Microfluidic System for Assessing the Controllability of MC-1 Magnetotactic Bacteria as Carriers in Micro-channels

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ABSTRACT

A special microfluidic system for assessing the controllability of the swimming direction of MC-1 magnetotactic bacteria in micro-channels is described in sight of its use as potential carriers in microsystems such as lab-on-chip. The proposed system is designed with embedded conducting wires and micro-electromagnets to characterize the behavior and responsiveness of the bacteria through controlled local magnetic fields. The microfabrication procedure of the system is described with simulation results.

Keywords: microfluidic system, magnetotactic bacteria, micro-electromagnet, micro-carriers.

1 INTRODUCTION

The methods used to manipulate microparticles or micro-objects potentially coated with antigens, proteins, or enzymes, to name but a few examples, are critical in microsystems such as lab-on-chip or micro-Total-Analysis-Systems (μTAS). Currently, micromanipulations in microfluidic systems rely mainly on techniques based on electrokinetics such as electro-osmosis or dielectrophoresis where the characteristics of the electrical signals required for inducing motion depend on the dielectric properties of medium and/or the micro-objects being manipulated. In several instances, specificity in micromanipulation cannot be achieved efficiently when the dielectric properties of various types of micro-objects or organisms within the same sample medium are similar.

To alleviate this problem, a new micromanipulation method where micro-objects are being pushed by Magnetotactic Bacteria (MTB) in a controlled manner, has been demonstrated by our group [1] as depicted in Figure 1b. In Figure 1a, the swimming direction of a swarm of MC-1 magnetotactic bacteria is controlled and showed that the typical swimming speed ranges from 100 to 200μm/s, making it the fastest magnetotactic bacteria known today.

Unlike previous work such as in [2] where MTB of type MS-1 (*Magnetospirillum magnetotacticcum*) were manipulated in a micro-chamber by inducing a force, the same manner as magnetic beads are manipulated under magnetic gradients, here, the MTB is not manipulated but instead used to manipulate other objects. Hence, the thrust provided by the flagella motor of the bacteria is exploited and directional control of the MTB is performed though magnetotaxis. In other words, by inducing with a computer controlled local magnetic field generated by electrical current flowing through conductors, a torque added directly on the chain of magnetosomes (acting like a compass) inside each MTB, a re-orientation the bacteria is performed such that the thrust of the flagella can be used in the desired direction.
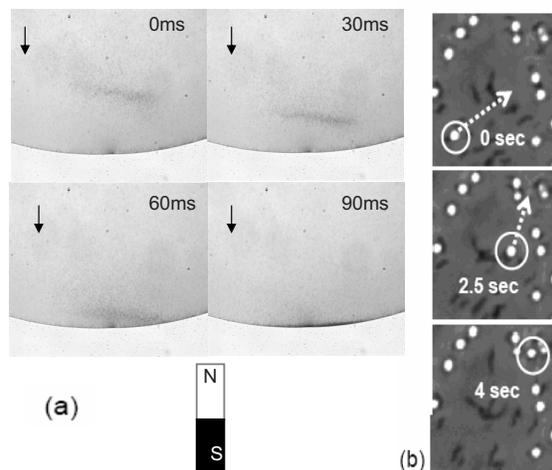

Figure 1: (a) Directional control of a swarm of MC-1 magnetotactic bacteria sweeping an aqueous solution where the black arrows indicate the direction of the controlled swimming path, and (b) directional control of a 3-micrometer bead being pushed by a single *Magnetospirillum gryphiswaldense* magnetotactic bacterium with a controlled change of the swimming path occurring after 2.5 seconds

Therefore, a special microfluidic system with microchannels having dimensions compatible with the size of the bacteria and structured by photolithography on a Pyrex glass wafer was necessary to allow detailed analysis of the movement of MC-1 bacteria operating under local magnetic fields and with minimum oxygen interference [3,

4]. More specifically, this microfluidic system allows us to assess the effect of the flow rate and the walls of the micro-channels on the motility and controllability of the MC-1 bacteria for determining design rules for future microsystems using such bacterial actuator.

2 DESIGN AND SIMULATION

2.1 Design

As shown by the simplified schematic in Figure 2, the system is designed to navigate the MTB from a left reservoir to a right reservoir through three different paths (P1, P2, and P3). The basic operation consists on directing the MTB through a pre-selected path between two reservoirs. For instance, when the MTB reach to the crossroad of the micro-channels, a micro-coil is first switched on to trap the MTB. Then, a specific conductor under the vertical channel and corresponding to the selected path is switched on. Concurrently, the micro-coil is switched off to release the MTB to allow them to swim in the selected micro-channel.
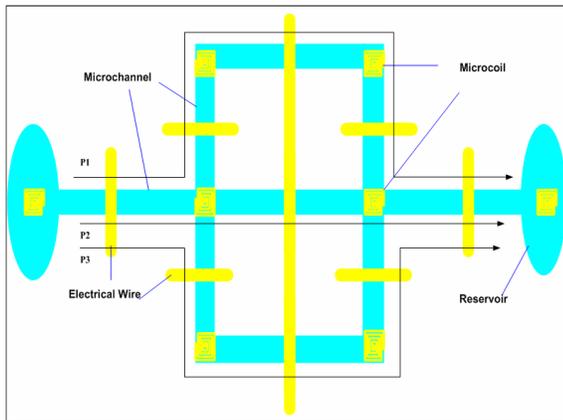
Figure 2: System diagram

The microfluidic device is fabricated with standard photolithography on a glass wafer. A layer of copper is deposited on the back side of the glass wafer to pattern the micro-conductor network required to generate magnetic field lines. In this particular implementation, micro-wires are patterned to navigate the MTB along a micro-channel (height: 3 µm, width: 100 µm) and micro-coils (height: 1µm, width: 100µm, with 100µm between successive turns) implemented to allow the collection and trapping of the bacteria. The electrical current in both the micro-coils and the micro-wires or conductors are controlled by electronic switches on a Printed Circuit Board (PCB) linked to a computer. The software is also developed to define the route of the MTB in the micro-channel through specific on/off sequences of the switches.

2.2 Simulation

The local magnetic field flux induced by the current carried by the conductors should be strong enough to navigate MTB in the micro-channels located above. Theoretically, MTB can be navigated under the magnitude of the geomagnetic field which magnetic flux density is 0.5 Gauss. The finite element analysis (FEA) simulation result for the conducting wires in our design is depicted in Figure 3. The magnitude of the magnetic field flux is ~20 Gauss in the micro-channel directly above, i.e. at a distance of 0.5mm above the conducting wire.
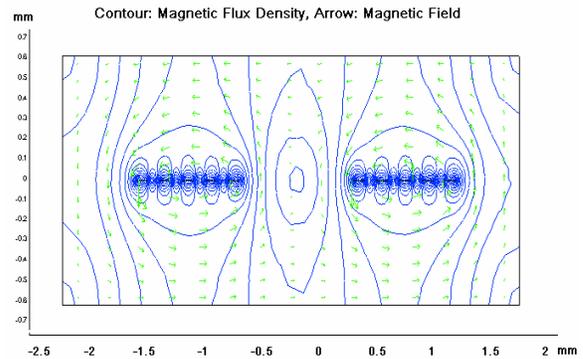
Figure 3: 2D simulation of magnetic field generated by electric wires (current density: $10^8 A/m^2$)

Micro-coils implemented under the intersections of the micro-channels are used to trap the MTB when they pass above the coil area to avoid random diffusion prior to conduct controlled navigation. The magnetic field generated in the coil was simulated using finite element analysis. The simulation results taking into account the geometry (planar coil), material (copper) and current density ($10^8 A/m^2$), show that the coil can generate hundreds of Gauss in the micro-channel.
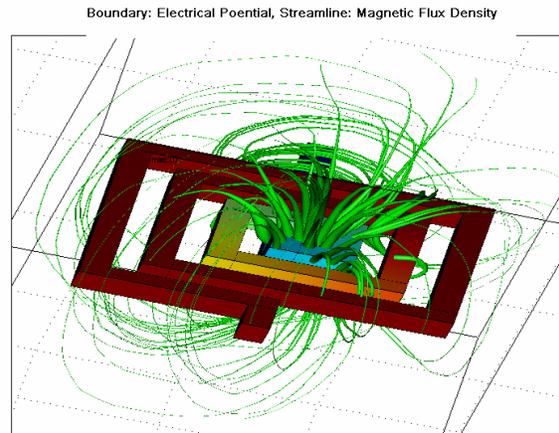
Figure 4: 3D simulation of the magnetic field generated by a micro-coil with a current density of $10^8 A/m^2$

3 FABRICATION

A glass wafer (Dow Corning, Pyrex 7740) is used to fabricate the microfluidic structures because of its excellent transparency, dielectric property, and good biocompatibility. The fabrication process (Figure 5) consists of three parts: 1. Fabrication of the micro-channels on one side of the Pyrex substrate; 2. Implementation of the pattern of micro-wires and micro-coils on the other side of the wafer; and 3. Bonding of the chip with another Pyrex wafer with outlets for fluid and bacteria injection.

For the fabrication of the micro-channel, a 20 nm thick chromium layer and a 250 nm thick gold layer are first deposited with e-beam technology then wet etched to form a metal mask for etching the micro-channels. HF etching process (HF: H_2O - 7:3) is used to etch 3 μm, 5 μm and 10 μm deep micro-channels. Another piece of Pyrex wafer is also mechanical drilled to form inlet and outlet with diameter of 2 mm. Then the two wafers are directly bonded together.
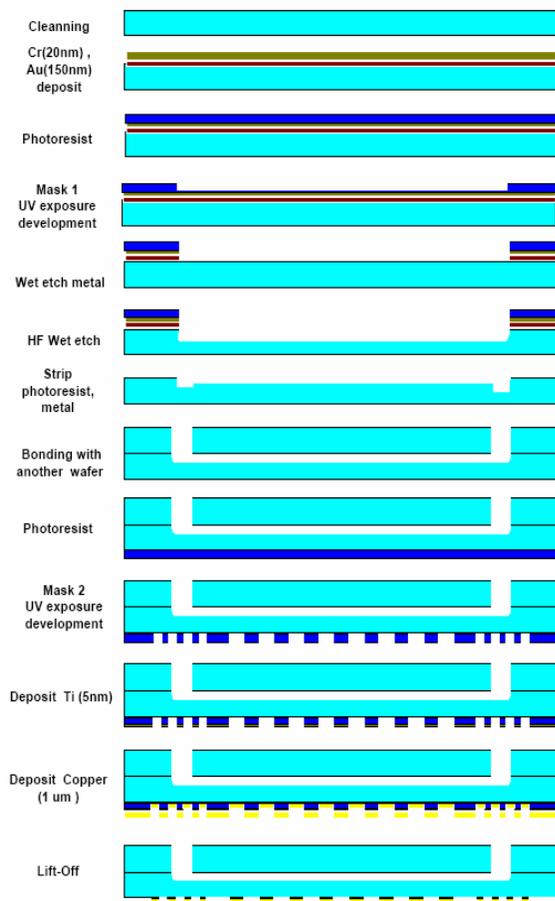
For implementing the conductors and micro-coils on the other side of the substrate, the standard lift-off process is used. After treatment with AP300 to increase the adhesion of the photoresist, a 2 μm thick photoresist (SP1813) is spin-coated and lithographically patterned to form the conducting wires and micro-coil arrays. Then, a 5 nm thick layer of titanium is deposited on the Pyrex substrate as a seeding layer. After that, a layer of copper with a thickness of 1 μm is sputtered. Finally, photoresist and unwanted metal are removed from the substrate.

Because of the capillary effect of the micro-channels, chemical solutions used during the procedure may contaminate the micro-channel and hence, an additional cleaning phase becomes necessary. The cleaning procedure consists of placing the device into an ultrasonic cleaner with DI water for 2 hour, followed by a period of 48 hours in oven for dehydration. Finally, the device is wire-bonded to a PCB for testing. A photograph of the microfluidic system is depicted in Figure 6.
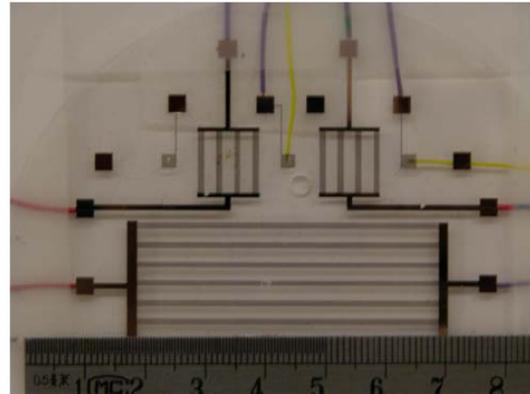

Figure 6: Photograph of the microfluidic system showing the conductors and the micro-coils used to control the swimming direction of the MTB.

4 EXPERIMENTAL SET-UP

The MC-1 bacteria are polar magneto-aerotaxis cocci [4, 5] which use the oxygen gradient of their surrounding to determine the direction in which they move relative to the magnetic field. To avoid the effects of oxygen in such an environment, the MC-1 bacteria are pumped into a microchannel by a syringe pump and then the system is entirely sealed with epoxy.

The device is then mounted on a fixture made of PMMA and placed under an optical microscope equipped with a CCD camera and an automatic controlled 3D stage used to trace the displacement of the MTB in the micro-channels. A simple diagram of the experimental set-up is depicted in Figure 7.
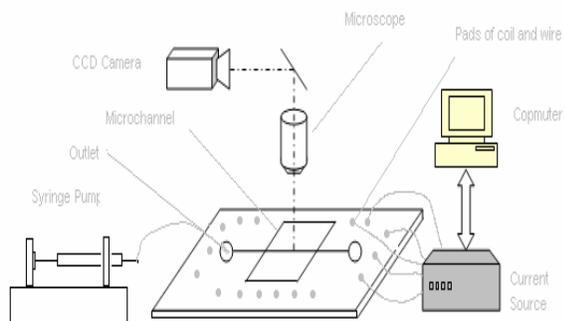

Figure 5: Microfabrication process

Figure 7: Experimental set-up

The MC-1 bacteria are first centrifuged to increase their concentration prior to be injected in the microfluidic system. Pure medium without MC-1 bacteria is first injected into the micro-channels with a syringe pump. Once the micro-channels are completely filled, the medium with concentrated bacteria is injected and a magnetic bar is typically placed under the reservoir at the same time to help collecting all bacteria. This is required to prevent them from dispersing randomly in the channels. When the computer controlled current source begins to output current in the wires and coils under the channels, the magnet bar is then removed in order to release the MC-1 bacteria in the microfluidic channels. Along the local magnetic field lines generated by the microfluidic system, the MC-1 bacteria would begin swimming to the first crossroad or intersection. With an pre-programmed on/off switching sequence of the control conductors, local magnetic fields are generated in order to influence the swimming path of the MC-1 bacteria along the magnetic field lines.

5 CONCLUSION

With the special microfluidic system described in this paper, we expect to gather many experimental results that may help us defining new design rules for future microsystems making use of magnetotactic bacteria as carriers. Among many experiments that can be performed with such a system, the effect, if any, of the density of the magnetic flux on the swimming speed of MC-1 bacteria when beyond some thresholds can be investigated. Another issue that will be investigated using the proposed system is the influence of implementing micro-channels with different diameters on the average swimming speed of the MC-1 bacteria, or more specifically, by investigating if the retarding effect caused by the walls of the channels plays an important role for variations of the swimming speed of the bacteria.

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