I. INTRODUCTION

Eukaryotic cells and unicellular prokaryotic microorganisms have been utilized as whole cell actuators for the controlled propulsion of objects of micro- and nanoscale characteristic dimensions [1,2]. Flagellated bacteria are well known for their superb swimming capabilities at speeds of up to 50 body lengths per second and their viability in a wide range of temperatures and pH. As such, they can be interfaced with microscale structures and be used as actuators [3–5]. These biotic and abiotic engineered systems, also known as biohybrid microrobots, are envisioned to be employed in large numbers for applications such as biosensing, transport and delivery of cargo, and minimally invasive treatment of diseases. Although the motility and taxis behavior of unicellular organisms have been extensively studied and modeled, the existing work is not readily applicable to biohybrid systems, as very often in these systems a large number of microorganisms are configured and constrained in a specific manner. In this work we have developed a computational stochastic model to investigate the emergent behavior of an ensemble of bacteria attached to a 10 μm spherical microbead in presence of a transient chemotactant gradient field. The stochastic model presented here encompasses the behavior of each individual bacterium attached to the microbead in a spatiotemporally varying chemoattractant field. The computational model shows that in a chemotactic environment, the ensemble of bacteria, although constrained, propel the bead in a chemotactic manner with a 67% enhancement in displacement to distance ratio (defined as directionality) compared to nonchemotactic propulsion. The simulation results are validated experimentally. Close agreement between theory and experiments demonstrates the possibility of using the presented model as a predictive tool for other similar biohybrid systems.

II. MODELING

The comprehensive model for the stochastic motion of the bacteria-propelled microbead presented here consists of the following modules.

A. Chemical concentration field model

The model concentration field used in this work is based on a classic chemotaxis assay initially developed by Pfeffer and later modified by Adler [6]: a schematic of the setup can be seen in Fig. 1. It comprises of a cylindrical capillary that contains a chemoattractant and is placed at the entrance of an enclosure containing the bacteria-propelled microbeads. The spatiotemporally varying chemical concentration field that is generated by diffusion of the chemoattractant from the capillary with initial chemical attractant concentration $C_0$ is given by [7]

$$C(r, t) = \frac{C_0 r_c^2}{2r \sqrt{\pi Dt}} \left[ \exp \left(-\frac{r^2}{4Dt}\right) \left(1 + \frac{3r_c r}{4Dt}\right) \right],$$

where $C_0$ is the chemoattractant concentration in the capillary, $D$ is the diffusion coefficient of the chemoattractant, $t$ is the time from the start of the simulation, $r_c$ is the radius of the capillary, and $r$ is the distance from the capillary to the point of interest in the experiment area. The diffusion coefficient of the chemoattractant (1% Casamino acid) $D = 8.5 \times 10^{-10}$ m$^2$/s is determined according to the method described in [8].
Bacteria-propelled micro-bead
Capillary

Cover slip U-tube Slide

FIG. 1. (Color online) A capillary-based chemotaxis assay was used to investigate the chemotactic behavior of bacteria-propelled microbeads. Capillary contains the chemoattractant solution (1% Casamino acid). Bacteria-propelled microbeads are enclosed within the experiment area.

B. Stochastic model of a bacterium

Flagellated bacteria such as Escherichia coli (E. coli) and Serratia marcescens (S. marcescens) possess between four and ten propulsive organelles known as flagella that are 20 nm in diameter and 10 μm long. The motility of bacteria comprises of two distinct states: run and tumble. During the run state, the flagellar motors rotate counterclockwise causing the flagella to coalesce and form a bundle which then produces a propulsion force and causes the bacterium to move forward at constant speed. Each bacterium’s run is followed by a tumble. Tumble occurs when one or more of the bacterium’s flagellar motors rotate in the clockwise direction causing the disruption of the bundle. During a tumble, the bacterium changes its heading direction randomly to begin a new run cycle. This leads to the stochastic motion of bacteria in three dimensions (3D) and can be modeled as a two-state Markov chain (as shown in Fig. 2) with state duration distributions occurring based on an exponential distribution [9]. Therefore, the run and tumble durations can be sampled through the following exponential distribution:

\[ f(t, \lambda_i) = \lambda_i e^{-\lambda_i t}, \]

where \( \lambda_i \) is the average rate parameter of the exponential distribution.

The average run and tumble durations are taken to be respectively \( \lambda_r \approx 0.9 \) s and \( \lambda_t \approx 0.1 \) s in an isotropic medium (no chemical gradient) [10,11]. However, in the presence of a positive chemoattractant gradient, bacteria tend to extend the duration of their runs which leads to a decrease in their tumbling probability. The tumbling probability of a bacterium in a chemical attractant gradient depends on the chemical concentration at the location of the bacterium and the change in chemical concentration the bacterium is subjected to from the start to the end of a run. The tumbling probability can be computed using the following equation [12]:

\[ P_t = P_0 \exp \left\{ -\sigma \frac{\partial}{\partial t} \left( \frac{N_T C}{K_d + C} \right) + \nu \nabla \left( \frac{N_T C}{K_d + C} \right) \right\}, \]

where \( P_0 \) is the tumbling probability in an isotropic medium and is equal to 0.1, \( \sigma \) is the chemotactic sensitivity and is equal to 75 000 μm²/s [13], \( N_T \) is the number of homogeneous receptors and is equal to 6, \( K_d \) is the dissociation constant which is equal to 0.00014 mol, \( \nu \) is the local speed of the bacterium, and \( C \) is the concentration sensed by the bacterium at any particular point in time.

At each time step, the location of each bacterium on the microbead with respect to a fixed reference frame is determined and subsequently, the chemical concentration sensed by each bacterium is calculated using Eq. (1). A bacterium is set to increase its running time and therefore decrease its tumbling probability, according to Eq. (3), when the chemical concentration it senses exceeds 5 nmol [14]. When in presence of a negative chemical gradient or in an isotropic medium, the bacterium maintains a constant tumbling probability of 0.1.

C. Stochastic model of the microbead

The location and orientation of the bacteria attachment on the bead will serve as basis for the development of the stochastic model for dynamics of the 10 μm bead propulsion by an ensemble of attached bacteria. In this model, bacteria were assumed to be uniformly distributed over the surface of the microbead. The attachment density was experimentally determined to be about 1 bacterium/7 μm² and 1 bacterium/11 μm² in the two sets of experiments conducted. Figure 3(c) illustrates the bacteria attachment configuration.

At the start of each simulation, bacteria are randomly assigned a state of 1 (run) or 0 (tumble). A bacterium with a state of 1 will exert a force at the attachment point that equates to 0.48 pN [1]. The initial direction of the force exerted by each bacterium at the point of attachment on the surface of the bead is randomly chosen. The dynamics of the system is assumed to be dominated by viscous effects and the inertial effects are neglected as the Reynolds number is of order of magnitude of 10⁻⁴. Therefore, the overall propulsion force which results from the contributions of all the attached bacteria must equate the translational drag \( F_D \). Similarly, the overall moment the microbead is subjected to is equal to the rotational drag \( M_D \). This model does not take into consideration the Brownian motion, as its effect on the dynamics of the microbead motion is negligible when compared to the effect of bacterial propulsion.
Hence, the equations of motion governing the dynamics of the microbead are

$$\vec{F}_D = 6\pi \eta R \vec{V} = \sum_b \vec{F}_{b,s_b}, \tag{4}$$  
$$\vec{M}_D = 8\pi \eta R^3 \vec{\Omega} = \sum_b \vec{r}_b \times \vec{F}_{b,s_b}, \tag{5}$$

where \(\vec{V}\) is the velocity of the propelled microbead, \(\eta = 8.9 \times 10^{-16}\) N s/μm² is the dynamic viscosity of the aqueous medium, \(R\) is the radius of the microbead, and \(\vec{\Omega}\) is the angular velocity of the microbead. \(\vec{F}_{b,s_b}\) and \(\vec{F}_b\) are the position vector and propulsion force of each bacterium, respectively. \(s_b\) represents the state of each bacterium, its value is 1 when running or 0 when tumbling. Dynamics of the microbead were determined by taking into account the effects of all bacterial forces on the translational and rotational displacement for every time step of the simulation. The initial tumble and run time durations are assigned based on the distribution function illustrated in Eq. (2). At the end of every time step, the change in position of each bacterium with respect to a fixed reference frame is determined. According to the change in the chemical concentration sensed by each bacterium from the start to the end of a run or tumble period, bacteria will individually sample a new run or tumble time duration. In case of an increase of chemical concentration sensed, the bacterium will sample a new run time from the exponential distribution shown in Eq. (2) with a higher average rate parameter \(\lambda_1\). The tumble average rate parameter does not change when the chemical gradient is either null or negative.

The emergent parameters utilized to effectively characterize and compare the motion of microbeads propelled by bacteria in both chemotactic and nonchemotactic settings are the mean velocity of the bead (\(\vec{V}\)), the total distance traveled by the bead (dist), the overall displacement value (disp), and the directionality. The mean velocity of every simulation run was computed by averaging the ratio of the distance traveled by the center of the bead during a time step to the duration of the time step. The mean velocity values are then averaged to determine a mean velocity value (\(\vec{V}\)) for the number of times the simulation was run. The total distance (dist) traveled by the bead is a measure of the total length traveled by the centroid of the bead from the start to the end of a single simulation, expressed as dist = \(\sum_{n=1}^{N_{\text{bead}}} \Delta r_{\text{bead},n}\). These values are then averaged over the total number of simulations to obtain an average overall distance. The overall displacement (disp) is a measure of the length of the vector from the start to the end of a single simulation run, expressed as disp = \(\|\vec{r}_{\text{end}} - \vec{r}_{\text{start}}\|\). Its final value is obtained by averaging the displacement values over the number of times the simulation was run. The trajectories of bacteria-propelled microbeads are of stochastic nature, therefore this parameter has not been utilized for comparison between theoretical and experimental results. In order to compare the propulsive behavior in chemotactic and nonchemotactic cases, all numerical simulations were carried out for time durations significantly shorter than the randomization time of the microbead. The randomization time is the minimum time required for a system to exhibit its random walk properties and can be obtained from \(\tau_R^{-1} = k_B T/8\pi \eta R^3\), where \(k_B\) is the Boltzmann’s constant, \(T\) is the absolute temperature, \(\eta\) is the dynamic viscosity, and \(R\) is the radius of the bead [15]. For a 10 μm microbead, the randomization time is \(\tau_R \cong 11\) min. Simulations with time durations of 5 s and longer time durations such as 30 s were run to observe the effect of simulation durations on the selected emergent parameters. The obtained results in both cases (5 and 30 s) showed that the duration of the simulation has no notable effect on the velocity and the directionality. Significantly shorter time duration of 5 s was then chosen for the simulations mainly to compare simulations with experimental results. Most videos recorded from experiments have microbeads in the same focal plane (minor changes in the z direction) for short time periods that range from 5 to 10 s.

Both chemotactic and nonchemotactic simulations were run 400 times to identify the average stochastic behavior of the system. The 400 runs were determined to suffice as there were no major differences in the results for a larger number of simulation runs. The capillary radius was taken to be 100 μm and the chemical concentration in the capillary was set to \(C_0 = 0\) and \(C_0 = 0.01\) mol, respectively, for the nonchemotactic and
the chemotactic runs. It was assumed that the chemotactarant concentration within the capillary remains constant throughout the simulation.

III. RESULTS AND DISCUSSIONS

A. Materials and methods

Wild-type *S. marcescens* (ATCC 274) was grown on L-broth (1% tryptone, 0.5% yeast extract, and 0.5% sodium chloride) culture plates containing 0.65% agar (Difco Bacto agar) and 5 g/l glucose. 10 μm polystyrene microbeads (Fisher Scientific for the 1 bacterium/7 μm² attachment density and Sigma-Aldrich for the 1 bacterium/11 μm² attachment density) were washed by repetitive centrifugation in DI water and were finally suspended in motility medium (0.01 M of potassium phosphate, 0.0067 M of sodium chloride, 10⁻⁴ M of EDTA, 0.01 M of glucose, and 0.002% of Tween-20, pH = 7.0). A 10 μl aliquot of 1% (w/v) bead suspension was pipetted behind the edge of the bacteria swarm on the plate and left at room temperature for about 5 min to allow bacteria to randomly interact with and adhere to the microbeads. At the end of the 5 min, the bacteria and bead suspension mixture was aspirated and subsequently pipetted in 1 ml of motility medium. A volume of about 200 μl was transferred in the experiment area as shown in Fig. 3(a). A one-end sealed capillary filled with a 1% Casamino acid solution, a commonly used chemoattractant, is then placed at the center of the opening of the experiment area. Figure 3(b) depicts a microscope image of the experiment area and the tip of the chemoattractant capillary.

The motion of the microbeads was captured using a Zeiss AxioObserver Z1 inverted microscope equipped with an AxioCam HSm camera at 20 frames per second. The images were analyzed using a two-dimensional (2D) particle tracking algorithm developed in MATLAB (The MathWorks, Natick, MA). Briefly, using cell segmentation and image restoration the artifacts existing in most of the captured imaged 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 segmented cells in successive frames and to determine the bacteria-propelled beads trajectories.

B. Experimental validation of stochastic model

The simulation results for both chemotactic and nonchemotactic cases are presented in Table I. All four characteristic parameters are affected by the presence of the chemoattractant concentration field. The microbeads’ mean velocity increased by about 12% from the nonchemotactic to the chemotactic environment. Over the simulation duration of 5 s, the directionality of the propelled microbead saw an increase of 67% when the microbead was in a chemotactic environment with an initial capillary concentration $C_0 = 0.01$ mol. A higher directionality value indicates a more directed motion of the bacteria-propelled microbead. The increase in the characteristic parameters is attributed to the fact that the chemical gradient sensed by the bacteria affects their tumbling probability $P_T$. A reduction in the tumbling probability implies an extension in the run period for each bacterium propelling the microbead, which will result in not only a larger overall distance but also a more directional path for the motion of the microbead.

In order to validate the stochastic model presented here, a chemotaxis assay for bacteria-propelled microbeads was conducted and the experimental data was compared with the computational results.

The trajectory information was used to determine the average speed, distance, displacement, and directionality over the 5 s duration of the experiments. Experimental results are presented in Tables I and II. Each data point represents an average of at least 10 experiments. The trajectories of the bead do vary between experiments because of the stochastic nature of bacteria motion. Representative examples of the bead trajectories are shown in Fig. 4. Addition of chemoattractant in the environment contributes to an increase in the directionality value as well as the overall speed of the microbead. This can be explained by prolonged force exertion by those attached bacteria which sense an increasing chemical attractant concentration. This will result in an overall extension of the displacement of the microbead in a directional manner. The experimental results obtained in both chemotactic and nonchemotactic cases closely match the results obtained from the computational model. The small difference between the computational and experimental results suggest that this computational model can be used as an effective prediction tool in both chemotactic and nonchemotactic environments.

C. Effect of bacteria attachment density on microbead motion

The effect of the density of bacteria attachment was also explored experimentally by constructing bacteria-propelled beads at two attachment densities of: 1 bacterium/7 μm² and 1 bacterium/11 μm². Tables I and II show the results for both bacteria attachment densities.

| TABLE I. Summary of results comparing simulations and experiments in chemotactic and nonchemotactic environments for a bacteria attachment density of 1 bacterium/7 μm². |
|-----------------|----------------|----------------|
| $C_0$ (mol)     | Model          | Experiment     |
| V (μm/s)        | 8.6 ± 0.8      | 8.5 ± 1.7      |
| disp (μm)       | 11.3 ± 4.4     | 13.4 ± 6.2     |
| dist (μm)       | 37.2 ± 2.7     | 42.7 ± 8.3     |
| disp/dist       | 0.3 ± 0.1      | 0.3 ± 0.1      |

| TABLE II. Summary of results comparing simulations and experiments in chemotactic and nonchemotactic environments for a bacteria attachment density of 1 bacterium/11 μm². |
|-----------------|----------------|----------------|
| $C_0$ (mol)     | Model          | Experiment     |
| V (μm/s)        | 8.0 ± 0.6      | 7.4 ± 3.3      |
| disp (μm)       | 7.4 ± 3.3      | 18.6 ± 7.5     |
| dist (μm)       | 34.2 ± 2.9     | 37.9 ± 3.7     |
| disp/dist       | 0.2 ± 0.1      | 0.5 ± 0.2      |

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Reducing the bacteria attachment density results in fewer bacteria attached to the microbead. In the case of fewer bacteria attached to the microbead (no significant nonuniformity in bacteria attachment), the overall force is expected to remain unchanged or slightly reduce (based on degree of variation in attachment density). Indeed, we observe only slight variations in speed and directionality values when the bacteria attachment density is changed from 1 bacterium/7 μm² to 1 bacterium/11 μm². More than the number of bacteria attached to the microbead, the location of attachment is expected to affect the overall behavior. If the attachment density becomes significantly nonuniform, we expect to see a change in the overall force and consequently observe a change in average speed and displacement compared to distance ratio. In a prior work, the effect of bacteria attachment site on overall speed in an isotropic (nonchemotactic) environment was investigated [16].

D. Effect of fluid viscosity and particle size on microbead motion

The viscosity of the fluid will affect the speed, the displacement, and the distance traveled by the microbead. However, the directionality will stay unchanged as long as all other parameters are kept the same. This is due to the fact that the speed of the microbead is proportional to the net force it is subjected to. An increase in the viscosity of the fluid will result in an increase in the drag force and a decrease in the net propulsion force. This will in turn affect the distance traveled for a given period of time. Similarly, the displacement will change with the same rate the distance varies by. Therefore, when the viscosity of the media is changed, the directionality of the microbead stays the same while the speed decreases.

The size of the microbead should not have a significant effect on the displacement to distance ratio for both a nonchemotactic and chemotactic cases assuming that the bacteria attachment density is kept constant. It has previously been demonstrated that for unpatterned particles the net propulsion force is linearly proportional to the radius [1,3]. According to the Stokes equation, the drag force is also linearly proportional to the radius. Therefore, for the same attachment density the drag and propulsion forces change as a function of radius and the net force should not change significantly. This assertion is supported by the simulation code, which shows that the speed and the ratio of displacement to distance vary slightly when the radius of the bead is changed and the bacteria attachment density is kept intact.

IV. CONCLUSION

In summary, a stochastic model for chemotactic propulsion of microbeads is presented in this study. The model encompasses key parameters including orientation and location of the attached bacteria, spatiotemporal variations in chemoattractant concentration field, and its effect on run and tumble rates of each of the tens of the attached bacteria. This numerical model was validated experimentally and it was shown that it can effectively describe the emergent dynamics of the motion of a 10 μm microbead propelled by an ensemble of flagellated bacteria homogeneously attached, in both chemotactic and nonchemotactic environments. The description of the motion of the microbead was based on four emergent parameters of average speed, displacement, distance, and directionality. It was determined that all four parameters increase from the nonchemotactic to the chemotactic case. Most notably, the results show that the presence of a chemoattractant gradient results in 67% larger directionality values. This proves the feasibility of directed autonomous movement of biohybrid microrobots through the use of chemotaxis. This model can be easily expanded to serve as a predictive tool for other biohybrid systems with different whole cell actuators, nonspherical geometries, heterogeneous whole-cell actuator attachment configurations, and different spatiotemporally varying chemical concentration gradients.

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