Controlled manipulation and actuation of micro-objects with magnetotactic bacteria

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Bacterial actuation and manipulation are demonstrated where *Magnetospirillum gryphiswaldense* magnetotactic bacteria (MTB) are used to push 3 μm beads at an average velocity of 7.5 μm s^{-1} along preplanned paths by modifying the torque on a chain of magnetosomes in the bacterium with a directional magnetic field of at least 0.5 G generated from a small programmed electrical current. But measured average thrusts of 0.5 and 4 pN of the flagellar motor of a single *Magnetospirillum gryphiswaldense* and MC-1 MTB suggest that average velocities greater than 16 and 128 μm s^{-1}, respectively could be achieved. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2402221]

The behaviors of bacteria in low Reynolds number hydrodynamics[1] suggest that they could be used to manipulate efficiently suspended micro-objects in fluids for potential applications in microsystems such as lab-on-a-chip and Micro-Total-Analysis Systems. Here, electro-osmosis[2] or dielectrophoresis[3] based on the principle of electrokinetics is used where frequencies and voltage amplitudes dependent on dielectric properties are required to induce a force. Our method referred here to as *bacterial manipulation* is independent of the dielectric properties and may prove to be suitable for many applications when low electrical power and compactness are required.

The integration of bacteria as functional components has been previously done,[4,5] where *Serratia marcescens* flagellated bacteria were attached to polydimethylsiloxane or polystyrene to form a *bacterial carpet* for moving fluid. Until then, bacteria were operating without external control appropriate for manipulation of micro-objects. Typical bacteria swims according to the so-called run-and-tumble pattern that can be explained by chemotaxis[6] models while remaining unpredictable for micromanipulation. We show here that magnetotactic bacteria (MTB) are more appropriate to carry out computer-based controlled micromanipulation or microactuation of micro-objects.

The exploitation of the motility of MTB has been done in the past such as in low field orientation magnetic separation[7] being a process, where motile, magnetic field susceptible MTB can be separated. Micromanipulation of MTB using microelectromagnets arrays has also been described.[8,9] In all these previous examples, MTB were the entities being manipulated instead of being used to manipulate other objects as described here.

Each MTB (Ref. 10) possesses a chain of magnetosomes which are membrane-based nanoparticles of a magnetic iron. Because of this chain, the swimming direction of MTB although influenced by chemotaxis and aerotaxis is mainly based on magnetotaxis,[11–13] being more "compatible" with electronics and computer-based software platforms. Although several types of MTB exist and can be found all over the world, in this study, *Magnetospirillum gryphiswaldense* bacteria[14] were used. This MTB has a length of ~1–3 μm with a swimming speed of ~40–80 μm/s. Magnetotaxis as chemotaxis[15–17] also influences the motility of MTB in search of nutrient gradients. To modify the paths of the MTB, magnetic field lines were generated from an electrical conductor network without inducing a propulsion force on the MTB or the beads.

The bacteria swimming speeds distribution and the effect of an applied magnetic field on the swimming direction of the MTB were assessed as depicted in Fig. 1. Active solutions of highly concentrated *Magnetospirillum gryphiswaldense* bacteria in a fresh culture medium were used. To "stick" the MTB to the microbeads, the bacteria were remixed in a medium poor in concentration of nutrients for 5 min prior to injecting a concentration between 1×10^6 and 1×10^8 MTB ml^{-1} in a fresh medium containing an average concentration of 5×10^6 microbeads ml^{-1}. Highly uniformed 3 μm beads[18] (density of 1.51 g/cm^3) made of melamine-formaldehyde resin showing hydrophilic properties and long term stability in an aqueous solution were used.

A drop of the solution of mixed MTB and microbeads was injected on a standard microscope slide. Two cover slips 150 μm thick were deposited apart on the slide and a third one on the top in order to form an observation pool placed on a custom-made electromagnetic grid made of 250 μm diameter parallel wires with a pitch of 500 μm arranged as two sets in parallel or oriented at right angle from each others. The grid was fixed to the *x-y* stage of a Zeiss Axiovert inverted microscope equipped with an acquisition charge-coupled device and set for observation in transmission (λ =530 nm) with phase contrast at 400×. The electrical current was adjusted and calibrated to provide a homogeneous magnetic field of 3.5 G through the observation pool.

After 5 min when placed in the fresh medium, ~1% of the bacteria already attached themselves to the microbeads and could even push them along the line of an applied magnetic field. To validate that the movements of the microbeads were due entirely by the pushing action of the motile MTB and not by any other forces, dead MTB, after being exposed to microwave irradiation and taken from the same cell cul-

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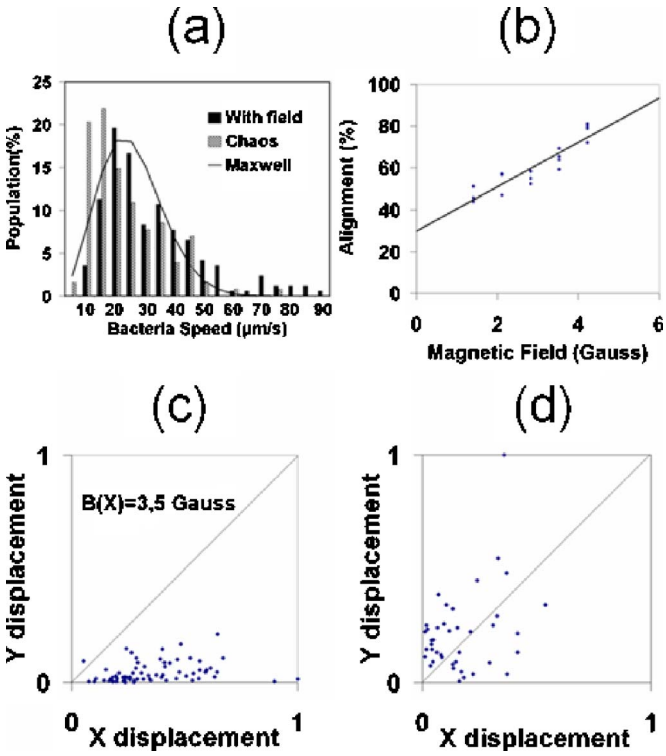

FIG. 1. (Color online) Motility and response to directional control using magnetic fields of two samples of 295 and 959 MTB. (a) Bacteria speed distribution from a sample of 295 MTB with and without the presence of an artificial magnetic field showing a Maxwell distribution. (b) Extrapolation line showing the percentage of the MTB oriented within ±30° along the line of an applied magnetic field at various intensities. (c) Swimming direction of the bacteria without an applied magnetic field. (d) Swimming direction with an applied field of 3.5 G along the *x* axes. The data have been normalized to unity and referred to the fastest bacterium with magnitudes corresponding to the swimming speeds of the MTB.

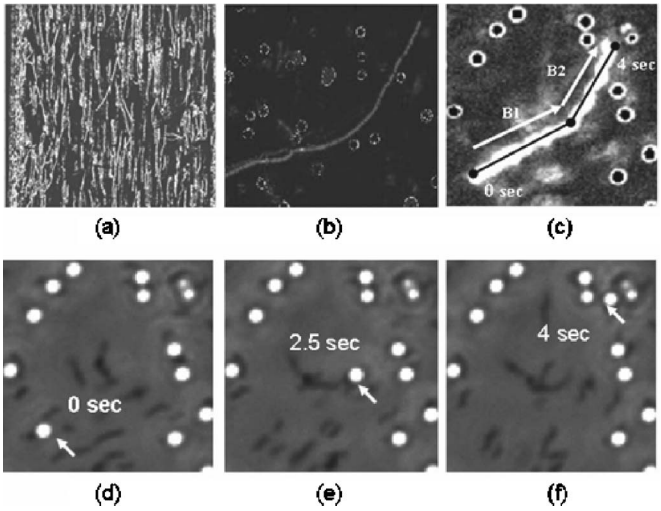

FIG. 2. Example of controlled manipulation of a microbead being pushed by one MTB. (a) Directional control of several MTB with an artificial magnetic field. (b) Movement path of one MTB not attached to a microbead during manipulation. (c) Trace showing the displacement path of a microbead being pushed by a single MTB with a voluntary change in the direction of the path through a change in the orientation of the magnetic field represented by B1 and B2. (d) Initial position of the MTB tagged microbead (at $t=0$ sec). (e) Position of the MTB tagged microbead when the direction of magnetic field is changed (at $t=2.5$ s). (d) Final position of the manipulated microbead pushed by one MTB. Images have edges of 36.0 μm.

ture, were added with microbeads not attached to MTB. No movements from either the dead MTB or the microbeads not being pushed by MTB were recorded during the experiments.

Figure 2(a) shows that when a magnetic field was applied, a directional controlled displacement of the MTB occurred from initial chaotic movements. Among many results, Fig. 2(c) shows a simple example that validates the controlled manipulation of a microbead by a MTB. In this particular example, the direction of a manipulated microbead being shifted intentionally ~30° anticlockwise after ~2.5 s was observed. Experiments were done with permanent magnets or using a simple program written in C++ to influence the chain of magnetosomes functioning as a navigational compass using the general torque.[19] Figure 3 shows and confirms that the microbead was actually pushed by a single MTB. The directions of the movement of the MTB tagged microbeads were similar to the movements of living MTB not attached to a bead as shown in Fig. 2(b).

Through a video analysis, an average speed of 7.5 μm s^{-1} and a peak velocity of 20 μm s^{-1} for a relatively large set of microbeads, each being pushed by a single MTB, were measured. When not attached to a bead, an average and a peak velocity of the MTB of 22.5 and 60 μm s^{-1} were also measured, respectively. But the average speed of this particular sample of MTB was lower than typical average speeds previously recorded for this type of bacteria (the bacteria already showed sign of weakness upon arrival from Germany to our laboratory in Canada) with expected swimming speeds of ~40–80 μm/s as mentioned earlier, as well as some other types of MTB. For instance, for the *Magnetospirillum* sp. AMB-1, an average swimming speed of 49 μm s^{-1} with a standard deviation of 20 μm s^{-1} has been recorded.[20] Hence from Stokes' law, it can be estimated that beads of 3, 10, and 100 μm in diameter could be pushed with an average speed of ~16.3, 4.9, and 0.49 μm s^{-1}, which correspond to a thrust of ~0.5 pN/MTB. Furthermore, since the low Reynolds

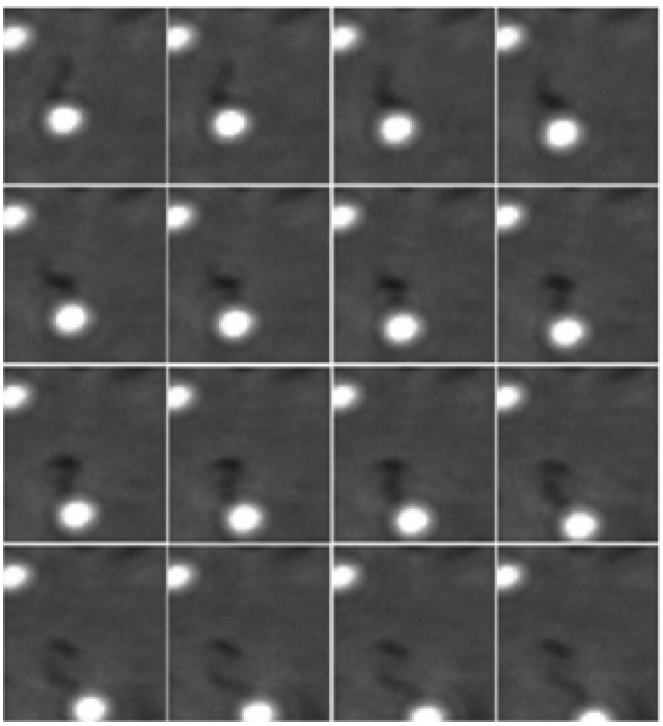

FIG. 3. Close-up view of a microbead being pushed by a single MTB.

number drag also scales like the size of the object, compensation with more MTB attached to larger objects can be expected. From experimental results, the bacteria could rotate 180° in water at room temperature at a frequency of ~2 Hz (~1 Hz for 360° rotation).

After ~5 min of pushing the microbeads, a percentage of the attached MTB had one side of the cell entirely glued to the microbeads resulting to an uncontrolled rotational motion. In other cases, the MTB tagged microbeads suddenly stopped when the flagella of the pushing MTB were "glued" to the beads. In sight of these issues and to improve the reliability of this technique, another attachment method using antibodies is being considered with promising initial results.

In current-carrying manipulation microcircuits,[21] Joule heating related to the induction of force on micro-objects being manipulated is often a critical issue. The proposed controlled *bacterial actuation* and *manipulation* require electrical current only to change the direction of motion of the MTB leading to a significant reduction of the required electrical current compared to other approaches while not being constrained to magnetic microbeads. Furthermore, since MTB can orient themselves and swim along the lines of the geomagnetic field (0.5 G) coupled with the fact that magnetic moment for MTB reaching 1×10^{-15} A m^2 has been measured[8] suggest that extremely small electrical energy would be required. Reduction of the operating current to less than 1 mA or a few hundreds of microamperes is possible through a preselection of the most responsive MTB, future genetic improvements of the bacteria, reduction of the feature sizes and the distance between conductors, synchronization between the motion of the MTB and ramp-shaped current signals multiplexed between neighbor conductors, and the use of a high magnetic permeability layer under the grid.

Other types of MTB can be considered. For instance, in more recent experiments conducted with MC-1 MTB cultivated in our laboratory, our group measured peak swimming speeds as high as ~300 μm/s, recorded maximum thrusts well above 4 pN/MTB for a small portion of the bacteria samples, and observed microbeads being pushed by a single MC-1 MTB.

Despite the fact that the experiments were done with open-loop control, the performances and possibilities of this method could be enhanced by including closed-loop control and decision-making algorithms in the computer software not only for controlled micromanipulation with possible release by the use of an intermediate MTB-tagged object but also possibly for controlled microswitches arrays, microvalves, micropistons, micromixers, micromotors, and autonomous microrobots propelled by bacteria, to name but a few examples.

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