Manipulation of Micro-Structure by Self-Powered Bacteria

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박테리아의 추진을 이용한 마이크로 구조의 조작

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Abstract

Flagellate bacteria such as *Escherichia* coli or *Serratia marcescens* possess a remarkable motility system based on a reversible rotary motor. We have employed *S. marcescens* as microactuators in low Reynolds number fluidic environments to move a larger engineering element around. Microstructures fabricated using conventional microfabrication techniques are blotted on the swarm plate, which leaves a bacterial monolayer on the surface of the microstructure. We have investigated microstructures powered by bacteria to determine how cell orientation on the microstructure surface relates to the swarming patterns as well as how the orientation is affected by the blotting process. This study will help to refine directional control of bacterial transporters by exploiting bacterial sensory mechanisms.

1. Introduction

As the field of engineered micro/nanoscale structures develops, so does the need for controllable methods of microactuation. The biomolecular motors embedded in the cell bodies of various strains of bacteria may be employed as actuators for such applications. Flagellated bacteria such as *Escherichia coli* and *Serratia marcescens* use rotating helical flagella to swim. The body of *Serratia marcescens* is rodshaped, about 1 μm in diameter by 2 μm long, and, typically has several flagella (5-6). Each flagellum has a rotary motor that can turn at approximately 150 Hz, either clockwise or counterclockwise. The motor is embedded in the cell wall and drives a short flexible hook connected to the helical filament that is about 10 μm long [1]. When all the flagella turn counterclockwise (when viewed from outside the cell body), they form a bundle that pushes the body forward in a run.

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When one or more of the motors reverses, the corresponding filaments unwind from the bundle, and the cell body moves erratically, or tumbles [2]. Flagellar motors offer many unique advantages as microactuators. Through well-established cell culturing techniques, ten of billions bacteria can be inexpensively cultured in a matter of hours. They draw chemical energy directly from their environment and are able to survive in a wide range of temperature and pH. Additionally, bacteria are controllable *en masse* through light and chemical sensory mechanisms as well as electrical and magnetic mechanisms.

The ability of cells to respond to chemical concentration gradients is referred to as chemotaxis [3], while phototaxis refers to the ability of cells to respond to light stimuli [4]. Galvanotaxis refers to the response of cells to seek an electrode due to the difference in electrophoretic mobility between the cell body and the flagella. This difference causes the cell to be mechanically oriented in the electric field. Different types of bacteria have been shown to demonstrate galvanotaxis toward the anode or the cathode depending on the surface properties of the cell membrane [5,6]. Galvanotaxis should be considered as a separate mobility from electrophoretic mobility, although these phenomena strongly contribute to the mobility of bacteria, especially with stronger electric fields [7,8].

Several lines of research have previously demonstrated the use of microorganisms to produce useful work. On/off control of microstructure movement using *Serratia marcescens* has been demonstrated by controlling exposure to ultraviolet light [4]. Such on/off control of bacterial actuation has also been demonstrated using copper ions and EDTA with microbeads [9]. Bacterial actuation and manipulation were also demonstrated using *Magnetospirillum gryphiswaldense* magnetotactic bacteria [10]. Additionally, bacteria have been demonstrated to self-coordinate when patterned in monolayer carpets, creating effective microfluidic pumps and mixers [11-13]. Other organisms have also been used for control and actuation. Microbeads have been transported using the phototactic movement of the algae *Chlamydomonas reinhardtii*[14]. A review of research in the field of bio-microactuation has been recently published covering many more examples [15]. Closely related research involves focuses on the control of inorganic microscale fluidic systems. It has been shown that various types of miniature semiconductor diodes floating in water act as self-propelling particles when powered by an external alternating electric field [16]. Additionally, it has been demonstrated that multiple polystyrene beads can be accurately steered and trapped at once using electroosmotic flows inside a microfluidic chamber [17]. It was also shown that fluorescent polystyrene nanospheres in solution can be trapped and manipulated with nanoscale resolution using an anti-Brownian electrophoretic trap [18]. Many works have employed electrophoretic and dielectrophoretic forces as a means of cell separation [19]. The present work combines aspects of these lines of research. The topic of this work is the enhanced mobility and controllability of inorganic micron scale objects using bacteria as configurable elements.

2. Materials and Methods

2.1 Cell Culturing

Swarming *S. marcescens* were cultured on a 0.6% agar plate. Swarming bacteria are especially useful as actuators due to their rigor and size. They are hyper-flagellated, elongated and migrate cooperatively [13]. To prepare agar plates for swarming, 5 g Difco Bacto tryptone, 2.5 g yeast extract, 2.5 g NaCl and 3 g Difco Bacto agar are dissolved into 500 ml of deionized water [14]. After autoclaving the solution was poured into smaller bottles for later redistribution to Petri dishes. This solution will solidify when stored at room temperature and can be reliquefied using a microwave on the lowest power setting.

Before pouring individual agar plates, the agar solution was mixed with 25 % glucose solution by adding 1 ml glucose solution for 100 ml of prepared agar solution. 50 ml of this new agar solution was pipetted into large 14 cm Petri dishes. The swarm plate was inoculated on one edge with 2 µl of *S. marcescens* saturated culture (Figure 1). Agar plates were incubated at 30 - 34 °C, and swarming began within 8-16 hours. The inoculation site generally turned pink shortly after the swarming motion developed. The swarms expanded across the plate in waves that appeared as concentric rings with the most active bacteria along the outermost edge of the swarm.

2.2 Manufacturing

Future studies and applications require precise microstructures that can be fabricated on large-scale, manipulated into the working fluid, and tracked using an algorithm with minimum processing time. To achieve these goals, fabricated structures should be biocompatible in the sense that materials preserve and promote bacterial motility and provide a surface to which bacteria attach readily. Additionally, the composite specific gravity of the structure should be similar to the working fluid and provide both chemical and thermal stability. It is additionally helpful if the fabricated structures are transparent and have a high refractive index to provide clearly defined boundaries which can be readily discerned by a tracking algorithm [15].

SU-8 Series 10 (MicroChem, Newton, MA) negative photoresist forms strong cross links on exposure to ultraviolet (UV) light, and the unexposed regions are easily removed using a developer solution. A glass slide was first cleaned in isopropanol. The slide was then dried with nitrogen gas, rinsed with deionized water, and dehydrated at 200 °C for 5 minutes. Once the slide was pre-treated, it was placed on the vacuum chuck of a spin coater. SU-8 10 negative photoresist was dispensed on the slide to cover 2/3 of the slide surface or 1 ml per inch of diameter. In order to achieve a final thickness of 10 µm, the spin coater was set to ramp to 500 rpm at 100 rpm/sec, held for 5-10 seconds, and was ramped to a final spin speed of 3000 rpm at 300 rpm/sec, held for 30 seconds at that speed, and stopped gradually. Upon completion of this process, the slide was soft baked in two steps. First, the slide was pre-baked for 2 minutes at 65 °C and then soft-baked at 95 °C for 5 minutes. The next fabrication step was UV exposure. On completion of exposure, the second step was to post-bake the slide. During post bake, the slide was baked at 65 °C for 1 minute then shifted to another hot plate to be baked at 95 °C for 2 minutes. Once the slide was cooled, an SU-8 developer was used to wash away regions of unexposed SU-8 from the slide and leave only the microstructures patterned on the surface. The slide was submerged in a container with SU-8 10 developer for approximately two minutes. The container was gently agitated to allow complete removal of unexposed SU-8 10. Isopropyl alcohol was then applied to wash away any developer left on the surface of the slide. This slide was once again rinsed with deionized water to remove any toxins that were present on the slide. The slide was then blow dried with a jet of Nitrogen gas, and the SU-8 pattern was ready for blotting and then extraction.

2.3 Micromanipulation

Current research depends on sophisticated and expensive equipment to perform micromanipulation. Considering that future application implementing bacterial actuators will require simpler, inexpensive micromanipulation process, a series of steps was developed to release microstructures into the working fluid without damaging the structure or attached bacteria. Henceforth, micromanipulation will be referred to as a procedure by which microstructures blotted with bacteria are extracted from the substrate and released into the working fluid with the aid of the microscope. After the fabrication process, the glass slide with microstructures was broken into sections approximately 10 mm × 5 mm, each of which contained several fully intact microstructures. To blot, the separated sections were washed with motility buffer (0.01 M potassium phosphate, 0.067 M sodium chloride, 10^{-4} M ethylenediaminetetraacetic acid (EDTA), 0.01 M glucose, and 0.002% Tween-20, pH 7.0) [16] then inverted onto the edge of the swarm plate. The section was removed from the swarm plate, transferred to a dish with motility buffer, and lightly agitated to remove unattached bacteria and excess agar. This process ensured that a monolayer of bacteria was attached to the microstructures with flagella free to move and untangled from other layers of bacteria and agar. The blotted section was then moved to a fresh Petri dish and submerged under a thin layer of motility buffer. The manipulation was performed using a stereo microscope for three-dimensional viewing, thus allowing individual microstructures to be selected and removed. After affixing the glass slide to the bottom of the Petri dish, a 25 gauge needle was used to select and remove structures along the longest side.

3. Results and Discussions

In an experiment of a 50 × 50 μm square microstructure, the results show a preferential orientation of 45º which closely resembles the average orientation of 48.42º. The total number of bacteria on the surface of this particular microstructure is 164 of which 112 bacteria are orientated between 20º to 70º measuring from the x-axis. Driven by its bacterial carpet, the microstructure moved along a curved path (Figure 1) and also displayed a clockwise rotating motion, suggesting the attached bacteria carried the structure in a manner consistent with their collective motion.

Careful observation revealed that one side of the microstructure in figure 1 moved at a slower rate. In figure 2, an arc path was traced suggesting a resultant torque. The bacterial carpet produced a resultant force pointing in the direction of the preferential orientation. Each bacterium contributed to the resultant force with a different magnitude and from a different location on the structure, causing the resultant force to be out of line with the centroid. This lack of balance of forces between two sides of the structure generates a torque about the centroid, resulting in a collective arc motion. Further experimentation showed similar rotational and arc motions.

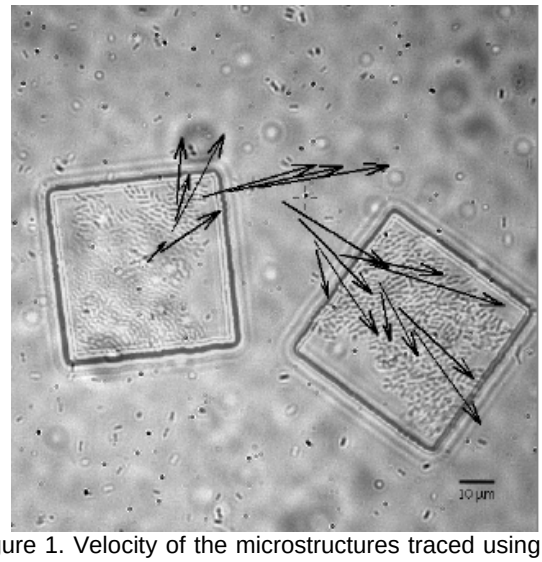

Figure 1. Velocity of the microstructures traced using the tracking algorithm.

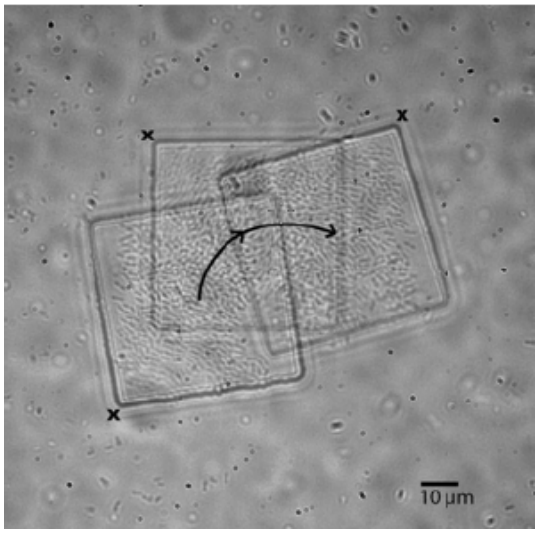

Figure 2. Arc motion due to torque created by the collective motion of bacterial carpet. A mark is place on a corner of the microstructure to follow the degree of rotation of the microstructure during this arc path.

4. Conclusions

A series of procedures was performed to reveal relationships between directional control of bacterial transportation and cell patterning on the SU-8 structure. Microstructures, driven by bacterial motors, displayed movement without any stimulation from external controls. The bacteria on the bacterial carpet generate a collective motion, carrying the microstructure accordingly. If the cell bodies are highly correlated, the direction of motion of a structure is correlated to the cell orientation. As data suggested, a resultant force in the direction of the preferential cell orientation was generated from the bacterial carpet. As a result, a net thrust was created giving the microstructure a curvilinear

arc motion.

However, if the structure shows little correlation between cell bodies, the resultant motion of the microstructures in unrelated to the cell orientation. This is likely due to the fact that thrust due to the many bacteria cancels out. That is, the resultant of many bacteria thrusting in an uncoordinated manner tends to be very small and unpredictable.

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References

[1] H.C. Berg and R.A. Anderson, "Bacteria swim by rotating their flagellar filaments", *Nature* **245**, 1973, pp. 380

[2] H.C. Berg, "The rotary motor of bacterial flagella", *Annu. Rev.Biochem.* **72**, 2003, pp. 19-54.

[3] L. Alberti and R. M. Harshey, "Differentiation of Serratia marcescens 274 into swimmer and swarmer cells", *Journal of Bacteriology* **172**, 1990, 4322.

[4] N. Darnton, L. Turner, K. S.Breuer, and H. C.Berg, (2004) "Moving fluid with bacterial carpets", *Biophys*. **86**, 2004, 1863

[5] E. Steager, C. B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, and M. J. Kim., "Control of microfabricated structures powered by flagellated bacteria using phototaxis", *Applied Physics Letters* **90**, 2007, 263901

[6] Madou M (1997) Fundamentals of microfabrication. CRC Press, NY

[7] E. Steager, J. Patel, C. B. Kim, D. K. Yi, W. Lee, and M. J. Kim, "A novel method of microfabrication and manipulation of precise bacterial teamsters in low Reynolds number fluidic environments", *Microfluidics & Nanofluidics*, in press, 2007

[8] M. Matsushita, in Bacteria as Muliticelluar Organisms, edited by J. A. Sharpiro and M. Dworkin, Oxford University Press, New York, 1997

[9]] E. Steager, C. B. Kim, and M. J. Kim, "Dynamics of pattern formation in bacterial swarms", *Physics of Fluids*, accepted, 2008

[10] R. M. Harshey, "Bacterial motility on a surface: many ways to a common goal", *Annual Reviews* **57**, 2003, pp. 249-73

[11] An YH, Friedman J., "Concise Review of Mechanisms of Bacterial Adhesion to Biomaterial Surfaces", *J Biomed Mater Res (Appl Biomater)* **43**, 1998, pp. 338-348

[12] W. Shi, B.A.D. Stocker, and J. Adler, "Effect of surface composition of motile Escherichia coli and motile Salmonella species on the direction of galvanotaxis", *J. Bacteriol*. **178**, 1996, pp. 1113-1119

[13] J. Henrichsen, "Bacterial surface translocation: a survey and a classification", *Bacteriol Rev* **36**, 1972, pp. 478-503.

[14] L. Eberl, G. Christiansen, S. Molin, and M. Givskov, "Differentiation of Serratia liquefaciens into swarm cells is controlled by the expression of the flhD master operon", *J Bacteriol* **178**, 1996, pp. 554-559.

[15] Z. Ling and K. Lian, "SU-8 3D microoptic components fabricated by inclined UV lithography in water", *Microsystems Technology* **13**, 2006, pp. 245-51

[16] M. J. Kim and K. S. Breuer, "Use of Bacterial Carpets to Enhance Mixing in Microfluidic Systems", *Journal of Fluids Engineering* **129**, 2007, pp. 319-324