Morphological Changes in Magnetotactic Bacteria in Presence of Magnetic Fields

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Nanomagnets manufactured by magnetotactic bacteria hold immense promise in magnetically directed drug delivery. In spite of discovery of these bacteria nearly three decades ago, it is not known how the bacteria are able to keep the nanomagnets trapped inside biological membranes (vesicles called magnetosomes). Understanding the physical nature of interactions, which these nanomagnets are capable of, is essential for envisaging any directed drug delivery application. We analyzed the morphology of two magnetic bacterial strains, *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense*, by defining the features of individual bacteria in two dimensions as length and width (in microns) under different magnetic fields using bar magnets. The control morphologies were taken to be the features of bacteria not under the influence of any magnetic field other than the earth's own. Using analysis of variance (ANOVA), we found statistically significant morphological changes in the *M. magnetotacticum* under different conditions. In contrast, there were no morphological differences observed for *M. gryphiswaldense* under any conditions. The width of *M. magnetotacticum* was found to be significantly higher for the control conditions compared to any magnetic condition. The length of *M. magnetotacticum* was found to be significantly lower when only south poles of the bar magnets (single or couple) were towards the bacteria. These results reflect a possible difference in packaging of magnetosomes inside two different strains of magnetic bacteria and imply that it may be important to select the right microbial source of nanomagnets (in contrast to using just any strain), trapped inside biological membranes, for potential targeted drug delivery applications, whereby enhanced sensitivity to external magnetic fields would be preferred.

Keywords: Magnetotactic Bacteria, Magnetosomes, Nanoparticles, Morphology, Video Microscopy, Membrane Packing.

1. INTRODUCTION

Magnetically targeted drug delivery promises to overcome the chemically invasive nature of existing strategies that are utilized for homing in delivery vehicles like liposomes or biodegradable nanoparticles to their specific target cells. The concept of magnetic drug delivery is quite fascinating1–4 in the respect that, given sufficient magnetism attributes to the targeting vehicles, one can direct them to the place of interest in the body by using mild external magnetic field variations and subsequent to delivery, the magnetic components can be removed from the system using the same external magnetic fields. However, to have the concept turn to practicality requires manufacturing of nanomagnets, which are small enough to be entrapped inside the drug-carrier vehicles, but also magnetic enough to be responsive to mild external magnetic fields. Further, magnetic materials are more or less inert, i.e., non reactive, so trapping them inside the target vehicles with the ability to move these target vehicles while staying inside them also poses a challenge.

There are certain bacteria, first isolated within the last 30 years,5,6 which have the ability to “manufacture” magnetic nanoparticles (magnetite particles of the size of tens of nanometers) by consuming iron salts. These bacteria, called magnetotactic bacteria, naturally use the magnetite nanoparticles to sense the earth’s magnetic field and survive. Magnetotactic bacteria are motile, mostly aquatic prokaryotes, and are gram negative in nature. All magnetotactic bacteria synthesize unique intracellular structures called magnetosomes,5–11 which comprise a magnetic mineral crystal (magnetite or greigite)7,8 surrounded by a lipid bilayer membrane about 3–4 nm thick. It is already known that the biological production of nanoparticles, by these bacteria, allows a better control over important nanomagnet parameters such as size, uniformity in shape,
concentration, quantity, anisotropy. Clearly, these bacteria have somehow evolved mechanisms to trap well-designed nano-magnets inside biological membranes that allow high magnetic sensitivity for these bacteria to be able to respond to even small differences in the earth’s magnetic field at different locations.

Therefore, understanding the physical nature of interactions, which these nanomagnets are capable of inside the bacteria, is of paramount importance for envisaging any directed drug delivery applications using nanomagnets. With the aim to understand the physical nature of bacterial cells trapping nanomagnets, that show high sensitivity to mild magnetic fields, we selected two magnetotactic bacterial strains, *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense* for investigating whether the overall morphology of the bacteria was sensitive to variations in mild external magnetic fields. We report that one of the selected strains experiences changes in morphology in presence of mild external magnetic fields. We found that the other strain was found to be “morphologically insensitive” to the external magnetic fields.

2. EXPERIMENTAL SECTION

2.1. Materials

Magnetic bacterial cultures were procured from DSMZ, Germany. All reagents purchased from commercial sources were used as received. Vitamin solution and trace element solution were freshly prepared. Vitamin solution components: biotin, folic acid, pyridoxine-hydrochloride, thiamine-hydrochloride dihydrate, riboflavin, nicotinic acid, D-calcium-pantothenate, and Vitamin B12 were used as received. Vitamin solution and trace elements were filter sterilized and added to autoclaved water. This solution was autoclaved separately and added to remaining autoclaved media components. TES and vitamin solution were filtered sterilized and added to autoclaved media. For cultivation of *M. gryphiswaldense*, medium 512 contained (per liter): 0.45 g Fe(III) citrate, 0.01 M Fe(III) quinate solution was prepared by dissolving 0.45 g FeCl3 × 6H2O and 0.19 g quinic acid in 100 ml distilled water. This solution was autoclaved separately and added to remaining autoclaved media components. For cultivation of *M. magnetotacticum*, medium 380 contained (per liter): 0.5 g KH2PO4, 1 g Na-acetate, 0.1 g NH4Cl, 0.1 g yeast extract, 20 μm Fe(III) citrate, and 0.5 g Na-thioglycolate. The pH was adjusted to 6.8 and the medium was prepared 2–3 days before use. For both media freshly prepared Na-thioglycolate was filter sterilized and added just before inoculation. The media was dispensed in anoxic vials with screw caps. Anoxic conditions were maintained by purging nitrogen for 15 minutes. Sterile air was added with a hypodermic syringe through the rubber cap to a concentration of 1% (v/v) in the vial. To prepare semi-solid medium, agar was added to a concentration of 1.3 g/1000 ml media (only for medium 380).

2.2. Media Preparation

Media 380 and 512, as per DSMZ (Germany) nomenclature, were used for *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense*, respectively. The media were prepared following standard protocol provided by DSMZ. Medium 380, used for cultivation of *M. magnetotacticum* contained (per liter): 10 ml vitamin solution, 5 ml trace elements, the Fe(III) quinate solution, 0.5 mg resazurin, 0.68 g KH2PO4, 0.12 g NaNO3, 0.05 g Na-thioglycolate, 0.37L(+)-Tartaric acid, 0.37 g succinic acid, and 0.05 g Na-acetate. All the ingredients, except Na-thioglycolate, were mixed and the medium was boiled for 3 mins, after adjusting pH to 6.5 with NaOH. The medium was autoclaved at 121 °C for 15 mins. 0.01 M Fe(III) quinate solution was prepared by dissolving 0.45 g FeCl3 × 6H2O and 0.19 g quinic acid in 100 ml distilled water. This solution was autoclaved separately and added to remaining autoclaved media components. TES and vitamin solution were filtered sterilized and added to autoclaved media. For cultivation of *M. gryphiswaldense*, medium 512 contained (per liter): 0.45 g KH2PO4, 1 g Na-acetate, 0.1 g NH4Cl, 0.1 g yeast extract, 20 μm Fe(III) citrate, and 0.5 g Na-thioglycolate. The pH was adjusted to 6.8 and the medium was prepared 2–3 days before use. For both media freshly prepared Na-thioglycolate was filter sterilized and added just before inoculation. The media was dispensed in anoxic vials with screw caps. Anoxic conditions were maintained by purging nitrogen for 15 minutes. Sterile air was added with a hypodermic syringe through the rubber cap to a concentration of 1% (v/v) in the vial. To prepare semi-solid medium, agar was added to a concentration of 1.3 g/1000 ml media (only for medium 380).

2.3. Inoculation

The bacteria were grown in 250 ml flasks, with custom-made rubber caps, and 30 ml anoxic vials, with screw caps and air tight rubber lining. 10 ml of inoculum was added to 150 ml media in 250 ml flasks and 1 ml inoculum was added to 10 ml media in 30 ml vials. Inoculum was added with a hypodermic syringe through the rubber cap taking care not to introduce air. The bacteria were allowed to grow at 30 °C for 2 weeks and growth was monitored by carefully withdrawing small samples with a hypodermic needle and observing under a microscope. Quantitative assessment of bacterial growth was not done as a part of this study, since it was not required for the purpose of the work done here.

2.4. Video Microscopy

1 ml of sample was withdrawn carefully using a hypodermic needle from growing cultures for video microscopy, which was done using minor modifications of the protocol described previously. Briefly, the cells were observed...
by placing a drop of sample on a flat glass slide, covering with a cover slip, and observing under oil immersion at 100X magnification. 10 seconds videos were captured at a 33 frames/ms resolution. To determine the control morphologies cells were first observed under the influence of only the earth’s magnetic field. Then the cells were observed under the influence of magnetic field of bar magnets placed in different orientations—Single bar magnet once with its north pole towards the slide and once with its south pole towards the slide; Two bar magnets placed at the opposite ends of the slide, once with same poles towards the slide and once with opposite poles towards the slide. Different magnetic field conditions were defined as: Control → no bar magnet placed, S–S → when two bar magnets were held at opposite ends of the slide with their south poles facing each other, 1-S → when only one bar magnet was present, with its south pole towards the slide, 1-N → when only one bar magnet was present with its north pole towards the slide, N–N → when two bar magnets were held at opposite ends of the slide with their north poles facing each other, N–S → when two bar magnets were held at opposite ends of the slide with the north pole of one magnet facing the south pole of the other magnet.

The size of the bar magnets used was 7.5 cm × 1.5 cm × 1 cm. The magnetic field strength, produced by the bar magnets, was measured using a Digital Gaussmeter (Model DGM 202, Scientific Instruments, Roorkee, India). It varied from 22 Gauss when opposite poles were towards the slide and 14 gauss for a single bar magnet to 7 gauss for two bar magnets with same pole towards the slide.

2.5. Measuring Morphological Changes and Statistical Methods

For analysis 300 frames, 33 ms apart, were extracted from each 10 s video. Using Scion Image (Scion Corporation), the changes in cell morphology were measured. The morphology was defined in terms of length and width of the bacteria, for the two dimensional images. The number of single bacterial cells observed under each condition varied from 10 to 30. The results were analyzed by Single Factor Analysis of Variance (ANOVA) with $\alpha = 0.05$ in Microsoft Excel.

3. RESULTS AND DISCUSSIONS

Both strains, *M. gryphiswaldense* and *M. magnetotacticum* were grown under microaerobic conditions. Samples were withdrawn for microscopy. Fast moving magnetotactic bacteria were easily visible with the 100X objective. Figure 1 shows two representative images for both strains. Presence of nanomagnetic particles inside the bacterial cells was confirmed by TEM, as shown in Figure 2. The images show chains of magnetosomes inside one cell each of both strains. Some of these vesicles were observed to be darker than the others. This is thought to be due to the entrapped nano-magnetite particles with varying densities. It can be seen from Figures 1 and 2 that there were no apparent morphological differences between either the nanomagnet packing inside the bacteria, or, the overall morphology of the two bacterial strains.

After successfully culturing the magnetotactic bacteria and ensuring the presence of nanomagnetic particles inside the bacterial cells, we observed the behavior of the bacteria under the microscope while subjecting the cells to different external magnetic fields. This was done by placing different combinations of bar magnets at opposite ends of
the microscope slide. Different conditions were defined as described in the experimental section on video microscopy. The arrangement of magnets is shown in Figure 3. For each of these conditions, 10 second videos were also captured to assess motility characteristics of the bacteria. Using Scion Image, imaging software, we measured the maximum and minimum lengths of each bacterial cell in separate frames, 33 ms apart. Figure 4 shows our definitions of the morphological features of the bacterial cells in terms of the “width” and “length” used by us.

Morphological features of the bacterial cells for both strains were compared under a variety of external magnetic conditions. Figure 5 shows the length (closed gray bars) and width (open bars) of 10–30 bacterial cells measured under each condition. In spite of the fact that a statistically relevant number of single bacterial cells were measured, to account for possible variations in measurements, the S–S condition was done in independent triplicate experiments, for the strain M. gryphiswaldense (as shown in Fig. 5), to confirm that possible variations within the same condition were statistically insignificant. All the results obtained were analyzed by single factor ANOVA (analysis of variance), to find out whether there was a difference within the groups and/or between the groups. The results are summarized in Table I, which contains the p-values obtained from ANOVA. The p-values indicate the probability that the data sets being compared are similar. By convention, for biological systems, a p-value greater than 0.01 indicates that the data sets are similar while a lower value implies statistically significant difference in the data sets.

For M. magnetotacticum, when the length of cells under all the conditions were compared with each other, the p-value obtained was 6.7 × 10^{-4} < 0.01. This indicated a strong dissimilarity in the length of cells under different magnetic field conditions. Further, by removing data for one set of conditions at a time, it was seen that the difference in lengths was arising due to the decrease in the length of bacteria under S–S and 1-S conditions (also shown by * in Fig. 5). Following the same procedure for the width, it was observed that the control set (no bar magnets) showed the significantly higher width as compared to cells subjected to external magnetic field (also shown by ** in Fig. 4). In contrast to these results, the data condition.
for *M. gryphiswaldense* did not show any such changes in morphology in the presence of external magnetic fields (*p* > 0.01).

The above results clearly show that the strain *M. magnetotacticum* reveals significant morphological sensitivity to the presence of mild external magnetic fields. While the length defined by us was found to be sensitive to presence of south-pole induced magnetic field (the length became shorter in presence of one or two south poles), the width became significantly shorter in presence of any magnetic field condition.

4. CONCLUSIONS

How is it possible to pack magnets inside phospholipids membranes? Theoretically, in absence of any chemical bonding, the nanomagnet should just “pop out” of the membrane boundary based on sheer magnetic force when attracted by another magnet. However, magnetotactic bacteria have evolved some strategy to physically hold the magnets inside membranous compartments. While exploring the physical nature of the interaction of these bacteria, driven by their nanomagnets, with mild external magnetic fields, we already have made an interesting observation. While one of the strains shows no morphological sensitivity to external magnetic field, the other strain shows sensitivity in both morphological features defined by us. In one of the features, this sensitivity is even more specific to just one kind of external magnetic field (that produced by south poles of our bar magnets). Our results may be explained by considering difference in the packaging of magnetosomes inside the bacteria. As *M. magnetotacticum* cells show changes in their morphology under different conditions, it may indicate that they contain a more flexible arrangement of magnetosomes as compared to *M. gryphiswaldense*. Since the magnetosomes contain nanomagnets, a flexible arrangement would allow the chain of magnetosomes to reorient in response to different magnetic fields. In nature, such an arrangement of magnetosomes might be useful where microaerobic conditions are harder to find. By rapidly reorienting their magnetosome chains the bacteria might be able to search for viable growth conditions more quickly.

While recent strain specific genetic studies have started addressing questions about packaging of magnetosomes in magnetotactic bacteria, the physical nature of interactions of the nanomagnets within these bacteria is still largely not understood. Whatever the reason(s) may be for the differences observed by us for the two different strains studied in this work, our results may have great importance in designing magnetically targeted drug delivery systems using nanomagnets from magnetotactic bacteria. In principle, our results clearly indicate that it may be important to have the right choice of nanomagnets (i.e., source of nanomagnets) for a particular delivery system, morphology of which can be modulated in presence of external magnetic fields based on the very source of nanomagnets (i.e., possibly the bacterial cultures) that are to be trapped inside the delivery vehicles. Further investigations on the differences observed by us promise to shed light on mechanisms by which magnetic targeted drug delivery vehicles with desired qualities can be prepared.

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References and Notes


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Nanomagnets manufactured by magnetotactic bacteria hold immense promise in magnetically directed drug delivery. By defining the features of single bacterial cells in two dimensions, while observing them under a microscope, we analyzed the morphology of two strains of magnetotactic bacteria under different conditions using bar magnets. While one strain showed remarkable morphological changes in response to external magnetic fields, the second strain was found to be morphologically insensitive under similar conditions. These results reflect a possible difference in packaging of magnetosomes inside two different strains of magnetic bacteria and imply that it may be important to select the right microbial source of nanomagnets, in contrast to using just any strain for potential targeted drug delivery applications.