Title

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Journal

Advanced Functional Materials, 29(38)

ISSN

1616-301X

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Publication Date

2019-09-01

DOI

10.1002/adfm.201904178

Peer reviewed

ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Nano and Microtechnologies for the Study of Magnetotactic Bacteria

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Magnetotactic bacteria (MTB) naturally synthesize magnetic nanoparticles that are wrapped in lipid membranes. These membrane-bound particles, which are known as magnetosomes, are characterized by their narrow size distribution, high colloidal stability, and homogenous magnetic properties. These characteristics of magnetosomes confer them with significant value as materials for biomedical and industrial applications. MTB are also a model system to study key biological questions relating to formation of bacterial organelles, metal homeostasis, biomineralization, and magnetoaerotaxis. The similar size scale of nano and microfluidic systems to MTB and ease of coupling to local magnetic fields make them especially useful to study and analyze MTB. In this Review, a summary of nano- and microtechnologies that are developed for purposes such as MTB sorting, genetic engineering, and motility assays is provided. The use of existing platforms that can be adapted for large-scale MTB processing including microfluidic bioreactors is also described. As this is a relatively new field, future synergistic research directions coupling MTB, and nano- and microfluidics are also suggested. It is hoped that this Review could start to bridge scientific communities and jump-start new ideas in MTB research that can be made possible with nano- and microfluidic technologies.

1. Introduction

Magnetotactic bacteria (MTB) are phylogenetically diverse organisms that can biomineralize and assemble linear chains of magnetite ($Fe(II)Fe(III)_2O_4$) or greigite ($Fe(II)Fe(III)_2S_4$) nanoparticles with different shapes and sizes. These nanoparticles that are bound in lipid membranes are known as magnetosomes.[1] Magnetosomes allow MTB to align with the Earth's magnetic field lines and navigate along oxygen gradients in bodies of water through a process referred to as magnetoaerotaxis.[2] MTB provide a model system for studying several important questions in cell biology including cellular organization, the formation of bacterial organelles, and metal homeostasis. It has been found that the formation of magnetosomes is controlled by a specific gene set collectively named as the magnetosome gene cluster (MAI).[2] Based on how much iron MTB are able to take up from their aquatic environments, it is also possible that MTB can play a significant role in global iron cycling.[3]

In addition to being of interest in basic cell biology, magnetosomes hold great promise for use in a variety of applications. Magnetosomes have homogeneous sizes and crystallography, high thermal and colloidal stability, and their surfaces can be functionalized with biocompatible organic molecules.[4] These properties have motivated research using magnetosomes in biomedical applications including targeted drug delivery,[5] magnetic resonance imaging (MRI),[6,7] magnetic hyperthermia,[8,9] and even neural modulation.[10]

Nano and microfluidic approaches hold great promise to transform and accelerate studies of MTB (**Figure 1**). These technologies utilize devices with fluidic channel dimensions approximately in the range of hundreds of nanometers (nm) to tens of micrometers (μm) that are suited to precisely control and manipulate fluids. As nano and microchannels have dimensions in the same length scale as biological cells including MTB, they are able to offer high spatial and temporal control in processes such as cell sorting and the study of cell mobility. For example, microfluidic technologies have been used for repeatable, high-throughput sample processing including cell lysis and nanoparticle functionalization.[11,12] They have also been integrated with other physical forces like magnetism to exploit differences in a particle's magnetic properties for separation.[13] Additionally, high throughput (≈1–10 mL min^{-1}) microfluidics

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DOI: 10.1002/adfm.201904178

has been used to monitor cell growth and isolate subpopulations of cells with desirable phenotypes from bioreactors.[14] Here, we provide a review of existing nano and microfluidic technologies that have been applied to the study and analysis of MTB. As this field is relatively new, we also offer ideas where such precise fluidic control may be applied to advance the MTB field. We hope that this review can encourage synergies among scientists working on MTB and microfluidics to improve our knowledge of this unique class of microorganisms.

2. Nano and Microfluidics for Cell Analysis

Nano and microfluidic technologies offer several advantages that make them ideal for cell analyses:[16] 1) channels with similar length scale (ranging from a few hundred nm to tens of μm) to particles and cells, which facilitates high spatiotemporal resolution for cell analyses, 2) ease of coupling with other modalities including electrical and magnetic fields to exploit properties of particles and cells for cell lysis, isolation, and purification, and 3) tunable flow rates that offer a range of precision for different cell analysis requirements.

2.1. Quantifying the Magnetic Properties of MTB

MTB are a phylogenetically diverse group of bacteria having different shapes (spirillum, vibrio, fava-bean, cocci, ovoid, and rod) and sizes (length: 1–20 μm, width: 0.2–2.2 μm). Furthermore, different species have also been found to produce different numbers (from <10 to even a thousand),[17] shapes (cubo-octahedral, elongated prismatic, bullet shaped, etc.), and sizes (35–120 nm in diameter) of magnetosomes.[6] There are a few methods to measure the outcomes of biomineralization and the magnetic response of MTB such as alignment to magnetic fields and magnetic content. These techniques include transmission electron microscopy,[18] C_{mag} (light scattering in the presence of a magnetic field) measurements,[19] and color inspection of brown colonies on agar gel.[20] During transmission electron microscopic imaging, MTB are transferred onto copper grid and can be visualized usually without the use of any contrast agents due to the presence of electron-dense magnetosomes. During C_{mag} measurement, MTB are placed parallel or perpendicular to an external magnetic field and the amount of light absorbance is detected using a spectrophotometer, typically at ≈400–600 nm. C_{mag} can be calculated by this equation: $C_{mag} = (A_{400\ nm,\ perpendicular}/A_{400\ nm,\ parallel}) - 1$. Note that the optical density should be at least 0.1 before C_{mag} measurement to ensure that there are sufficient MTB for reproducible readings. Color inspection is a simple technique where the color of MTB colonies is visualized by naked eye. Generally, the darker the color of colonies with the same sizes, it is assumed that the MTB have more magnetosomes. However, these techniques may suffer from limitations such as being nonquantitative, labor intensive, and low throughput (**Table 1**). A few other light-based techniques including optical magnetic imaging,[21] fluid cell scanning transmission electron microscopy,[22] and confocal Raman micro-spectrometry[23] have been described in the literature but as they typically require expensive set-ups, their use has been relatively limited.

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Dino Di Carlo received his B.S. in bioengineering from the University of California, Berkeley in 2002 and received a Ph.D. in bioengineering from the University of California, Berkeley and San Francisco in 2006. From 2006 to 2008, he conducted postdoctoral studies at the Center for Engineering in Medicine at Harvard Medical School. He joined UCLA in 2008 and is now Professor and Vice Chair of the department of Bioengineering. His work spans numerous fields of biomedicine and biotechnology, including inertial microfluidics, cell analysis for rapid diagnostics, directed cellular evolution, single-molecule assays, next-generation biomaterials, and phenotypic drug screening.

Nano and microfluidic technologies have been developed to separate cells based on cellular properties such as size, deformability, and magnetic content.[13] Recently, Myklatun et al. reported the use of a magnetic microfluidic platform to isolate MTB suspended in a ferrofluid.[24] The team immobilized MTB which had fast flagellar motion using 75 °C heated media for 15 min but did not demonstrate viability of heat-treated MTB postseparation. While this technology is useful as a single use magnetic separation and quantification of MTB, it

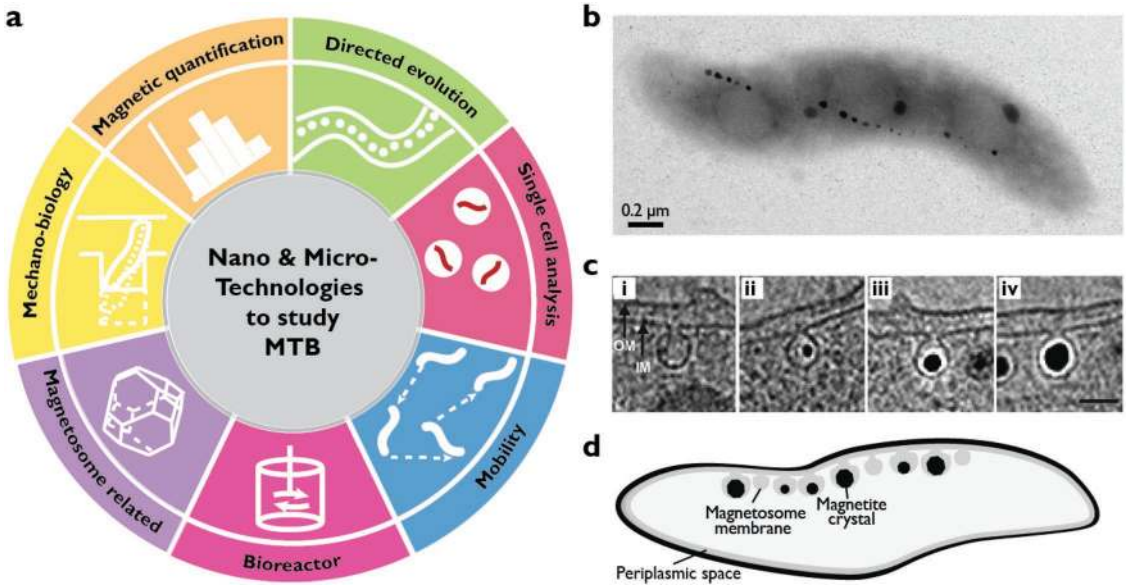

Figure 1. Applications of nano/microfluidics to study MTB. a) Ideas include using nano and microfluidics for quantification of magnetic content, directed evolution and single cell analyses. Microfluidics can also be applied for industrial purposes such as on-chip purification and functionalization of magnetosomes. We also anticipate microfluidics technology as useful platforms to study biological processes like magneto-aerotaxis. b) Transmission electron microscopic (TEM) image of a wild-type AMB-1. c) Electron cryotomography of a wild-type AMB-1 magnetosome. b,c) Reproduced with permission.[15] Copyright 2006, American Association for the Advancement of Science. (i) AMB-1 with magnetosome membrane and no magnetite. (ii) small magnetite crystal. (iii) growing magnetite crystal. (iv) full-sized magnetosome. Outer membrane (OM) and inner membrane (IM) are indicated. d) Schematic of wild-type AMB-1 with empty magnetosome, growing magnetite crystals and full-sized magnetosomes.

is not likely applicable for sorting live MTB due to the use of heated media.

This problem was recently overcome by inhibiting rapid flagellar movement with transient cold, alkaline treatments which preserved cell viability.[25] Next, by exploiting the balance between frictional drag in fluids and magnetic field gradient in a microchannel, a mixed population of live, wild-type *Magnetospirillum magneticum* (AMB-1) was separated from mutants producing about 2.2× more magnetosomes with efficacy similar to theoretical estimates.[26] The same platform was also adapted to isolate wild-type *Magnetospirillum gryphiswaldense* (MSR-1) from its Δ*mamAB* mutant counterparts, which do not produce magnetosomes, with sensitivity up to 80% and isolation purity up to 95% as confirmed with a gold standard, fluorescent-activated cell sorter (FACS) technique (**Figure 2**a).[25] The magnetic microfluidic platform also offers 25-fold higher throughput than the one fabricated by Myklatun et al. (25000 cells min^{-1} vs 1000 cells min^{-1}).[24]

Another application of microfluidic or other micromagnetic-based separation devices is to distinguish MTB producing magnetosomes of different sizes, shapes, and elemental compositions. This is because these physical properties are known to affect how magnetosomes interact with magnetic fields. For instance, assuming everything being constant, a larger magnetosome is expected to provide more magnetic force than a smaller magnetosome. A magnetosome with higher purity of iron content will also provide more magnetic force than a magnetosome, which has lower iron content. These physical properties affect the magnetic volume of magnetosome and hence their interactions with magnetic fields.[25,27] The ability

Table 1. Techniques for magnetic estimations of MTB.

	C-mag[19]	Color inspection[20]	Electron microscope[18]	Optical magnetic imaging[21]	Magnetic ratcheting[32]	Magnetic microfluidic[24,25]
Time needed	Fast [min]	Slow (≈2 weeks)	Slow (≈2–3 h)	Very fast [≈s]	Fast [min]	Fast [min]
Subjective	No	Yes	Yes	No	No	No
Automated	No	No	No	Yes	Semi	Semi
Quantitative	Yes	No	Yes	Yes	Yes	Yes
Throughput	N.A.	N.A.	Low	Low	High	High
Possible to reculture	Yes	Yes	No	Yes	Yes	Yes
Potential for single cell selection	No	No	No	No	Yes	Yes
Potential for continuous flow	N.A.	N.A.	N.A.	N.A.	No	Yes

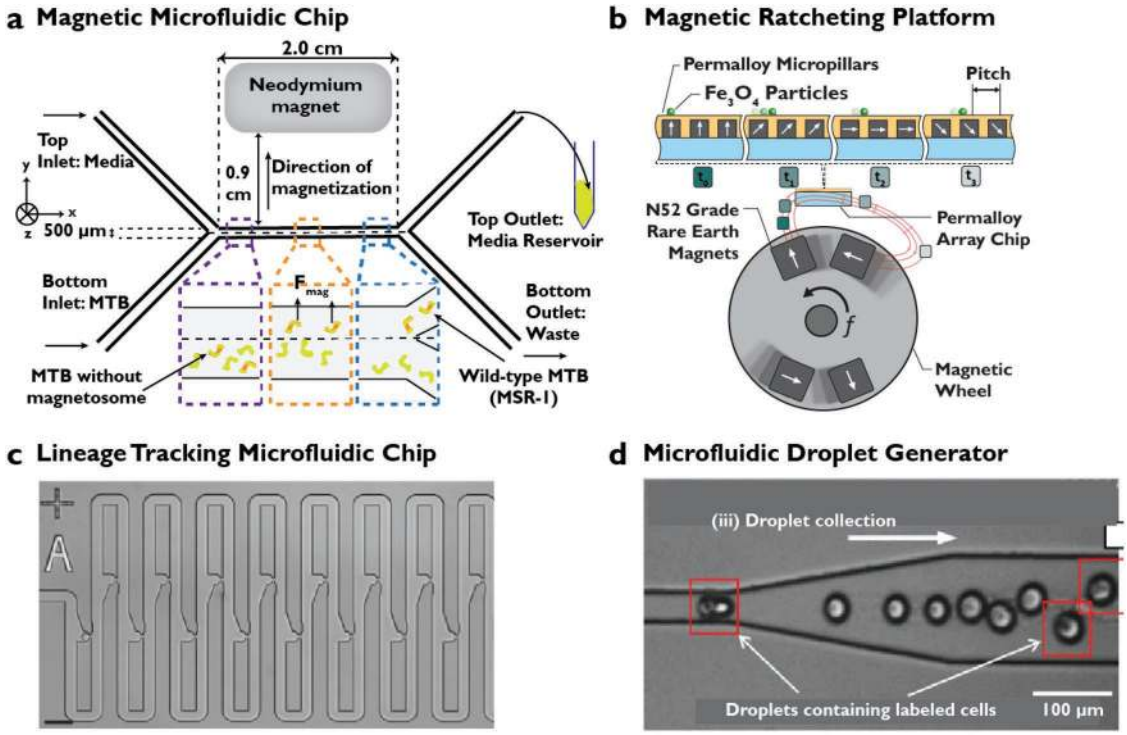

Figure 2. Microfluidics for magnetotactic bacteria (MTB) analysis. a) Magnetic microfluidic chip that separates MTB based on their magnetic contents. MTB which are more magnetic are deflected by the magnetic field gradient across the fluid streams and exit through the selection outlet. Reproduced with permission.[25] Copyright 2018, American Society of Microbiology. b) Magnetic ratcheting platform separates MTB based on the balance between magnetic forces and Stokes' drag. Combining random chemical mutagenesis and this device, AMB-1 over-producers producing 2.2-fold more magnetosomes than wild-type were generated. Reproduced with permission.[27] Copyright 2017, Wiley-VCH. c) Lineages of cells can be tracked with this device as a newly dividing daughter cell is flushed to the next chamber for isolation. Reproduced under the terms and conditions of the Creative Commons Attribution 4.0 International License.[55] Copyright 2016, Nature Publishing Group. d) Cells encapsulated in droplets can be better separated by magnetic fields due to reduced impact of cell motility.[65] Furthermore, droplets with MTB can be selected based on magnetic content and growth rates. Reproduced with permission.[65] Copyright 2013, Royal Society of Chemistry.

to differentiate magnetosomes in this way will be of great utility in biomedical applications, especially because magnetic nanoparticles with different physical properties, including size, shape, and magnetic properties[28] are known to affect biological processes like endocytosis[29] and cytotoxicity.[30] For instance, smaller nanoparticles are more likely to be endocytosed, and hence more likely to induce cytotoxicity when a large quantity is internalized by cells. Furthermore, the composition of the magnetosomes, whether it is magnetite or greigite, can also affect their magnetic and thermal properties for purposes like magnetic hyperthermia.[31]

2.2. Directed Evolution

MTB are fastidious bacteria that grow at an extremely slow rate (a few hour per cell division compared to as fast as 20 min per cell division for *Escherichia coli*)[33] even in optimized conditions. Kolinko et al. addressed this growth limitation by transferring 30 key genes from MSR-1 to *Rhodospirillum rubrum*, a faster growing photosynthetic prokaryote, showing the possibility of endogenous magnetization in non-magnetic organisms.[34] Liu et al. also introduced a mutation upstream of an ATPase gene to generate MSR-1 that overproduced magnetosomes.[35] In addition, Lohβe et al. demonstrated the use of gene amplification to create MSR-1 mutants that produce 2.2-fold more magnetosomes than wild-type cells.[36] These studies are indicative of the rising interest within the community to engineer or evolve organisms that produce magnetosomes more rapidly. Directed evolution offers great implications for studying MTB biology and the use of MTB and magnetosomes. For instance, MTB that overproduce magnetosomes can be cultured in large numbers to harvest magnetosomes for biomedical applications. MTB overproducer mutants may also be more useful than wild-type MTB as microrobots for drug delivery purposes as they can be more easily manipulated with magnets. Furthermore, by combining directed evolution and genetic dissection, the magnetic gene cascade may be understood to advance the technique of magnetogenetics for wireless magnetic cell manipulation.[37]

2.2.1. Selection of MTB Overproducers

To demonstrate the use of nano and microfabrication technologies for directed evolution of MTB, random chemical mutagenesis and a quantitative magnetic ratcheting technique were combined to select overproducing mutants (Figure 2b).[27] Existing magnetic methods for cell separation such as

magnetic-activated cell sorting (MACS) that accumulates all cells with any magnetic content did not offer sensitivity high enough to differentiate MTB mutants with small differences in magnetosome numbers. Thus, the authors employed a ratcheting system consisting of magnetized permalloy magnetic elements, which offer high sensitivity up to ±5 magnetosomes. AMB-1 was randomly mutated with chemical mutagens to generate mutants with different magnetosome producing abilities followed by selection using the magnetic ratcheting platform. The mutated AMB-1 can be distinguished based on the balance between the magnetic force on each bacterium and the fluid drag force that each bacterium experiences. Using this strategy, AMB-1 mutants with different number of magnetosomes were generated. For instance, there were mutants without magnetosomes and mutants overproducing approximately twofolds more magnetosomes than control AMB-1. The magnetosomes synthesized by the over-producers were also comparable in terms of size, shape, and magnetic properties to those produced by wild-type, control AMB-1.

We also like to highlight that although the working principles, i.e., balance between magnetic force and Stokes' drag of magnetic microfluidic platform (see Section 2.1. and Figure 2a) and magnetic ratcheting system are similar, they offer different levels of precision and throughput. Magnetic microfluidic system provides much higher throughput (25 000 cells min^{-1} vs <10000 cells min^{-1}) in quantifying magnetic properties of MTB. On the other hand, the magnetic ratcheting system offers higher quantitative accuracy (±5 magnetosomes versus (±10–15 magnetosomes) for more precision selection of MTB overproducers. A useful future development of the magnetic ratcheting technique is to enable sterile, closed-loop, continual generation of diverse MTB variants with desirable properties to enhance its throughput while preserving its higher precision.[38]

2.2.2. Genetic Engineering

Over a few decades of research, microbiologists have elucidated the roles of the *mamAB* gene cluster in magnetosome formation.[39–46] Other operons, such as *mms6,* that play a role in determining the crystal structure of magnetosomes have also been identified.[43,46] However, it remains a challenge to introduce large plasmid constructs all at one time. For instance, Loh*β*e et al. doubled the genes in the magnetosome island of MSR-1 to generate mutants producing double the number of magnetosomes.[36] But this was achieved through a laborious process involving random conjugation of MTB with competent *E.coli* for one plasmid construct transfer at a time. Furthermore, this process may be challenging to control and offer variable frequencies depending on the plasmids.[47] There is a biological limitation in the plasmid size (typically less than 50 kilobases (kbp)) that can be transferred through the conjugation pili.[47]

Nano and microtechnologies can be used to introduce exogenous materials like plasmid constructs more efficiently into organisms with user-defined conditions. One example is through nano and microchannel penetration and electroporation.[48] Unlike the use of conjugating *E. coli* or other biological methods, penetration and electroporation makes use of physical forces to locally penetrate through the cell or transiently open pores in the cell wall/membrane for DNA delivery. Bacteria centrifuged at fast speed can be mechanical penetrated onto nano- and microstructures. Electroporation makes use of steep voltage differences to create localized electrical potential differences across cells. This disrupts the cell wall and membrane, causing formation of transient pores for entry of biomolecules such as DNA for transformation.[49] The properties of nano- and microstructures such as length, diameter, and voltages can be more easily controlled and optimized by users to achieve increased uniformity in genetic engineering.

Paulo et al. made use of computational simulation to determine the critical electric field just sufficient to induce electroporation and performed microfluidic electroporation to amplify and better control the spatiotemporal properties of the electric field strength.[50] This technique can potentially transform MTB with greater precision in the number of copies of DNA/bacterium as compared to random conjugations with *E. coli,* especially for MTB strains that are difficult to transform. For instance, Okamura et al. made use of bulk liquid electroporation to introduce DNA plasmids into MTB and we speculate that this process could be possibly improved using microfluidic electroporation.[51]

Recently, there is also increasing interest in using nano and microwires or pillars for direct penetration through cell walls for DNA delivery.[52] Several groups have also developed nanochannel electroporation platforms which integrate localized electric fields to draw large DNA, up to tens of kbp, into cells.[53] These nanochannels are typically a few hundred nm and are larger in magnitudes than plasmids. The use of electric fields facilitates active electrophoretic delivery of negatively charged DNA; it also reduces the possibility that the nanochannels would be clogged. This can also be useful to genetically or synthetically incorporate magnetic properties to non-magnetic cells as genes of the magnetosome island's operons can be up to tens of thousands of base pairs.

2.2.3. Lineage Tracking

Microfluidic devices can also facilitate lineage tracking to understand environment-dependent and environment-independent phenotypic variations and cell-fate switching. Such platforms have improved our understanding of stochastic phenotypic changes in bacteria and temporal control of gene circuits encoding for bacterial oscillatory cell fate behaviors.[54] For instance, Wang and co-workers designed a microfluidic device consisting of multiple channels that accommodated only one *E. coli* bacterium at each channel (Figure 2c).[55] Each newly generated bacterium was flushed by microfluidic flow to the next available channel, which allowed easy monitoring of cell shape and size. As *E. coli* and MTB share similar size range, and it will be simple to adapt this device design to separate sister MTB of different generations in their respective microchannels. For instance, single MTB can be isolated for physical phenotyping and genetic screening to assess the mutational frequency in MTB that is known to affect their magnetic properties.[56] Another potential application of this device is to monitor the production of magnetosomes by MTB overproducers over multiple generations to understand the role of specific genes

in phenotypic reversions. It may also find utility in the fundamental understanding of magnetosome splitting between daughter MTB at the single cell level especially because in some MTB strains, there can be uneven magnetosome numbers (e.g., *Magnetospirillum magneticum* AMB-1) or multiple magnetosome chains (e.g., "*Candidatus* Magnetobacterium bavaricum"),[57] which complicate equal magnetosome distribution.[58] We note that although most microfluidic platforms are designed to study *E. coli* and other micro-organisms with health implications, they can be conveniently modified to study MTB which has similar size range.

2.2.4. Bacterial Ecosystems

It is possible that MTB in their natural habitats interact with other micro-organisms through processes like iron and phosphate recycling.[59] Microfluidic devices have been employed to construct and replicate environments to elucidate the role of bacterial ecosystems in regulating cellular biology.[54] For instance, Kim et al. cocultured three strains of bacteria. Each strain had a specific role: supplying nitrogen, providing carbon, or degrading antibiotics.[60] The bacteria flourished in the coculture with finite inter-chamber distance but perished when separated or when positioned too far away for sufficient diffusion of resources. As MTB are able to store iron, a coculture of MTB may reveal their ecological dynamics with other micro-organisms, especially in iron cycling. Additionally, the ability of MTB to take up toxic, heavy metals such as cobalt and manganese from their natural habitats[61] has intriguing future implications for using MTB as tools for ecological waste water treatment or heavy metal removal.

2.3. Single-Cell Analysis

Single-cell analysis has revealed how heterogeneity in cell populations affects cellular functions and responses to stimuli.[62] Single-cell analysis techniques have been applied to understand the similarities in genetic makeup of uncultivated MTB and those that are cultured in order to determine the preferred electron donors and acceptors of uncultivated MTB strains.[63] However, even with knowledge about the genetic makeup of uncultivated MTB, it can be a laborious and costly process to generate multiple, different media compositions in large (liters) quantities to cultivate newly found MTB strains.

Microfluidics can be helpful to screen a multitude of culture conditions in small volumes (μL to mL). Single-cell droplet microfluidics can generate droplets each encapsulating a single cell for analysis.[64] Using this method, libraries of droplets containing different media conditions can be used to optimize media composition by screening for essential nutrients and elements in a small-volume environment. This automated approach could greatly save on resources and manpower, but also enable signaling molecules to accumulate within a droplet (with sub-nanoliter volumes) at much high concentrations compared to multi well-plates (with tens of microliters to milliliter volumes) approaches. Such techniques may also be useful to progressively isolate MTB mutants that have adapted to different carbon/electron sources for culturing in laboratories.

Currently, droplets are generated in microchannels using water and oil emulsification controlled precisely by flow rate or geometry. However, the magnetic microfluidic droplet generator created by Chen et al. can exploit the inherent magnetic properties of MTB to potentially facilitate more accurate downstream analyses with single-cell droplets (Figure 2d).[65] This microfluidic platform can also be coupled with additional functions for automatic cell lysis, nucleic acid extraction, and polymerase chain reactions for single-cell analysis.[66] Another noteworthy design is by Brouzes et al. who introduced a magnetic microfluidic droplet methods to analyze mRNA in single cells.[67] Using a technique called droplet splitting, a single cell with adsorbed magnetic beads was encapsulated in each droplet. After attracting the magnetized cell to one end of the microchannel, the droplet was cut into two halves. With the volume of the droplet containing the magnetized cell halved, the concentration of mRNA that can be isolated from the cell is doubled, potentially improving the accuracy of downstream analyses. This droplet-splitting strategy can be applied to isolate naturally magnetic MTB in droplets with smaller volume to concentrate samples (DNA/mRNA/proteins) for more sensitive single-cell analysis. It can also be useful for high-throughput genetic screening of mutants in order to learn about the functions of specific genes and their roles in magnetosome formation.

2.4. Mobility

There is interest in studying the tactic abilities of MTB—magnetotaxis, chemotaxis, phototaxis, and aerotaxis[68–70]— because MTB can be used as model organisms to study competing modes of tactic behaviors. There is also potential to use MTB taxis to develop microrobots for transport and on-chip diagnosis.[5,71–73] Microfluidic devices can be used to advance taxis research. Besides offering better spatiotemporal control of gradients, the transparency of glass or PDMS-based microfluidic devices also facilitate convenient monitoring of biochemical gradients using colored/fluorescent dyes.[74] Such devices have been employed to understand the mobility of bacteria. For instance, Waisbord et al. found that *Magnetococcus marinus* in growth media display mobility governed by the balance between magnetic torques and fluctuations of thermal energy.[75] However, in a microchannel devoid of nutrients, *M. marinus* demonstrated run-and-tumble dynamics. This behavior is also observed when *M. marinus* encountered geometrical constraints such as sediments in their natural habitats. Recently, Loehr et al. also demonstrated the possibility of magnetically guiding *M. gryphiswaldense* along lines of instability, revealing a type of mobility not displayed by other bacteria like *E. coli*.[76] Microfluidic flow and mixing can also simulate microparticle suspensions to imitate the presence of agitation[77] to understand how MTB may respond to similar situations in their natural habitats. The data obtained will be useful for modelling of MTB behaviors to create physical models of processes such as magnetotaxis, which can aid in the design of magnetic robots for in vivo drug delivery.[69]

Aerotaxis refers to directional motion in response to oxygen gradients. Unipolar, dipolar, and axial magnetoaerotactic

behaviors have been observed in different MTB strains by Lefèvre et al., who monitored the motions of MTB in capillary tubes.[70] The team also found that different MTB strains preferred different oxygen levels. Popp et al. looked at the behavior of MSR-1 in oxygen gradients and found that MSR-1 display swimming polarity through oxygen sensory pathways regulated by CheOp1.[78] Lately, Felfoul et al. described the use of MTB to deliver drug-containing nanoliposomes to tumor hypoxic regions.[5] In the future, microfluidic devices can also be used to enrich MTB with different sensitivity to oxygen gradients for targeted drug delivery to body tissues with different oxygen levels.

Most of the existing literature on the aerotactic behaviors of bacteria made use of distinct bands, corresponding to specific oxygen concentrations, formed by the bacteria in capillary tubes with oxygen gradients.[79–81] However, it is challenging to accurately measure oxygen level in capillary tubes, especially when taking the spatial distribution of the bacteria and the rate of oxygen consumption into account. To overcome this technical limitation, Adler et al. created a microfluidic device with stable linear profiles of oxygen, ranging from 0% to 0.5% oxygen to up to 16%.[82] The same group also demonstrated the possibility of generating linear, exponential, and nonmonotonic shaped oxygen gradients,[83] which can be applied to understand both the effects of geometry and oxygen gradients in MTB mobility. For instance, Li et al. integrated valves in their microfluidic device and observed that they could induce AMB-1 migration by mixing different concentrations of oxygen and nitrogen gases.[84]

Microfluidic chips have also been employed for studying chemotaxis in bacteria. Mao et al. first introduced microfluidics into this field by arguing that the conventional capillary assay has limited sensitivity as the chemical concentration gradient becomes shallower with time.[85] However, with a microfluidic device, they were able to maintain steady concentrations of chemoattractants/repellents at a fixed spot due to constant replenishment. Ahmed et al later proposed a mathematical model to quantify the chemotactic behaviors of cells in steady, nonlinear microfluidic gradients of arbitrary shapes.[86] Englert et al. also used flow-based microfluidic systems to generate a steady chemical gradient along the entire channel length as long as flow is maintained.[87] Using devices like these, a wide range of chemical gradients can be created to study the response of MTB to various chemicals of interest. This could be of utility to select MTB attracted to chemical signals secreted by different body tissues or tumors for targeted in vivo drug delivery. Other microfluidic devices used to study the thermo-[88] and phototaxis[89] behaviors of bacteria/algae can also be easily adapted for MTB.[90]

3. Nano and Microfluidics for Industrial Applications

Nano and microfluidic technologies coupled to bioreactors can also be useful for high throughput monitoring of cell growth and magnetosome production rates. Furthermore, they can be integrated with ultrasound or chemical on-chip cell lysis, magnetosome extraction, purification, and functionalization approaches.

3.1. Bioreactor

MTB can be cultured in large-scale bioreactors with optimal culture conditions.[91,92] Parallelized microfluidics with multiple devices running simultaneously can offer high throughput sorting at a range of 10–1000 mL min^{-1}.[93] This may offer advantages over the use of filters for isolating subpopulations of MTB to minimize clogging/biofouling and repeated need for changing expensive filters.[94] Microfluidic bioreactors may also be used to monitor the response of MTB to environmental changes such as pH, temperature, presence of polyethylene glycol (PEG),[95] oxygen, and shear stresses[96] in real time. Furthermore, microscale versions of microfluidic bioreactors can be constructed to monitor how certain processes such as the fluid dynamics of mixing occur in scaled-up versions.[97] Motivated by the use of multiplexed microfluidic systems for biotechnological purposes, we propose that a highly parallelized magnetic microfluidic system to quantify magnetic contents in MTB may also be used as microfluidic reactors (**Figure 3**a),[25] highlighting the flexibility of microfluidic devices for use in biotechnology. Mach and Di Carlo also proposed the use of inertial microfluidic platform for scalable blood cell filtration (Figure 3b).[98] The platform was able to achieve a processing speed of 240 mL h^{-1}. It could be adapted for high throughput magnetic isolation by patterning nano-/micromagnets within the channels and magnetizing them remotely with a large external magnetic field.[99]

3.2. Magnetosome Purification and Functionalization

One of the goals of generating MTB overproducers is to harvest their magnetosomes for use in biotechnology or biomedical applications. The current techniques to isolate magnetosomes are ultrasound, chemical, or mechanical lysis followed by collection using a magnet. These methods are time consuming and have user-dependent performance. Furthermore, to recover magnetosomes, substantial wash steps are necessary that increase the loss of magnetosomes.[100] Microfluidics can be coupled with various modalities to construct a μTAS (total analysis system) for on-chip cell lysis, magnetosome extraction, purification, and even functionalization.[101] A μTAS could allow for careful control of cell lysis to avoid any damage to the lipid membranes encapsulating the magnetic nanoparticles and can potentially enhance the recovery of magnetosomes (Figure 3c).

Numerous microfluidic platforms have been described for cell lysis. Bao and Lu made use of electric fields to break up bacterial cells.[102] Lu et al. also made use of electric fields that only disrupt external cell membranes and not membranes of organelles, which is important to prevent aggregation of magnetosomes.[11] Microfluidic platforms can be used to functionalize magnetosomes with antibodies or biocompatible polymers for biological applications (Figure 3d).[12] The advantages of using such a system in contrast to bulk magnetosome functionalization include saving on expensive reagents like antibodies and obtaining magnetosomes with more uniform and reproducible functionalization.[103] Furthermore, in biomedical applications, functionalization of magnetic nanoparticles with radioactive probes/molecules are usually performed on site.[103]

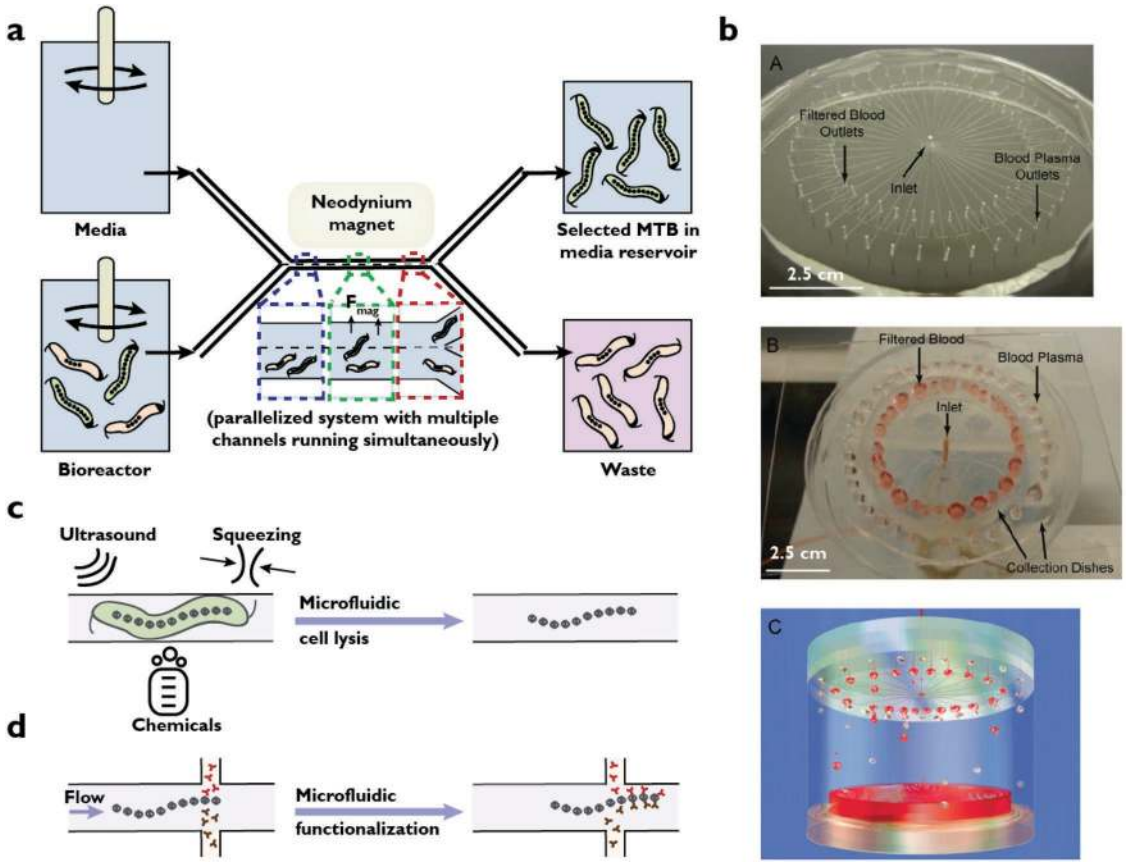

Figure 3. Microfluidics for industrial manipulation of MTB. a) A parallelized version of a magnetic microfluidic chip[25] with multiple channels running simultaneously may be coupled to a bioreactor to investigate the effects of supplements in culture media on MTB growth and magnetosome production rate. b) Example of systems incorporating radial microfluidic channels for scalable cell separations. Microfluidic channels can be patterned with nano/micromagnets remotely magnetized by large external magnetic fields for high through-put MTB processing. Reproduced with permission.[98] Copyright 2010, Wiley Periodicals, Inc. c) Microfluidic lysis of MTB with ultrasound, chemicals, or mechanical stresses. By performing cell lysis in microfluidic channels, there is potentially less magnetosome loss than bulk methods such as French-press. d) Microfluidic functionalization of magnetosomes with antibodies or bio-polymers for biotechnological applications. This method helps to reduce waste of costly reagents like antibodies, thus leading to cost-savings. Furthermore, external magnetic fields may be used to orientate magnetosomes for homogenous functionalization.

A miniaturized microfluidic system capable of processing 10–25 mL could therefore facilitate timely functionalization of magnetosomes for clinical purposes. However, one caveat is that prototypical microfluidic platforms may not always be compatible with harsher solvents/chemicals used in functionalization,[103] and PDMS may need to be substituted with materials such as glass.

4. Microfluidics for Mechanobiology

Geometrical constraints in the natural habitat of MTB can influence the diffusion of nutrients, metabolic waste, and signals that trigger adaptation in the form of growth and mobility. Different microfluidic devices have been fabricated to investigate the role of spatial geometry on the ecological and evolutionary properties of bacteria.[54] Cho et al. showed that *E. coli* oriented and grew to respond to chamber shapes in a microfluidic device to minimize mechanical stresses induced by cell growth and promote efficient nutrient diffusion.[104] Takeuchi et al. also demonstrated the possibility of shaping *E. coli* into patterned shapes.[105] These microfluidic designs may be adapted to explore the mechanobiology of MTB. It will be interesting to understand how the rate of cell division, rate of magnetosome production, and regulation of shape and mobility of MTB are influenced by mechanical forces.[106] How MTB respond to mechanical forces may help inform the design of bioreactor geometry, surface roughness, and stirring rates to influence metabolism or magnetosome production. A recent study also found that the proportion of fine to coarse sand in the environment may influence the dominant species of MTB. Microfluidic devices may be useful for experimental validations of this observation.[107]

Microfluidic platforms have also been developed to investigate differences in the stiffness and deformability of bacterial cells such as *E. coli* and their resistance to antibiotics.[108] These platforms offer much higher throughput than conventional tools like optical tweezers and atomic force microscopy.[108] Similar devices can be used to understand whether MTB with different numbers of magnetosomes have different stiffness, which may enable high throughput isolation of subpopulations of interest using deformability-based cell cytometry.[109]

5. Conclusions and Outlook

Nano and microfluidic technologies are increasingly used for purposes such as single-cell analysis, nanoparticle functionalization, and investigations of cellular processes. Many of the described platforms have been used for cells of relevance to human health applications, like *E. coli*. However, the platforms can be easily adapted for under-studied and useful organisms like MTB through appropriate scaling and creative repurposing. For instance, magnetic microfluidic chips were first described for isolating cancer cells bound to magnetic beads but we have adapted this platform for isolating MTB mutants with different magnetosome numbers. Similar adaptation can be performed to generate microfluidic coculture systems to understand the ecological roles of MTB. Or microfluidics can be used to analyze MTB behaviors in chemical and oxygen gradients. Microfluidics also has huge potential to improve our understanding of MTB biology and to expand the translational applications of MTB and magnesotomes. For instance, devices for lineage tracking can be used to understand magnetosome splitting during MTB cell division. Microtechnologies can also be used for directed evolution to generate MTB mutants that overproduce magnetosomes for biomedical applications. High-throughput microfluidic bioreactors are equally useful for industrial-scale culture of MTB and functionalization of magnetosomes. Our review aims to introduce MTB to researchers developing microtechnologies and vice versa. Our goal is that through more conversations between the two communities, there can be new microtools to advance our understanding of MTB and to manipulate MTB for scientific applications.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

magnetic particles, magnetosome, magnetotactic bacteria, microfluidics, microsystems

Received: May 24, 2019
Revised: June 14, 2019
Published online:

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