CONTROLLED BIO-CARRIERS BASED ON MAGNETOTACTIC BACTERIA

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Abstract: Magnetotactic bacteria (MTB) can be controlled by an externally applied magnetic field. Their fast migration speed and quick response time, along with their capability to achieve 4 pN thrust force, make them well suited for micro-bio-actuators and bio-carriers. Here, we use conventional methods of microlithography to construct a microfluidic device to evaluate the activity of MTB in microchannels. Experiments also prove that a single MC-1 MTB can push a 2μm bead in a well controlled manner. Finally, we discuss the advantages of MTB based bio-carriers and prospect for future applications.

Keywords: Bio-carrier, Magnetotactic Bacteria, Microfabrication, μTAS

1. INTRODUCTION

Transportation and manipulation of micro- and nano-particles, especially the ones coated with antigens, proteins, or enzymes, are important for biological and chemical analysis, pathogen detection, and drug delivery, to name but a few applications. Currently, most of the Lab-On-Chip (LOC) or Micro-Total-Analysis Systems (μTAS) requiring particle transportation typically use techniques relying on electrokinetics [1], such as electro-osmosis or dielectrophoresis [2], where motion depends on the electrical properties of the medium and/or the particles being manipulated. In some cases, specificity between objects being manipulated cannot be achieved effectively due to similar dielectric properties among the objects. An alternative technique is to use magnetic beads [3, 4], which can be manipulated by an external magnetic gradient. However, to achieve sufficient local magnetic gradients for this type of micro-device, a relatively large electrical current is required, bringing potential concerns related to Joule heating and the level of miniaturization that is possible. To overcome these constraints, a novel bio-carrier based on MC-1 Magnetotactic Bacteria (MTB) [5-9] is proposed. Magnetotactic bacteria propel themselves in an aqueous medium by rotating their flagella, as illustrated in Fig.1 (a). Permanent magnetic nano-particles, called magnetosomes, are synthesized in their cells and connected to form a magnetic chain as shown in Fig. 1(b). The torque induced on the magnetic chain by a directional magnetic field (typically above 0.5 Gauss) provides directional control of the bacteria. When the orientation of the external magnetic field is reversed, the bacteria respond immediately by executing a "U turn", and then swim along the magnetic field lines. According to their behavior in a magnetic field, MTB can be classified as polar or axial. The MC-1 bacterium used in our microsystems is a north-seeking polar MTB. With a swimming speed reaching 300μm/s, they are known to be the fastest MTB ever found.

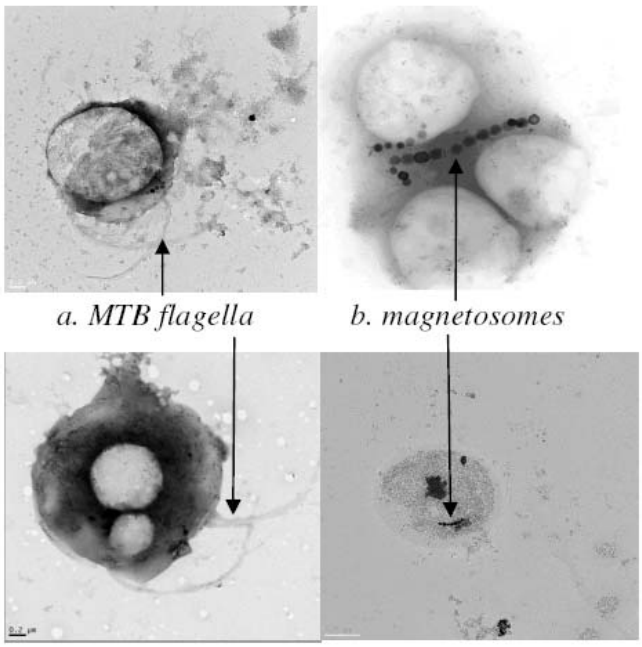

Fig. 1 MTB flagella and magnetosomes

Table 1 Characteristics of MC-1 bacteria

Type of MTB	*MC-1*
Living environment	marine water
Shape	Cocci
Size	1~2μm(in Diameter)
Magnetosome size	~20nm
flagella	One side
Swimming speed	50~300μm/s
Magnetosome number	Up to 20

Table 1 presents some characteristics of the MC-1 MTB.

In this paper, we present a microfluidic device developed to validate the concept of exploiting MTB as controllable bio-carriers. A simple schematic of the MTB transportation system is shown in Fig. 2. Preliminary experimental results show an average thrust force of 4 pN/MTB [7]. The results presented here were obtained with MC-1 MTB being controlled using a 10 Gauss external magnetic field.

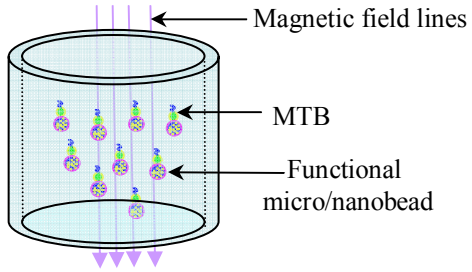

Fig. 2 Schematic representing the MTB transporting the functional micro/nanobeads under the control of a directional magnetic field.

2. DESIGN AND MICROFABRICATION

The purpose of this design is to verify whether the MC-1 MTB can be precisely navigated in the microchannel with a quick response to the direction of external magnetic field lines. The present chips are microfabricated on microscope glass slides (1.1mm thick). First, slides are cleaned with Pirahna, RCA1 and RCA2 solution respectively. Next, a 10 nm chromium seed layer and a 100 nm gold film are deposited by E-Beam deposition. Then, a 1.2μm layer of positive photoresist is spun on to the top of the metal layers. After exposure and development, the exposed metal layers are etched individually by gold etchant and chromium etchant. The patterned metal layers are deployed as metal masks in preparation for HF glass etching. The microchannels are etched with a depth of 20μm and a width varying between 50μm and 120μm. Both of the metal layers are stripped off after the glass etching procedure. A piece of PDMS (1mm thick) is used as a transparent cover to seal the microchannels. With biopsy tools, two 2 mm holes were punched onto the PDMS block to provide outlets. The PDMS block and the etched slide are put into a RIE chamber for oxygen plasma treatment. The PDMS block and the slide are aligned and firmly pressed together manually. Finally, lure fittings are tightly inserted into the outlets on the PDMS. The detailed microfabrication steps are depicted in Fig. 3.

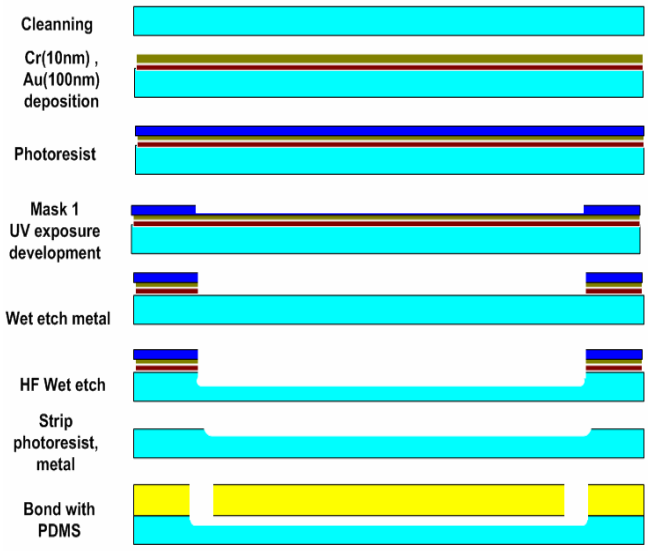

Fig. 3 Microfabrication procedure for the microchannels.

3. EXPERIMENT SETUP AND RESULTS

The microfabricated microfluidic device was placed on a custom-made fixture where the local magnetic field was generated by inputting electric current into metal wires or coils. An optical microscope was used to observe the movement of the MTB in the microchannels. Pictures and movies were recorded by a CCD camera connected to the microscope. A simple schematic of the whole system is illustrated in Fig.4.

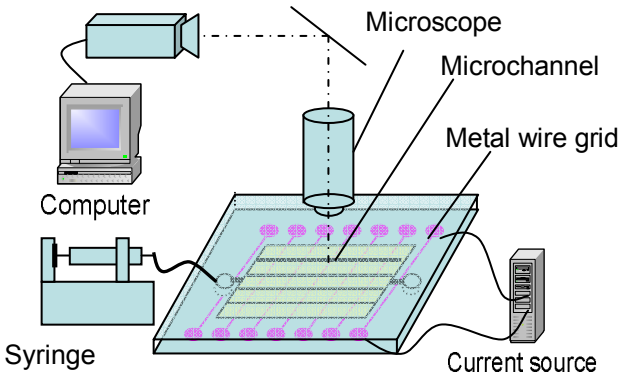

Fig. 4 Experimental setup for observing and recording the activity of MTB in the microchannels.

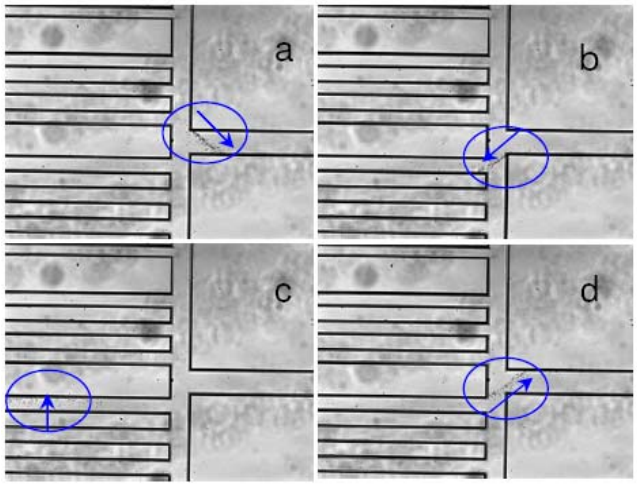

Fig. 5 A group of MC-1 MTB is manipulated in microfluidic channels by re-orienting the direction of a 10 Gauss magnetic field.

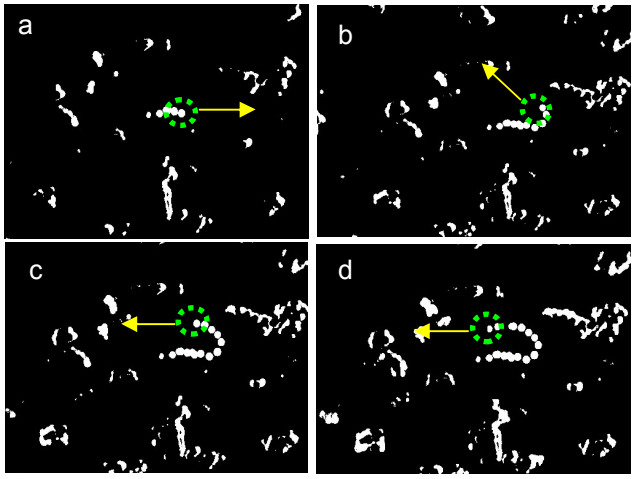

Fig. 6 A single MC-1 pushes a 2μm fluorescent microsphere in an aqueous medium. When the external magnetic field reverses, the MC-1 bacterium still pushes the microsphere while executing a "U-turn". The arrows give the direction of the swimming path of the MTB and the magnetic field.

First, MC-1 bacteria are injected in the microchannel with a syringe. After stabilization of the liquid (no flow being observed) in the microchannel, the MC-1 bacteria are navigated in the magnet field lines. A field intensity of 10 Gauss used during the experiments was validated with a Gaussmeter (Lakeshore model 450). At 10 Gauss, most of the bacteria could be controlled for effective navigation in the microchannels. By changing the orientation of the magnet, a corresponding change in the swimming direction of the MC-1 bacteria in the microchannels results, with an immediate response time. As depicted in Fig. 5 (a), a swarm of MC-1 bacteria is first navigated into the upper corner of the microchannels and when the magnetic field lines are reversed, the bacteria leave the corner and migrate into the microchannel. After the magnetic field is set to the original direction, the bacteria then swim back to the corner. Then in (b), the magnet field is set to -45^{o} with respect to the parallel channel, and the bacteria begin to swim into the central microchannel (width of 100 μm) in the middle from the upper corner. Then in (c), the magnetic field is switched to -135^{o} and immediately, the bacteria in the central channel begin to migrate into the lower channel (width of 50 μm). The Bacteria reverse their swimming direction after the magnetic field is set to 45 ^{o} and swim back to the central channel, as shown in (d).

In many cases and particularly for bio-carriers, an appropriate attachment technique is essential. Without surface modification of the microbeads, the naturally attachment efficiency is very low (less than 1% in our case). Therefore, under these conditions, the observation microbeads being pushed by MTB in the microchannels, represents a very challenging task. Nonetheless, other attachments techniques relying on biochemical methods are likely to provide better results but

still need further investigations. Here, we give an example of a 2μm fluorescent microsphere being pushed by a single MC-1 MTB in a water drop under unbounded condition (see Fig. 6). Swimming speeds of MTB pushing 2μm sphere of 100μm/s have been recorded experimentally. These preliminary results demonstrated that MC-1 MTB can be used to implement controllable bio-carriers for micro/nanoparticle transportation.

4. DISCUSSION

MTB based bio-carriers offer three main advantages. First, their manipulation is independent of their dielectric properties allowing the MTB to push or carry any type of nano/microparticles. Second, the use of high magnetic gradients to move magnetic beads is avoided since a DC magnetic field of only a few Gauss is required, potentially facilitating both integration and the miniaturization level that can be achieved while avoiding Joule heating. Third, highly controllable displacements of MTB can also be suitable for separation, positioning, transportations, sorting, and assembly of micro-objects. Within microchannels of different diameters (50μm to 120μm in this case), we noticed that the swimming speed of MC-1 MTB didn't change significantly due to an increase of the drag force caused by the channel wall effect. In some cases, the MC-1 MTB could maintain their velocity even when swimming in very thin liquid gap between air bubbles and the walls of the microchannels, which may indicate that MC-1 MTB has potential to increase their thrust force under some conditions. Our next step is to investigate the behaviors of MC-1 MTB in microchannels of less than 10μm in diameter.

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REFERENCES

[1] A. Ramos, and H. Morgan, N.G. Green and A. Castellanos, "AC electrokinetics: a review of forces in microelectrode structures," J. Phys. D Vol. 31, pp. 2338–2353, 1998.

[2] B. Malyan, and W. Balachandran, "Sub-micron sized biological particle manipulation and characterization," Journal of Electrostatics Vol. 51-52 , pp. 15-19, 2001.

[3] E. Verpoorte, "Beads and chips: new recipes for analysis," Lab on chip,Vol 3, pp. 60-68, 2003.

[4] H. Kawaguchi, "Functional polymer microspheres," Progress in Polymer Science, Vol. 25, pp. 1171-1210, 2000.

[5] R. B. Frankel, R. P. Blakemore, R. S. Wolfe, "Magnetite in Freshwater Magnetic Bacteria," Science. Vol. 203, pp. 1355-1357, 1979.

[6] R. B. Frankel, D. A. Bazylinski, M. S. Johnson and Barry L.Taylor, "Magneto-aerotxis in Marine Coccoid Bacteria," Biophysical Journal, Vol. 73, pp. 994-1000, 1997.

[7] Z. Lu and S. Martel, "Preliminary Investigations of Bio-carriers Using Magnetotactic Bacteria," In Proc. of the 28th Annual Int. Conf. of the IEEE Engineering in Medicine and Biology Society, New York, USA, Aug. 30-Sept. 3, 2006.

[8] Z. Lu and S. Martel, "Microfluidic System for Assessing the Controllability of MC-1 Magnetotactic Bacteria as Carriers in Microchannels," In Proc. of The Nanotechnology Conference and Trade Show (NSTI) Nanotech, Boston, USA, May 7-11, 2006.

[9] S. Martel, C. C. Tremblay, S. Ngakeng, and G. Langlois," Cotrolled manipulation and Actuation of Micro-objests with Magnetotactic Bacteria," Applied Physics Letters, Vol. 89, No.1 , pp. 233804-6, 2006.