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Controlled Bacterial Micro-actuation

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Abstract

The integration and exploitation of biological components onto micro- nanorobots and modern engineered microsystems such as Micro-Electro-Mechanical Systems (MEMS), lab-on-a-chip, or Micro-Total-Analysis Systems (μTAS) offer new possibilities. In particular, the flagellar motor found in many bacteria is a compact and extremely effective biological micro-actuator that is presently impossible to match with modern engineering techniques while requiring no electrical power to operate, a significant advantage especially in the conception of untethered microsystems. Previously, because chemotaxis-based bacteria such as E. Coli have been used for the exploitation of the flagellar motor, the number of possible applications have been very limited due to the absence of an effective method to control the motion of the bacteria. Controlling the direction of propulsion of the flagellar motor with computer software is demonstrated by exploiting magnetotaxis inherent in Magnetotactic Bacteria (MTB). The possibilities and advantages of this method are shown through a simple experiment where the controlled manipulation of microbeads is performed by MTB.

Keywords: Magnetotactic bacteria, controlled bacterial actuation, magnetotaxis, microsystems

1 INTRODUCTION

Bacteria are very effective for many operations in low Reynolds number hydrodynamics [1] as it is the case in microfluidic systems. In particular, the ability to manipulate by pushing suspended microparticles or micro-objects and potentially objects at the sub-micrometer level in liquid has significant potential for applications in micro- nanorobotics and microsystems such as lab-on-a-chip and in μTAS technology. In these microsystems for instance, micro-manipulation or micro-actuation methods rely mainly on electro-osmosis [2] or dielectrophoresis (DEP) [3] based on the principle of electrokinetics where electrical power is required to induce a force using relatively high frequencies and voltage amplitudes that depend on dielectric properties. The resulting voltage amplitudes are most often incompatible with the embedded electronics that tend towards lower operating voltages, resulting in larger devices through added voltage converters with further decrease of the electrical power efficiency. As such, controlled micro/nano-manipulation and micro-actuation methods independent of the dielectric properties of the micro- and sub-micro-objects are needed.

As an initial proof of concept, the magnetotactic bacteria (MTB) *Magnetospirillum gryphiswaldense* [4] were used to push microbeads in a controlled manner. The experimental results suggest that MTB could be controlled to operate as micro-actuators for applications in micro-manipulation, and in microsystems such as micro-switches, micro-valves, micro-pistons, or micro-motors to name but a few, and independently of the dielectric properties of the medium or the objects being manipulated or actuated. Furthermore, the preliminary experimental results also suggest that the same technique could potentially be adapted to objects with dimensions in the order of a few hundreds nanometers. Unlike most bacteria that are based on chemotaxis to detect nutrient gradients and hence influence their motility [5-7], the direction of displacement of MTB [8] with their chain of magnetosomes which are membrane-based nanoparticles of a magnetic iron, although influenced by chemotaxis and aerotaxis, is mainly based on magnetotaxis [9-11] which represents a more suitable interface with electronics and computer-based software.

Since this chain of magnetosomes acts like a compass and enables the bacteria to orient themselves and swim along the lines of a magnetic field, the control method consists of modifying the migrating paths of the MTB with the generation of magnetic field lines using small programmed electrical current passing through a special embedded conductor network. Compared to other traditional methods, any voltage amplitudes can be used, hence heat generation is minimized since electrical current is only used to change the direction of the bacteria and not to induce a force on the MTB itself. As such, the power requirement can be minimized by reducing the space between the conductors and by exploiting the motility of MTB.

The integration of bacteria as functional components has been previously done when *Serratia marcescens* flagellated bacteria were attached to polydimethylsiloxane or polystyrene to form a *bacterial carpet* for moving fluid or to move a bead or chip at ~5 μm s^{-1} when attached to it [12]. In previous examples [12,13], bacteria were acting without external control. Without coordination, not only the propulsion force F generated by N bacteria attached to an object of size L will scale like $F \alpha N^{1/2}$ assuming $N \alpha L^2$, but the displacement paths would result into the so-called run-and-tumble strategy that can be explained by a chemotaxis model [14] while remaining unpredictable. But controlling the direction of motion of N bacteria attached to an object of size L may scale like F α N. The motility of MTB have been exploited in the past through the use of permanent or electromagnets, typically in mass-scale applications such as low field orientation magnetic separation (OMS) [15] being a process in which motile, magnetic field susceptible MTB can be separated. Although controlled micromanipulation of MTB using micro-electromagnets arrays where the MTB are the entities being manipulated have also been described [16,17], the integration of MTB as functional components or controlled micro-actuators within a system and especially for the controlled manipulation of micro-objects or micro- nano-particles has not been reported.

In our knowledge, the concept of magnetotactic bacteria-based systems (MBS) is introduced for the first time and it refers to the integration of living MTB as integral components in engineered systems (or microsystems) capable of orienting their motion according to a particular task.

2 SELECTION OF THE BACTERIA

Several types of MTB exist and can be found all over the world. Presently, the selection process for the type of MTB is constrained since only a few types can be cultured in artificial or laboratory conditions. The three main types of MTB that we are presently considering in MBS are known as the *MV-4*, *MC-1*, and *Magnetospirillum gryphiswaldense*. The *MV-4* bacterium is the smallest MTB with a length of ~0.5 µm and swimming speeds in the range of ~30-80 µm/s. The *MC-1* bacterium has a diameter of ~1.0 µm and it is the fastest MTB that we know so far with swimming speeds in the range of ~80-200 µm/s. Both previous types have two bundles of flagella on one side of the cell and they are classified as polar MTB because they swim (in salt water) persistently in one direction along the magnetic field. The *Magnetospirillum gryphiswaldense* bacterium which usually lives in fresh water has a length varying between ~1 and 3 µm with a width of ~0.5 µm. This MTB has swimming speeds in the range of ~40-80 µm/s. The cell has two bundles of flagella on both ends. These MTB swim in both directions along the magnetic field lines with frequent reversals of swimming directions with approximately the same number of bacteria swimming in each direction. Because of this behavior, they are classified as axial MTB. *Magnetospirillum gryphiswaldense* bacteria typically show the best result in term of synthesis of magnetosomes with a longer chain than the ones typically found in other MTB and hence, may show a faster response to a directional change of a low magnetic field. This long chain of magnetosomes imparts to the MTB a magnetic moment that generates sufficient torque so that the bacteria can align themselves to magnetic field lines. Compared to *MC-1* and *MV-4* with a number of magnetosomes varying typically between 5 and 12 or 14, the chain can have up to ~50 cubo-octahedral magnetosomes (Fe_3O_4) when grown by micro-aerophilic *Magnetospirillum* strains and a micro-aerobic fermentation procedure [18]. Bacteria showing the most motility from observation under a microscope with the best response to magnetic field from a permanent magnet or from experimental platforms such as the one depicted in Fig. 1 can be selected as the best candidates to be embedded in MBS.

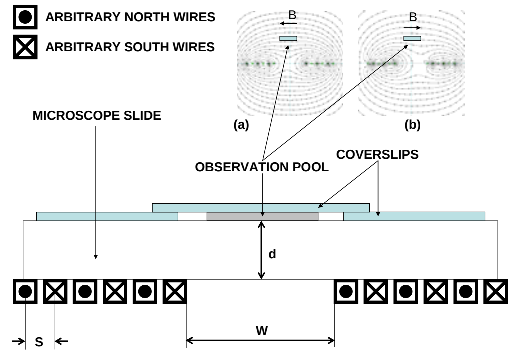

Fig. 1. Schematic (not to scale) of an experimental counter-directional electromagnetic grid, S = 500 µm, W = 2 mm, and d = 1 mm. The distance W is used to let the light to pass through the observation pool located in the middle for observation by the microscope in transmission mode which is essential in achieving better phase contrast for the detection of the bacteria. Arbitrary North and South wires have electrical currents circulating in opposite directions and controlled by computer software. The simulated distribution of the electromagnetic field is also shown (top right).

3 PROOF OF CONCEPT

Initially, chaotic displacements (Fig. 2a) are observed when no magnetic field is applied. A simple example (Fig. 2c), among many successful results, validate the controlled manipulation of microbeads by MTB using *Magnetospirillum gryphiswaldense* bacteria in this particular case. In this specific example, the direction of a manipulated microbead that was self-attached to the bacteria through a special process was shifted ~30° anticlockwise after ~2.5 seconds. The traces of the movements of the microbead or bacteria were obtained simply by the superposition of images taken at different time. The control of the movement of the MTB was performed in some cases using permanent magnets and in other experiments a simple program written in C++ and compiled prior to the experiment was coded to change the direction of the MTB through special electro-magnetic grids on platforms similar but adapted from the one depicted in Fig. 1.

The experimental grids were simulated and conceived to achieve a uniform field within the observation pool. A constant magnetic field through the observation pool guaranteed that the magnetic moment of the magnetosomes chain would not interact with the magnetic field gradient, but rather functions as a navigational compass using the general torque [19]. Observation under an optical microscope also confirmed that each microbead was pushed by a single and not by several MTB. The directions of the movements of the mobile microbeads were similar to MTB in the same medium but not attached to a bead (Fig. 2b)

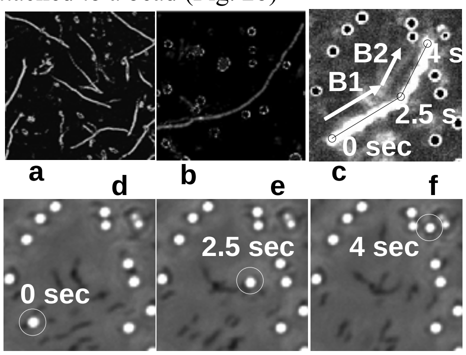

Fig. 2. Example of controlled manipulation of a microbead being pushed by one MTB in fresh water at room temperature. (a) Chaotic movements of the MTB when no artificial magnetic field is applied. (b) Trace showing the path of one MTB not attached to a microbead during manipulation. (c) Trace showing the displacement of one 3 μm microbead pushed by a single MTB with a controlled change in the direction of the path. (d) Initial position of the mobile microbead. (e) Position of the mobile microbead when directional change is applied. (f) Final position of the manipulated microbead. Images have edges corresponding to 36.0 μm.

The video analysis gives an average speed of 7.5 μm s^{-1} with a peak velocity of 20 μm s^{-1} for a set of microbeads being manipulated by a single MTB. Since the viscous drag on a sphere is proportional to its radius and knowing that the cell body of the MTB is smaller than the diameter of the microbead being pushed, an average speed of 7.5 μm s^{-1} and a peak speed of 20 μm s^{-1} would correspond to an average and a peak velocity of the MTB without the bead of 22.5 μm s^{-1} and 60 μm s^{-1} respectively. These values appear to be consistent with previously measured speeds of the MTB but the recorded average speed of this particular sample of MTB was below typical average speeds of other samples of *Magnetospirillum gryphiswaldense* bacteria or other types of MTB such as *Magnetospirillum* sp. AMB-1 cells where an average swimming speed of 49 μm s^{-1} with a standard deviation of 20 μm s^{-1} has been recorded [20]. Many factors could explain such low average motility but were not investigated in this particular study. Nonetheless, there is no apparent reason to believe that higher average velocities could not be achieved. This suggests that a single MTB could move a bead of 3 μm, 10 μm, and 100 μm in diameter with an average speed of ~16.3, 4.9, and 0.49 μm s^{-1} respectively, corresponding from Stokes' law, to a thrust of ~0.5 pN per MTB. Furthermore, since the low Reynolds number drag also scales like the size of the object, we can expect higher velocities with more MTB attached to larger objects. The experimental results also show that the frequency response of *Magnetospirillum gryphiswaldense* bacteria to rotate 180° was ~2 Hz (~1 Hz for a 360° rotation). Preliminary experimental tests also showed that a much higher thrust would be available with the use of *MC-1* bacteria and more interesting is the fact that preliminary experiments also showed that they could swim efficiently in blood but for a limited amount of time apparently because the temperature of the blood is well above room temperature.

Measurements by video analysis also showed some small variations in velocity of the mobile microbeads. Although not confirmed yet, we believe that these variations in velocity may be attributed to some extents to collisions with other microbeads and the effects of neighbor bacteria. For instance, from previous observations [12], we can expect that the speed of a bacterium or a mobile microbead will be affected by any bacteria within a range of ~50 μm ahead of it with a larger probability of occurrences with higher concentrations of bacteria.

This example alone of a microbead being pushed by a MTB may lead to many applications. For instance, by coating the beads with targeting agents, many applications in bio-engineering, genomics, and medicine, to name but only a few fields, may become candidates for this technology. This is particularly true for miniature detection and analysis systems and field instruments

4 CARACTERISTICS OF THE AQUEOUS MEDIUM

The motion behavior of the MTB can be influenced by many factors. For instance, such motion behavior could in some cases vary through the life span of the bacteria which can be extended for relatively very long periods of time depending on living conditions. But the medium itself can also have an impact on the motion behavior. The main potential mediums where MTB could operate in the context of micro-robotics and in micro-systems are fresh or salt water, and potentially blood. As such, a proper type of MTB must be selected to operate in one of these particular mediums. Furthermore, the level of oxygen in the medium must typically be kept low if longer operations are suitable. Other main characteristics of the medium that will typically have an impact on the motion behavior of the MTB are the level of toxicity, the viscosity, and the temperature. This also may lead to the use of MTB as biological sensors based on variations of their motion behaviors due to changes occurring for one of these characteristics. It appears that there is a linear relationship between the concentration of toxic chemical and swimming speed, a reliable parameter for measuring the total environmental toxicity [20].

Beside the environmental temperature fluctuations, the energy dissipated by the embedded electronics (although it would typically be very small) could cause a rise in temperature that could indeed be a potential concern for extremely low volume of aqueous medium in proximity. In [21], torque-speed curves for a flagellar motor are given taking into account the viscosity of the fluid and variation in temperature. At low rotation speeds (such as in more viscous mediums), the motor appears to operate near thermodynamic equilibrium where the rate of displacement of internal mechanical components or translocation of protons are not limiting. The transition between the low speed and high-speed regimes (the knee) shifts to lower speeds at lower temperatures, and the rate of decline of torque with speed steepens. Hence, for lower viscosity medium (e.g. water instead of water with a percentage or concentration of solution of Ficoll for instance), the loss of torque is strongly temperature dependent up or close to a survival limit of the MTB. It should be noted also that a loss in viscosity due to an increase in temperature could be compensated by techniques such as the addition of Ficoll in the aqueous solution. Other factors may also influence the motion behavior of the MTB including but not limited to the concentration of nutrients, the presence of other entities sharing the same medium and illumination. It is known that the motility of bacteria can be damaged by intense blue lights such as xenon arc used in dark-filed microscopy for instance or with tungsten-halogen lamps.

5 DISCUSSION AND CONCLUSION

Based on recent current-carrying manipulation microcircuits [22] that result into sufficient heat to be problematic for many applications, controlled *bacterial manipulation* and *bacterial actuation* may reduce the required current by at least 100 folds while not being restricted to superparamagnetic microbeads. Since MTB can orient themselves and swim along the lines of the geomagnetic field (0.5 gauss) coped with the fact that magnetic moment for MTB reaching 1×10^{-15} A m^2 has been measured [16] suggests that a minimum current amplitude of less than 1 mA or even a few hundreds micro-amperes could be envisioned with a pre-selection of the most responsive MTB, by genetic improvements of the bacteria, by reducing the feature sizes and the distance between conductors, by synchronizing with the motion of the MTB ramp-shaped current signals with multiplexing to neighbor conductors, and/or by inserting a high magnetic permeability layer just under the grid, to name just a few approaches. Although the initial experiments were done with open-loop control, the use of sensors or imaging systems to detect the position of the microbeads being manipulated would allow closed-loop control and decision-making algorithms to be included in the computer software.

This new proposed micro-actuation method based on magnetotactic bacteria capable to operate efficiently in low Reynolds conditions and under software algorithms offers many advantages compared to other micro-actuation methods especially for micro-systems where electrical power must be minimized. Although initial tests were performed with microbeads, many other micro-structures may be envisioned.

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