Synthesis and magnetic characterization of microstructures prepared from microbial templates of differing morphology

Rakesh Mogul a,*, John J. Getz Kelly b, Morgan L. Cable a, Arthur F. Hebard b

a Harriet L. Wilkes Honors College, Florida Atlantic University, Jupiter, FL, 33458, USA
b Department of Physics, University of Florida, Gainesville, FL 32611, USA

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Abstract

In this study, we demonstrate the use of bacterial templates of differing morphology for the facile synthesis of magnetic micron-sized spheres, tetrad of spheres, filaments, and coils. These microstructures were synthesized by a platinum catalyzed electroless deposition of nickel under mild aqueous conditions using Deinococcus radiodurans, genetically elongated Escherichia coli, and Rhodospirillum rubrum as templates, respectively. Analysis by SEM/EDS, TEM, and SQUID magnetometry indicates the formation of superparamagnetic metallic microfilaments, which possess a blocking temperature of ~70 K with no observable exchange anisotropy. XPS analysis of the metallic microfilaments supports the presence of NiB, which is diamagnetic, thus supporting the hypothesis that the morphologically unique microbial templates are encapsulated within a biosurface-bound NiB shell that contains embedded nickel nanoparticles (12–20 nm). The use of microorganisms as templates, which may be recombinantly modified, may therefore serve as alternative means for magnetic microstructure and core–shell synthesis.

The integration of biology into materials research is projected to bring forward significant advances in microfabrication and assembly [1–3]. Namely, the self-assembling and self-reproducing properties of biological systems are amenable to the ordering and repair requirements for advanced materials. In the work presented herein, we describe the use of microorganisms of differing morphology as templates for the fabrication of magnetic microspheres, microfilaments, and microcoils (Fig. 1). These structures have many potential applications in the development of micro-electromechanical, fluidic, injection, and optical systems [4,5]. Moreover, microorganisms are attractive alternatives to lipid tubules and vesicles [6,7], DNA [8], and inorganic templates [9,10] due to their low costs, varying shapes, and self-replicative properties.

Bacteria were chosen, out of the vast selection of microorganisms, as model templates due to their differing morphologies (i.e. cocci, bacilli, and spirilla), commercial availability, ease of preparation/handling, and potential for genetic manipulation. By utilizing the metal-binding properties of bacterial surfaces, we have developed an aqueous electroless deposition procedure that yields monomeric magnetic nickel microstructures that retain the shape of the template’s morphology. Deinococcus radiodurans [11], elongated Escherichia coli [12], and Rhodospirillum rubrum were chosen as model bacterial templates to yield particle shapes of spheres, filaments, and coils, respectively.

All microbiological and chemical experiments were performed under appropriate sterile and preparative conditions to avoid interferences from biological contamination and chemical side products. E. coli, which are normally ~1 μm in length, were elongated using genetic techniques by controlled expression of a recombinant ftsZ gene. Overexpression of this gene [12], which is associated with assembly and function of the cytoskeleton, typically resulted in lengths of 10–80 μm, though lengths of several hundreds of microns could also be obtained.

* Corresponding author. Current address: Chemistry Department, Cal Poly Pomona, Pomona, CA, 91768, USA. Tel.: +1 909 869 3662.
E-mail address: rmogul@csupomona.edu (R. Mogul).

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Prior to all deposition experiments [13], the microbial cultures (1–2 mL) were harvested by centrifugation (2 min, $\approx 3000 \times g$) and washed by fully resuspending the cell pellet in 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5. This process was repeated a total of three times to insure complete removal of all constituents of the growth media. Cells ($10^6$ – $10^8$ cell/mL) were then activated using 2 mM K$_2$PtCl$_4$ in 25 mM HEPES (pH 7.5) for 40 min, then harvested by centrifugation, and the cell pellet washed with buffer. Metallizations of $10^6$ – $10^8$ cells/mL were performed using 25 mM NiSO$_4$ and 42 mM (2.5 g/L) dimethylamine borane in 25 mM HEPES (pH 7.5). Reactions were carried out at room temperature for $\approx 35$ min or until a black suspension was formed. Upon completion, the metallized cells were harvested, resuspended, and washed twice using both buffer and water. Aqueous suspensions of the metallized microstructures were dried onto microscope slides, aluminum stubs, formvar grids, or Si substrates under moderate vacuum and characterized by light microscopy, SEM/EDS, TEM, XPS, and SQUID magnetometry [14].

Light microscopy was used as a crude measure of reaction progress due to significant increases in opaqueness of the nickel deposited cells. SEM/EDS analysis revealed the synthesis of metallized spheres, tetrads of spheres, filaments, and coils (Fig. 1A–C) with EDS showing the presence of both nickel and carbon (Fig. 2), thereby confirming the deposition of nickel onto the bioorganic template. Typical dimensions for the metallized filaments were $\approx 1$ µm in width and $\approx 45$–80 µm in length (Figs. 1B and 3A), and $\approx 1$ µm in width and $\approx 9$ µm in length for the metallized coils (Fig. 1C). As judged from the rough edges of the imaged filaments (Figs. 1C and 3B), a width of 40–50 nm for the metallized membranes was obtained, which suggests metallization of both the inner and outer membranes of *E. coli* (which typically spans 30–40 nm). Surface substructures also observed in the SEM images (Fig. 3B) were possibly due to metallization of surface nanostructures such as the pili or flagella. Furthermore, TEM analysis of the metallized filaments (Fig. 3C) showed no significant transmission, supporting the formation of a fairly thick metallic coating on the exterior of the microbial template. Dried samples showed no significant degradation as measured by SEM analysis over the course of a few weeks. As expected, degradation of the samples was observed by light microscopy when suspended in $\approx 0.01\%$ aqueous dextrin solutions after 3–5 days. Aggregation of the metallic structures was also dependent on solute identity and concentration with HEPES, dextrin, and cysteine having favorable stabilization properties.

The magnetic properties of the metallic microstructures were assessed using a SQUID magnetometer (MPMS-5S, Quantum Design) and by crude attraction studies using a permanent magnet. Magnetic measurements were carried out between 10 and 300 K at varying applied fields. All data were corrected for background from the diamagnetic Si substrate but were not normalized to sample mass or volume. The effect of applied field on the total magnetic moment for the microstructures (Fig. 4) was temperature dependent with an appearance of hysteresis occurring below 100 K (Fig. 4A). This is indicative of superparamagnetic behavior where hysteresis appears below a blocking temperature ($T_b$) [15]. The blocking temperature for the metallized filaments, using an applied field of 100 Oe, was determined to be $\approx 70$ K from the peak in the zero-field cooled (ZFC) magnetization versus temperature plot (Fig. 4B).
4B). The approximate size of the magnetic particles was also estimated using the relation:

\[ T_b = \frac{(KV)}{30k} \]  

where \( k \) is Boltzmann’s constant, \( V \) is the volume of the particle, and \( K \) is the anisotropy constant. For these calculations, \( K \) is roughly estimated by Eq. (1) utilizing the relation:

\[ K = \frac{K_1}{12} \]  

which assumes that the magnetic particles are non-interacting, spherical bcc particles with a <111> easy axis [16], and by Eq. (2) using literature-accepted values for the first order magnetocrystalline anisotropy constant \( K_1 \) (\(-8 \times 10^5 \) and \(-15 \times 10^5 \) erg/cm\(^3\)) [16–18]. Note, that the \( K_1 \) value of \(-8 \times 10^5 \) erg/cm\(^3\) applies for bulk Ni at low temperatures [16], while \(-15 \times 10^5 \) erg/cm\(^3\) is an experimentally determined value using Ni nanoparticles [17,18]. Using these assumptions, nickel particle sizes of \(~12–20\) nm are obtained. Since particle interactions generally act to increase blocking temperatures when measured by the ZFC techniques [19], we can view these sizes as rough upper bounds. It is reasonable to assume, therefore, that the Ni nanoparticles are encapsulated in a matrix of either diamagnetic NiB or anti-ferromagnetic NiO due to our use of a borane reductant under aerobic aqueous conditions [20,21]. The lack of observed exchange anisotropy [16,17] for the magnetic samples (Fig. 4C), however, is not consistent with a NiO shell since the anti-ferromagnetic NiO should exchange bias the Ni nanoparticles. In contrast, initial XPS analysis (Fig. 5) shows the detection of dual peaks at \(~187\) and \(~196\) eV, which is suggestive of the presence of Ni–B and O–B bonds, respectively [20,22]. Further spectral analyses on the bio-metallic structures are currently underway.

Together, the combined data and analyses support the formation of a superparamagnetic material, which consists
of nanoscale Ni particles embedded within a biosurface-bound NiB shell or matrix. In further support, light microscopy and infrared spectroscopic analysis of reaction products isolated in magnetic attraction studies, show the presence of microstructures possessing the correct morphologies and functional groups (CH and amide stretches) associated with bacteria; thus supporting the formation of magnetic bio-metallic microstructures.

To conclude, the outlined methods and data represent an alternative and rapid approach towards synthesizing magnetic microstructures of differing shapes. Microbes (both prokaryotic and eukaryotic) are inexpensive starting materials and exist in variety of shapes and arrangements (e.g. tetrads, cubes, chains of cells, and ordered mats); hence their uses as templates have yet to be fully realized. Potential examples include metallized cocci, bacilli, and spirilla as precursors for ferrofluids, microtubes, micron-sized springs, heat exchangers, and solenoid coils. Furthermore, our use of a recombinant E. coli strain as a template also demonstrates the use of bacterial genetics as a means to confer morphological and/or compositional changes within the cell. Metallization or encapsulation of recombinant bacteria may therefore lead to novel strategies for the development and synthesis of new micron-sized core–shell materials.

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References

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Fig. 5. XPS analysis (B 1s) of the metallized filaments.