HIGH THOUGHPUT CONTROLLED BACTERIAL TRANSPORT USING GEOMETRICAL FLUIDIC MICROCHANNELS OR 3D MICROFIBERS STRUCTURES

S. Martel and M. Mohammadi
NanoRobotics Laboratory, Department of Computer Engineering, École Polytechnique de Montréal, Montréal, CANADA. (correspondence: sylvain.martel@polymtl.ca)

ABSTRACT
A novel method of transport for samples in aqueous medium is proposed and validated. The method makes use of Magnetotactic Bacteria (MTB) as controlled carriers to transport samples in fluidic channels. Furthermore, we demonstrate that to achieve higher density transport channels, the swimming paths of MTB can be controlled in fluid retained through capillary forces in geometrical fluidic channels or though a thin layer of aqueous solution on microfibers that can be constructed onto various 3D structures. This approach provides an alternative to other transport methods used in microsystems with specific characteristics that can be advantageous to many applications.

Keywords: Magnetotactic Bacteria, Bacteria Transport, Microchannel, Microfiber.

1. INTRODUCTION
Transport methods typically used in lab-on-a-chip or in Micro-Total-Analysis Systems (µTAS) such as pumping fluid containing samples to be analyzed using micro-pumps are often relatively bulky and often difficult to integrate in very constrained spaces, and/or require relatively high power to operate. Other popular techniques such as electro-osmosis or dielectrophoresis (DEP) are based on the principle of electrokinetics where relatively high frequencies and voltage amplitudes dependent on dielectric properties are required to induce a force of the samples to be transported. Our proposed method referred to as bacterial transport in this paper, minimizes electrical power requirements and can be easily integrated in very small spaces while being independent of dielectric properties. As such, bacterial transport may prove to be suitable for many applications when low electrical power, compactness and/or specificity among micro-objects with similar dielectric properties are required. When coupled with fluid retention through capillary force in various geometrical fluidic microchannels or 3D microfibers structures, high density bacterial transport can be embedded in various microsystems including µTAS.

2. THEORY AND METHOD
Because flagellated bacteria are very effective in low Reynolds number hydrodynamics, the flagella motor embedded in each bacterium can be exploited to provide a propulsion force sufficient to transport samples being attached to the cells. Directional control is achieved by inducing a torque on a chain of nanoparticles called magnetosomes acting like a compass embedded in each MTB. Such directional torque can be provided by a permanent magnet or by using an electrical current. Although MTB can be oriented with directional magnetic field intensities equivalent to the geomagnetic field (0.5 Gauss), higher directional magnetic field intensities in the order of 3 Gauss provide better and highly predictive responses and hence optimal computer-based control by making magnetotaxis the dominant
factor over other influencing factors in the motion behavior of the MTB such as chemotaxis and aerotaxis. Here, MTB of type MC-1 cultivated in our laboratory are used for several reasons but particularly beside being a polar bacteria, because of its higher swimming speed (speeds up to 300 µm/s have been recorded by our group) compared to other flagellated bacteria, superior thrust (>4 pN compared to 0.3-0.5 pN for many other bacteria).

A solution that can be used in combination with narrower channels to increase channels density is to exploit capillary retention in angular regions of the microchannels. Simple examples using a square shape microchannel or using two fibers in parallel are shown in Fig. 1. In Fig. 1, a single fluidic microchannel for instance is replaced by four channels by exploiting capillary force and channel geometry. Depending upon different factors such as surface tension and the level of complexity in fabrication of microchannels with more angles along the periphery, a higher density of channels can be realized.

The same approach can be applied between two or more microfibers as depicted in Fig. 1 and even on a single fiber when a thin layer of fluid is retained. In the latter case, we can show that the layer thickness of only a few micrometers is sufficient for the MTB (cell diameter of MC-1 MTB is approximately 2-3 µm) to swim along providing efficient yet very dense transport channels where bacterial transport can be done in two reciprocal directions simultaneously.

Attachments to polymer microbeads or nanoparticles that may be coated with samples being transported are typically done using antibodies developed by our group and specific to the MC-1 bacteria.

3. EXPERIMENTAL RESULTS

As a simple example that proves the concept is the one in [1] where we demonstrated a single MTB pushing a microbead (that could be coated with reactive agents or other samples) following a pre-determined swimming path (see Fig. 2a. In [2], MC-1 MTB have been controlled to navigate inside microfluidic channels. An example is depicted in Fig. 2b. In this example, all bacteria follow the same channels. If different paths are required, several fluidic channels would be required at the cost of decreasing the channels density unless more advanced microfabrication techniques are used to reduce the width of the channels. Another alternative is depicted in Fig. 2b. A proof of concept showing MC-1 MTB swimming along four fluidic channels formed by capillary retention along the four corners of a simple square microchannel is depicted in Fig. 3. In this example, the average swimming speed of the bacteria measured by dark-field microscopy using analysis of video images was 180µm/s. Experimental results related to Fig. 3 showed that bacteria swim to the left in 50% of the channels with the remaining bacteria swimming to the right. The microchannel depicted in Fig. 3 was made of Pyrex glass. Only a thin layer of water at the corner of the fluidic channel was used by the bacteria to swim in both directions. Here a 10 Gauss DC magnetic field was used during the experiments. Much lower magnetic field intensities could also be used.
More complex paths are also possible. Fig. 4 for instance shows bacteria swimming along right-angle paths. Although relatively complex computer-based control can be used when bacteria are tracked and the directional magnetic field is changed in real-time accordingly, sophisticated travel paths for bacterial carriers along 2D or 3D fluidic structures are possible using fixed permanent magnets. For instance, by increasing the DC magnetic field intensity to above approximately 120 Gauss using stronger permanent magnets or placing the magnets closer to the fluidic structure, the bacteria will automatically reverse their swimming directions when reaching the end of a swimming path, which is not the case when the field intensity is lower. Hence, by integrating permanent magnets providing high low field intensities throughout the fluidic structures and constraining the swimming paths of the bacteria with thin layers of water, complex micro-transport systems requiring no electrical power can be implemented.

4. DISCUSSION AND CONCLUSIONS

Our initial experimental results show that MTB can be used as an effective means of transport that is well adapted to the requirements of many applications in microsystems and particularly in µTAS. By exploiting the motility of bacteria, less electrical energy is required leading to further miniaturization while avoiding problems such as Joule heating in small fluid samples. A binding techniques to such bacterial carriers based on antibodies have been implemented successfully. The use of MTB allows us to control its displacement in various ways that can be implemented in computer software. Although this novel approach has many advantages, it also has limitations such as limited operation in higher temperature, constraints related to the type of aqueous medium used, etc. Nonetheless, this approach still may be more appropriate in many applications used in µTAS or at least providing a complementary alternative to other existing transport techniques used in fluidic microchannels.

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