Enhancing Magnetic Nanostructures for Biomedicine

Undergraduate Researcher
Jagannath Sam Nayak
Illinois Mathematics and Science Academy

Faculty Mentor
Vinayak Dravid
Department of Materials Science and Engineering, Northwestern University

Postdoctoral Mentor
Mohammed Aslam
Department of Materials Science and Engineering, Northwestern University

Abstract

Magnetic nanostructures are of great interest as platforms for medical applications, including biomedical imaging and targeted cancer therapy. However, current magnetic nanostructures are limited by magnetic strength and insufficient biocompatibility. In this research project, significant progress was made to address these confines, including 1) increasing magnetic responsiveness by replacing the core of the nanostructures with metals or their alloys (i.e., FePt, $CoFe_2O_4$, and $MnFe_2O_4$); and 2) increasing biocompatibility by separating the fluorescent dye and silica into two separate outer shells of the nanostructure to prevent the potentially harmful dye from coming in direct contact with the body.

Introduction

It is estimated that more than 500,000 Americans die from cancer each year.[1] Cancer is the second leading cause of death in the United States, and no cure has yet been found. Scientists and engineers are hoping the nanotechnology can soon be harnessed to provide better cancer detection and therapeutics.

Biofunctionalized magnetic nanostructures are among the most promising candidates for the development of platforms capable of extremely early detection of cancer. These structures are composed of a magnetic core, a shell containing fluorescent dye and silica, and antibodies that allow the structures to attach specifically onto cancer cells. These nanostructures should be capable of entering individual cells, imaging those cells, and providing targeted therapeutics as needed. Although these nanostructures have great potential, they are currently limited by low magnetism and bioincompatibility. Two approaches were undertaken in this project to improve the quality of the nanostructures. The first objective was to make the core structure a stronger magnet, which could enhance contrast in magnetic resonance imaging (MRI). The second objective was to create an inner shell of fluorescent dye, which provides another method for imaging and tracking, and an outer shell of silica, to ensure biocompatibility.

Background

Magnetic nanostructures are made of three main components. The first is the core magnetic nanostructure, usually consisting of a metal or metal oxide. The second is a thin shell layered on top of the core particle, which consists of a fluorescent dye. The third is the silica coating of the nanostructure, which is a versatile platform for bioconjugation and makes the structure highly biocompatible. Because the structures are biocompatible, they can enter the human body, and antibodies on the silica surface can attach onto cells. By varying the

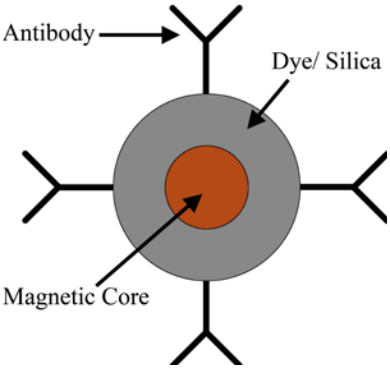


Figure 1. The traditional structure of a magnetic nanostructure.

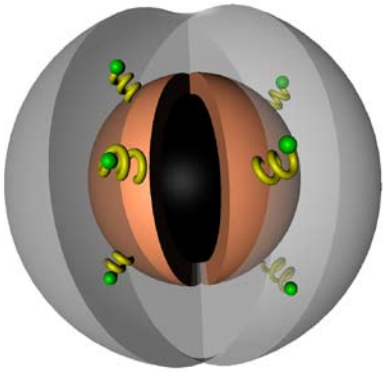


Figure 2. The desired magnetic nanostructure, made up of core magnetic structure (FePt, $CoFe_2O_4$, or $MnFe_2O_4$) shown in black, Fe_3O_4 shell shown in brown, dopamine shown in yellow, fluorescent dye shown in green, and a SiO_2 shell shown in gray.

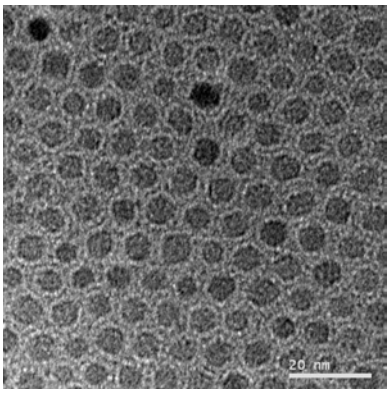


Figure 3. A TEM image of nonaqueous Fe_3O_4 particles.

antibodies attached to the silica surface the nanostructures could have the ability to target different cancer cells.

Once these structures enter the cells, they can be tracked through a magnetic field and can be detected with the help of the magnetic core and fluorescent dye. The magnetic core would enhance MRI contrast[2] by increasing the magnetic force of the protons present in tissue, which is measured through the MRI. The FITC fluorescent dye also provides another medium for imaging the cells that can be used to confirm MRI images at specific locations. The magnetic nanostructures could also be used as a drug delivery method. For example, chemotherapeutic drugs could be attached to the silica shells of the magnetic nanostructures. Once the nanostructures target the specific cancer cells they could deliver therapeutics in a highly highly localized area. Also, when the magnetic structure is subjected to an AC magnetic field, the particles quickly heat up and kill the tumor on which it is attached through a process called hypothermia.[2] These properties of magnetic nanostructures make them one of the most promising tools for cancer diagnostics and therapeutics.

In the past, the core of the magnetic nanostructures were made with iron oxide, which does not have the magnetic strength that many other metals possess. Conventional Fe_3O_4 nanostructures are coated with a thick polymer layer of dextran, which aids in dye and silica coating, but diminishes MRI contrast enhancement. These traditional nanostructures are coated with a shell of fluorescent dye and silica. However, the porosity of silica allows the toxic dye to leach out, thus reducing biocompatibility.

Approach

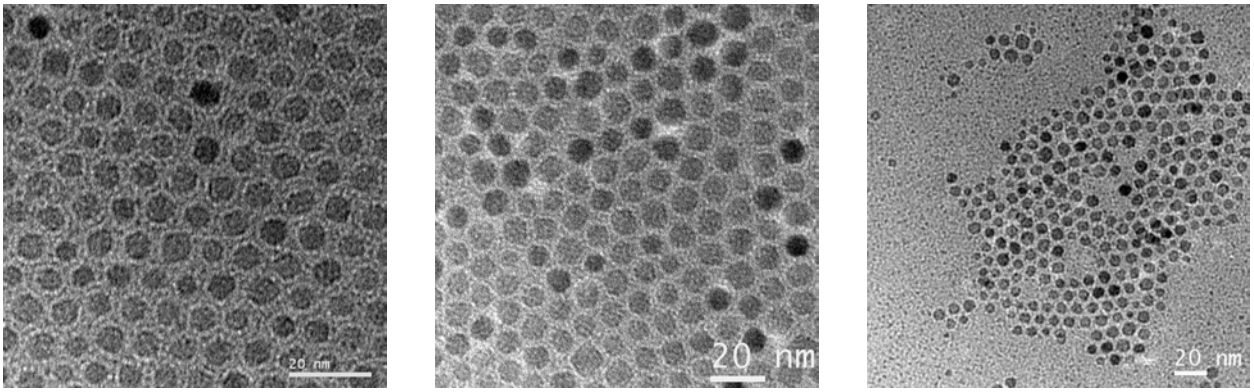
An optimal magnetic nanostructure, described in terms of the core magnetic structure, dye attachment, and silica coating, is depicted in Figure 2.

Core Magnetic Structure

One approach to enhancing the magnetic nanostructure is to substitute metals with greater magnetic force for the traditional iron oxide core.[4] Some of the metals considered to replace Fe_3O_4 were FePt, $CoFe_2O_4$, and $MnFe_2O_4$. Although these metals have a stronger magnetic susceptibility, one inherent limitation is their poor biocompatibility when compared with Fe_3O_4. This obstacle can be overcome by coating the FePt, $CoFe_2O_4$, or $MnFe_2O_4$ seed with a thin shell of Fe_3O_4. This method of synthesizing the magnetic core should enhance the magnetic properties of the structure while maintaining its biocompatibility.

Dye Attachment

The dye is a key component of these nanostructures because of its fluorescent imaging capabilities. Attachment of the FITC fluorescent dye directly to the iron oxide, rather than mixing it with silica, would enhance biocompatibility. In order to affix the FITC fluorescent dye to the iron oxide of the magnetic core, an anchor molecule such as dopamine must first be attached to the surface of the iron oxide. For the dopamine to be attached to the iron oxide, the magnetic core must be stable in a phosphate buffer. The pH of the buffer must be around 7.5 for biocompatibility. In order to stabilize the iron oxide surface, it was coated with Tetramethylammonium 11 aminoundecanoic acid.[5] This acid would act as a bipolar surfactant that would replace any other surfactant on the iron oxide surface, allowing the normally organic



A B C

Figure 4. TEM images of A) $MnFe_2O_4$ particles with an average size of 5.5 nm; B) $MnFe_2O_4$ particles with an average size of 9.5 nm; C) $CoFe_2O_4$ particles with an average size of 6.5 nm.

particles to be soluble in aqueous conditions, consequently stabilizing the structure for dopamine and dye attachment.

Silica Coating
Silica coating around the entire magnetic nanostructure provides a final layer of biocompatibility for the magnetic nanostructure. It was desired that the silica coat only individual particles without aggregation. This type of coating allows the magnetic nanostructures to target cells more specifically and to image them more accurately.

Results

Core Magnetic Structure
To begin making the core magnetic structure, the traditional nonaqueous Fe_3O_4 was made. The resulting Fe_3O_4 particles were highly uniform, around 5.5 nm in diameter, as shown in Figure 3. Next, a variety of seed particles were created to be coated with an Fe_3O_4 shell. These seeds consisted of FePt, $CoFe_2O_4$, and $MnFe_2O_4$. $CoFe_2O_4$ was grown with an average diameter of 6.5 nm, as shown in Figure 4. $MnFe_2O_4$ was grown at two different sizes, at 5.5 nm and 9.5 nm, also shown in Figure 4. FePt seeds were grown, with an average diameter of 3 nm.

X-ray diffraction (XRD) analysis of these particles is shown in Figure 5. Each metal or metal alloy has specific diffraction peaks. From the graph four distinct peaks can be seen, at angles of 40, 46, 68, and 82 degrees. These FePt seeds were then used to grow a shell of Fe_3O_4. The resulting particles are shown in Figure 6. There is a large amount of excess Fe_3O_4. However, some FePt seeds were well coated.

Dye Attachment
Dye attachment began with tetramethylammonium 1,1 aminoundecanoic acid being added to nonaqueous Fe_3O_4 particles in a dichloromethane solvent. The solution was then stirred, so that tetramethylammonium 1,1 aminoundecanoic acid could coat the particles and replace the excess surfactant. The coated particles were then dispersed in a phosphate buffer of pH 7.5. Figure 7 shows the coated particles after sitting for several hours and the same particles after being shaken. The particles that sat for a long period of time aggregated and settled to the bottom of the vile. After being shaken, these same particles were resuspended in the phosphate buffer.

Silica Coating
Finally, silica coating was attempted on both the $CoFe_2O_4$, and $MnFe_2O_4$ particles, as shown in Figure 8. Both these images show a core and shell structure. Both images also show excess silica that does not encapsulate any particles and particle conjugation.

Because Fe_3O_4 would eventually coat the core FePt, $CoFe_2O_4$, or $MnFe_2O_4$ seeds, to prepare for coating these structures with silica, silica was coated onto Fe_3O_4 structures. This resulted in highly uniform and individual coating of particles with minimal excess silica, as it can be seen in Figure 9.

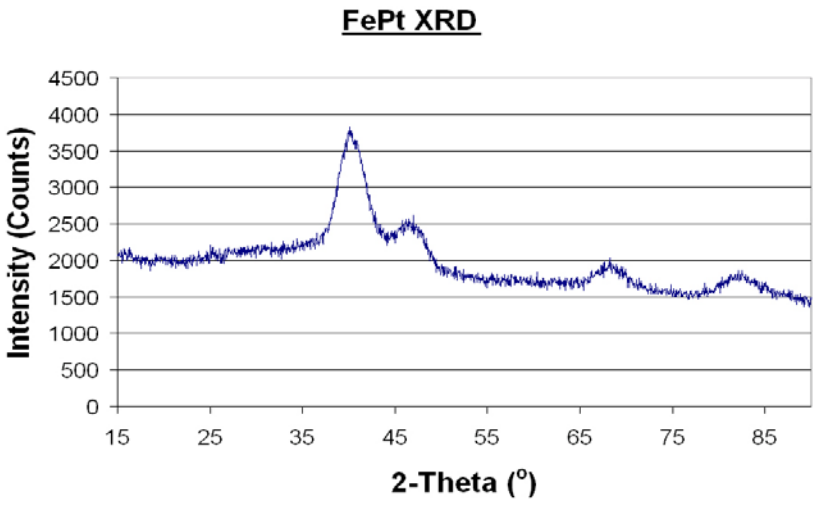


Figure 5. An XRD graph of FePt nanoparticles.

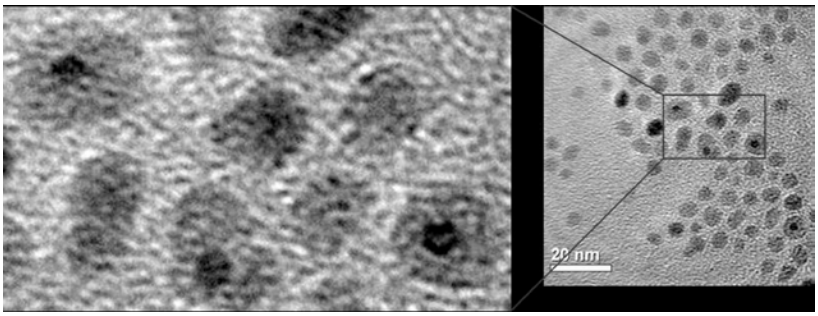


Figure 6. A TEM image of FePt seeds coated with Fe_3O_4.

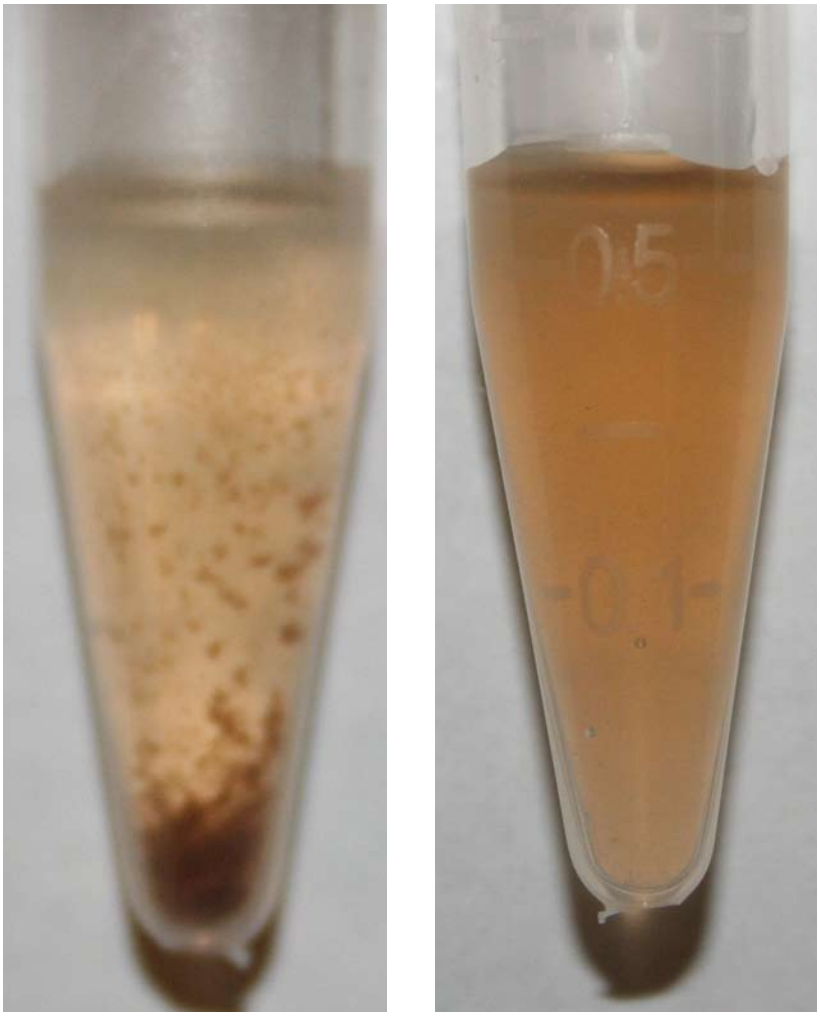
Discussion

Core Magnetic Structure

The magnese ferrite and cobalt ferrite nanostructures created in this project were highly uniform and of a suitable size, which would be very effective magnetic cores once coated with iron oxide. The FePt phase of the iron platinum seeds synthesized was confirmed, because the XRD graph contained the four major peaks associated with FePt.[6] The yield produced from Fe_3O_4 coating of FePt, was very low, because of excess iron oxide present in the environment. A higher yield of core shell magnetic structures (such as isolated FePt @ Fe_3O_4) could be obtained by altering the ratio of seed FePt particles to the amount of Fe_3O_4 precursors. The FePt core magnetic structure could also be enhanced by making larger seeds on top of which iron oxide could grow. The larger seed would make the overall structure a stronger magnet, which would improve the MRI contrast enhancement and the rate of hypothermia.

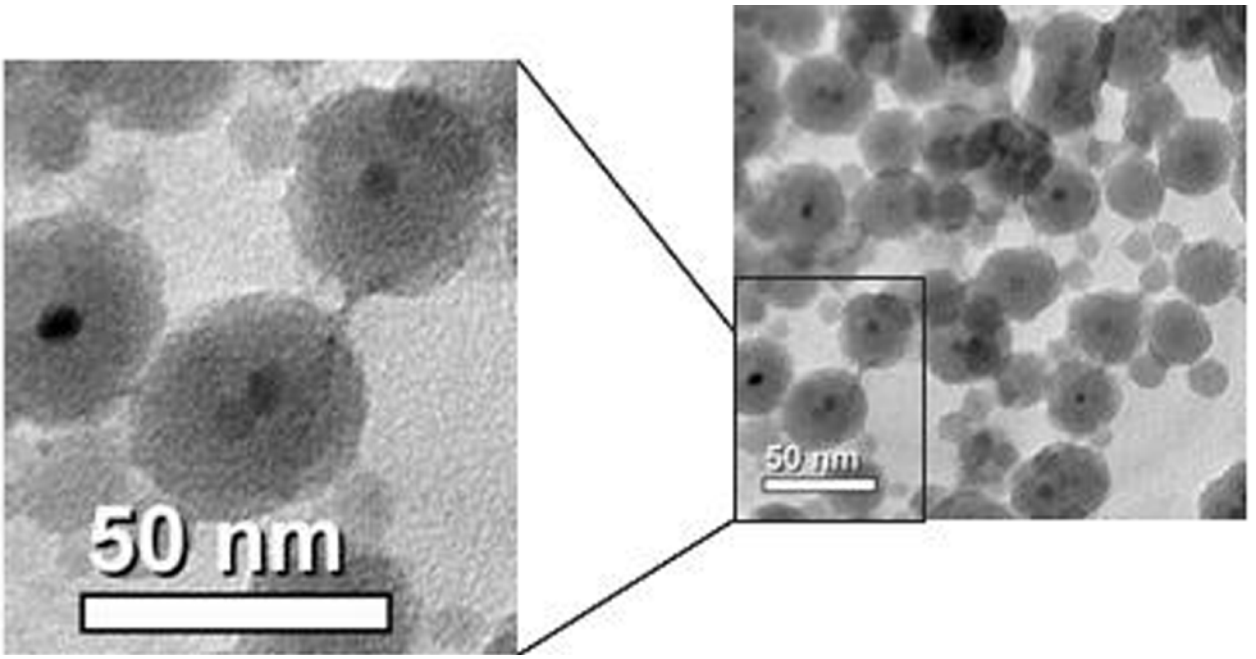
Dye Attachment

The surfactant on the surface of the particles causes the particles to aggregate in solution. However, if fully coated with the tetramethylammonium 11 aminoundecanoic acid (TTMAAD), the particles should be suspended in aqueous solution because TTMAAD acts as a bipolar surfactant that replaces any other surfactant on the particle's surface. This allows the normally organic particles to be soluble in aqueous conditions and consequently stabilizes the structure for dopamine and dye attachment. The rapid resuspension of the structures into the phosphate buffer suggests that the structures are only partially coated with



A B

Figure 7. Fe_3O_4 particles coated with Tetramethylammonium 1,1 aminoundecanoic acid A) after sitting and B) after being shaken.



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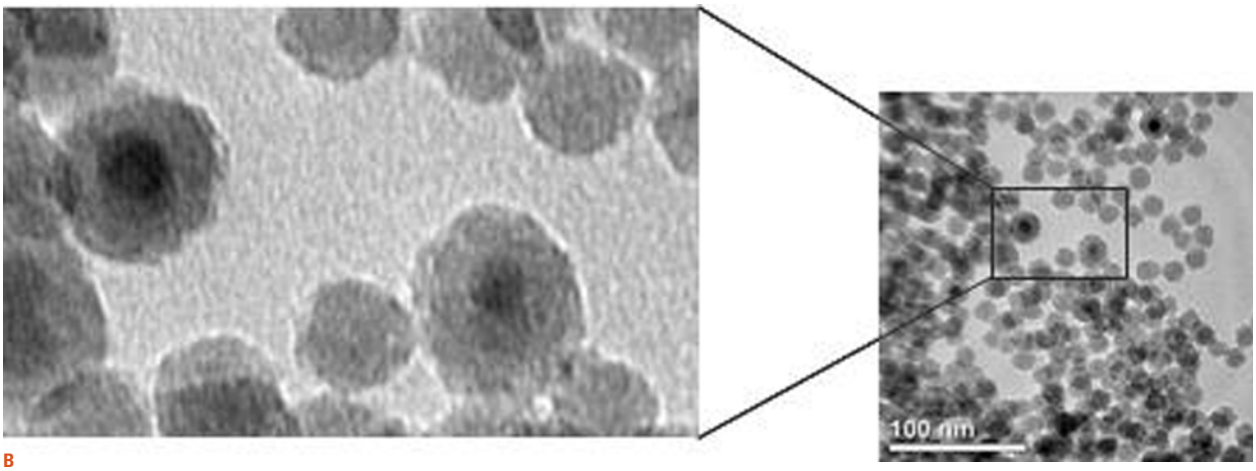

B

Figure 8. TEM Images of A) $CoFe_2O_4$ coated with silica and B) $MnFe_2O_4$ coated with silica.

the TTMAAD. This may be because the procedure used to synthesize the acid may have provided a very small yield. Commercially produced tetramethylammonium 1,1 aminoundecanoic acid could be used in the future. Also, the amount of coating on each structure can be increased by adjusting the ratio of the number of structures to the amount of acid used. These adjustments would ideally increase the particle stability and facilitate dye attachment.

Silica Coating

The $CoFe_2O_4$ and $MnFe_2O_4$ structures both have excess silica growth due to the instability of the structures. However, excess silica growth can easily be removed through magnetic separation. Sometimes two magnetic structures were encapsulated in the same silica shell. This may also have been due to the stability of the particles. Thus, increasing the stability of the particles could increase the yield of the silica coating by preventing aggregation. The stability and individuality of the silica coating on Fe_3O_4 particles suggest that once the core particles are coated with Fe_3O_4, they will easily be silica coated.

Conclusions

Although the optimal biofunctionalized magnetic nanostructures were not synthesized, many significant steps were made towards this goal. New information was obtained regarding the ratios of precursors and the importance of particle stability could lead to a higher magnetic susceptibility of the structures and the separation of the dye and silica shells. Once these modifications are optimized, biofunctionalized magnetic nanostructure could be a very useful tool in biomedicine.

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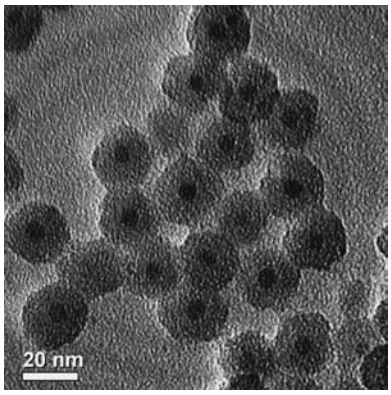

Figure 9. A TEM image of Fe_3O_4 coated with silica.