

# Bacterial Flagella-Based Propulsion and On/Off Motion Control of Microscale Objects

Bahareh Behkam and Metin Sitti\*

NanoRobotics Laboratory, Department of Mechanical Engineering,  
Carnegie Mellon University, Pittsburgh, PA 15213, USA

**ABSTRACT.** Miniaturization of the power source and on-board actuation is the main bottleneck for development of microscale mobile robots. As a possible solution, this paper proposes the use of flagellar motors inside the intact cell of *Serratia marcescens* bacteria for controlled propulsion of swimming robotic bodies. The feasibility of the proposed idea is demonstrated by propelling  $10\ \mu\text{m}$  polystyrene beads at an average speed of  $15 \pm 6\ \mu\text{m}/\text{s}$  by several bacteria randomly attached on their surface. On/off motion control of the bead is achieved by introducing copper ions to stop the bacteria flagellar motors and ethylenediaminetetraacetic acid to resume their motion.

**KEYWORDS.** Bacterial flagellar motor, bioactuators, micro/nanofluidics, swimming microrobots

\*E-Mail: sitti@cmu.edu

[ Final version of paper can be found in [Applied Physics Letters](#) ]

Recent developments in micro/nanoscale engineering have led to realization of various miniature mobile robots<sup>1,2</sup>, however, the most significant bottleneck for further miniaturization of mobile robots down to micrometer scale is the miniaturization of the on-board actuators and power sources required for mobility. Biomotors are deemed to be one of the most promising choices for on-board actuation. They are advantageous over man-made actuators because they are much smaller (micro/nanometer overall sizes), and are capable of producing more complicated motions. More importantly, they convert chemical energy to mechanical energy very efficiently. The main drawback of the biomotors is that isolating and reconstituting them is a complicated task with low yield<sup>3</sup>. Also it is difficult to interface them with electronic circuitry. On the other hand, using biomotors inside the intact cells is simpler because no purification and reconstitution is necessary; simple nutrient such as glucose is provided and ATP or ion gradients are generated by the cell. Most importantly, sensors are already present in the cell and integrated with the motors. Lastly, more complex organelle can be used; hence more sophisticated motion can be generated<sup>4,5</sup>. Therefore, in this work, flagellar motors inside the intact cells are used for actuation. The power and actuation are harvested from the bacteria while they are provided with the required chemical energy source and preferred environmental condition<sup>6</sup>.

To demonstrate the feasibility of using bacterial flagella as bioactuators,  $10\ \mu\text{m}$  polystyrene (PS) beads are propelled by several *S. marcescens* bacteria attached to them. PS beads are tracked and their displacements are compared with the diffusion length for  $10\ \mu\text{m}$  spherical particles to prove that the beads are propelled by bacteria and their displacement is not due to Brownian motion. Moreover, the on/off motion control of the

mobile beads is demonstrated by stopping and resuming the motion of the flagellar motors of the attached bacteria by using copper ions ( $\text{Cu}^{+2}$ ) and ethylenediaminetetraacetic acid (EDTA), respectively.

The bacterium *S. marcescens* (ATCC 274, American Type Culture Collection, Manassas, VA) was grown in Luria broth (L-broth) to saturation. A  $1.8 \mu\text{l}$  aliquot of a  $10^{-6}$  dilution of the saturated L-broth culture was used to inoculate a  $9 \text{ cm}$  swarm plate (L-broth containing  $0.6\%$  Difco Bacto-agar and  $5 \text{ g/l}$  glucose) off-center. The Petri plate was incubated for  $19$  hours at  $30 \text{ }^\circ\text{C}$ . The result was a swarming colony approximately  $7 \text{ cm}$  in diameter.

PS beads (G1000, Duke Scientific, Palo Alto, CA) suspended in deionized (DI) water were introduced into  $1 \text{ ml}$  of motility medium ( $0.01 \text{ M}$  potassium phosphate,  $0.067 \text{ M}$  sodium chloride,  $10^{-4} \text{ M}$  EDTA,  $0.01 \text{ M}$  glucose, and  $0.002\%$  Tween-20, pH 7.0)<sup>7</sup>. The solution was vortexed and subsequently centrifuged at  $800g$ . The beads were then concentrated five-fold. A  $10 \mu\text{l}$  aliquot of the final suspension was pipetted onto the leading edge of the swarm plate. After 5 minutes, the region was pipetted back into  $1 \text{ ml}$  of motility medium. During these 5 minutes, micro-beads randomly interact with the bacteria swimming on the surface of the swarm plate. Several of these bacteria adhere to the micro-beads. A  $10 \mu\text{l}$  sample of the final suspension was placed in an imaging enclosure which is an approximately  $500 \mu\text{m}$  thick PDMS ring placed on a No. 1 microscope glass slide. Once the sample is deposited in the enclosure, it was covered with a coverslip to prevent evaporation.

The motion of the PS beads was observed with a  $60\times$  oil immersion phase objective. Figure 1 depicts a PS bead at  $t=0$  and the same bead at  $t=6 \text{ s}$ . The total

displacement of the bead was measured to be approximately  $90 \mu\text{m}$ . On the other hand, the diffusion length,  $L_d$ , for a  $10 \mu\text{m}$  spherical particle is computed to be  $0.9 \mu\text{m}$  from  $L_d = \sqrt{4Dt}$ <sup>8</sup>, where  $D = k_B T / (6\pi\eta R) = 4.93 \times 10^{-14} \text{ m}^2/\text{s}$  is the diffusion coefficient,  $t=6 \text{ s}$  is the time,  $k_B$  is the Boltzman's constant,  $T$  is the absolute temperature of the solution,  $\eta$  is the dynamic viscosity of water, and  $R$  is the radius of the particle. This value is about 100 times smaller than the observed displacement of the bead and this confirms that the PS bead is actually propelled by the attached bacteria.

The average velocity of the bead shown in Fig. 1 is calculated to be  $15 \mu\text{m/s}$  and there were 10 bacteria attached to the bead. From Stokes' law, drag force of a sphere at low Reynolds number ( $Re \ll 1$ ) is  $F_{drag} = 6\pi\eta R U$ , where  $F_{drag}$  is the drag force and  $U$  is the velocity. Drag force of the sphere shown in Fig. 1 is calculated to be  $1.4 \text{ pN}$ . Net propulsion force of the  $N$  randomly attached bacteria can be approximated as  $F_{propulsion} \cong \sqrt{N} f_{bacterium}$ <sup>5</sup>, where  $f_{bacterium}$  is the propulsion force generated by each bacterium. For low  $Re$  flow,  $F_{drag} = F_{propulsion}$ , because inertia is negligible. Using this relation, it is concluded that  $f_{bacterium} = 0.45 \text{ pN}$ ; which is consistent with the value predicted by applying the Stokes' law to the geometry of *S. marcescens* bacteria, approximately modeled as a  $0.5 \mu\text{m}$  radius sphere.

During the experiments, different beads demonstrated different behaviors. Some of the beads did not have any bacteria attached to them and were not mobile. For the 35 experiments performed, on average, 60% of the beads were mobile. Although significant displacement, compared to the diffusion length, of the PS bead was consistently observed, the net displacement and speed of the beads were random and not identical among the experiments. For a sample of 8 experiments the average bead displacement

over 6 seconds was  $92 \pm 35 \mu\text{m}$ . Firstly, this is largely due to the fact that the flagellar motors of the wild type bacteria used in the experiments demonstrated random *run* and *tumble* behavior which changes the magnitude of the net force and in turn the direction of motion<sup>9</sup>. Secondly, since the beads were pipetted onto the swarm plate, the quantity, orientation and spacing of the adhered bacteria were not controlled. Basically, bacteria adhere to the beads at random sites and in random directions. This leads to various net propulsion forces and speeds for different beads also smaller net propulsion compared to the case where all the bacteria are attached in a unidirectional fashion.

After demonstrating the propulsion of the bead by bacteria, devising a speed control technique is the next significant step towards developing a microscale robotic system. Chemical and optical stimuli can be used to modulate the speed of the *S. marcescens*<sup>10</sup>. However, neither of these two stimuli can cause the flagellar motors to halt. It was previously reported by Adler<sup>11</sup> that absence of a chelating agent in bacterial suspensions leads to paralysis of the bacteria. A chelating agent is a substance whose molecules can form several bonds to a single metal ion. The reason for the observed phenomenon is that heavy metal ions, naturally present in water, bond to the rotor of the flagellar motors of the bacteria and prevent their motion. Here, this fact is taken advantage of and the bacteria are purposefully paralyzed only temporarily and in a reversible fashion. It is important to note that contrary to the chemical stimuli which interact with chemoreceptors causing them to signal to the motor to modulate its speed, heavy metal ions directly bond to the rotor of the flagellar motor, impairing its motion instantaneously.

Mobile PS beads were fabricated using the procedure described earlier. A  $10\ \mu\text{l}$  sample was placed in the imaging enclosure and was observed using an  $60\times$  oil immersion phase objective. A  $10\ \mu\text{m}$  PS bead which had approximately 8 bacteria attached was tracked for 6 seconds. Fig. 2(a) depicts the bead at intervals of 0.6 seconds for the entire 6 seconds. To stop the bead,  $5\ \mu\text{l}$  of  $5\times 10^{-3}\ \text{M}$   $\text{CuSO}_4$  solution (source of heavy metal ions) was added to the enclosure. The PS bead stopped moving almost instantaneously. Fig. 2(b) depicts the bead at intervals of 0.6 seconds for 6 seconds. It is shown that the bead does not move at all. To resume the motion of the bead,  $7.5\ \mu\text{l}$  of  $5\times 10^{-3}\ \text{M}$  EDTA solution was added to the enclosure. The PS bead resumed its motion immediately. Fig. 2(c) depicts the bead at intervals of 0.6 seconds for 6 seconds. It can be seen that the bead has resumed its motion. This experiment was repeated for several other beads and the above results were consistently observed. The repeatability of the control method was tested by stopping and resuming the motion of a bead 3 times. This control method is highly advantageous because it does not damage the bacteria and can be repeated as many times as required. Most importantly, the flagellar motors are not controlled through a sensory input. Normally when bacteria are controlled by introducing a stimulus, there exist a latency phase during which the chemoreceptors relays the information to the flagellar motors causing the motor to regulate its speed. This can take up to 2 seconds. However, here the only factor which may delay the bacteria response is the diffusion time of the copper ion or EDTA which is insignificant for the small test volumes.

In conclusion, the feasibility of using bacterial flagella for controlled propulsion at microscale is demonstrated in this paper. It is shown that several *S. marcescens*

bacteria, randomly attached to a 10  $\mu\text{m}$  PS bead, produce a large enough propulsion force to propel the bead forward. On/off motion control of the bead is achieved by introducing copper ions and subsequently EDTA prompting the bacterial flagellar motor to halt and resume motion without causing any damage. This method of motion control is reversible and can be used repeatedly. Bacteria demonstrate immediate response to the metal ions and chelating agents. However, when the sample volume is large, the response time is affected by the diffusion rate of both chemicals. This problem can be overcome by local introduction of the chemicals. This propulsion method will be used to develop hybrid (biotic/abiotic) swimming microrobots for potentially biomedical applications such as non-invasive screening for diseases and targeted drug delivery within areas of the body with stagnant or low velocity fluid flow.

This work is supported in part by the ICES-DOWD fellowship. The authors thank Philip Leduc for allowing the use of the Cellular Biomechanics Laboratory facilities, Rahel Strässle for help with the experiments, and Cagdas Onal for help with particle tracking.

## REFERENCES

- <sup>1</sup> B. Donald, G. Levey, C. McGray, I. Paprotny, and D. Rus, *J. of Microelectromechanical Systems*, **15**, 1 (2006)
- <sup>2</sup> K. B. Yesin, K. Vollmers, and B. J. Nelson, *The Int. Journal of Robotics Research*, **25**, 527 (2006)
- <sup>3</sup> R. Soong, G. D. Bachand, H. P. Neves, A. G. Olkhovets, H. G. Craighead, and C. D. Montemagno, *Science*, **290**, 1555 (2000)
- <sup>4</sup> D. Weibel, P. Garstecki, D. Ryan, W. R. DiLuzio, M. Mayer, J. E. Seto, and G. M. Whitesides, *PNAS*, **102**, 11963 (2005)
- <sup>5</sup> N. Darnton, L. Turner, K. Breuer, and H. Berg, *Biophysical J.*, **86**, 1863 (2004)
- <sup>6</sup> B. Behkam and M. Sitti, *Proc. of IEEE Int. Conf. of Eng. in Med. and Bio.*, 2421 (2006)
- <sup>7</sup> J. Adler, and B. Templeton, *J. of General Microbiology*, **46**, 175 (1967)
- <sup>8</sup> P. Hiemnez and R. Rajagopalan, *Principles of Colloid and Surface Chemistry*, 85 (1997)
- <sup>9</sup> H. Berg, *Annual Review of Biochemistry*, **72**, 19 (2003)
- <sup>10</sup> F. Neidhart, J. Ingraham, K. Low, B. Magasanik, M. Schaechter, and H. Umberger, (ed.) *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*, 732 (1987)
- <sup>11</sup> J. Adler, *J. of General Microbiology*, **74**, 77 (1973)

## FIGURE CAPTIONS

FIG. 1. Phase-contrast optical microscope images of a mobile  $10\ \mu\text{m}$  PS bead with several *S. marcescens* bacteria attached to it at (a)  $t=0$  and (b)  $t=6\ \text{s}$ . PS bead's path is shown with rings.

FIG. 2. Phase-contrast optical microscope images of the positions of a mobile  $10\ \mu\text{m}$  PS bead at intervals of 0.6 seconds for 6 seconds: (a) before  $\text{CuSO}_4$  is added, (b) after  $\text{CuSO}_4$  is added, and (c) after EDTA is added to the medium.



