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Enhanced Directionality of Bio-Hybrid Mobile Microrobots Using Non-Spherical Body Geometries*

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Abstract— **Mobile microrobots are envisioned to be employed for several applications including drug delivery, diagnostic imaging and environmental monitoring. In the bio-hybrid microrobot that is presented here, microparticles are used as the body of the microrobot and bacterial cells are utilized to realize on-board actuation. In this work, the importance of body shape on the dynamics of bacteria-propelled swimming microrobots (BacteriaBots) is investigated. We have shown that, with the use of non-spherical microparticles, average directionality of the BacteriaBots is enhanced compared with the spherical BacteriaBots.**

I. Introduction

Mobile microrobots can access small spaces and have the potential to be employed in distributed network systems for swarm robotic applications. Such microrobots are envisioned to impact a diverse range of applications including minimally invasive diagnosis and localized treatment of diseases, environmental monitoring, and homeland security [1]. Amongst the most significant obstacles to realization of mobile robots at micro-scale are the miniaturization of onboard actuators, power sources, and communication and control modules. Bio-hybrid approaches can be employed to address these challenges by using prokaryotic and eukaryotic cells within robotic systems [2-4]. BacteriaBot, a bio-hybrid microrobot, is constructed here by interfacing a microfabricated robot body with a group of live bacteria for onboard actuation, sensing, communication and control. Motile behavior of flagellated bacteria is well understood and falls under two characteristic modes of *run* and *tumble*. This behavior results in a three-dimensional random walk for bacteria and consequently a stochastic motion for the micro-objects propelled by bacteria.

Motility of spherical microstructures actuated by an ensemble of attached bacteria has been characterized in previous literature [5-8] but a systematic study of the effect of microstructure geometry on propulsive behavior is currently missing. Mobile microrobots with optimal body geometries are envisioned to significantly impact drug delivery and environmental monitoring. Limited particle diffusion and directional coefficient of drag are enhanced through using such bio-hybrid systems. In this work, we have utilized a low-cost and high throughput technique to obtain non-spherical microparticles and investigate the effect of particle shape on the motile behavior of the BacteriaBots.

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II. Materials and Methods

A. Fabrication of Non-Spherical Particles

A high throughput spherical particle casting and mechanical stretching under heat treatment, as previously described in [9], was used to produce non-spherical polystyrene (PS) particles which are the engineered synthetic body of the microrobots. Briefly, 6 μm PS spheres (Polysciences) were casted to make a 35 μm thick polyvinyl alcohol (PVA) film. The film was uniformly stretched in one dimension and PS beads were made viscous using a bath of hot mineral oil. For making prolate ellipsoid-shaped particles, 2% glycerol was added to the PVA film as a plasticizer. The fabrication parameters are summarized in Table 1. After removal from bath and cooling, the deformed PS particles were released from the PVA film by soaking in 30% isopropyl alcohol (IPA): DI water solution at 80˚C, and washed two more times using the same solution.

B. Bacterial culture

Escherichia coli (E. coli) strain k-12 (MG1655) was cultured in 10 ml of Luria Broth (1% tryptone, 0.5% yeast extract and 0.5% NaCl). The culture was grown to an optical density (OD_{600}) of 0.5 at 37 ˚C. Bacteria were centrifuged at 3000×g for 5 min and the pellet was resuspended in motility medium (0.01M potassium phosphate, 0.067M sodium chloride, 10^{-4}M EDTA, 0.01M glucose, and 0.002% Tween-20) [10].

C. BacteriaBot Construction

The mixture of Poly-$_L$-lysine (PLL) and micro-particles was incubated on a vortex mixer for one hour. The PLL-coated microparticles were then added to the bacteria allowing the bacteria to self-assemble on the BacteriaBot body.

Table 1: Fabrication parameters for manufacturing non-spherical particles

Particle	Stretching aspect ratio	Liquefaction method	Plasticizer (Glycerol)
Bullet	1.1-1.3	140˚C oil bath	No
Prolate Ellipsoid	1.2-1.5	130˚C oil bath	Yes

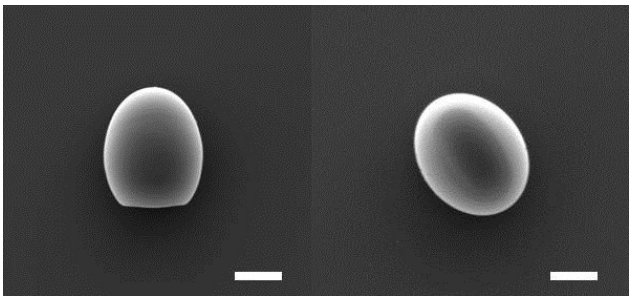


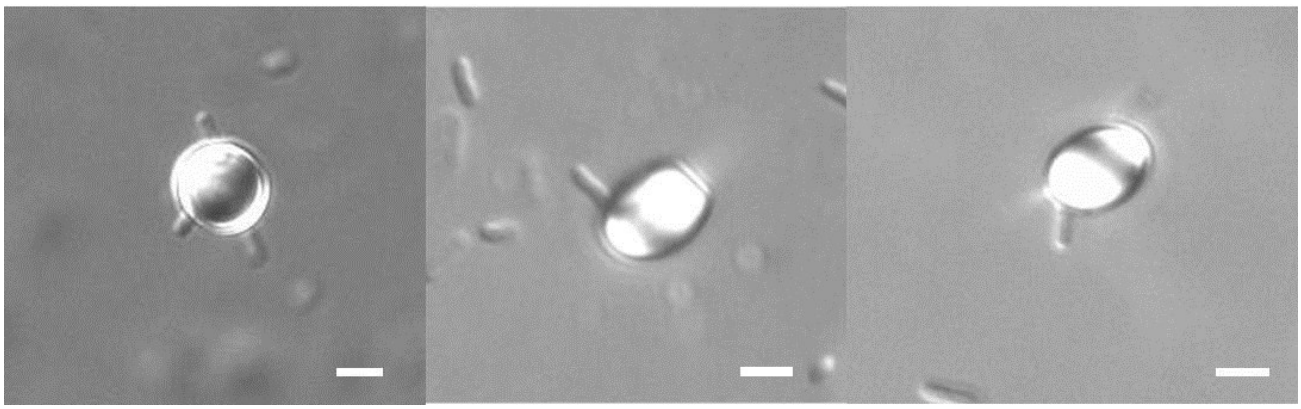
Figure 1: Scanning electron microscopy (SEM) images of non- spherical polystyrene geometries produced by casting and mechanical stretching of spherical particles. Scale bars are 2 micron.

D. Two-dimensional Single Particle Tracking

The motion of the microparticles was captured at 20 frames per second using a Zeiss AxioObserver Z1 inverted microscope equipped with an AxioCam HSm camera. The images were analyzed using a two-dimensional (2D) particle tracking routine developed in MATLAB (MathWorks, Natick, MA). Briefly, using cell segmentation and image restoration, the artifacts existing in most of the captured images were removed. This was followed by noise removal and cell boundary recognition using a border following algorithm. Finally, the nearest-neighbor method was used to link BacteriaBots in successive frames and generate the trajectories.

III. Results and Discussions

Polymeric micro/nanoparticles are currently employed in drug delivery applications and more specifically, microparticles are mostly used for local drug delivery. Microparticles have shown passive targeting to antigen presenting cells using the special cellular uptake mechanism present at the target site for this size of particles: they can only enter phagocytic cells which can be beneficial for specific applications [11]. Two different particle shapes, prolate ellipsoid and bullet, were fabricated here using the PS micro-sphere casting and mechanical stretching method described earlier. Representative scanning electron microscopy (SEM) images of the resulting geometries used for BacteriaBot Experiments are given in Fig. 1. The BacteriaBot experiment was conducted as to explore how the body shape will affect directionality of the motion of BacteriaBot. Directionality is defined as the ratio of the magnitude of the displacement vector to the total distance traveled. Representative images of the BacteriaBots with different geometries are also given in Fig. 2. The 2D image tracking routine described earlier was utilized to characterize the motion and particularly investigate the directionality of the BacteriaBots. Also, to prove that the bacteria attached to the mobile microparticles are the main source of propulsion, control experiments were performed. Minimal displacement of the control microparticles confirmed that the bacteria attached to the microparticles are the main source of propulsion. Therefore, any directed movement observed for the microparticles would be neither due to diffusion nor due to the flow field generated by the free-swimming bacteria present in the background. The experimental results for directionality of the BacteriaBots with spherical, bullet and prolate ellipsoid-shaped BacteriaBots are shown in Fig. 3. It can be seen that the bullet and prolate ellipsoid-shaped microparticles are propelled with a higher directionality compared with their spherical counterparts.



(A) (B) (C)

Figure 2: (A) A spherical BacteriaBot with three bacteria, (B) a bullet-shaped BacteriaBot with one bacterium, and (C) a prolate ellipsoid-shaped BacteriaBot with one bacterium attached. Scale bars are 2 micron.

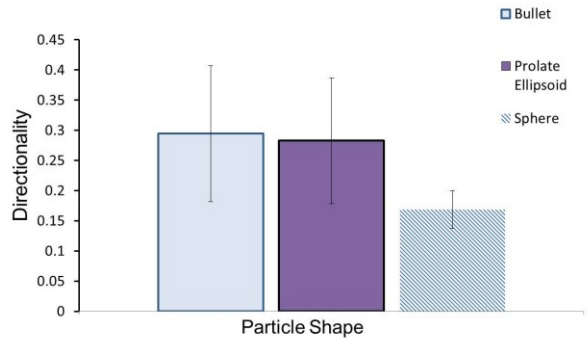


Figure 3: Directionality as a function of BacteriaBot body shape.

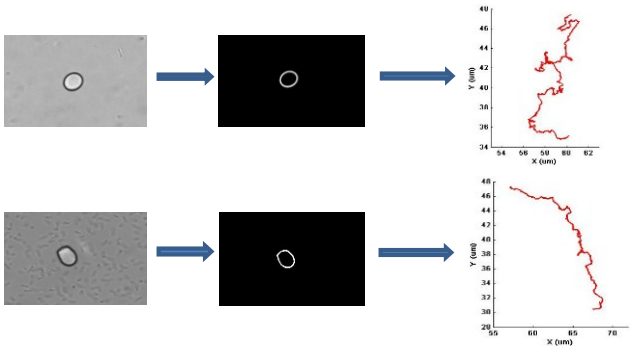


Figure 4: Two representative trajectories of BacteriaBots provided by the custom-made image processing routine.

Two representative trajectories of the BacteriaBots are shown in Fig. 4. For the elongated particles, the enhanced directionality of the motion for non-spherical particles is believed to be due to high resistance to rotation around any of the short principal axes for the BacteriaBots with such body shape. Also, the location of the attachment of bacteria is expected to affect the motile behavior of BacteriaBots significantly [6]. Number of attached bacteria for all geometries varies between 1 and 6; however, our experimental results did not seem to be dependent upon the number of bacteria attached. This is consistent with our previous study where the exerted force on the microparticles was unchanged despite the changing number of attached bacteria which were uniformly distributed over the body [7].

IV. Conclusion

Variation in motile behavior of BacteriaBots due to their body shape can be very complex because non-spherical geometries have varying coefficients of drag depending on their aspect ratio and the direction of motion. By utilizing a high throughput PS microparticle manufacturing method, we characterized the motile behavior of BacteriaBots with different shapes of sphere, bullet and prolate ellipsoid. It was shown that body shape strongly affected the average directionality of the motion. We are currently investigating the effect of particles size on the observed trend.

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