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TOWARDS HYBRID SWIMMING MICROROBOTS: BACTERIA ASSISTED PROPULSION OF POLYSTYRENE BEADS

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Abstract— **Compactness and efficiency of biomotors makes them superior to man-made actuators and a very attractive choice of actuation for micro/nanorobots. However, biomotors are difficult to work with due to complications associated with their isolation and reconstitution. To circumvent this problem, here we use flagellar motors inside the intact cell of *S. marcescens* bacteria. An array of bacteria is used as propeller for a $10\ \mu m$ polystyrene (PS) bead. PS bead is tracked for several seconds and its displacements is compared with diffusion length of a $10\ \mu m$ particle. It is shown that the bead moves with an average velocity of $17\ \mu m/s$. Orientation of adhesion of *S. marcescens* to polydimethylsiloxane (PDMS) chips and microscale PS fibers was also investigated. It is shown that for both substrates; only bacteria from farther behind the leading edge of the swarm, adhere in end-on configuration.**

I. INTRODUCTION

The most significant bottlenecks for miniaturization of mobile robots down to microscale are miniaturization of the power source required for mobility and on-board actuation. Biomotors are attractive actuators in micro/nanotechnology. They are advantageous over man-made actuators mainly because they are capable of converting chemical energy to electrical or mechanical energy very efficiently. The main drawback of the biomotors is that they are difficult to work with outside the cell [1]. Using biomotors inside the intact cells is simpler because no purification and reconstitution is necessary. Moreover, 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 motor. Lastly, more complex organelles can be used hence more sophisticated motions can be produced [2], [3]. Therefore, in this research, we use flagellar motor inside the intact cell. Here, we propose a hybrid swimming microrobot which is propelled by helical flagella - only about 20 nanometers in diameter - of the bacteria attached to an inorganic robot body. Conceptual drawing of the robot is depicted in Fig. 1.

The advantages of the hybrid robots include: (i) They run on a small amount of nutrient for an extended period of time (miniature and efficient), (ii) Components of the propulsive element, *i.e.* bacteria, self-replicate; therefore no microfabrication is required. However, there are numerous challenges associated with realization and characterization of these robots. Some of them are: (i) Repeatability and yield, (ii) control of the speed of the bacteria, (iii) Orientation and configuration of the adhered bacteria.

In this paper, preliminary experimental results on the development of a hybrid swimming microrobot are presented. To demonstrate the feasibility of the idea, $10\ \mu m$ polystyrene (PS) beads are propelled by an attached array of *S. marcescens* bacteria. PS beads are tracked and their displacements are compared with diffusion length for $10\ \mu m$ particles. The large difference between the two values for displacement proves that the beads are propelled by bacteria and their displacement is not due to Brownian motion. In order to study these mobile beads in further detail, it is imperative to seek methods in which we can repeatedly fabricate mobile beads with similar density and orientation of bacteria attached. As the first step, experiments were performed to investigate whether there exists a preferential orientation of adhesion for the bacteria grown in different media. The experiments were carried out using small polydimethylsiloxane(PDMS) chips and a grid of mircoscale PS fibers as substrates. Orientation and density of the adhesion for the bacteria from L-broth culture and swarm plate was studied.

II. MATERIALS AND METHODS

Bacteria: The bacterium *S. marcescens* (ATCC 274, American Type Culture Collection, Manassas, VA) was grown in Luria broth (L-broth) to saturation. A $1.8\ \mu l$ aliquot of a 10^{-6} dilution of the saturated L-broth culture was used

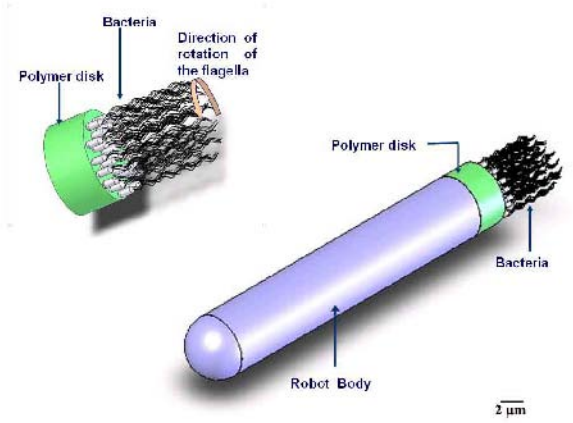

Fig. 1. Conceptual drawing of the hybrid swimming robot; robot body bonded to the propulsive element. Inset: magnified image of the propulsive element: An array of bacteria attached to a polymer micro-disk.

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to inoculate a swarm plate (L-broth containing 0.6% Difco Bacto-agar and 5 g/l glucose). The Petri plate was incubated for 20 hours at 30 $^{\circ}C$. This resulted in a swarming colony approximately 5 cm in diameter.

PS Bead Sample Preparation: 10 μm PS beads (G1000, Duke Scientific, Palo Alto, CA) suspended in deionized (DI) water were introduced into 1 ml of motility medium (0.01 M potassium phosphate, 0.067 M sodium chloride, 10^{-4} M EDTA, 0.01 M glucose, and 0.002% Tween-20, pH 7.0) [4]. The solution was vortexed and then centrifuged at $800g$. The beads were then concentrated five-fold. A 10 μl aliquot of the final suspension was pipetted into the leading edge of the swarm plate. After 5 minutes, the region was pipetted back into 1 ml of motility medium. A sample was sealed between a glass slide and a coverslip. An approximately 500 μm thick PDMS ring was used as a spacer between the glass slide and the coverslip.

PS Bead Tracking: The motion of the PS bead was recorded by taking sequential images with frequency of 20 Hz for 4 seconds. A particle detecting program written in Visual C was used to track the bead. MATLAB image processing toolbox was used for image operations. The algorithm detects beads in successive frames [5]. For each frame, image gradients and their directions are extracted using a sobel filter. Then, an adaptive threshold according to the maximum gradient value is used to pick the pixels that may be of interest. Using an accumulator, pairs of pixels that satisfy the conditions of a circle mentioned above are used to vote for a circle center and radius. This voting mechanism is used to avoid false positives. Finally, maximum values on the accumulator are given as circles. This technique is based upon the algorithm proposed by Rad *et al.* [6] for fast detection of circles utilizing the gradient directions of a grayscale image.

PDMS Chip Preparation: Approximately 10 gr of PDMS solution (Sylgard 184, Dow Corning,Midland, MI) was mixed according to the manufacturer's instruction and degassed in a vacuum chamber. The solution was then poured into a 3" plastic petri-dish and cured at 100 $^{\circ}C$ for 45 minutes. This yields an approximately 1 mm thick PDMS film. A 2×2 mm^2 pieces were cut out with an Xactor knife. The PDMS chips were either placed onto the swarm plate or were introduced into the saturated L-broth culture. In both cases, the chip was left to be in contact with bacteria for 5 minutes. The chip was then washed free of unattached bacteria by floating it over motility medium and swirling it around. The procedure was repeated three times. This method of transferring bacteria to PDMS is referred to as "blotting" [3].

PS Fiber Grid Preparation: PS fibers were pulled across a stainless steel washer , 2 mm ID, with spacing of 75 μm. The fibers were pulled using the approach outlined in [7]. Molecular weight of the PS solution was 2×10^6 gr/mol. The bacteria were blotted onto the PS grid.

III. BACTERIAL ADHESION

Mechanism of adhesion of bacteria to surfaces is not well understood, though it is speculated that the bacterial adhesion occurs in two steps: (1) Reversible adhesion occurs within few seconds. This is due to van der Waals, electrostatic forces, or acid-base interactions. (2) Irreversible attachment happens after the reversible attachment is made. This is through surface conformational changes and formation of protein-ligand bonds and production of extracellular polymers (lipopolysaccharides, phospholipids, proteins). Bacteria attachment is also mediated by flagella and pili [8], [9]. If bacteria attach to the surfaces with their bodies not their flagella, they adhere in one of the two possible configurations: end-on or lying flat. In end-on configuration long axis of the body of the bacteria is perpendicular to the surface and in lying flat configuration, long axis of the body of the bacteria is parallel to the surface.

A. ADHESION OF THE BACTERIA TO THE PDMS CHIPS

Orientation of the bacteria adhered to the PDMS chips for three different cases are investigated. Fig. 2 depicts the bacteria from saturated L-broth culture attached to the PDMS chips. It is shown that almost all bacteria are in lying flat configuration. Fig. 3 shows the bacteria from the leading edge of the swarm attached to a PDMS chip. Due to higher viscosity of the agar plate medium compared to liquid broth, bacteria grown on the plate are larger in size. This bacteria are also in lying flat configuration. Fig. 4 shows the bacteria from farther behind the leading edge of the swarm attached to a PDMS chip. These bacteria adhere in end-on configuration.

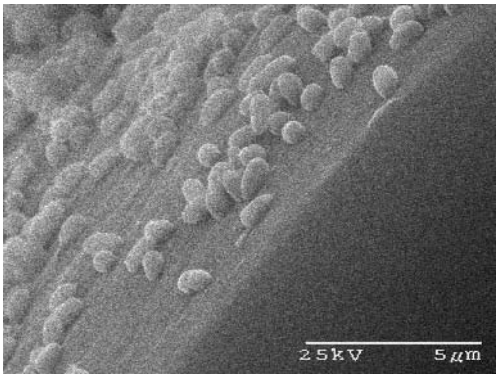


Fig. 2. Scanning electron microscopy (SEM) image of *S. marcescens* from saturated L-broth culture attached to a PDMS chips. Majority of bacteria are in lying flat configuration.

To further examine the repeatability of orientation of adhesion of bacteria, a grid of microscale PS fiber was introduced to the swarm plate at the location behind the leading edge of the swarm where it was speculated to yield adhesion in end-on configuration. The density of the attachment was low, nevertheless; most of the bacteria were observed to be attached in end-on configuration (shown in Fig. 5). To facilitate the adhesion of bacteria to PS fibers and

perhaps increase the number of bacteria attached, another PS grid was coated with Poly$_{-L}$lysine. The coated grid was then introduced onto the swarm plate at a location close to the location of the experiment for uncoated grid. The bacteria attached to the coated grid of fibers were still mainly in end-on configuration but the number of attached bacteria increased substantially. Fig. 6 demonstrates the Poly$_{-L}$lysine coated fiber before and after bacteria attachment.

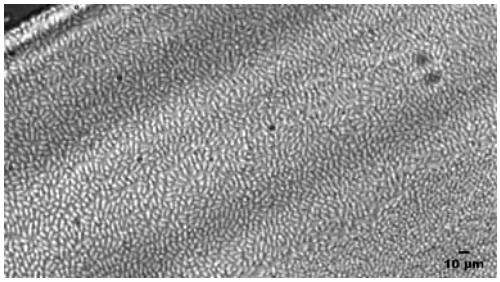


Fig. 3. Phase contrast image (60× oil immersion phase objective) of *S. marcescens* from the leading edge of the swarm attached to a PDMS chip. Majority of bacteria are in lying flat configuration.

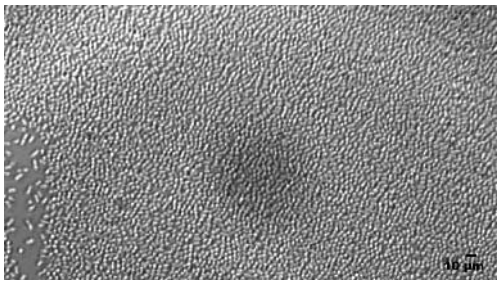


Fig. 4. Phase contrast image (60× oil immersion phase objective) of *S. marcescens* from behind the leading edge of the swarm attached to the PDMS chip. Majority of bacteria are in end-on configuration.

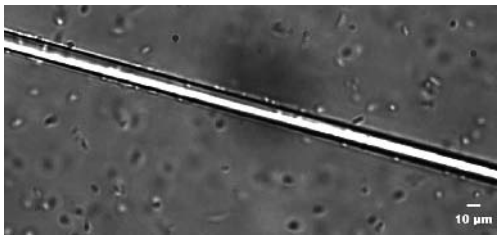
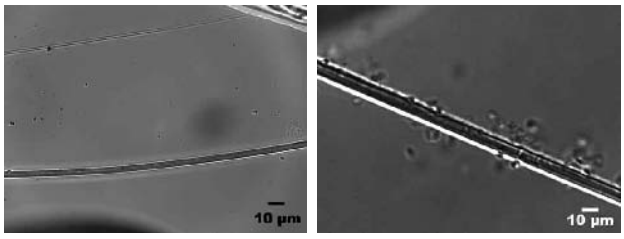
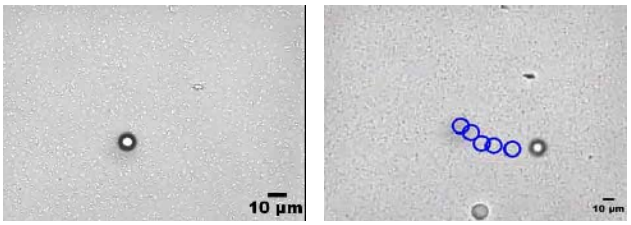


Fig. 5. 60× phase-contrast image of the uncoated PS fiber with few bacteria attached. Most of the bacteria attached in end-on configuration.



(a) **(b)**

Fig. 6. 60× phase-contrast image of a Poly$_{-L}$lysine coated PS fiber **(a)** before attachment of bacteria and **(b)** after attachment of bacteria. Most of the bacteria adhered in end-on configuration.



(a) **(b)**

Fig. 7. 60× Phase-contrast image of a 10 μm PS bead with *S. marcescens* attached to it at **(a)** $t = 0$ s and **(b)** $t = 4$ s. PS bead's path is shown with blue rings.

IV. AUTONOMOUS PS BEADS

The motion of the PS beads was observed with a 60× oil immersion phase objective (Zeiss Axiovert 200). Different beads demonstrated different behaviors. Some of the beads were not mobile. Across several experiments performed, an average of 60% of the beads were mobile. The velocities of the different beads were neither identical nor uniform. This was expected considering that the adhesion of the bacteria to the beads was not controlled and the bacteria were demonstrating their characteristic "run" and "tumble" behavior [10]. Fig. 7 depicts a PS bead at $t = 0s$ and the same bead at $t = 4s$. The total displacement of the bead was measured to be 70 μm. This value is significantly larger than the diffusion length for a 10 μm particle. Diffusion is the random migration of molecules or small particles due to thermal energy [11] and diffusion length is the characteristic length scale for diffusion. It is shown as $L_d = \sqrt{4Dt}$, where D is the diffusion coefficient and t is the time. D depends on the size and to a lesser extent the shape of the object. For spherical particles in water, $D = k_B T/6\pi\eta R$ [12], where k_B is the Boltzmann's Constant, T is the absolute temperature of the solution, η is the dynamic viscosity of water, and R is the radius of the particle. For 10 μm PS beads, $D = 4.93 \times 10^{-14}$ m^2/s, therefore, the diffusion length after $t = 4$ s is roughly equal to 0.9 μm which is 79 times smaller than the observed displacement of the bead. This confirms that the beads are actually propelled by the attached bacteria. The average velocity of the bead shown in Fig. 7 is calculated to be 17 $\mu m/s$.

V. CONCLUSIONS

In this work, the feasibility of propelling microscale objects by bacteria is investigated. It is shown that *S. marcescens* bacteria can effectively propel a 10 μm PS bead in liquid medium. The experiment was repeated multiple times. Although significant displacement (compared to diffusion length) of the PS bead was consistently observed, the net displacement and speed of the beads were not identical across multiple experiments. Firstly, this is largely due to the fact that wild type bacteria were used in these experiments, and their flagellar motors demonstrate random "run" and "tumble" behavior. In addition, for any given bead, there are different number of bacteria attached to the bead and flagellar motors of each bacterium demonstrate different behaviors at

any instant in time, hence it would be nearly impossible to have two beads move on similar paths. Secondly, since the bacteria were pipetted onto the swarm plate, the orientation and spacing of the adhered bacteria was not controlled. Basically, bacteria adhere to the beads at random sites and in random directions. This leads to (a) smaller net propulsion of the bead compared to the case when all the bacteria are attached in a unidirectional fashion, and (b) various net propulsion forces and speeds for different beads. To alleviate this problem, bacteria need to be attached to the substrate in an oriented fashion. To investigate the possible existence of preferential orientation in bacterial adhesion, bacteria from different parts of the swarm plate as well as bacteria from L-broth culture were attached to PDMS chips and PS fibers. It was observed that for both substrates, most of the bacteria from behind the leading edge of the swarm adhered in end-on configuration. For all other cases, bacteria were attached mainly in lying flat configuration. We are currently studying the adhesion results to gain a better understanding of the preferential orientation of adhesion. We are also working on controlling the density of the adhered bacteria by patterning the substrate.

VI. ACKNOWLEDGMENTS

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