Artificial magnetotactic motion control of *Tetrahymena pyriformis* using ferromagnetic nanoparticles: A tool for fabrication of microbiorobots

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We induce artificial magnetotaxis in *Tetrahymena pyriformis*, a eukaryotic ciliate, using ferromagnetic nanoparticles and an external time-varying magnetic field. Magnetizing internalized iron oxide particles (magnetite), allows control of the swimming direction of an individual cell using two sets of electromagnets. Real-time feedback control was performed with a vision tracking system, which demonstrated controllability of a single cell. Since the endogenous motility of the cell is combined in one system with artificial magnetotaxis, the motion of artificially magnetotactic *T. pyriformis* is finely controllable. Thus, artificially magnetotactic *T. pyriformis* is a promising candidate microrobot for microassembly and transport in microfluidic environments. © *2010 American Institute of Physics*. [doi:10.1063/1.3497275]

Artificial and biological microrobots capable of operating in low Reynolds number environments have been investigated for microscale applications such as drug delivery and microassembly. For artificial microrobots, magnetic control has been widely utilized because it is easy to implement. Zhang *et al.*[1] designed an artificial microrobot which mimicked flagellar motions using a helical tail and a magnetically controllable head. Sakar *et al.*[2] utilized the force created by a magnetic field gradient to control a microrobot fabricated with magnetite-doped SU-8 photoresist.

Other researchers have utilized microorganisms as actuators for microrobots because of the propulsive efficiency of cellular motors such as flagella and cilia. Martel *et al.*[3] have investigated magnetic control of magnetotactic bacteria, which have nanometer-sized magnetosomes inside of their cells. Steager *et al.*[4,5] have studied on/off and electrokinetic directional control of *Serratia marcescens* using ultraviolet light and electric fields on cells which have been blotted onto microstructures. Additionally, some tactic movement of eukaryotic cells such as galvanotactic or phototactic motion of *Tetrahymena pyriformis* (*T. pyriformis*)[6] and *Paramecium*[7] has been studied and demonstrated.

In this paper, we propose a method to create an artificially magnetotactic organism with *T. pyriformis* using iron-oxide particles (magnetite), and demonstrate controllability of such organisms via magnetotaxis. Magnetotactic *T. pyriformis* is easily created artificially and is finely controllable. In addition, *T. pyriforms* moves much more quickly than many other microorganisms, using the approximately 600 cilia covering its body. As the signaling mechanisms of *T. pyriformis* have high homology to those of more complex eukaryotes, it is frequently used as a model cell in biochemistry and cell biology.[8] Applying chemotactic selection to these cells provides the option of creating *T. pyriformis* subpopulations possessing increased chemotactic responsiveness based on the expression of selected receptors in the surface membrane.[9] Consequently, *T. pyriformis* may be used as a highly specific actuator to provide propulsion and sensing for microrobots in microfluidic environments.

T. pyriformis, a ciliate protozoan, is cultured to the logarithmic phase in a culture medium which includes 0.1% yeast extract (Difco, Michigan, USA) and 1% Bacto tryptone (Difco, Michigan, USA) in distilled water.[10] To create artificially magnetotactic *T. pyriformis*, a 0.1% concentration of 50 nm spherical iron oxide particles (Sigma Aldrich, MO, USA) are added to the culture medium. *T. pyriformis* internalizes these particles through the oral apparatus located at its anterior end.[11] After addition of the magnetite particles, the culture medium is gently agitated and then allowed to stand for about 10 min to ensure that most cells ingest the magnetite.

Microscopic evaluation of the internalized particles shows that they are stored in membrane bounded vesicles which are formed when the cell ingests food particle (e.g., microorganisms). The swimming behavior of the magnetite-loaded *T. pyriformis* is normal and identical to physiologically intact cells.[11] Our previous results showed that internalized small, physiologically non-self, test particles (e.g., ink particles) are released normally through the cytoproct after a few hours.[12] Figures 1(a) and 1(b) show a normal *T. pyriformis* cell and a *T. pyriformis* cell with internalized magnetite, respectively. The normal cell does not have any magnetite inside of the body and the circular vesicles are clean. How-

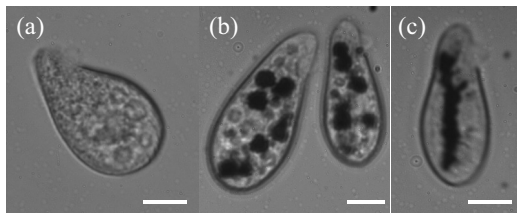

FIG. 1. Images of *T. pyriformis* (a) normal status (b) after internalization of iron oxide particles (c) after magnetization of internalization particles. The scale bars are 10 μm.

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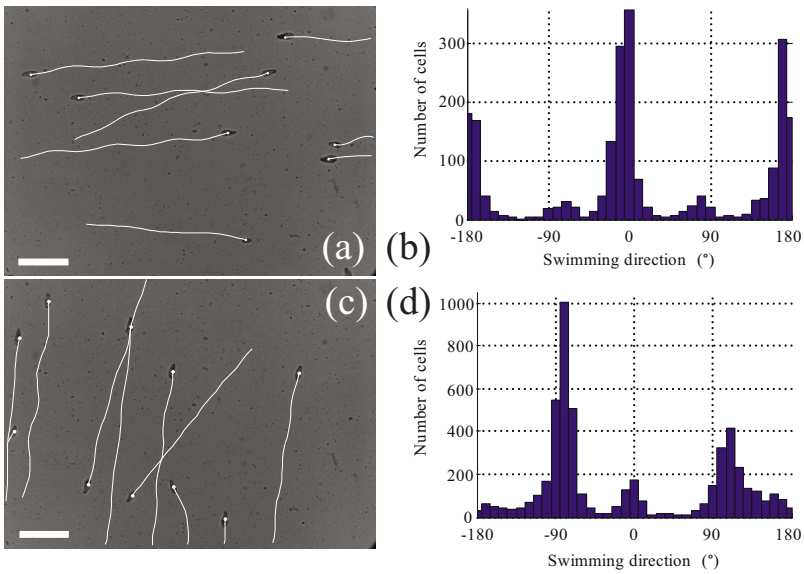

FIG. 2. (Color online) Magnetotactic motion of *T. pyriformis:* (a) paths when the magnetic field is applied along the *x* axis and (b) associated velocity direction histogram, (c) paths when the magnetic field is applied along the *y* axis and (d) associated velocity direction histogram. The scale bars are 250 μm. (enhanced online). [URL: http://dx.doi.org/10.1063/1.3497275.1]

ever, the magnetite-bearing cell has some dark circular vesicles which contain the internalized magnetite particles.

To magnetize the internalized magnetite, a rectangular neodymium-iron-boron (NdFeB) magnet with a surface field of 1964 G (K&J Magnetics, PA, USA) was applied on the cell culture for about 1 min. Due to the ferromagnetic nature of magnetite, the resulting induced magnetic dipoles in the internalized particles will remain even after the external magnetic field is removed but will diminish slowly over a period of time.[13] The cells which respond to the magnetic fields were observed for 1 h after magnetization so we assumed that the magnetic dipoles are saturated during the experiment. Although, the internalized particles are randomly distributed in the vesicles of cells before exposure to the external field, magnetization induces in most cells an aggregation of the magnetite-containing vesicles along the major axis [Fig. 1(c)]. We suspect that spatial limitations along the minor axis of the cell induces the particles to aggregate along the major axis, so that magnetic dipoles are characteristically formed along the major axis when the magnetite aggregation is magnetized.

The electromagnet used for this experiment is an approximate Helmholtz coil. Two sets of approximated Helmholtz coils are installed along the *x* and *y* axes, creating a maximum magnetic field strength of 2 mT at the center of the stage. The magnetic field strength within a 3.19 mm radius from the center is essentially constant, with a variation of only 2% between the maximum and minimum field values, allowing us to neglect magnetic forces and only consider the torque acting on each magnetite particle. The stage with the electromagnets was installed on the inverted microscope and cell motions were observed through the vision system at a low magnification (10×).

Since the magnetic dipoles are formed along the major axis of the cell, the net torque on the cell ($\boldsymbol{T}$) can be computed from their total magnetic moment ($\boldsymbol{m}$) and the applied magnetic field ($\boldsymbol{B}$)

$$\boldsymbol{T} = \boldsymbol{m} \times \boldsymbol{B} = mB \sin\theta, \tag{1}$$

where θ is the angle between the direction of the cell's motion (which is identical to its magnetic dipole direction) and the direction of the magnetic field. When the cell's motion is not aligned with the magnetic field, a torque is applied to the cell and the cell changes direction to reduce the angle between the applied magnetic field and the magnetic dipole moment of its ingested magnetite aggregation.

Figure 2 shows the cell's motion in a microfluidic channel when a magnetic field is applied along either the *x*- or the *y*-axis. Figure 2(a) shows a case in which most cells are moving horizontally due to a magnetic field applied along the *x*-axis. Figure 2(b) shows a histogram of the cell orientations, where the peaks at 0° and 180° indicate that most cells did indeed move in either the positive or the negative *x*-directions. Figures 2(c) and 2(d) demonstrate similar results for a case in which the magnetic field was applied along the *y*-axis; most cells moved in the positive or negative *y*-direction, as shown by the histogram peaks at ±90°. We measured the average speed of our magnetotactic *T. pyriformis* to be 786.7 μm/s, with a standard deviation of 190.7 μm/s.

Our result that magnetotactic *T. pyriformis* will move either in alignment or in antialignment with an external magnetic field could be misinterpreted as a case of axial magnetotaxis, which is movement along an axis without regard to the positive or negative direction.[14] However, during the original magnetization step, the orientations of the cells are randomly distributed, so that in some cells the resulting magnetic dipole will point from head to tail while in others it will point from tail to head. Each individual *T. pyriformis* cell will therefore still display polar magnetotaxis (preferential migration along the direction which aligns its magnetic pole with the external magnetic field). Polar magnetotaxis is a better method to control cells than axial magnetotaxis because the motion of polar-magnetotactic cells is much easier to predict.

To verify the controllability of polar-magnetotactic *T. pyriformis*, a simple feedback control algorithm was implemented to maneuver a cell to the desired position. Since the cell preferentially swims along the direction of the magnetic fields, the magnetic fields are applied in the direction toward the destination point from the current cell position, while the strength of the magnetic field is held constant

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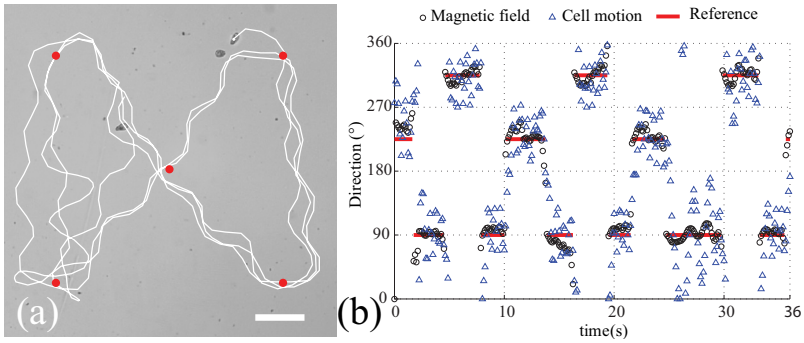

FIG. 3. (Color online) Real-time feedback control of *T. pyriformis* using the electromagnet system (a) path through five sequential destination points (b) direction of motion of *T. pyriformis* (triangle), magnetic field direction (circle), and reference between previous and current targets (red line). The scale bars are 250 μm. (enhanced online).
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$$\theta_{MG} = \text{atan } 2(y_{\text{target}} - y_{\text{cell}}, x_{\text{target}} - x_{\text{cell}}), \quad (2)$$

where θ_{MG} is the direction of the magnetic fields, and x and y represent the position coordinates of the destination and the cell. The above feedback control algorithm is not perfect because the speed of the cell is not controllable. Ideal control of the position and orientation of the cell would require both the velocity and steering angle to be controllable, as in a car. However, in this case, only the steering angle is controllable, which means the cell cannot be reliably directed toward the destination point when the direction toward the destination point is at a right angle to its current direction of motion.

To compute position information for a cell, a tracking algorithm to find the centroid was used.[6] Each cell was guided through a sequence of five destination points, as shown in Fig. 3(a). Once the cell travels to all five destination points, it will return again to the first destination point. In the case shown in Fig. 3(a), the cell approached from the top of the image and the vision system began tracking to detect its position. Then, the feedback control was initialized and the cell was controlled to move the destination point on the bottom left. For real-time feedback control, the images are captured at 8 frames/s. Figure 3(b) shows the relationship between the direction of the cell's motion (triangles) and the direction of the applied magnetic field (circles). The red reference line indicates the direction from the previous destination point to the next destination point.

The magnetic fields were applied to guide the cell to the destination points and consequently were roughly aligned with the reference direction, with some fluctuations to compensate for the oscillations of the cell. The average speed of the cell was 448.9 μm/s, with a standard deviation of 111.0 μm/s. The speed of this cell was smaller than that of the cells in the experiments in Fig. 2 because of its turning motions during the feedback control.

In this paper, we have introduced artificial magnetotactic motion control of *T. pyriformis*. This artificial magnetotaxis is created by magnetization of magnetite particles internalized by *T. pyriformis* cells. The direction of motion of the cell is controlled with two sets of approximate Helmholtz coils on the x and y axes. Combining this setup with simple feedback control allowed a *T. pyriformis* cell to be repeatedly directed through a sequence of five destination points. This enhancement of a motile microorganism with artificially created magnetotaxis, which allows these cells to be finely controlled, makes artificially magnetotactic *T. pyriformis* a promising candidate as a microrobot to address a variety of significant engineering and biotechnological tasks in microfluidic environments.

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[1]L. Zhang, J. J. Abbott, L. Dong, B. E. Kratochvil, D. Bell, and B. J. Nelson, Appl. Phys. Lett. **94**, 064107 (2009).
[2]M. Sakar, E. Steager, M. Kim, G. Pappas, and V. Kumar, Appl. Phys. Lett. **96**, 043705 (2010).
[3]S. Martel, M. Mohammadi, O. Felfoul, Zhao Lu, and P. Pouponneau, Int. J. Robot. Res. **28**, 571 (2009).
[4]E. Steager, C.-B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, and M. J. Kim, Appl. Phys. Lett. **90**, 263901 (2007).
[5]M. S. Sakar, E. B. Steager, D. H. Kim, A. A. Julius, M. J. Kim, V. Kumar, and G. Pappas, Int. J. Robot. Res. (2010) (accepted).
[6]D. H. Kim, D. Casale, L. Kőhidai, and M. J. Kim, Appl. Phys. Lett. **94**, 163901 (2009).
[7]N. Ogawa, H. Oku, K. Hashimoto, and M. Ishikawa, J. Theor. Biol. **242**, 314 (2006).
[8]G. Csaba, Int. Rev. Cytol. **95**, 327 (1985).
[9]L. Köhidai and G. Csaba, Cytokine **10**, 481 (1998).
[10]L. Kőhidai and G. Csaba, Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol. Endocrinol. **111**, 311 (1995).
[11]J. L. Rifkin and R. Ballentine, Trans. Am. Microsc. Soc. **95**, 189 (1976).
[12]L. Köhidai, B. Lovas, and G. Csaba, Zoolog Sci. **12**, 277 (1995).
[13]J. William, H. Hayt, and J. A. Buck, *Engineering Electromagnetics*, 7th ed. (McGraw-Hill, New York, 2006).
[14]D. A. Bazylinski and R. B. Frankel, Nat. Rev. Microbiol. **2**, 217 (2004).

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