Targeting of Cell Mockups using Sperm-Shaped Microrobots In Vitro

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Abstract—Sperm-shaped microrobots are controlled under the influence of weak oscillating magnetic fields (milliTesla range) to selectively target cell mockups (i.e., gas bubbles with average diameter of 200 μm). The sperm-shaped microrobots are fabricated by electrosprinning using a solution of polystyrene, dimethylformamide, and iron oxide nanoparticles. These nanoparticles are concentrated within the head of the microrobot, and hence enable directional control along external magnetic fields. The magnetic dipole moment of the microrobot is characterized (using the flip-time technique) to be $1.4 \times 10^{-11}$ A.m$^2$, at magnetic field of 28 mT. In addition, the morphology of the microrobot is characterized using Scanning Electron Microscopy images. The characterized parameters and morphology are used in the simulation of the locomotion mechanism of the microrobot to prove that its motion depends on breaking the time-reversal symmetry, rather than pulling with the magnetic field gradient. We experimentally demonstrate that the microrobot can controllably follow S-shaped, U-shaped, and square paths, and selectively target the cell mockups using image guidance and under the influence of the oscillating magnetic fields.

I. INTRODUCTION

During their journey towards the ovum, sperm cells undergo a wide variety of swimming patterns by a beating tail. The sperm cell propagates planar or three-dimensional traveling wave (that breaks time-reversal symmetry) along the tail. This microorganism consists of a head and a flagellum that contains a midpiece and an actively beating tail. The traveling waves are generated by local bending moment along the flagellum. Dreyfus et al. [1] have mimicked the locomotion mechanism of the sperm cells by colloidal magnetic particles that are connected together using DNA and attached to a red blood cell. The external magnetic fields have allowed this biologically-inspired microrobot to be adjusted and driven using a flagellated swim. A microrobot that resembles the morphology of sperm cell has been fabricated using SU-8 polymer for the tail and a cobalt-nickel layer on the head to provide a dipole moment [2]. These microrobots are driven using external oscillating magnetic fields. The flagellated swim of the E. coli bacteria has also been used to design artificial bacterial flagella with rigid helical structures by Zhang et al. [3], [4]. These artificial bacterial flagella are driven by rotating magnetic fields using electromagnetic configuration and under microscopic guidance. Ye et al. [5] have used multiple flexible artificial flagella to increase the propulsive force of the microrobot, as opposed to microrobot with a single flexible flagellum. It has also been demonstrated that the swimming speed increases linearly with the number of flagella. The propulsion mechanism of microscopic and miniature swimmers [6] with flagellar motion have been modeled through measurement of the propulsive forces by Tony et al. [7] and Behkm et al. [8], respectively. The cellular uptake of micro-particles has been studied by Gratton et al., and it has been demonstrated that micro-particles with high aspect ratios are more prone to cell uptake [9].

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achieved by incubation with the cells for longer than 24 hours. In this work we expand on our previous studies [11] and achieve the following:

1) characterization of the morphology and magnetic properties of sperm-shaped microrobots (Fig. 1) that are fabricated using electrospinning [11];

2) motion control of the microrobots using external magnetic fields and under microscopic guidance;

3) selective targeting of cell mockups using the sperm-shaped microrobots.

The remainder of this paper is organized as follows: Section II provides information pertaining to the morphology and the magnetic dipole moment of the sperm-shaped microrobots. The morphology and magnetization of the microrobot are characterized using Scanning Electron Microscopy (SEM) images and the flip-time approach [12], respectively. Motion control of the sperm-shaped microrobots and targeting of cell mockups are included in Section III. A discussion pertaining to the use of sperm-shaped microrobots in targeting of U-373 MG human astrocytoma cells is included in Section IV. Finally, Section V concludes and provides directions for future work.

II. CHARACTERIZATION OF THE SPERM-SHAPED MICROROBOTS

The sperm-shaped microrobots consist of an ultrathin fiber and a magnetic head. This head contains iron-oxide nanoparticles (45-00-252 Micromod Partikeltechnologie GmbH, Rostock-Warnemuende, Germany) that provide the magnetic dipole moment to the microrobot. The equation of motion of the microrobot (based on the force-free swimming condition [13], [14]) is given by [11]

\[
\begin{bmatrix}
V \\
\Omega
\end{bmatrix} = -B_{sw}^{-1} \begin{bmatrix}
F_{mag} + F_{add} \\
T_{mag} + T_{add}
\end{bmatrix},
\]

(1)

where \( V \) and \( \Omega \) are the linear and angular rigid-body velocities of the microrobot, respectively. Further, \( F_{mag} \) and \( F_{add} \) are the magnetic force and inertial force due to added mass and forces due to unstationary far field (history effect) on the microrobot, respectively. Further, \( T_{mag} \) and \( T_{add} \) are the magnetic torque and the inertial torque due to added mass and torques due to unstationary far field acting on the microrobot, respectively. In addition, \( B_{sw} \) is the resistance matrix of the microrobot that consists of the resistance matrices of the body and the tail. The magnetic force \( F_{mag} \) and torque \( T_{mag} \) exerted on the microrobot are given by

\[
\begin{bmatrix}
F_{mag} \\
T_{mag}
\end{bmatrix} = \begin{bmatrix}
R_{sw}^{-1} B (M \cdot V) B (P) \\
VM \times B (P)
\end{bmatrix},
\]

(2)

In (2), \( V \) is the volume of the magnetic nanoparticles of the microrobot and \( M \) is its magnetization in the frame of the laboratory [15]. Further, \( B (P) \) is the magnetic flux density vector generated by the electromagnetic configuration (Fig. 1), at point \( P \). Furthermore, \( B_{sw} \) is the rotation matrix between the frames of the microrobot and the electromagnetic configuration. The transient hydrodynamic force due to the added-mass and history effects are calculated using [16]-[19]

\[
\begin{bmatrix}
F_{add} \\
T_{add}
\end{bmatrix} = \begin{bmatrix}
\phi \int_{-\infty}^{t} \frac{dV}{dt} \frac{d\tau}{\sqrt{t-\tau}} - \frac{2}{3} \pi R^3 \rho_l \frac{dV}{dt} \\
S_b F_{add}
\end{bmatrix},
\]

(3)

where \( \rho_l \) is the density of the liquid medium and \( R \) is the radius of the body. In (3), \( \phi \) is calculated using, \( \phi = 6 \pi R^2 \sqrt{\mu \rho_l} \), and \( \mu \) is the dynamic viscosity of the medium. Further, \( S_b \) is a skew-symmetric matrix for the cross-product required to calculate the resultant torque on the microrobot [11]. Equations (1), (2), and (3) indicate that the magnetization of the microrobot and its morphology are essential in the modelling. Therefore, the morphology and magnetization of the microrobot are characterized.

A. Characterization of the Morphology of the Microrobots

Morphology of the sperm-shaped microrobots is determined using microscopic and SEM imaging (Fig. 2). First,
the microrobots are fabricated using 20% polystyrene in dimethylformamide, and iron-oxide nanoparticles. The distance between the collector and the tip of the syringe needle is adjusted to be 10 cm, and voltage of 20 kV and flow rate of 20 μl/s are applied using high voltage power supply a syringe pump (CMA 402 Syringe Pump, CMA Microdialysis, Kista, Sweden), respectively. Second, the microrobots are extracted from the beaded fibers using nano-tweezers under microscopic guidance. Table I provides the characterized dimensions of the microrobot from 30 samples. These samples are fabricated using the mentioned electrospinning parameters. The morphology of the microrobots is used in the calculation of its magnetic dipole moment.

B. Characterization of the Magnetic Dipole Moment

Under the influence of magnetic field reversals, the sperm-shaped microrobots undergo flip-turns, as shown in Fig. 3. The flip-time of the microrobot depends on its magnetization, external magnetic field, morphology, and the properties of the fluid, and is given by

\[ \tau = \frac{\alpha}{|m| |B(P)| \ln \left( \frac{2 |m| |B(P)|}{kT} \right)} \]

(4)

where \( \tau \) is the elapsed flip-time, and \( k \) and \( T \) are the Boltzmann constant and the temperature of the fluid, respectively. Further, \( m \) is the magnetic dipole moment of the microrobot, and \( \alpha \) is the rotational drag coefficient and is approximated using [22]

\[ \alpha = \frac{\pi \eta d^3}{3} \left[ \ln \left( \frac{l}{d} \right) + 0.92 \left( \frac{d}{l} \right) - 0.662 \right]^{-1} \]

(5)

where \( d \) and \( l \) are the major diameter and length of the sperm-shaped microrobot, respectively, and \( \eta \) is the dynamic viscosity of the fluid. The microrobot is contained inside a petri dish with a medium of 80% Glycerine and 20% water. Therefore, we assume that \( \eta = 0.95 \text{ Pa.s} \). We use 2 opposite electromagnetic coils (Fig. 1) to generate uniform magnetic fields, as shown in Fig. 3. These fields allow the magnetic fields to align along the \( x \)-axis. We reverse the direction of the magnetic field and observe that the microrobot undergoes a flip-turn towards the negative \( x \)-axis. The measured flip-time is calculated and used in (4) to calculate the dipole moment at different magnetic fields. The fields are measured using a calibrated 3-axis digital Teslamer (Senis AG, 3MH3A-0.1%−200mT, Neuhofstrasse, Switzerland).

Fig. 3 provides the calculated magnetic dipole moment at a range of magnetic fields from 12 mT to 28 mT. Magnetic fields of less than 12 mT does not exert enough torque to allow the microrobot to flip. In addition, we observe that the calculated magnetic dipole moment converges to an asymptote \( (1.4 \times 10^{-11} \text{ A.m}^2) \) above magnetic field of 28 mT. At each magnetic field, the average magnetic dipole moment is calculated using 10 different sperm-shaped microrobots. These microrobots are extracted from the same electrospinning sample.

The characterized morphology and dipole moment of the sperm-shaped microrobots are used in the realization of the dynamical model (1). Fig. 4 shows the simulation of the tail deformation of the sperm-shaped microrobot at different time instants. This simulation is done using the following current inputs to the electromagnetic coils [11]:

\[ I_{CA} = I_{CC} = I_{max} \sin \left( \Omega_z + \frac{\pi}{4} \cos(2\pi ft) \right) \]

(6)

where \( I_{CA} \) and \( I_{CC} \) are the current inputs to electromagnetic coils A and C, respectively. Further, \( I_{max} \) and \( f \) are the maximum input current and the frequency, respectively. The current inputs to electromagnetic coils B \( (I_{CB}) \) and D \( (I_{CD}) \) are given by

\[ I_{CB} = I_{CD} = I_{max} \cos \left( \Omega_z + \frac{\pi}{4} \cos(2\pi ft) \right) \]

(7)

The simulation result shown in Fig. 4 demonstrates that the microrobots breaks time-reversal symmetry [23], [7], [24], and hence locomotion is achieved at low Reynolds number fluids. We also compare our analysis to experiments and
prove that the locomotion of the microrobot is only a consequence of asymmetric flagellar waves. Fig. 5 provides the measured deflection of the tail of the microrobots at different time instances. The deflection is measured at 7 representative points along the tail between time, \( t = 0.03 \) seconds and \( t = 0.18 \) seconds. The microrobot used in this experiment has a tail length of 80 \( \mu \text{m} \), and the major and minor diameters of its magnetic head are 25 \( \mu \text{m} \) and 16 \( \mu \text{m} \), respectively. The deflection is calculated off-line after recording the oscillation of the tail using a high speed camera (avA1000-120kc, Basler Area Scan Camera, Basler AG, Ahrensburg, Germany). The solid lines are plotted to represent the tail by interpolating cubic splines to the experimental data.

III. MOTION CONTROL AND TARGETING OF CELL MOCKUPS

Motion of the sperm-shaped microrobot is achieved by orienting the magnetic fields towards a desired orientation, while oscillating the field lines [20], [21]. Figs. 6 show representative motion control results of sperm-shaped microrobots along S-shaped, U-shaped, and square paths using oscillating magnetic field of slightly greater than 12 mT. The microrobot swims along an S-shaped path at an average speed of 200 \( \mu \text{m/s} \), at frequency of 10 Hz. This path is achieved by two consecutive U-turns with diameter of 210 \( \mu \text{m} \), as shown in Fig. 6(a). A single U-shaped path with diameter of 550 \( \mu \text{m} \) is followed at an average speed of 225 \( \mu \text{m/s} \), at frequency of 10 Hz (Fig. 6(b)). Fig. 6(c) shows the flagellated swim of the microrobot along a square path with an edge length of 800 \( \mu \text{m} \). In this representative experiment, the microrobot swims at an average speed of 132 \( \mu \text{m/s} \), for oscillating magnetic field of 10 Hz. The paths shown in Figs. 6 show that the microrobot can controllably follow different trajectories in two dimensional space, without exerting a magnetic force on its dipole, and hence we benefit from the larger projection distance of the magnetic field, as opposed to the magnetic field gradient.

The electromagnetic configuration (shown in Fig. 1) generates maximum magnetic field gradient of 5 T/m, and the maximum magnetic dipole moment of the microrobot is characterized to be \( 1.4 \times 10^{-11} \text{A.m}^2 \), at magnetic field of 28 mT. Therefore, the maximum pulling magnetic force exerted on the dipole of the microrobot is \( 7.0 \times 10^{-11} \text{N} \). This force is one order-of-magnitude less than the drag force \( F_d(\mathbf{P}) \) exerted on the microrobot. This drag force is calculated using the following approximation [25]:

\[
F_d(\mathbf{P}) = \frac{\eta l}{\ln \left( \frac{d}{2r} \right)} - 0.81 \mathbf{\dot{P}},
\]

where \( \eta \) is the dynamic viscosity of the medium, and \( l \) and \( d \) are the length of the microrobot and the major diameter of its head (Table I). Using (8), the drag force on the microrobot is calculated to be \( 2.1 \times 10^{-10} \text{N} \), at average speed of 200 \( \mu \text{m/s} \). Therefore, the locomotion of the microrobot is achieved by breaking the time-reversal symmetry of the flagellum (Figs. 4 and 5), not by the magnetic field gradient.

We also achieve selective targeting of cell mockups (gas bubbles), as shown in Fig. 7. The gas bubbles are injected inside the fluid in different places to demonstrate that the microrobot can achieve selective targeting. These mockups are not influenced by the magnetic field or the field gradient, and have an average diameter of 200 \( \mu \text{m} \). In this representative experiment (Fig. 7), the red and blue dashed circles and arrows represent the desired and undesired cell
for the targeting operation, respectively. In targeted therapy, it is likely that the mockups (indicated using the red and blue dashed circle) resemble diseased and healthy cells. The sperm-shaped microrobot achieves flagellated swim towards the desired mockup, at an average speed of 107 \( \mu \text{m/s} \), in this representative trial. At time \( t=8 \) seconds, the head of the microrobot comes into contact with the desired mockup without affecting its neighbour. The accuracy of this targeting experiment is essential in targeted therapy to avoid any permanent damage to healthy cells, while targeting the diseased cells. Please refer to the accompanying video that demonstrates the selective targeting of a cell mockup using the sperm-shaped microrobot.

Another representative selective targeting is shown in Fig. 8. At time, \( t=5.0 \) seconds, the sperm-shaped microrobot reaches within the vicinity of the desired and undesired mockups, that are indicated using the red and blue dashed circles, respectively. The average swimming speed of the microrobot is calculated to be 118 \( \mu \text{m/s} \). At time \( t=7 \) seconds, the microrobot changes its orientation and swims between the two mockups, and undergoes a U-turn to target the desired cell (red dashed circle). At time, \( t=12 \) seconds, the microrobot moves and partially engulfs the mockup using its flexible tail. This selective targeting experiment is repeated 5 times and we observe consistent results. Please refer to the accompanying video that demonstrates the selective targeting of a cell mockup using the sperm-shaped microrobot.

IV. DISCUSSION

The length of the sperm-shaped microrobots used herein is approximately 2 orders-of-magnitude greater than the diameter of the U-373 MG human astrocytoma cells. We do expect that these microrobots will be used in biomedical application such as targeted therapy, in vitro fertilization, cell sorting, and cell manipulation. The current size of the microrobot does not allow for cell uptake of single micro-robot, as shown in Fig. 9. In this representative experiment, U-373 cells are cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) (Lonza, 14-802F) and 1% penicillin-streptomycin (Lonza, 17-602E). They are incubated at 5% CO\(_2\) and 37\(^\circ\)C until they reach 80-90\% confluency. The U-373 cells are washed twice using phosphate buffered saline (PBS) (Lonza, 17-516F), followed by trypsinization (Lonza, CC-5002) and re-suspension in 10 ml of the DMEM (Lonza, BE12-707F). The U-373 cell suspension is centrifuged at 121xg for 5 minutes at room temperature. The supernatant is aspirated, and the cell pellet is re-suspended in fresh DMEM. Finally, the suspended cells in medium are contained within the electromagnetic configuration with the microrobots, as shown in Fig. 9.

It is likely that the current size of the sperm-shaped microrobot would enable targeting of tissue or large group of cells, rather than individual cells. In order to achieve cell uptake of single microrobot, its size has to be decreased by at least 2 orders-of-magnitude. This decrease will influence the exerted magnetic torque on the microrobot since the magnetic dipole moment and magnetic torque will decrease faster than the decrease in the friction force on the surface of the microrobot. We do expect that this problem can be overcome by increasing the external magnetic field to generate similar behaviour to that provided in this study. It has been reported by Gratton et al. [9] that the cellular uptake and mechanism of internalization are influenced by the shape (aspect ratio), size, surface charge of the microrobot and other physicochemical factors of the cells. Therefore, the aspect ratio of the head of the microrobot (ratio between the major and minor diameters) has to be increased in addition to the external magnetic field strength to increase the cell uptake. We do not yet have a good understanding of the influence of the electrospinning parameters (concentration, voltage, flow rate, distance) on the shape of the head of the microrobot. Fig. 2(b) shows a microrobot with a higher aspect ratio than that of the microrobot shown in Fig. 2(a) although they are extracted from the same sample. However, we can select microrobots with highest aspect ratio from the electrospinning sample before cutting under microscopic

![Fig. 6. Motion control of sperm-shaped microrobots using weak oscillating magnetic fields along different paths.](image-url)

(a) Flagellated swim along an S-shaped path  
(b) Flagellated swim along an U-shaped path  
(c) Flagellated swim along a square path

*Please refer to the accompanying video that demonstrates the selective targeting of a cell mockup using the sperm-shaped microrobot.*
guidance. Also we do not yet have a clear understanding of the relation between the amount of nanoparticles and the magnetization of the microrobot. This will also be the subject of future study.

V. CONCLUSIONS AND FUTURE WORK

This work expands on our previous study [11] and proves through simulations and experimental results that the sperm-shaped microrobots achieve locomotion by breaking the time-reversal symmetry, in low-Reynolds number fluids. These microrobots are fabricated using electrospinning and their average magnetic dipole moment is characterized to be $1.4 \times 10^{-11}$ A.m$^2$, at magnetic field of 28 mT, using the flip-time technique. We also show that the microrobot can achieve flagellated swim controllably along S-shaped, U-shaped, and square paths using oscillating magnetic field of greater than 12 mT. This motion control enables selective targeting and penetration of the cell mockups at an average speed of 0.25 body-lengths-per-second, at frequency of 4 Hz.

As part of future studies, the size of the sperm-shaped microrobots will be decreased (to less than 20 μm) and the microrobots will be used in targeting of cancer cells. This decrease will be achieved by decreasing the molecular weight of the polystyrene and optimizing the electrospinning parameters (decreasing the molecular weight will enable generation of sperm-shaped microrobot without cutting the ultra-thin fibers using tweezers after electrospinning). The cell uptake will be investigated in vitro inside stationary fluid and inside microfluidic channels in the presence of time-varying flow rates. The microrobots will be coated with chemotherapeutic agents and the physiological conditions of the drug release will be studied in the presence of time-varying flow. In addition, we will study the locomotion of sperm-shaped microrobots using planar and helical propulsion, and investigate the parameters that will enable transition between planar to helical propulsion of the microrobot, in low-Reynolds number fluids. This investigation will be done using a magnetic-based robotic system with open-configuration that will provide the planar and helical propulsion of the flexible tail through rotating dipole fields [28].

REFERENCES


Fig. 8. Targeting of a cell mockup (gas bubble) with diameter of 200 μm using a sperm-shaped microrobot. The microrobot moves controllably towards a cell mockup (red dashed circle) using a flagellated swim under the influence of oscillating magnetic fields at 10 Hz. The microrobot swims inside a medium of 80% Glycerine and 20% water. The average speed of the microrobot is calculated to be 118 μm/s, at frequency of 4 Hz. Two gas bubbles are produced in the medium using a syringe needle. The bubbles are indicated using the red and blue dashed circles and arrows. The red arrows represent oscillation of the flexible tail of the microrobot. In targeted therapy, the red blue dashed circles can indicate diseased and healthy cells, respectively. Please refer to the accompanying video that demonstrates the selective targeting of a cell mockup using a sperm-shaped microrobot.

![Sperm-Shaped Microrobot](image)

Fig. 9. Microscopic image of a sperm-shaped microrobot and groups of U-373 MG human astrocytoma cells. The length of the microrobot has to indicate very small group of cells and individual cells. U-373 MG cancer cells. The current size of the microrobot enables targeting of tissue and large groups of cancer cells. The white arrows indicate very small group of cells and individual cells.


