NSOM/HRTEM Characterization of Biologically Derived Cubo–Octahedral Nanomagnets

Mohit Naresh1, Kalon Gopinadhan2, Srivats Sekhar2, Prabhjot Juneja2, Manish Sharma3, and Aditya Mittal4

1Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi 110016, India
2Department of Physics, Indian Institute of Technology Delhi, New Delhi 110016, India
3Centre for Applied Research in Electronics, Indian Institute of Technology Delhi, New Delhi 110016, India
4School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi 110016, India

Nanomagnets synthesized by the magnetotactic bacterium *Magnetospirillum gryphiswaldense* have been characterized. Transmission electron microscopy (TEM) shows that the nanomagnets have a mean diameter of 38 nm ± 10 nm. Structural analysis done using high-resolution TEM (HRTEM) indicates that, while the nanomagnets appear spherical in shape, crystal planes of magnetite are observed on the cubo–octahedral facets. Microdiffraction analysis suggests that the magnetic nanoparticles have a composition close to Fe3O4 and are crystalline. Utilizing a near-field scanning optical microscope (NSOM), we report insights into the magnetic properties of the nanomagnets inside bacterial cells with imaging done on single cells. The experimental design relied on performing magnetization measurements using the magneto–optic Kerr effect (MOKE). The NSOM-MOKE image clearly shows the magnetization of the nanomagnets, while still aligned in continuous chains inside the bacterial cells.

Index Terms—Biomagnetics, electron microscopy, magnetooptic Kerr effect, magnetotactic bacteria, *magnetospirillum gryphiswaldense*, nanomagnets, near-field scanning optical microscope (NSOM).

I. INTRODUCTION

ANODIMENSIONAL magnetic materials hold immense promise in a variety of applications. These range from development of biomedical techniques to utilization in memory and energy storage devices, and conceptualization of products useful in translational biological work such as biosensors and biomarkers [1]–[5]. Control over physical and chemical properties such as shape and crystal anisotropy, size distribution, sensitivity to magnetic fields, existence of a single magnetic domain, and biocompatible surface functionalization for biological and biomedical purposes is essential for envisioning potential applications of magnetic nanoparticles. While different methods of chemical synthesis of magnetic nanoparticles do exist, they do not allow for precise control over one or more of the aforementioned properties. Moreover, chemical synthesis is required to be carried out under harsh conditions (e.g., high temperature and pressure) and results in nanoparticles that can be transiently magnetized at best. In contrast, there is a class of natural bacteria, called magnetotactic bacteria, that synthesize nanomagnets by consuming iron from the environment [6], [7]. Magnetotactic bacteria are aquatic prokaryotes, with microbiological properties of being gram negative and obligate microaerophilic in nature. They synthesize nanomagnets of magnetite or greigite by consuming iron salts, with a very robust control over physicochemical properties. Most importantly, biofriendly synthesis of nanomagnets by these bacteria occurs at very mild conditions (at room temperature and atmospheric pressure) compared to chemical methods [8], [9].

In this work, we employ high-resolution transmission electron microscopy (HRTEM) and near-field scanning optical microscopy (NSOM) to initiate development of a methodology for rigorously understanding the magnetic behavior and characteristics of magnetotactic bacterial cultures on a single cell as well as population levels [10]. We report, for the first time, NSOM measurements on the magnetic behavior of the nanomagnets while still inside bacterial cells, on the level of single cells. This work promises to lead to a thorough understanding of magnetic characteristics of individual bacterial cells. It will also help in correlating magnetic characteristics with the observed population features of bacterial cultures. In addition, it will also certainly allow the development of better strategies for developing large scale production of biologically derived nanomagnets.

II. MATERIAL AND METHOD

A. Media Preparation and Culture Cultivation

Magnetic bacterial cultures were procured from DSMZ, Germany. *Magnetospirillum gryphiswaldense* was grown in anoxic vials. Medium for culturing the bacteria was prepared as described previously [4], [11]. After adjusting pH to 6.5 with NaOH, the medium was autoclaved at 121 °C for 15 min. Filter sterilized vitamin solution, autoclaved trace element solution (TES), and freshly prepared 0.01M Fe(III) quinate solution were added to the autoclaved medium. The medium was dispensed in anoxic vials with screw caps and anoxic conditions were maintained by purging with nitrogen for 15 min. To prepare semisolid medium, agar was added to a concentration of 1.3 g/1000 mL media. Sterile air was added with a hypodermic syringe through the rubber cap to a concentration of 10% (v/v) in the vial and kept for 24 h to set redox gradient. Freshly prepared 0.2-μm filter sterilized Na-thioglycolate was added to the vials prior to inoculation. The bacteria were allowed to grow in the incubator at 28 °C and, as seen in Fig. 1(a), it was observed that the bacterial growth happens in a layer that moves with respect to redox gradient. The growth and purity of the culture was monitored by performing gram staining [Fig. 1(b)] and observing under optical microscope at different time inter-
Fig. 1. Cultivation of *M. gryphiswaldense*. (a) Anoxic 10-mL vials with semisolid medium at the end of sixth day. (b) Optical micrograph of gram-stained bacteria. (c) Electron micrograph of a single cell of *M. gryphiswaldense* showing nanocrystals of magnetite as magnetosomes arranged in a long chain.

Fig. 2. TEM image of nanomagnets collected after lysing bacterial cells. The size distribution (inset (a)) of the nanomagnets is 38 nm ± 10 nm.

vals. The grown bacteria were then examined using electron microscopy and found to contain chains of magnetosomes [Fig. 1(c)]. This is described in more detail in Section III.

B. Specimen Preparation for HRTEM and NSOM

Approximately $10^8$ cells/mL were centrifuged (10 000 r/min; 15 min) for the analysis. The bacterial cells were washed five times in buffer solution, fixed with Karnovsky’s fixative and kept at 4 °C for 4 h. After fixing, the cells were washed five times by suspending in buffer and the cell suspension was loaded on 300 mesh copper grid, stained with 2% (w/v) ammonium molybdate (Merck, Darmstadt, Germany) (pH 7 for 1 min), air dried, and examined under transmission electron microscope (TEM) operating at 80 kV. High-resolution transmission electron microscope (HRTEM) images for crystal studies were done on a separate electron microscope operating at 200 kV.

For NSOM, approximately $10^8$ cells/mL were centrifuged and the pellet was washed five times by suspending in buffer solution. Cell sample of 10 μL was loaded on glass slide, dried at room temperature, and analyzed under the microscope. A Nanonics NSOM/AFM system was used and images collected simultaneously by operating in the atomic-force microscope (AFM) mode for surface profile imaging and in the magneto–optic Kerr effect (MOKE) mode for magnetization contrast imaging of the sample [10]. The system had provision of an electromagnet for applying a direct current (dc) magnetic field to the sample to influence the bacteria.

III. RESULTS AND DISCUSSION

A. Structural Characterization of Magnetosomes by HRTEM

It has been previously established that magnetotactic bacteria contain inorganic crystals of Fe₃O₄ in the range ~ 25–120 nm in diameter, which is in permanent single magnetic domain size range [12], [13]. The analyses of TEM images [Fig. 1(c)] of the cultured magnetotactic bacteria have been done for 15 cells and concluded that individual cells contain roughly 12–40 magnetosomes beautifully aligned in chains. In addition, after lysing the cells, nanomagnets were collected together and a large number were analyzed for size. As seen in Fig. 2, each magnetosome has an average diameter size of 38 nm ± 10 nm. For biologically synthesized nanomagnets, a size distribution of 10 nm can be considered very good, although other synthesis techniques
such as high-temperature decomposition [14] or coprecipitation [15] can be used to get much tighter size distributions.

These results are in strong agreement with the previously reported uniform size distributions with a narrow size range for the bacterial nanomagnets. They have also been reported to consist of a permanent, single magnetic domain of magnetite [12], [13]. However, the extent of homogeniety (or heterogeniety) in the morphology of these crystals has not been investigated in detail. During our investigations, we noticed the presence of presumably immature magnetite (Fe$_3$O$_4$) crystals at the edges of the chains, as indicated by their smaller crystal size, in comparison to other crystals as also seen in Fig. 1(c).

Therefore, for structural characterization of the nanomagnets synthesized by the magnetotactic bacteria, we carried out detailed HRTEM studies. Data obtained from these studies, i.e., the images, show that depending on the orientation of crystals of bacterial nanomagnets with respect to the electron beam, they appear to be of slightly different shapes (such as dodecahedron, octahedron, or cubic) in the diffraction contrast (see Fig. 3).

The varying contrasts in the magnetite crystals [Fig. 3(a)] may also be due to different mineral density. Thus, while our results confirm that the bacterial nanomagnets are uniform as reported previously, the morphological distributions in the chains have variations towards the end of the chain compared to the crystals placed at the center of the chain. Our results indicate the possibility of positional variations in magnetic crystal synthesis by the bacterial cells, and raise the question of whether they nucleate and mature in similar manner to the central matured crystals. However, detailed investigations regarding this are beyond the scope of this work.

The HRTEM image data were also used to index the nanomagnets for their crystallographic phase and lattice constants in the reciprocal space. The calculated values match well with standard $d$ values of face-centered cubic spinel Fe$_3$O$_4$ structure (Fd3m space group). The calculation of $d$ for 30 different crystals was repeated. We found that, for each nanomagnet in the chain, selected area electron diffraction (SAED) data at the edges and at the center have similar $d$ values, thus indicating that, regardless of the minor differences in size, crystals have the same morphology.

Having found similar crystal morphologies, the next step was to understand the magnetic behavior of crystals inside the bacterial cells. The mild variations in the electron diffraction patterns for individual crystals observed by us would be expected to lead to correspondingly mild variations in the magnetic moments of the nanomagnetic crystals inside the bacterial cells (regardless of the reasons for the variations). This is confirmed from the AFM and NSOM data acquired simultaneously on the same samples, with representative images shown in Fig. 4.

The AFM images [Fig. 4(a)] do not show much variation in bacterial cell morphologies. However, mild variations are observed in the dark-light pixel contrast [Fig. 4(b)], due to variations in magnetic moments.

### B. NSOM Imaging of Magnetosomes

After studying crystal sizing and morphology of the nanomagnets, we now discuss their magnetic characterization. In TEM, specimen preparation is a major problem in imaging magnetotactic bacteria as samples need to be thin enough for the electron beam to pass through. In addition, the TEM environment precludes imaging of live bacteria. NSOM imaging comes handy in this situation since it can image both living and dead bacteria with the same accuracy. To measure the magnetization signal from the nanomagnets inside the bacteria, we performed NSOM-MOKE imaging directly on air dried magnetotactic bacteria after washing with buffer solution. As can be seen in Fig. 4, in the AFM image, the shapes of the bacteria are clearly visible, although the exact location of the chains cannot be discerned. In the NSOM-MOKE image, both the shape and the presence of the chains inside the bacteria are clearly visible. It should also be noted that the strong black-and-white contrast is much broader than the physical size of the individual nanomagnets inside the chains [Fig. 1(c)]. It should be noted that this is only partly indicative of the extent of the magnetic signal present inside the bacteria since the NSOM tip has a curvature of 80 nm, which sets a limit on the resolution of the NSOM-MOKE image.

To understand the influence of external magnetic field, the NSOM tip was positioned on a single bacterium and NSOM-MOKE imaging performed in the presence of an external magnetic field of up to 80 kA/m. In Fig. 5(a), it can be clearly
observed that no chain or signal has been observed in AFM, and correspondingly when NSOM has been performed, a clear change in magnetization contrast has been detected when the magnetic field was reversed [Fig. 5(b) and (c)]. This verifies the existence of magnetic dipoles in the bacteria and that their magnetization can be reversed by applying a magnetic field. Considering the proximity of the tip and the sample, and the pole pieces of the electromagnet, significant care is needed to avoid the strong field from the electromagnet moving the substrate stage or causing local heating. In our NSOM system, to get the best image in each scan, slight adjustments have to be made in the polarizer/analyzer present before the photodetector collecting the NSOM-MOKE signal. These appear as slight contrast shifts in the nonmagnetic regions between Fig. 5(b) and (c). However, since the nanomagnet signal occurs with much stronger contrast (significantly darker/brighter areas), it is reasonable to consider these background shifts as artifacts.

IV. CONCLUSION

We have synthesized nanomagnets with a mean diameter of 38 nm and narrow size distributions. Structural analysis done using HRTEM techniques indicates the nanomagnets are monocristalline and have cubo–octahedral facets. Using NSOM-MOKE, we characterized the nanomagnets while still inside magnetosomes in the bacteria. The NSOM-MOKE images clearly show the magnetic field produced by the long chains of nanomagnets. We are currently developing the technique further to quantify the magnetic field produced by the nanomagnets and observe the motion of live bacteria when influenced by external magnetic fields.

ACKNOWLEDGMENT

This work was supported by the Department of Biotechnology India, SERC Department of Science and Technology India, and the IRD Unit at Indian Institute of Technology Delhi (IIT Delhi), New Delhi, India. HRTEM work was done with the help of V. Singh at the HRTEM Facility of IIT Delhi.

REFERENCES

[9] A. Mittal, “Women are from venus, are magnetic bacteria from mars?,” Nature India DOI: 10.1038/nindia.2008.216.