An Investigation of the Sensing Capabilities of Magnetotactic Bacteria

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***Abstract*— We investigate the sensing capabilities of magnetotactic bacteria (*Magnetospirillum gryphiswaldense* strain MSR-1) to MCF-7 breast cancer cells. Cancer cells are allowed to grow inside a capillary tube with depth of 200 μm and motion of magnetotactic bacteria is investigated under the influence of oxygen gradient and geomagnetic field. The influence of cancer cells is modeled to predict the oxygen gradient within the capillary tube in three-dimensional space. Our experimental motion analysis and count of motile magnetotactic bacteria indicate that they migrate towards less-oxygenated regions within the vicinity of cancer cells. Bands of magnetotactic bacteria with average concentration of $18.8\pm2.0\%$ are observed in close proximity to MCF-7 cells ($h = 20$ μm), whereas the concentration at proximity of 190 μm is $5.0 \pm 6.8\%$.**

I. Introduction

Recently, there has been a growing interest in the use of biological microrobots to achieve various non-trivial tasks at low-Reynolds numbers. The realization of these biological microrobot has been achieved in mainly two ways: Coupling of motile microorganisms to a magnetic structure to provide a hybrid micro-bio-robot [1]-[3] and through magnetotactic bacteria (MTBs) [4]. In hybrid micro-bio-robots, the microorganism provides a propulsive force and the magnetic structure enables directional control under the influence of an external magnetic field. In contrast to hybrid micro-bio-robots, MTBs develop magnetite nanocrystals inside their cells, and hence their magnetic dipole moment enables directional control using external field in milliTesla range. Micromanipulation [5], microassembly [6], microactuation [7], directional control and maneuvering [8], [9], and targeted drug delivery have been demonstrated using biological microrobots. For instance, Felfoul *et al.* have utilized *Magnetococcus marinus* strain MC-1 to transport drug-loaded nanoliposomes into hypoxic regions of a tumour *in vivo* [10]. On the other hand, Magdanz *et al.* have suggested novel assisted fertilization technologies and nanomedicine applications based on hybrid micro-bio-robots (Spermbots) [11].

MTBs have the following practical advantages compared to hybrid micro-bio-robots: first, the size of MTBs (500 nm in diameter) enables them to access the smallest capillary (5 μm in diameter) within the human body; second, culturing of MTBs is relatively easier than the coupling between microorganisms and a magnetic structure; third, the coupling causes a reduction in the swimming speed of the hybrid micro-bio-robot compared to MTBs; fourth: MTBs have an advantage as drug carriers over any micro-scale delivery system owing to their biocompatibility and low immunogenicity; fifth, MTBs have the capability to swim deep into hypoxic regions created by rapid proliferated cancer cells to overcome the drawbacks of systematic application of liposomes [12], as shown in the schematic representation in Fig. 1(a). The implication of these advantages is that MTBs can be steered

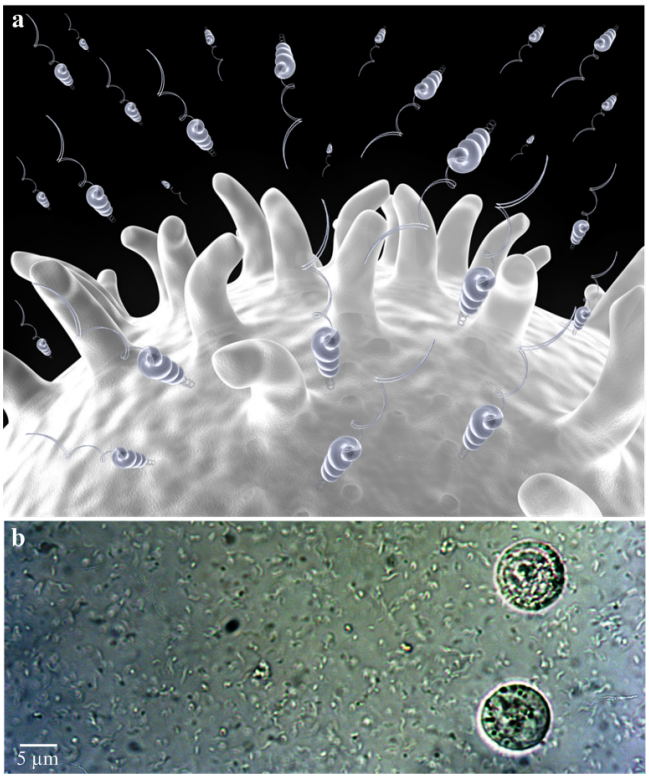

Fig. 1. Magnetotactic bacteria (MTBs) align their helical bodies along external magnetic field lines and swim towards less-oxygenated regions. (a) A schematic representation demonstrates the helical propulsion of MTBs towards a cancer cell. (b) Our experiments are conducted using magnetotactic bacteria (*Magnetospirillum gryphiswaldense* strain MSR-1) and MCF-7 breast cancer cells. Motion of the bacteria is studied under the influence of geomagnetic field without applied external fields. MTBs form a band in close proximity to cancer cells owing to the oxygen-gradient caused by cancer cells and the magneto-aerotaxis characteristic of MTBs.

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towards a deep seated region within the human body using external magnetic fields, and then released to migrate to preferred oxygenated regions based on the gradient created by the cancer cells and their magneto-aerotaxis characteristic [13]. Martel *et al.* have used *Magnetococcus marinus* strain MC-1 to visualize less-oxygenated levels in planar and three-dimensional fluidic environments [14]. This experiment suggests that directional control can be implemented only to guide MTBs to a region under investigation. Once MTBs are positioned at this region, magnetic field can be decreased and MTBs can be considered as self-propelled oxygen sensors.

In this work, we investigate the influence of MCF-7 breast cancer cells on the motion of MTBs (*Magnetospirillum gryphiswaldense* strain MSR-1) in the absence of an applied external magnetic field (MTBs are influenced by geomagnetic field). First, we model the diffusion of oxygen in the growth medium to predict less-oxygenated regions caused by cancer cells. Second, we use motion analysis to measure the number of MTBs attracted towards less-oxygenated regions [Fig. 1(b)]. The remainder of this paper is organized as follows: Section II provides analysis pertaining to the diffusion of oxygen in a medium that contains MTBs and cancer cells. Section III presents our experimental results and discussions. Finally, Section IV concludes and provides directions for future work.

II. Magnetotactic Bacteria and Cancer Cells

In oxygen-gradient medium, MTBs form bands at less-oxygenated regions. This oxygen-gradient is generated in a medium by cancer cells [15]. We consider cancer cells with diameter of 20 μm in a capillary tube with depth of 200 μm and width of 1 mm [Fig. 2(a)]. The oxygen distribution is modified passively by diffusion and actively by cancer cells. The diffusion of the oxygen is governed by Fick's second law as follows [16]:

$$\frac{\mathrm{d}c_\mathrm{o}}{\mathrm{d}t} = D_\mathrm{o}\nabla^2 c_\mathrm{o} = D_\mathrm{o}\sum_{i=x,y,z}\frac{\mathrm{d}^2 c_\mathrm{o}}{\mathrm{d}i^2} \approx D_\mathrm{o}\sum_{i=x,y,z}\frac{c_\mathrm{o}(i+\mathrm{d}i)-2c_\mathrm{o}(i)+c_\mathrm{o}(i-\mathrm{d}i)}{\mathrm{d}i^2}, \quad (1)$$

where c_o and D_o are the concentration and diffusion constants of the oxygen, respectively. Cancer cells consume oxygen based on the following relation:

$$\frac{\mathrm{d}V_\mathrm{o}}{\mathrm{d}t} = -k_\mathrm{o}A_\mathrm{c}, \quad (2)$$

where V_o is the volume of oxygen in the medium. Further, k_o is the consumption coefficient of oxygen, and A_c is the surface area of a cancer cell. The cells are assumed to be located within $\mathbf{xy}$-plane ($z = 0$) as they adhere to the bottom surface of the capillary tube.

Simulation of the oxygen distribution is calculated using a finite-difference computation of (1), for $c_\mathrm{o}(t = 0) = 1\%$, $D_\mathrm{o} = 2.06 \times 10^{-9}$ m^2/s, $k_\mathrm{o} = 1 \times 10^{-9}$ m/s (0.033×10^{-15} mol/s/cell), $\mathrm{d}i = 10$ μm, and $\mathrm{d}t = 5.4$ ms [17]. At $t = 0$ minutes, the oxygen distribution of 110 cancer cells is uniform within $\mathbf{xy}$-plane inside the capillary tube, as shown in Fig. 2. At the interval $0 \leq t \leq 60$ minutes, cancer cells generate oxygen gradient and less-oxygenated regions are observed, as shown in Fig. 2(c). The capillary tube contains cell sediment at its bottom surface [Fig. 2(a)]. Therefore, we observe a slight decrease in the calculated oxygen concentration towards the bottom of the capillary tube. Fig. 3(a) shows a uniform oxygen concentration at $t = 0$ minutes. A slight decrease in the oxygen concentration of 0.065% is also observed at $t = 20$ minutes [Fig. 3(b)]. At $t = 40$ minutes and $t = 60$ minutes, the difference between oxygen concentrations at the proximal sides of the tube are calculated as 0.09% and 0.1%, respectively [Figs. 3(b) and 3(d)]. Therefore, cancer cells cause a slight difference in the oxygen concentration within the capillary tube. The ability of MTBs to sense this difference is experimentally investigated.

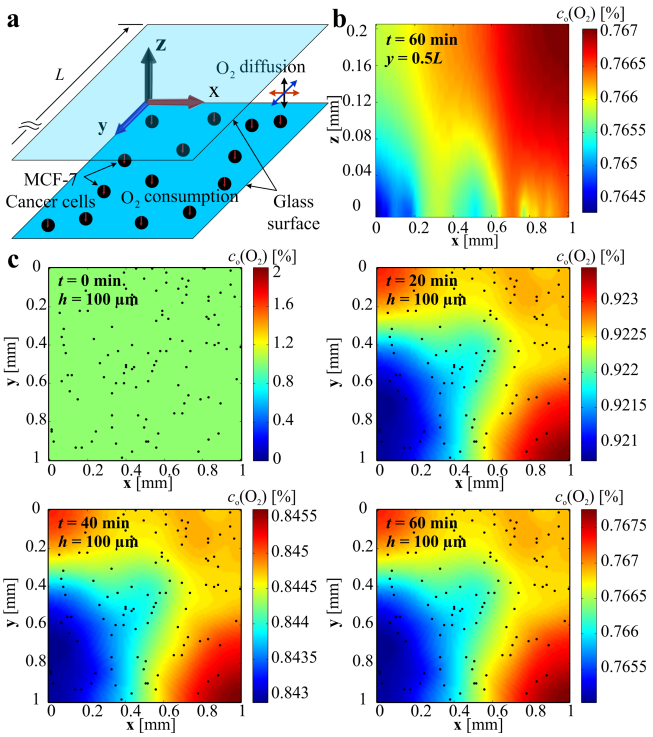

Fig. 2. Distribution of oxygen is calculated using (1) and (2), for $c_\mathrm{o} = 1\%$, $D_\mathrm{o} = 2.06 \times 10^{-9}$ m^2/s, $k_\mathrm{o} = 1 \times 10^{-9}$ m/s, $\mathrm{d}i = 10$ μm, and $\mathrm{d}t = 5.4$ ms [17]. (a) Schematic representation of the experimental setup. It consists of a capillary tube that contains MCF-7 breast cancer cells and magnetotactic bacteria. (b) Oxygen concentration c_o is calculated along the vertical $\mathbf{xz}$-plane. (c) Oxygen concentration is calculated along $\mathbf{xy}$-plane at different time instants. The small black circles indicate the positions of the cancer cells, and are assigned based on our microscopic observation of cancer cells inside the capillary tube.

III. Experimental Results

Magnetospirillum gryphiswaldense strain MSR-1 is used in this study to investigate the influence of cancer cell on their behaviour. This strain is obtained from the German collection of microorganisms and cell cultures (DSM 6361, Deutsche Sammlung von Mikro-organismen und Zellkulturen, Brunswick, Germany). The strain is inoculated in magnetospirilum growth medium (ATCC medium: 1653 Revised magnetic Spirillum growth medium) with an oxygen

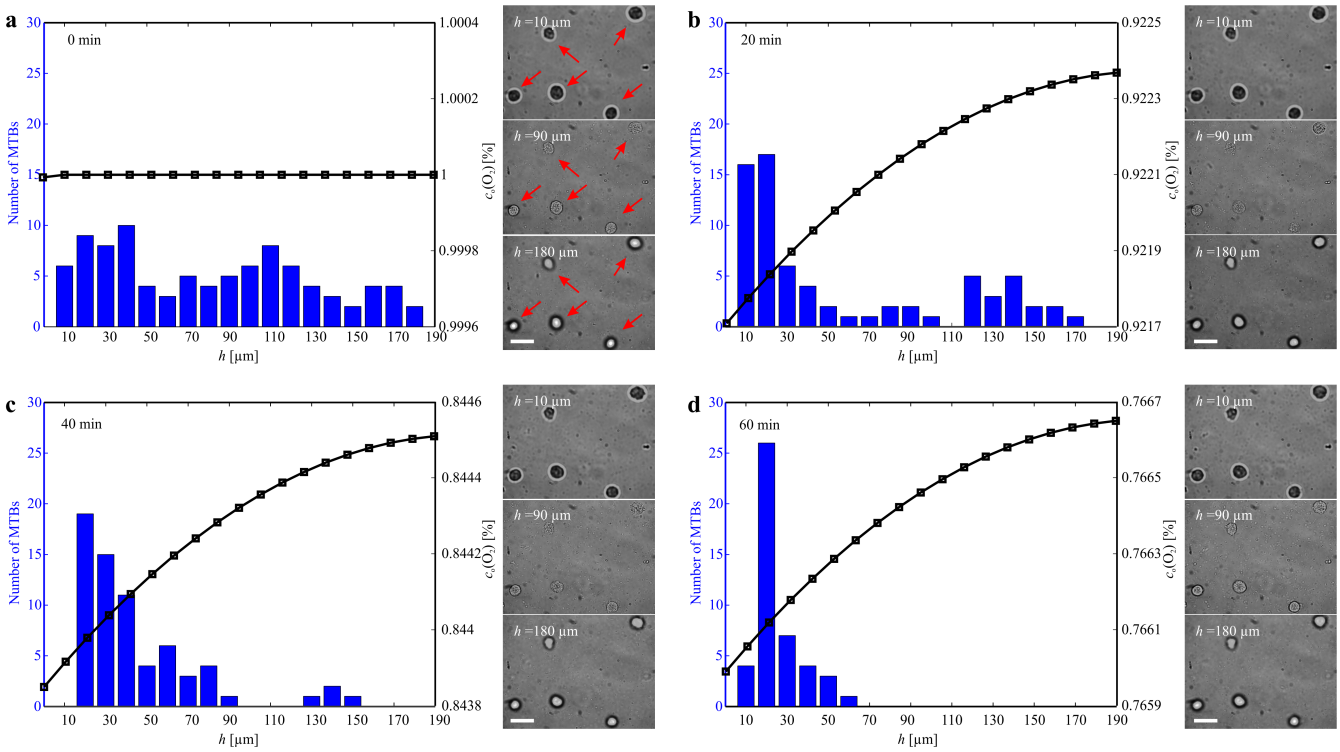

Fig. 3. Migration of magnetotactic bacteria (MTBs) towards less-oxygenated region within the vicinity of MCF-7 breast cancer cells is observed. Cancer cells are allowed to grow on the lower surface of a capillary tube. The tube is sealed and contains MTBs in growth medium of the MCF-7 breast cancer cells. The number of motile MTBs is determined within a range of 0 to 200 μm with a step of 10 μm. (a) An almost uniform cell count is observed upon insertion of MTBs inside the capillary tube. The red arrows indicate the cancer cells. (b), (c), and (d) At $t > 20$ minutes, a band of MTBs is observed within the vicinity of the cancer cells. The oxygen concentration c_o is calculated using (1), for $c_o(t = 0) = 1\%$, $D_o = 2.06 \times 10^{-9}$ m^2/s, $k_o = 1 \times 10^{-9}$ m/s, $\mathrm{d}i = 10$ μm, and $\mathrm{d}t = 5.4$ ms [17]. Scale bar is 60 μm.

concentration of 1%. The cultures are then cultivated at 26°C for two to four days [19]. 1.5 ml of the medium is centrifuged at 13400 RPM for 20 minutes, and pallets are formed and suspended in 1.5 ml of cancer medium. The MCF-7 cells are cultured in RPMI-1640 media (Lonza, 12-702F) containing 10% Fetal Bovine Serum (Lonza, 14-802F) and 1% penicillin-streptomycin (Lonza, 17-602E). The cells are incubated at 5% CO_2 and 37°C (Galaxy 170R) until they reach 80-90% confluency. Cells are washed twice using phosphate buffered saline (Lonza, 17-516F), followed by trypsinization (Lonza, CC-5002) and re-suspension in 10 ml of the RPMI-1640 media. The cell suspension is then centrifuged (MIKRO, 22R) at 121xg for 5 minutes at 18°C. The supernatant is aspirated, and the cell pellet is re-suspended in fresh medium [18].

Motion of MTBs inside the capillary tube is investigated using a microscopic unit (Axio Scope.A1, Zeiss, Oberkochen, Germany). Motility of MTBs decreases with time. Therefore, they are observed during the first hour of the experiment in cancer cell medium. The tube is filled with MTBs and MCF-7 medium with volume concentration of 16.67% (v/v). Videos are acquired using a camera (Axiocam 105 Color, Zeiss, Jena, Germany) and a 50× N-Achroplan objective. First, we acquire videos at depth (h) of 10 μm. At this depth, MTBs and cancer cells are almost in the same plane. The depth is increased by a step of 10 μm based on the depth-of-focus of the objective (< 10 μm) to avoid counting the same cells twice. This procedure is repeated for a range of 10 μm to 200 μm and the acquired videos are processed off-line. Fig. 3 shows a representative trial for counting motile MTBs at different layers with respect to cancer cells. Videos are also acquired each 20 minutes to investigate the influence of cancer cells on the MTBs. We observe an almost uniform distribution of motile MTBs within the capillary tube, as shown in Fig. 3(a). For $t > 20$ minutes, a band of motile MTBs is observed in close proximity to cancer cells, as shown in Figs. 3(b), 3(c), and 3(d). We repeat this experiment using the same culture ($n = 3$). The maximum average concentration is measured as $18.8 \pm 2.0\%$ at $h = 20$ μm (Fig. 4), whereas the concentration is measured as $5.0 \pm 6.8\%$ at $h = 190$ μm.

Cancer cells are characterized by several abnormal characteristics that prevent them from functioning in the same manner as normal cells such as rapid replication, genetic abnormality, resistance to cell death, and relatively high nutrients consumption. Hypoxic environments are formed due to cancer cell growth and consumption of nutrients and oxygen [20]. Our experiments are conducted using relatively low volume concentration of MCF-7 cancer cells. Nevertheless, our measurements demonstrate the ability of MTBs to sense and migrate towards less-oxygenated regions using their magneto-aerotaxis characteristic [21]. Unlike polar MTBs,

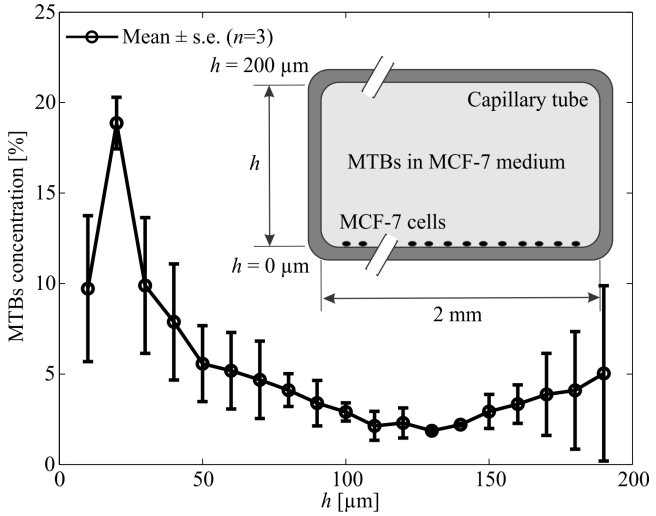

Fig. 4. Magnetotactic bacteria (MTBs) are counted at different depths (h) in the presence of MCF-7 breast cancer cells. MCF-7 cells are allowed to grow on the bottom (inset) of the capillary tube and MTBs are contained in MCF-7 medium. The ends of the capillary tube are sealed to ensure that the oxygen-gradient is only caused by cancer cells. The depth-of-focus of the microscope is < 10 µm and motile MTBs are only counted in each trial. Mean and standard error of the mean (s.e.m.) are calculated using 3 trials.

our *Magnetospirillum gryphiswaldense* strain MSR-1 exhibit axial magneto-aerotaxis behaviour and swim along magnetic field lines in both directions. In the presence of oxygen gradient, MTBs swim parallel and anti-parallel to field lines and aerotaxis determines the direction of migration. This behaviour is demonstrated in this study despite the relatively low oxygen gradient generated by the MCF-7 cells.

IV. Conclusions and Future Work

We investigate the sensing capabilities of magnetotactic bacteria (*Magnetospirillum gryphiswaldense* strain MSR-1) to MCF-7 breast cancer cells with volume concentration of 16.67% (v/v). Our experiments show that bands of motile MTBs are formed in close proximity to MCF-7 cells with concentrations of $9.7 \pm 5.7\%$, $18.8 \pm 2.0\%$, and $9.8 \pm 5.3\%$ at proximities of 10 µm, 20 µm, and 30 µm, respectively. This concentration decreases to $5.0 \pm 6.8\%$ as the distance to MCF-7 cells is increased to 190 µm.

As part of future studies, we will investigate the influence of the volume concentration of MCF-7 cells on the ability of MTBs to form bands. It is essential to detect cancer early before spreading. Therefore, the ability of MTBs to form bands in close proximity to cells, before they develop into a tumor, holds a promise in early detection and more effective treatment. It is also essential to achieve motion control of MTBs in a non-uniform flow-field and study their behaviour along and against the flowing streams of bodily fluids.

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