Snowman Swimming and Robotics inside Synthetic Mucus

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Abstract— **This paper introduces microrobot swimming of two bead 'snowman' microswimmers inside synthetic mucus solutions. In Newtonian fluids, two beads bonded together would be unable to propagate in low Reynolds number environments due to their apparent symmetry. The non-Newtonian interactions and heterogeneity of synthetic mucus allow snowman structures to maneuver under actuation from rotating magnetic fields without this geometric requirement. Two experiments were carried out in this paper, the first determined the directional controllability of these snowman swimmers inside synthetic mucus, while the other analyzed their velocity response to increasing frequency. The results indicate that snowman swimmers can swim omnidirectionally, however, their trajectories were offset due to self-generated internal flows. The velocity of the snowman swimmers increased linearly with frequency and were successful in overcoming small internal flows within the surrounding medium.**

I. Introduction

Microswimmers are constantly being developed for use in vivo applications. The ultimate dream of Microrobotics is to one day perform complex medical tasks such as minimally invasive surgery, targeted drug delivery, and tissue fabrication. One key issue associated with these endeavors is understanding how fluidic interactions associated with Non-Newtonian environments can alter the nature of well understood microrobots that were previously tested inside Newtonian environments. Most important among these interactions are the induced by structured fluids composed of complex heterogeneous fiber networks. Other groups have made substantial progress in examining micro and nanoscale robots in these mediums. In one study, nanopropellers were found to have an advantage, due to their length scale being comparable to the mesh density of surrounding fluidic media, allowing them to ignore bulk viscosity [1]. Another group found that even though bulk fluids, like low concentration methyl cellulose, were Newtonian, interactions from their structural fluidic properties allowed for improvements in microswimmer performance [2]. Even bacteria have been shown to have maneuverability increases when inside structured fluids like methyl cellulose at low concentration [3].

Human mucus, a non-Newtonian fluid, is of particular interest to Microrobotics research as it introduces a host of fluidic uncertainties not previously accounted for in microswimmer design. Mucus consists primarily of a protein called mucin, which is responsible for the creation of it's structured heterogeneous fiber network [4]. This fiber network is often responsible for causing microparticles to become entangled within the fluid and severely limit localized transport phenomenon [4]. Designing microrobots to take advantage of mucus's structural interactions will be paramount for manipulation in the human gastrointestinal track or anywhere else in the human body with high mucus concentrations.

In the past we tried to introduce achiral microrobots into synthetic mucus formulations. These swimmers are composed of three or more magnetic beads chemically bonded together using avidin-biotin compounds. Development of these microswimmers is outlined in references [5-12]. However, the formation of achiral microswimmers inside mucus environments has proven to be difficult, since avidin-biotin chemical bonding is interfered with inside high concentrations of mucin. At high concentrations of mucin, achiral swimmers break apart or are impossible to form at all [13]. However, three bead achiral microswimmers, or other complex microrobots, may be unnecessary for medical applications inside human mucus. In this paper, we explore the possibility of using two bead 'snowman' microswimmers for use in these mucus environments. Unlike achiral microswimmers, snowman swimmers possess three planes of symmetry, and therefore would not be able to propagate in Newtonian environments [14-15]. Additionally, these structures are observed to possess reciprocal motion and have non-flexible bodies, neither of which allows swimming in Newtonian low Reynolds number environments [16]. As will be shown here, the synthetic mucus under investigation is non-Newtonian and structured, allowing the snowman swimmers to interact with the mucin fibers and produce swimming like properties. An example of both achiral and snowman swimmers can be seen in Fig. 1. The main contributions of this paper include:

- Demonstrating the directional controllability of snowman microswimmers
- Characterizing the performance of snowman under increasing frequencies of rotational magnetic fields.

II. Methodology

A. Synthetic Mucus

Human mucus is primarily composed of mucins, salts, lipids, DNA, and cellular debris [3]; A slight variation of any of these components could radically change a mucus samples viscos-elastic properties. This heterogeneity of human mucus makes it almost incomparable between samples and unreliable for repeatability tests. To address this, our lab formulated synthetic mucus that is a mixture between deionized water and rehydrated mucin glycoproteins. Mucin glycoproteins alone are responsible for most of mucus's viscos-elastic

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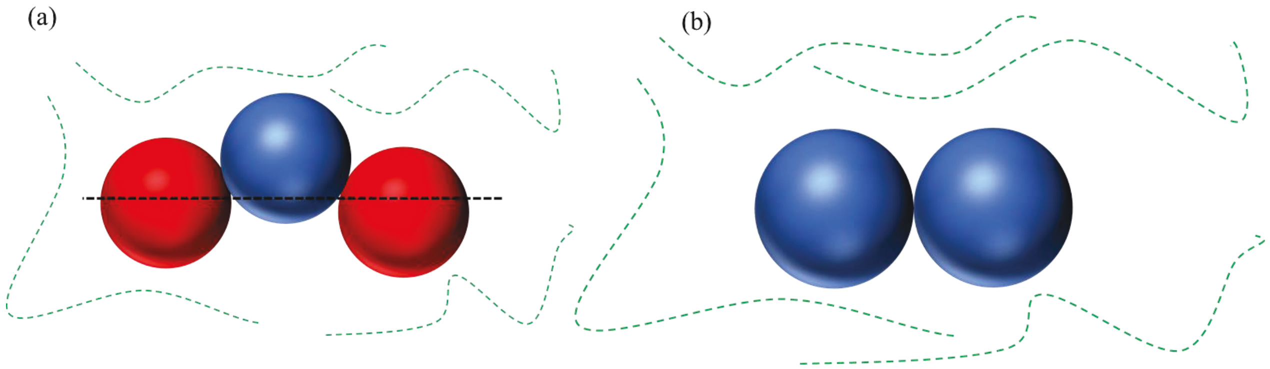

Figure 1: (a) achiral microswimmer composed of one avidin coated particle and two biotin coated particles. Achiral microswimmers can swim along their long axis when exposed to rotating magnetic fields. This propagation is caused by the fact that there is only two planes of symmetry and one plane of asymmetry (black dashed line), allowing for non-reciprocal motion. (b) snowman swimmer composed of two avidin beads. While the snowman is not chemically bonded, there is still a significant magnetic attractiveness keeping them together. Since the snowman has no plane of asymmetry, it cannot propagate in Newtonian Fluids like deionized water. However, the mucin fibers (green dashed lines) present in mucus can allow snowman enough interaction with the fluid to swim.

properties and is solely responsible for the creation of its fiber network. This fiber network is filled with voids that allow small particles to translocate through the network and causes larger particles to become 'entangled.' A picture of these voids can be seen in in Fig. 2. While these voids can be on the order of microns, the microparticles examined in this paper are 10.4 µm in diameter, and are large enough for purely entanglement interactions.

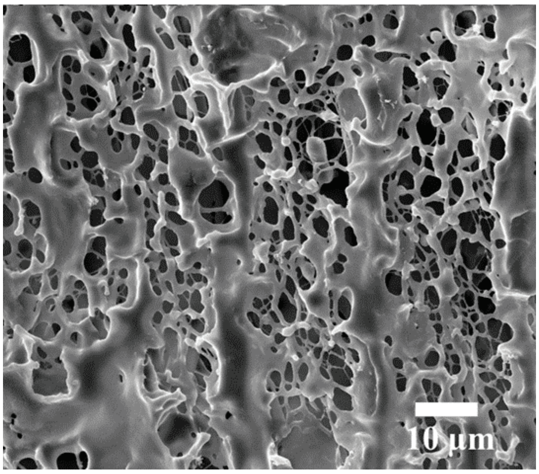

Figure 2: SEM image of Synthetic mucus. Voids can be easily distinguished from mucoidal structural fibers.

We examined the viscosity of synthetic mucus samples with different mucin concentrations. This data was obtained using a DHR-3 Rheometer from TA instruments. Three different mucin samples of each concentration were exposed to a viscosity-shear rate test and the results were then averaged together. As can be seen in Fig. 3, when mucin concentration increases, the viscosity of each respective curve noticeably shifts to a higher range of viscosities. Between 0.5%-2% mucin concentration, there is some overlap early on in the curves, but this is most likely caused by internal flows of water during the Rheological tests. For the experiments carried out in this paper, we will examine snowman swimmers embedded within 4% mucin solutions; this concentration was chosen due to it's ability to keep swimmers in suspension for prolonged periods of time without sinking. This was also chosen since swimmers were still able to be actuated under our current experimental setup.

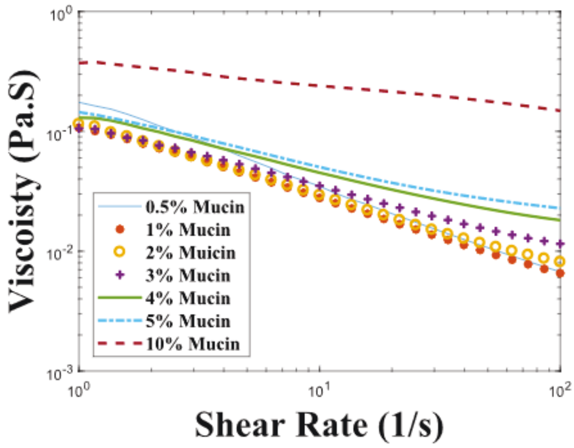

Figure 3: The viscosity vs. shear rate of different synthetic mucus samples. Synthetic mucus was fabricated by mixing mucin glycoproteins with deionized waters at varying concentrations. As mucin concentration increases, the overall viscosity curve shifts upward but each maintains a noticeable shear thinning effect. 4% mucin samples were selected for study in this paper.

B. Control System

The entangled microswimmers were actuated using an approximate Helmholtz coil system arranged in a three-dimensional configuration. Using bipolar power supplies, the system generates rotating magnetic fields in three dimensions. The governing equations for these magnetic fields can be seen in both (1) and (2). Where B_r is the maximum amplitude of the rotating magnetic field, B_s is the magnitude of the static field, ω is the rotational frequency of the field, θ is the direction of rotation, and t is the time. Equation (1) shows the direction of the rotating magnetic fields while (2) is a static magnetic field that governs the heading angle orienting the

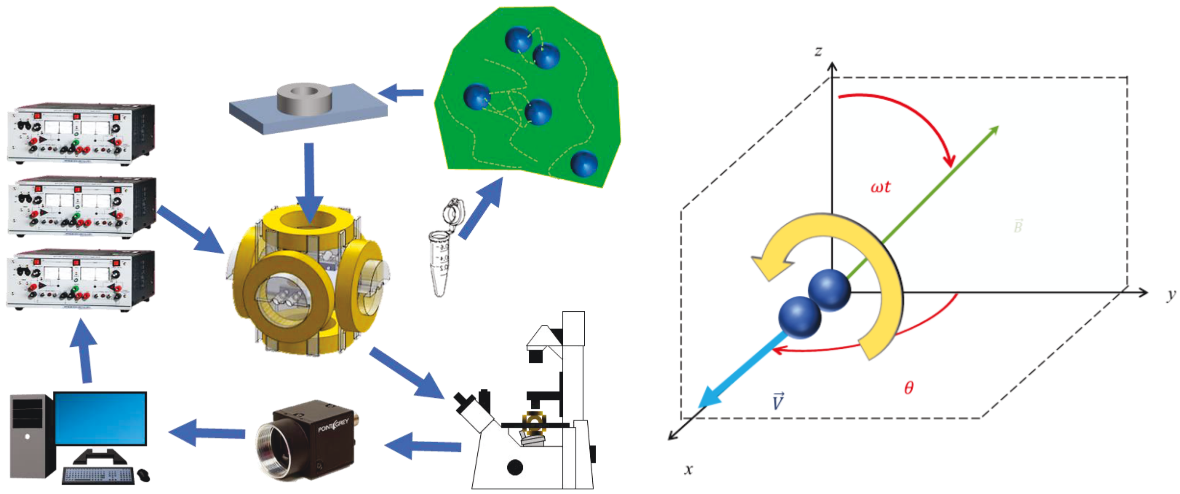

Fig 4: (a) shows the control system set up for the experiments performed. The tri-axial Helmholtz coil produces rotating magnetic fields in three-dimensional space. The mucin samples with entangled particles are loaded into a PDMS chamber and placed in the center of the coils. The coils are attached to an inverted microscope with a CMOS camera for image acquisition at 30 fps. Using a customized LabVIEW program, and DAQ boards to interface with the bipolar power supplies, rotating magnetic fields are produced to actuate the swimmer. (b) shows how Eq. (1) and Eq. (2) manipulate the swimmer under investigation. Ideally, the swimmer will travel perpendicular to the rotating magnetic field corresponding with angle θ.

swimmer. The only two parameters being actively changed during experiments are θ and ω, either to change the direction of the investigated swimmer or alter how fast it moves. A diagram of the experimental system set up can be seen in Fig 4.

$$B = \begin{bmatrix} -B_s\cos(\theta) + B_r\sin(\theta)\cos(\omega t) \\ B_s\sin(\theta) + B_r\cos(\theta)\cos(\omega t) \\ B_r\sin(\omega t) \end{bmatrix} \quad (1)$$

$$n = [-\cos(\theta) \quad \sin(\theta) \quad 0] \quad (2)$$

III. Experimental Procedure

Synthetic mucus of 4% concentration by weight was fabricated by mixing mucin from a porcine stomach (Type II, Sigma Aldrich) with deionized water. First 100 ml of deionized water was added to a beaker with a stirring rod. The magnetic stirrer was set to 1500 Hz and the hot plate temperature was set to 35°C. Six grams of mucin was then added to the beaker. Once all of the mucin was sufficiently wetted, an additional 50 ml of deionized water was added to the beaker. After 30 minutes, the mucin formulation was then centrifuged for 10 minutes at 3000 rcf; this was done to remove any undissolved particulates and allow for easy viewing with an optical microscope. The supernatant was then transferred to clean tubes and stored at 4°C. For this experiment, 1 ml of 4% synthetic mucus was combined with 1-2 µL of 10.4 µm streptavidin beads (Spherotech, SFM-100-4) and vortexed thoroughly. A sample chamber was prepared by placing a piece of PDMS, with a 3/8 inch diameter hole punched into it, onto a clean glass slide. The mucus and bead solution was then added to the hole in the PDMS until slightly overfilled. Then a clean glass cover slide was placed on top of the PDMS chamber carefully making sure that no air bubbles were present in the chamber. Any excess mucus solution was carefully wiped away from the sample. The prepared chamber was then placed in the Helmholtz coil system. A manual control system based on equations 2-3, was used to control the beads. The user selected a desired location for the target bead to travel, and then adjusted the frequency and magnetic field strength produced from the coils to the desired experimental parameters. When selecting beads for testing, only beads sufficiently far away from the boundaries of the sample chamber were examined, and only ones that exhibited a multi-bead composition. Constant adjustments were made to the focal plane of the microscope during the experiment as the beads sank over time from magnetic rotation. When performing experiments, the length of experiments were kept under 5 minutes, to ensure the temperature of the coils remained reasonable, and prevent any damage to the system from overheating. Two sets of experiments were performed, the first tested the ability of two bead entangled swimmers to swim in user defined patterns, the second test showed the velocity vs. frequency relationship of the swimmers.

IV. Results

A. Directional Controllability

The results of this study were promising. The first conclusion was that snowman swimmers can swim quite well through the synthetic mucus mediums, despite their symmetric geometry. When directed to perform directional changes, the swimmers oriented themselves to their magnetic dipoles and propagated along their long axis. Figure 5 shows the directional controllability; (a) shows a box pattern while (b) shows an up-right-up pattern. While the intention of (a) was to create a perfect box, that was clearly not the result; the slanted trajectory was caused by slight internal flows from the snowman interacting with mucin fibers. The intention of (b)

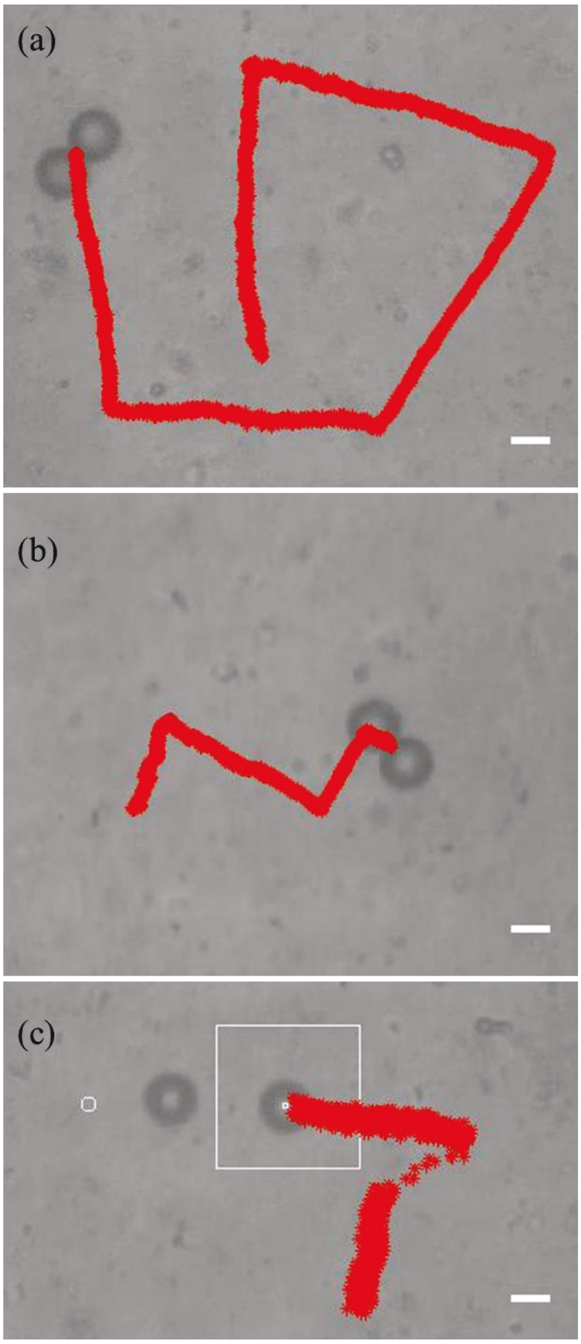

Figure 5: (a) a box like pattern with the swimmer moving 90º,0º,270º, 180º, then 90º back to the start position. The frequency during this experiment was 19 Hz. The box like pattern is visible, but due to internal flows caused by the rotation of the swimmer, the path is skewed towards the direction of rotation. (b) a step like pattern, but for the same reasons as (a), the path was skewed. Both (a) and (b) demonstrate significant directional controllability of double bead entangled swimmers. (c) shows the path of a non-contact double bead swimmer, while much rarer then the ones in (a) or (b), this swimmer was still surprisingly viable in the fluidic medium, and could be controlled just as well as it's counterparts. The scale bar in (a), (b), and (c) is 10 μm.

was to create a step like appearance, but it ended up being more of a zig-zag for the same reasons. This variation due to internal flow can be modulated by implementing a simple feedback control algorithm, like was previously done for achiral microswimmers[17-19].

During testing, a separated two bead microswimmer behaved in a much similar manner to the connected swimmers. The separation was caused by mucin fibers entangled between the two particles. A small sample of it's path planning can be seen in Fig. 5 (c). Due to it's separation, it was difficult to track using traditional techniques, and often the program switched between following one bead or the other. The separation of the beads remained mostly constant throughout the trials but the swimmers themselves would slightly contract when not exposed to magnetic fields. When a change in direction was performed, the swimmer would 'flip' along it's long axis and reorient itself to the correct dipole orientation. This motion was not as smooth as the connected snowman swimmers. While these separated swimmers were rare, they never the less show the versatility of natural microswimmers that can be formed in such environments.

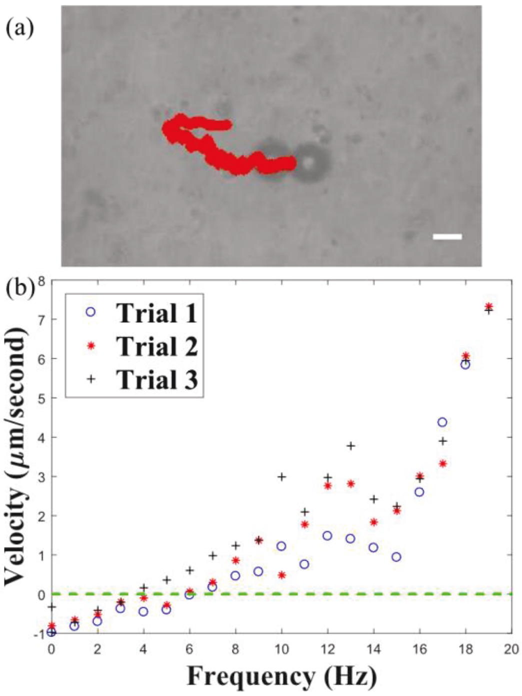

Figure 6: (a) shows the path of a snowman swimmer moving to the right. The initial reverse motion was caused by an internal flow of 0.8 μm/s to the left. (b) shows the trials conducted on the swimmer, all of which show consistent upward trends. The green line represents the point where the swimmer reached equilibrium with the internal flow. The scale bar is 10 μm.

B. Velocity vs. Frequency Analysis

The second set of tests revolved around understanding how snowman swimmers responded to increasing rotational frequency under a constant directional input. The results of this experiment were surprising, instead of a noticeable step out frequency, the snowman swimmers maintained a linear trend in velocity as frequency increased. As an added point of interest during the trials, a slight internal flow was present in the surrounding medium, pushing the particles the left at a constant rate of 0.8 μm/s. The microswimmers in all three trials was able to overcome this flow after about 7 Hz and propagate to the right. The results of three trials of the same snowman swimmer can be seen in Fig. 6 (b) where (a) is visualization of one of the trials. With slight variations, these results were repeatable among other snowman swimmers suspended in the fluid. However, finding additional separated swimmers was difficult aside from one or two additional cases and were even harder to track using image processing techniques. Overall, these are promising developments, and leads to the belief that even simpler geometries can be actuated inside mucus environments

V. Conclusion

This paper demonstrates that snowman microswimmers can be viable in mucus environments when actuated by rotating magnetic fields. When exposed to directional controllability tests, snowman swimmers could easily propagate in any desired direction, but would often be offset by self-generated internal flows. Velocity vs. frequency curves for snowman swimmers showed a clear linear trend upwards and also demonstrated that these swimmers could overcome internal flows present within the chamber. Future work will be to see if these same interactions can occur within biological mucus from rats and mice. As well, these experiments will be expanded to determine if even simpler structures can be actuated in these environments..

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References

[1] D. Schamel, A. G. Mark, J. G. Gibbs, C. Miksch, K. I. Morozov, A. M. Leshansky, “Nanopropellers and Their Actuation in Complex Viscoelastic Media,” ACSNANO, Vol. 8, No. 9, 2014

[2] B. J. Nelson, “Micro- and Nanorobots Swimming in Heterogeneous Liquids,” ACSNANO, Vol. 8, No. 9, 2014

[3] H. C. Berg, L. Turner, “Movement of Microorganisms in Viscous Environments,” Nature, 278, 349-351, 1979

[4] S. K. Lai, Y. Wang, D. Wirtz, J. Hanes, “Micro- and macrorheology of mucus,” Advanced Drug Delivery Reviews, 61, 86-100, 2009

[5] U.K. Cheang, M.J. Kim, “Fabrication and control of the simplest low Reynolds number microswimmers,” Appl. Phys. Lett., Vol. 109, 034101, 2016

[6] U.K. Cheang, M. J. Kim, “Self-assembly of robotic micro- and nanoswimmers using magnetic nanoparticles,” J. Nanopart. Res., Vol. 17, 145, 2015

[7] U.K. Cheang, M.J. Kim, “Fabrication and control of the simplest low Reynolds number microswimmers,” Appl. Phys. Lett., Vol. 109, 034101, 2016

[8] E. P. Diamandis, T. K. Christopoulous, “The Biotin-(Strept)Avidin System: Priniples and Applications in Biotechnology,” CLIN. CHEM 37/5, 625-636 (1991)

[9] U.K. Cheang, F. Meshkati, Hoyeon Kim, K. Lee, H.C. Fu, M.J. Kim, “Versatile microrobotics using simple modular subunits,” Sci. Rep., Vol. 6, 2016

[10] F. Meshkati, U.K. Cheang, Min Jun Kim, H. Fu, “Magnetic microswimmers: Controlling particle approach through magnetic and hydrodynamic interaction,” The 68th Annual Meeting of the APS Division of Fluid Dynamics, Boston, MA, U.S.A., 2015.

[11] U.K. Cheang, K. Lee, A.A. Julius, Min Jun Kim, “Multiple agent drug delivery strategy through coordinated teams of robotic microswimmers,” Appl. Phys. Lett., Vol. 105, 083705, 2014

[12] P.S.S. Kim, A. Becker, Y. Ou, A.A. Julius, M. J. Kim, “Imparting magnetic dipole heterogeneity to internalized iron oxide nanoparticles for microorganism swarm control,” J. Nanopart. Res., Vol. 17, 144, 2015

[13] L.W. Rogowski, H. Kim, X. Zhang, S. Sheckman, D. Kim, Min Jun Kim, “Swimming in synthetic mucus,” The 14th International Conference on Ubiquitous Robots and Ambient Intelligence, Jeju Island, Korea, 2017

[14] F. Meshkati, H.C. Fu, “Modeling Rigid Magnetically rotated microswimmers: Rotation Axes, Bistability, and Controllability,” Physical Review, E90, 063006, 2014

[15] U. K. Cheang, F. Meshkati, D.H. Kim, M.J. Kim, H. Fu, “Minimal geometric requirements for micropropulsion via magnetic rotation,” Phys. Rev. E, Vol. 90, 033007, 2014

[16] E. M. Purcell, “Life at low Reynolds number,” American Journal of Physics, 45,3, 1976

[17] U.K. Cheang, H. Kim, D. Milutinovic, J. Choi, L. Rogowski, M.J. Kim, “Feedback Control of Three-Bead Achiral Robotic Microswimmers,” URAI 2015

[18] U. K. Cheang, M. Dejan, J. Choi, M. Kim, “Towards Model-Based Control of Achiral Microswimmers,” ASME 2017 Dynamic Systems and Control Conference, 2014

[19] U.K. Cheang, J. Ali, H. Kim, L. Rogowski, M.J Kim, “On-Surface Locomotion of Particle Based microrobots using magnetically induced oscillation,” Micromachines, Vol. 8, 46, 2017