wait (0.3);
            number++;
            for (int i = 0; i < 17; i++)
                n[i] = 0;
        }
        else
        {
            dout = 0;
            aout = 0;
        }

        wait(0.01);
        //pc.printf("Number = %d\n", number);
    }
}

```

Code S2

File "/Actuator/main.cpp" printed from mbed.org on 3/29/2017

```
#include "mbed.h"

AnalogIn ain (p15);
AnalogOut aout(p18);
DigitalOut dout(LED1);

int delay[1000] = {0};
int da = 0;
int number = 0;
int sw = 0;

float a =0;
float b = 0;

int main()
{
    while(1)
    {
        b = ain;
        if (b > 0.75 && a < 0.75)
            number++;

        for (int i = da; i < number; i++)
        {
            delay[i]++;
            if (delay[i] > 950)
                sw = 1;
            if (delay[i] >=1050)
                da = i+1;
        }

        if (sw == 1)
        {
            aout = 1;
            dout = 1;
        }
        else
        {
            aout = 0;
            dout = 0;
        }

        a = b;
        wait(0.005);
        sw = 0;
    }
}
```

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