Magnetically guided micro-droplet using biological magnetic material for smart drug delivery system

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***Abstract*— Biodegradable polymer droplet containing magnetosome demonstrates active propulsion by magnetic field. Magnetosome is extracted from magnetotactic bacteria, AMB-1. Mixture of magnetosome and sodium alginate composes into droplet using the microfluidic device applied Plateau-Rayleigh instability principle. The magnetosome-contained droplet selects its route at the bifurcate microchannels by magnetic field. This shows tissue targeting potential of the proposed drug delivery system.**

I. Introduction

Conventional drug administration methods, while widely utilized, have many problems such as inconstant drug concentration in the administration period, unexpected effect to healthy tissue, and low delivery rate to the target tissue. In addition to these issues, high cost and long period for new drug molecule development expedite research for the effective drug delivery system [1].

The drug delivery system (DDS) is the system to ensure that drugs get into the body and reach the area where they are needed [2]. This system improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. It includes magnetic nano-capsule [3], bacteria-driven biodegradable polymer [4], microsponges [5], immune-conjugates [6], thermo-triggered squirting [7], responding to light [8], and microelectromechanical systems (MEMS) [9]. Besides magnetic nano-capsules and bacteria-driven bio-degradable polymer, most of the above studies utilize blood circulation as transport agent. Therefore, it is hard to reach speed of more than blood flow and control moving direction at blood vessel bifurcations. It means that they reach the target tissues arbitrarily. On the other hand, magnetic nano-capsule and bacteria-driven biodegradable polymer have active propulsion by bacteria and external magnetic field respectively. Autonomous mobility effectively attributes targeting capability of those DDSs.

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In this paper, we propose an actively propulsive drug delivery system composed in the shape of droplet using mixture of sodium alginate and magnetosome which is extracted from magnetotactic bacteria. Figure 1 shows conceptual schematics for composing process of the calcium alginate droplet which contains magnetosome (Fig. 1 (a)) and propulsion testing of the composed droplet at the bifurcate microchannels (Fig. 1 (b)).

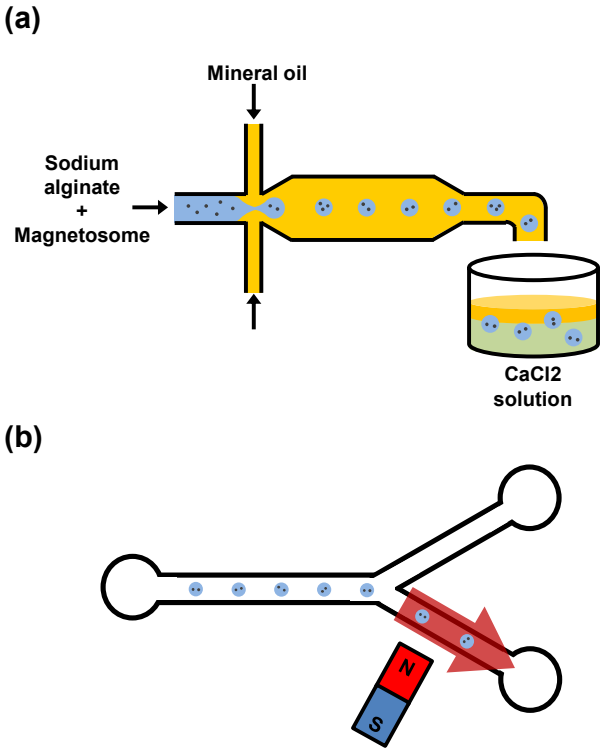

Figure 1. (a) Conceptual schematic of the droplet fabrication. (b) Ceonceptual schematic of the droplet guidance.

Magnetotactic bacteria are a polyphyletic group of bacteria that orient along the magnetic field lines of Earth's magnetic field by its organelles called magnetosomes [10]. Magnetosome chains are membranous prokaryotic structures containing 15 to 20 magnetite crystals that together act like a compass [11]. For this study, Magnetospirillum magneticum strain, AMB-1, is utilized to extract magnetosome.

Sodium alginate is utilized as droplet composing material for containing the extracted magnetosome. It is gellized when mixing with calcium chloride ($CaCl_2$) [12]. It is widely used for drug delivery system because of its biodegradability. Its biodegradability enables to release the contained therapeutic compound with the constant rate.

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Mixture of sodium alginate and the extracted magnetosome is composed as droplet by the microfluidic device applied Plateau-Rayleigh instability principle. The composed droplets test their propulsion potential in the bifurcate microchannels.

II. METHODS

A. Bacterial Culture

Magnetospirillum sp. AMB-1 (ATCC 700264, USA) was used for magnetosome. For AMB-1 cultivation, a revised magnetic spirillum growth medium (MSGM) was prepared until ready to use an AMB-1 vial. The revised MSGM contained 1L distilled water, 10 mL Wolfe's vitamin solution, 5 mL Wolfe's mineral solution, 2 mL ferric quinate solution (0.27 g $FeCl_3$ and 0.19 g quinic acid in 100 mL water), 0.45ml of 0.1 % resazurin solution, 0.37 g succinic acid, 0.12 g sodium nitrate, 0.68 g potassium phosphate, and 0.05 g sodium acetate. The MSGM measured using a pH meter and was adjusted to pH 6.75 with sodium hydroxide. If the pH of the MSGM is proper, the broth color turned pale pink. After autoclave the medium at 121^{o}C for 15 minutes, AMB-1 cells were inoculated into a screw cap test tube containing 45mL of the broth and empty space of the tube was filled with nitrogen gas to achieve microaerophilic conditions. This AMB-1 was cultured in the incubator at 30^{o}C for 6 days. Later 6 days, the broth color was transparent and became cloudy.

B. Magnetosome Extraction

Magnetosome chains can be extracted from AMB-1 cells using sonication. Several 1mL of AMB-1 broth tubes were centrifuged at 7,000rpm for 10 min at 4^{o}C and the supernatant liquid was discarded. The centrifuged cells were suspended into 10mM Tris · HCl buffer (pH 7.4) and sonicated for 30 minutes at 60W. After sonication, magnetosome chains lyse in AMB-1 because the cell membrane had been broken. The magnetosome chains were separated by a strong magnet next to the tube. The remainders were removed except magnetosome chains. The magnetosome chains were washed several times with 10 mM Tris · HCl buffer (pH 7.4) to break chains and were finally suspended in distilled water.

C. Droplet Generator Fabrication

The microfluidic device for droplet generation was fabricated with polydimethylsiloxane (PDMS) using well-known soft lithography process. Negative photoresist, SU-8 (MicroChem, MA, U.S.A.), was patterned as master mold with the shape of 3 microchannels merging into 1 micro-channel. On the processed mater mold, PDMS was poured and cured at about 80 °C. The cured PDMS was detached and attached on slide glass using O_2 plasma. Holes for inlet and outlet tubes were introduced using commercial punch. Plastic tubes were connected from the fabricated PDMS microfluidic device to pumps.

D. Droplet Generation

The design of the microfluidic device is cross channels structure with width of 100 μm, height of 100 μm. Syringe pumps (NE-1000, New Era Pump Systems Inc., USA) were employed for injection into the microfluidic system. The microfluidic device was linked to syringes through Fluoropolymer (FEP) tubes. Sodium alginate solution (1.5 wt%, No. 918, Duksan, Korea) mixing magnetosome was slowly injected into inlet of the middle of device at a 0.2 μL/min. The immiscible liquid (a mineral oil) was injected into the upper inlet as the continuous phase at a 30 μL/min. Sodium alginate solution was forced into the oil phase at the junctions of the cross channels to droplets. The microchannels at a cross point formed the droplets of the alginate solution. This channels offer a convenient and promising method for providing finer size- and shape-controlled sodium alginate droplets by varying the flow conditions. Through tube, the droplets dropped into a beaker of calcium chloride solution (200 mM, No. 07058-73, Kanto chemical Co., Inc., Japan). After few minutes, alginate droplets sank to the bottom of the beaker, being collected by a 5 mL syringe and filtered by micro sieve (mesh of 50 μm size).

E. Propulsion Test

We designed mimetic blood vessels microchannel using AutoCAD (Autodesk Inc., MA, U.S.A.). The dimensions of channel were width of 200 μm, height of 200 μm taken from the size of small arteries. The device was designed by one inlet channel and two bifurcation channels as 'Y'. Due to the same dimension of channels, submerged particle could move both sides of channel randomly. Flow rate of arterial branches is about 0 - 60 cm/sec [13]. Based on it, we controlled syringe pump rate, which has maximum velocity with 1.44 μL/min. Neodymium magnet (25-50MGOe) is placed near one of the divided channels to guide magnotosome droplets. Micro-scope (SMZ745T, Nikon, Japan) having image sensor was used to monitor behavior of the guided droplets in the Y-shaped microchannels.

III. RESULT

The extracted magnetosome from AMB-1 is shown in Fig. 2. At the non-magnetic circumstance magnetosome is distributed uniformly, so that the solution in Fig. 2 (a) looks opaque all around the vial. When a magnet is placed near the magnetosome vial, dark stain near the magnet is formed and the other part of the vial becomes little bit clearer (Fig 2 (b)). Uniformly distributed magnetosome seems to gather near the magnet. It shows that the extracted magnetosome remain their magnetic property after the extraction process.

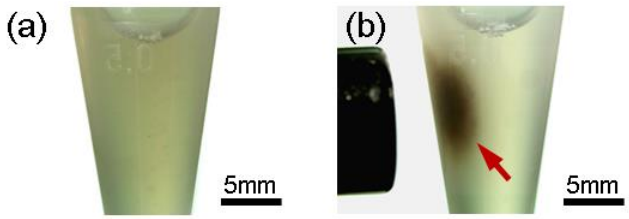

Figure 2. (a) Extracted magnetosome in a vial without any magnetic field. (b) Gathered magnetosome by a magnet (red arrow).

Figure 3 (a) shows the fabricated PDMS microfluidic device, which is colored to elucidate the microchannel part. As shown in Fig. 3 (a) and (b), in the junction of the micro-channels, droplets were generated uniformly. According to velocity of the injected fluids, size of the composed droplet was varied, as shown in Table 1. Its diameter was adjustable from 36 μm to 185 μm approximately. Considering size of the propulsion evaluation microchannel, 60 μm diameter droplets were tested.

The magnetosome droplet was dragged by neodymium magnet, as shown in Fig. 4 (a) and (b). The red arrow in Fig. 4 (b) indicates the dragged magnetosome droplet. When the magnetosome droplet is 736 μm away from the maganet, its velocity to the magnet is 40 μm/sec.

TABLE I. DROPLET SIZE VARIATION

Sodium alginate (μL/min)	*Oil (μL/min)*	*Droplet diameter (μm)*
0.2	50	36
0.3	40	60
0.13	20	185

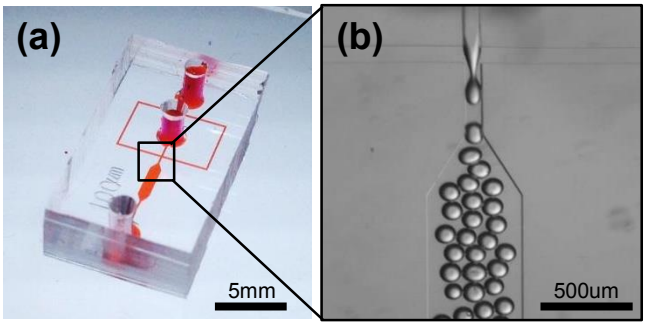

Figure 3. (a) The droplet composing device. (b) Droplet composing.

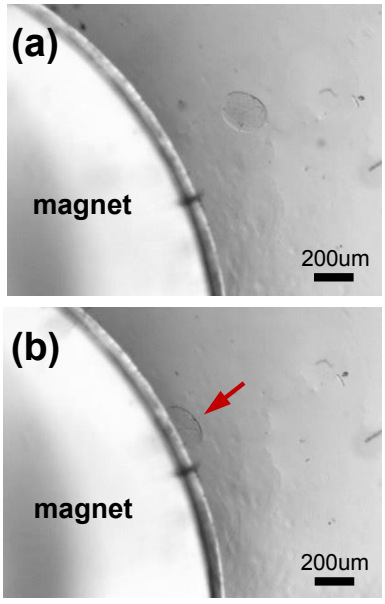

Figure 4. Dragging the composed droplet by magnet (red arrow).

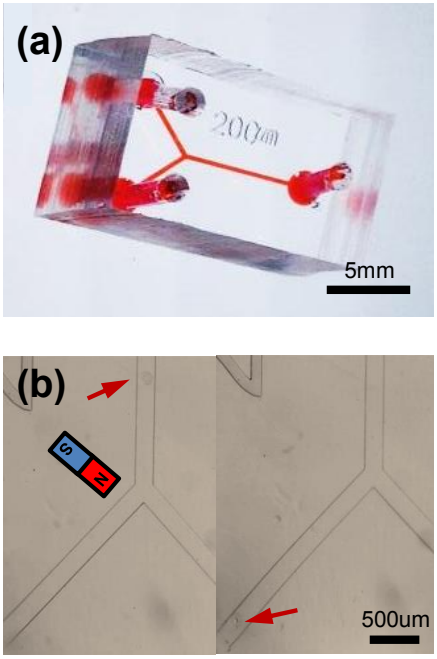

Figure 5. (a) The fabricated PDMS microchannel for the propulsion test. (b) The direction of the magnotosome droplet (red arrow) is guided by magnetic field.

The bifurcate microchannel is fabricated using the same process with the droplet generating device, as shown in Fig. 5 (a). To test active propulsion property of the magnetosome droplet, diluted solution with the droplet was flowed with 0.5 μL/min. The flowed droplet determined its route by magnetic field. This is demonstrated in Fig 5 (b) by the red arrow.

IV. CONCLUSION

We composed calcium alginate droplet containing magnetosome which is extracted from magnetotactic bacteria AMB1. The magnetosome droplet demonstrated to make selection at the bifurcate microchannel by magnetic field. It shows potential for the tissue targeted drug delivery system.

Calcium alginate can embrace more than one type of drug as well as magnetosome. Furthermore, bilipid layer of magnetosome has capability to bind therapeutic compound directly [11]. Therefore, at least two kind of drug can be released using the proposed DDS at the target tissue.

Alternating magnetic field induces heat at the magnetosome [14]. The induced heat can control drug release rate of the droplet as well as operate the heat therapy to cancer in the body. Currently we are developing the versatile drug delivery system which releases multi-drugs with controlled rate and operates the heat therapy at the targeted tissue using the magnetosome droplet.

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