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A PubMed Search: Application of Magnetotactic Bacteria in Cancer Treatment

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Introduction

Magnetotactic bacteria (or MTB) are a polyphyletic group of bacteria discovered by Richard P. Blakemore in 1975, that orient along the magnetic field lines of Earth's magnetic field. To perform this task, these bacteria have organelles called magnetosomes that contain magnetic crystals. The biological phenomenon of microorganisms tending to move in response to the environment's magnetic characteristics is known as magnetotaxis (although this term is misleading in that every other application of the term taxis involves a stimulus-response mechanism). In contrast to the magnetoception of animals, the bacteria contain fixed magnets that force the bacteria into alignment, even dead cells align, just like a compass needle. The alignment is believed to aid these organisms in reaching regions of optimal oxygen concentration[1].

History

These remarkable bacteria were first discovered by Salvatore Bellini in 1962. Bellini noticed that a group of bacteria swam towards the direction of the North Pole and dubbed this bacteria group “magneto sensitive bacteria”. In 1975, Blakemore named these bacteria “magnetotactic bacteria” by deriving from the biological term “magneto taxis” . The discovery of MTB caused a significant impact on research fields such as microbiology, physics, biophysics, chemistry, biochemistry, geology, limnology, crystallography, and even in astrobiology. Although they can be found in any environment, especially in lake and seawater habitats, due to their unique life, it is not easy to isolate and cultivate MTB [19]. However, the fast advances in biotechnology, magneto technology and in other fields have eased the cultivation of MTB in laboratories, and sped up the researches[2].

Magnetotactic bacteria, described by Richard Blakemore, have long intrigued researchers because they synthesize intracellular nanoscale (40-100 nm) magnetic particles composed of Fe3O4, termed magnetosomes. It is reported that the extensively studied strains of magnetotactic bacteria include *Magnetospirillum gryphiswaldense MSR- 1, M. magnetotacticum MS-1, M.*

Magneticum AMB-1, Magnetococcus sp. MC-1, and magneto-ovoid strain MO-1. Interestingly, a variety of higher organisms, including bees, algae, pigeons, eels, and humans, are also capable of synthesizing intracellular magnetite. The formation and physiological function of magnetic crystals in these organisms are still not known sufficiently. However, thorough understanding of bacterial magnetosome formation will serve as a model to uncover the mechanism of magnetosome formation and function in other species. It is stated that current research focuses on the molecular mechanisms of bacterial magnetosome formation and its practical applications in biotechnology and medicine. It is also noted that functional analysis of several genes involved in magnetosome formation, e.g., mamJ, mamK, magA have revealed the roles of membrane associated proteins in transport and bio mineralization processes required for the installation of magnetosomes[3].

Biology

Several different morphologies (shapes) of MTB exist, differing in number, layout and pattern of the bacterial magnetic particles (BMPs) they contain. The MTBs can be subdivided into two categories, according to whether they produce particles of magnetite (Fe_3O_4) or of greigite (Fe_3S_4), although some species are capable of producing both. Magnetite possesses a magnetic moment three times that of greigite. Magnetite-producing magnetotactic bacteria are usually found in an oxic-anoxic transition zone (OATZ), the transition zone between oxygen-rich and oxygen-starved water or sediment. Many MTB are able to survive only in environments with very limited oxygen, and some can exist only in completely anaerobic environments. It has been postulated that the evolutionary advantage of possessing a system of magnetosomes is linked to the ability of efficiently navigating within this zone of sharp chemical gradients by simplifying a potential three-dimensional search for more favourable conditions to a single dimension (see the "Magnetism" subsection below for a description of this mechanism). Some types of magnetotactic bacteria can produce magnetite even in anaerobic conditions, using nitric oxide, nitrate, or sulfate as a final acceptor for electrons. The greigite mineralizing MTBs are usually strictly anaerobic[4].

It has been suggested MTB evolved in the early Proterozoic Era, as the increase in atmospheric oxygen reduced the quantity of dissolved iron in the oceans. Organisms began to store iron in some form, and this intracellular iron was later adapted to form magnetosomes for magnetotaxis. These early MTB may have participated in the formation of the first eukaryotic cells. Biogenic magnetite not too different from that found in magnetotactic bacteria has been also found in higher organisms, from Euglenoid algae to trout. Reports in humans and pigeons are far less advanced. Magnetotactic bacteria produce their magnetic particles in chains. The magnetic dipole of the cell is therefore the sum of the dipoles of each BMP, which is then sufficient to passively orient the cell and overcome the casual thermal forces found in a water environment. In the presence of more than one chain, the inter-chain repulsive forces will push these structures to the edge of the cell, inducing turgor[5].

The diversity of MTB is reflected by the high number of different morphotypes found in

environmental samples of water or sediment. Commonly observed morphotypes include spherical or ovoid cells (cocci), rod-shaped (bacilli), and spiral bacteria of various dimensions. One of the more distinctive morphotypes is an apparently multicellular bacterium referred to as the many-celled magnetotactic prokaryote (MMP). Regardless of their morphology, all MTB studied so far are motile by means of flagella are Gram-negative bacteria of various phyla: Despite the majority of known species' being proteobacteria, e.g. *Magnetospirillum magneticum* an alphaproteobacterium, members of various phyla possess the magnetosome gene cluster, such as *Candidatus Magnetobacterium bavaricum* a Nitrospira. The arrangement of flagella differs and can be polar, bipolar, or in tufts. The first phylogenetic analysis on magnetotactic bacteria using 16SrRNA gene sequence comparisons was performed by P. Eden et al. in 1991. Another trait that shows considerable diversity is the arrangement of magnetosomes inside the bacterial cell. In the majority of MTB, the magnetosomes are aligned in chains of various lengths and numbers along the cell's long axis, which is magnetically the most efficient orientation. However, dispersed aggregates or clusters of magnetosomes occur in some MTB, usually at one side of the cell, which often corresponds to the site of flagellar insertion. Besides magnetosomes, large inclusion bodies containing elemental sulfur, polyphosphate, or poly-β-hydroxybutyrate are common in MTB.
The most abundant type of MTB occurring in environmental samples, especially sediments, do coccoid cells possess two flagellar bundles on a somewhat flattened side. This "bilophotrichous" type of flagellation gave rise to the tentative genus *"Bilophococcus"* for these bacteria. In contrast, two of the morphologically more conspicuous MTB, regularly observed in natural samples, but never isolated in pure culture, are the MMP and a large rod containing copious amounts of hook-shaped magnetosomes (*Magnetobacterium bavaricum*).

Magnetosome and magnetite

Magnetosomes are a type of vesicles that is found in the cell. This structure which is surrounded by a membrane and consists of inorganic crystals containing magnetic iron is vital for MTB. Magnetosomes are vesicles that are in a membrane within the cytoplasm that is full of magnetite and greigite, which help the bacteria align themselves according to the Earth’s magnetic field. *Magnetospirillum magneticum* and *Magnetospirillum gryphiswaldense*, which have approximately 15-20 magnetosomes along the cell’s center line aligned horizontally are magnetotactic organisms that have been researched the most. This line-up along the central line creates a structure similar to a compass needle that has the magnetic moment that is equal to the sum of each magnetosome’s magnetic moment. Electron chromatographic analysis shows that there is a filament that accompanies the magnetosome chains, and that the magnetosomes are connected to this filament by acidic MamJ proteins. In various electron microscopes, magnetosome crystals of various shapes were observed in MTB, such as cubic octagonal, bullet-shaped, prismatic and rectangular[4].
Magnetite (Fe_3O_4) is the main chemical component of magnetosomes characterized by the high

chemical purity, fine grain size uniformity, and good biocompatibility, which can be used as a new kind of nano-magnetic materials applied in many fields of biochemistry, magnetic materials, clinical medicine and wastewater treatment etc. Magnetosome formation is the mineralization process under strict biochemical mechanisms control, including four steps: iron accumulation, membrane formation, transportation and controlled bio mineralization of Fe_3O_4. Magnetosomes are generally organized in linear chains and orient the cell body along geomagnetic field lines while flagella actively propel the cells, resulting in so-called magnetotaxis[6].

Behavior of magnetosomes in east and north hemisphere of earth

Magnetotactic bacteria, which are affected by the Earth's magnetic field face north in the northern hemisphere, and face south in the southern hemisphere. MTB from the Southern Hemisphere swim antiparallel to the vertical component of the geomagnetic field toward the South and are termed South-seeking MTB (SS-MTB). In contrast, MTB from the Northern Hemisphere swim parallel to the vertical component of the geomagnetic field lines and are predominantly North-seeking (NSMTB). The inclination of the geomagnetic field lines is believed to direct cells downwards away from toxic concentrations of oxygen in surface waters, thereby helping them locate and maintain an optimal position in vertical gradients which is usually at or near the oxic-anoxic interface. However, there are reports of SS-MTB and NS-MTB in both hemispheres[7].

Applications of magnetosome

Nano-technology has been identified as an area which will bring about new evolutions in material, devices and processes. New challenges of nano-biotechnology entail manufacturing more sophisticated and highly efficient biosensors and biomaterials at the nano-scale level for use in interdisciplinary fields. Matsunaga and Okamura stated that magnetic bacteria synthesize intracellular magnets which are encapsulated by lipid bilayer membranes. Sizes of bacterial magnetic particles (BMPs) vary from 50 - 100 nm in diameter, and number over 10 per cell. BMPs are composed of magnetite (Fe_3O_4) with a single magnetic domain. Easy aqueous dispersion of BMPs enables development of highly sensitive chemiluminescence enzyme immunoassays by the chemical coupling of antibodies on BMP surfaces. BMPs can likewise be used as drug delivery systems employing magnetoliposomes with high capture volumes. After the discovery of MTB, it was noticed that they possess unique magnetic, physical and optical characteristics. Due to these characteristics, their use in various scientific and technological applications has been a current issue. It was claimed that by means of magnetic separation, the MTB could separate heavy metals and radionuclides. As MTB can be retained with a magnetic recorder, the samples in water can be easily removed and the magnetically labeled cells and nanoparticles can be retained. Another application field of live MTB is nanorobotics. A routing magnetic field is used to orient MTB and a torque is applied to the magnetosome chain in

bacteria to propel them at a predetermined location[8].

The magnetite magnetosomes in bacteria are used to block relatively large bioactive substances. These substances can later be controlled by a magnetic field. Magnetite particles of MTB are used as carrier genes in determining nucleic acids. With the help of the organized magnetosomes, nucleic acids can be isolated. In addition, it was found that magnetosomes are helpful in determining biomolecular interactions in medicine and diagnosis. In recent times, magnetosomes gained importance as a potential drug-carrier for tumor treatment and as contrast material for MRI. The fact that MTB can swim by following the magnetic field direction and their efficient activity provides a possibility to deliver drugs to tumors. Development of deformed capillaries, heterogeneous blood flow, high pressure between cells and other micro-environment parameters of tumors affect drug transfer to the tumors. Microorganisms who act as micro carriers help distribute drugs evenly while ailing the aforementioned conditions[9].

Again, MTB are used in magnetic hyperthermia, which is used in tumor therapy. Magnetic hyperthermia is a method in which magnetic nanoparticles are sent to the tumor and are heated by applying alternate magnetic fields. The heat generated by the nanoparticles has an anti-tumor effect. This technique can be used in the treatment of some tumors such as in lung cancer. Up to now, chemically synthesized iron oxide nanoparticles display super paramagnetic or ferromagnetic characteristics in normal human body temperature. In a magnetic field less than the threshold value, the chemically synthesized nanoparticles' specific absorption ratio (SAR) is much less than that of magnetosome nanoparticles. For this reason, it is claimed that it would be more advantageous to use magnetosomes. Hence, the interest in the "magnetic hyperthermia" by using magnetosomes for cancer treatment has increased[10].

Practical applications

of magnetosomes are based on their ferrimagnetism, nanoscale size, narrow size distribution, dispersal ability, and membrane-bound structure. Bacterial magnetosomes have been used experimentally as carriers of enzymes, antibodies for highly sensitive immunoassay, and as efficient sorbents for isolation and purification of DNA or RNA. Artificial magnetic nanoparticles have been used as carriers for cancer diagnosis and targeted therapy in experimental animals. Similarly, magnetic nanoparticles enclosed in biological membranes can be linked to genes or drug molecules and thus could be used as carriers of drugs for targeted therapy of tumors. Several recent reports indicate that purified, sterilized magnetosomes from M. gryphiswaldense MSR-1 are non-toxic for mouse fibroblasts in vitro, and may be useful as carriers of genes, or drugs for cancer therapy or other diseases. However, the applications of magnetosomes have not yet been developed commercially, mainly because magnetotactic bacteria are difficult to cultivate and consistent, high yields of magnetosomes have not yet been achieved[11].

Membrane and proteins

The formation of the magnetosome requires at least three steps:
1. Invagination of the magnetosome membrane (MM)
2. Entrance of magnetite precursors into the newly formed vesicle
3. Nucleation and growth of the magnetite crystal

During the first formation of an invagination in the cytoplasmic membrane is triggered by a GTPase. It is supposed this process can take place amongst eukaryotes, as well.
The second step requires the entrance of ferric ions into the newly formed vesicles from the external environment. Even when cultured in a Fe^{3+} deficient medium, MTB succeed at accumulating high intracellular concentrations of this ion. It has been suggested that they accomplish this by secreting, upon need, a siderophore, a low-molecular-weight ligand displaying an elevated affinity for Fe^{3+} ions. The "Fe^{3+}-siderophore" complex is subsequently moved in the cytoplasm, where it is cleaved. The ferric ions must then be converted into the ferrous form (Fe^{2+}), to be accumulated within the BMP; this is achieved by means of a transmembrane transporter, which exhibits sequence homology with a Na^{+}/H^{+} antiporter. Furthermore, the complex is a H^{+}/Fe^{2+} antiporter, which transports ions via the proton gradient. These transmembrane transporters are localized both in the cytoplasmic membrane and in the MM, but in an inverted orientation; this configuration allows them to generate an efflux of Fe^{2+} ions at the cytoplasmic membrane, and an influx of this same ion at the MM. This step is strictly controlled by a cytochrome-dependent redox system, which is not yet fully explained and appears to be species-specific.
During the final stage of the process, the magnetite crystal nucleation is by action of transmembrane proteins with acidic and basic domains. One of these proteins, called Mms6, has also been employed for the artificial synthesis of magnetite, where its presence allows the production of crystals homogeneous in shape and size.
It is likely that many other proteins associated with the MM could be involved in other roles, such as generation of supersaturated concentrations of iron, maintenance of reducing conditions, oxidization of iron, and partial reduction and dehydration of hydrated iron compounds[12].

Biotechnology applications

In certain types of applications, bacterial magnetite offers several advantages compared to chemically synthesized magnetite. Bacterial magnetosome particles, unlike those produced chemically, have a consistent shape, a narrow size distribution within the single magnetic domain range, and a membrane coating consisting of lipids and proteins. The magnetosome envelope allows for easy couplings of bioactive substances to its surface, a characteristic important for many applications.
Magnetotactic bacterial cells have been used to determine south magnetic poles in meteorites and rocks containing fine-grained magnetic minerals and for the separation of cells after the introduction of magnetotactic bacterial cells into granulocytes and monocytes by phagocytosis.

Magnetotactic bacterial magnetite crystals have been used in studies of magnetic domain analysis and in many commercial applications including: the immobilization of enzymes; the formation of magnetic antibodies, and the quantification of IgG; the detection and removal of Escherichia coli cells with a fluorescein isothiocyanate conjugated monoclonal antibody, immobilized on magnetotactic bacterial magnetite particles; and the introduction of genes into cells, a technology in which magnetosomes are coated with DNA and "shot" using a particle gun into cells that are difficult to transform using more standard methods.
However, the prerequisite for any large-scale commercial application is mass cultivation of magnetotactic bacteria or the introduction and expression of the genes responsible for magnetosome synthesis into a bacterium, e.g., *E. coli*, that can be grown relatively cheaply to extremely large yields. Although some progress has been made, the former has not been achieved with the available pure cultures.

Biophysics of magnetotactic bacteria

Each MTB has iron particles that can be seen with an electron microscope. Before knowing the chemical condition of the iron within, it was understood that every cell has a constant magnetic dipole moment. When the bacteria that were obtained from an environment rich with mud were subjected to a magnetic pulse of a few hundred Gaus for 1 μs anti-parallel to the swimming direction, it was seen that the cells were momentarily magnetized in magnetic pulses of 200-800 Gaus pulse magnitudes. It was observed that the magnetized cells made a U-turn and began to swim in the opposite direction. This result shows that each bacterium has a specific magnetic moment. In order to understand how the interaction between MTB and the Earth's magnetic field takes place, the cell's magnetic moment should be taken as basis. The main factor that orientates the bacterium in the direction of the magnetic field is the interaction energy between the bacterium's magnetic moment and the outer magnetic field. The bacterium's thermal energy is the factor that makes the bacterium swim randomly, and it is directly proportional of the environment's temperature (thermal energy=kT: k-Boltzmann constant, T-Temperature). In order for the MTB to orient themselves in the direction of the magnetic field, the interaction energy and the outer magnetic field must be greater than the thermal energy. The Langevin function can be used to compare these two energies[13].

In the classical paramagnetic theory, the Langevin function defines the average array of the magnetic dipole groups that do not interact with each other within a magnetic field. The Langavin function is defined with the following equation. $\text{Cos}\theta=L\ (MH/kT)$.

In the equation above, θ is the angle between the (H) direction of the environment's magnetic field and the bacteria's magnetic moment direction (M). The average array in an outer magnetic field is determined by the ratio of the applied magnetic field and the bacterium's magnetic field (MH) to the thermal energy of the bacterium (kT). When the MH/kT ratio exceeds 10, the

magnetic moments of the particles in the bacterium become aligned with the magnetic field. Therefore, the MTB become aligned with the magnetic field direction[14].

Different approaches have been used to determine the dipole moment magnitude of MTB. The studies showed that some magnetosomes contain magnetic iron oxide, also known as lodestone. In another study, the shape, size and average number of magnetosomes in a cell were studied. It was determined that each cell contains approximately 20 magnetosomes with an aligned edge length of 420 A°. However, it is not completely clear how the MTB limit the magnetosome dimensions to a single active domain, and why they are in a chain. In addition, it is thought that this limitation is caused by the interaction between the membrane that encompasses the magnetic particle and the cell. The magnetic domains that are in a single chain are aligned such that the magnetic axes are parallel to each other. Therefore, the total magnetic energy of the magnetosome is the sum of all the magnetic moments of each particle. The magnetic moment of MTB is responsible of aligning according to the Earth's magnetic field. These bacteria behave as ferromagnetic biomagnets[15].

Conclusion

In the treatment of many diseases, especially cancer, it is very important that the drugs only reach the tumoral cells (as a target cell), and that a point-shot is directed at these ailing cells. In order to avoid surgery and side effects of drugs, a target subject among current researchers in having bacteria and small scale robots cooperate. The idea that the drug to be carried to problematic cells by bacteria, in other words, using bacteria as little robots have been met with enthusiasm in the scientific community. Especially since they can swim in blood and have magnets within them, the use of magnetotactic bacteria by directing them to target cells with an applied magnetic field has been raised as a topic. As a result, the aim of this paper is to attract attention to these microorganisms that have become the spotlight in research and their medical applications. The increase number of studies and supports in this research field may open up new horizons.

Realization of point-to-point positioning of a magnetotactic bacterium (MTB) necessitates the application of a relatively large magnetic field gradients to decrease its velocity in the vicinity of a reference position. We investigate an alternative closed-loop control approach to position the MTB. This approach is based on the characterization of the magnetic dipole moment of the MTB and its response to a field with alternating direction. We do not only find agreement between our characterized magnetic dipole moment and previously published results, but also observe that the velocity of the MTB decreases by 37% when a field with alternating direction is applied at 85 Hz. The characterization results allow us to devise a null-space control approach which capitalizes on the redundancy of magnetic-based manipulation systems. This approach is based on two inputs. The first controls the orientation of the MTB, whereas the second generates a field with alternating direction to decrease its velocity. This control is accomplished by the redundancy of our magnetic-based manipulation system which allows for the projection of the second input onto

the null-space of the magnetic force-current map of our system. A proportional–derivative control system positions the MTB at an average velocity and region of convergence of 29 μm s^{-1} and 20 μm, respectively, while our null-space control system achieves an average velocity and region of convergence of 15 μm s^{-1} and 13μm, respectively.

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