Smart Therapy: The Multivariate Potentials of Iron Oxide Nanoparticles in Drug Delivery

Undergraduate Researcher
Darrius Guiden, Northwestern University

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

Postdoctoral Mentors
Ming Su and Lei Fu
Department of Materials Science and Engineering, Northwestern University

Abstract

In modern medicine, physicians destroy otherwise healthy tissues in patients' bodies because of the lack of site-target specificity and sensitivity in detecting and attacking cancerous cells. Many cancer patients are left with the difficult task of weighing the benefits against the harm of undergoing current cancer treatments. Members of the Nanoscale Science and Engineering Center (NSEC) are seeking ways to significantly increase the specificity and sensitivity of drug delivery. Examination of magnetic nanoparticles as a means for drug delivery offers new possibilities for enhanced, specific targeting of diseased cells and thus for potential cancer treatments.

Past research indicating that iron oxide (commonly called magnetite) nanoparticles can be coated with organic and biological molecules forms the basis of this experiment, which attempts to characterize and develop efficient ways of immobilizing biological molecules (e.g., medicines) onto the surfaces of magnetic iron oxide particles. Combined transmission electron microscopy (TEM) images and thermal gravity analyzer (TGA) data determined that nanoparticle size is, at most, insignificantly affected by varying temperature and pH. Furthermore, the immobilization of a biological molecule onto a coated iron oxide nanoparticle was compared for two different organic couplers, Ethylenediaminetetraacetate ion (EDTA) and Dextran. TGA data confirmed that EDTA proved more efficient in immobilizing the biological protein Bovine Serum Albumin (BSA) onto the magnetic nanoparticles. This information adds new insight into the mechanisms and the functional groups needed to equip a suitable organic coupler to most efficiently immobilize medicines.

Introduction

New methods in the fight against cancer are needed. The majority of cancer patients are cured of primary tumors, but secondary tumors, also referred to as metastasis, continue to perplex physicians. In 2003, more than half a million people in the United States will die of cancer,[1] the second-leading cause of death in the country. Focusing on the conventional modes of treatment (e.g., chemotherapy and radiation therapy), this research questions how to develop treatments with increased sensitivity and specificity.

Chemotherapy is often employed to attack cancerous cells. Chemical toxins alter the tumor's cell cycles (e.g., cell divisions, manufacturing processes), resulting in cell death. In a case where two or more chemicals are needed (called combinational chemotherapy), chemotherapy has more pronounced effects and frequently harms healthy cells. Although chemotherapy treatments must be administered at high initial doses, the likelihood of harm to healthy cells is considered an acceptable sacrifice to preserve, for example, a particular organ.

Site specificity is also limited in radiation therapy. The high-frequency X-rays used to damage cancerous cells often injure healthy tissues as well.

Now researchers are developing novel ways to combat cancer using the emerging science of nanotechnology. The Nanoscale Science and Engineering Center (NSEC), headquartered at Northwestern University, unites some of the country's leading academic institutions, industries, and national laboratories in an effort to develop nanoscale equipment to revolutionize current biological sensing techniques. Northwestern researchers are searching diligently for methods to employ nanoparticles as a tool for treating cancer, adding increased sensitivity and specificity to therapies.

Background

In a summer 2003 project funded by the National Science Foundation, conditions that might allow magnetic nanoparticles to serve as a means of more sensitive and highly specific drug delivery were investigated. Iron oxide (commonly called magnetite) nanoparticles are relatively abundant and, because of their superparamagnetic properties, have long been researched as suitable particles for in vivo drug delivery. In a 2001 paper published in the *Journal of Magnetism and Magnetic Materials*,[2] researchers at the University of Western Australia showed that iron oxide particles coated with two different organic polymers displayed differing superparamagnetic properties depending on particle size. Employing transmission electron microscopy (TEM), the study further showed that the polymer used also played a role in the size of the resulting nanoparticles.

In another study published in the same journal in 2002, researchers at the Institute of Experimental Physics[3] showed that by using an organic coupling agent (1-[3-dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride - CDI), they could

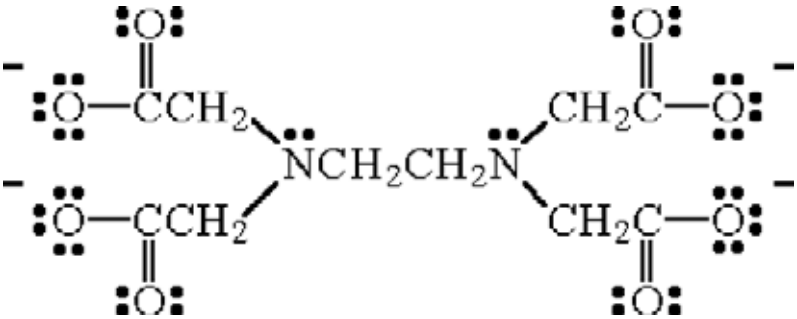


Figure 1: EDTA molecule.

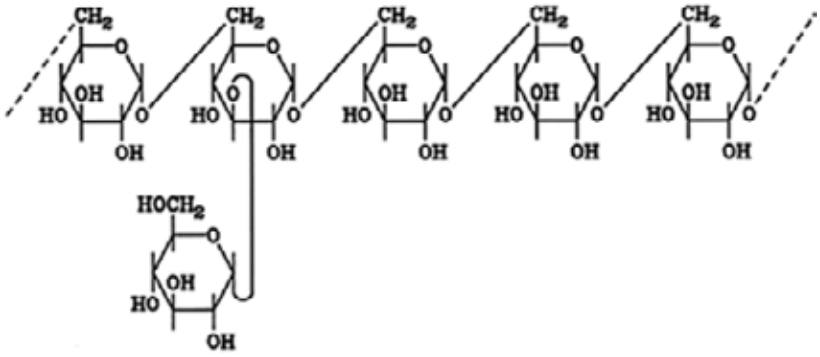


Figure 2: Dextran polysaccharide.

immobilize proteins and enzymes onto iron oxide nanoparticles.

Combining techniques from these two experiments, a research project at Northwestern University set out to test the efficacy of two different copolymers, or coupling agents, in immobilizing protein BSA and the proteolytic enzyme Streptokinase onto coupler-coated iron oxide nanoparticles. The experimental environment was also altered to test particle-size effects in varying pH conditions and temperatures. Results enhance understanding of the conditions that optimize bioimmobilization.

Approach

If nanoparticles are to serve as a means for drug delivery, treatment using the particles must increase site specificity and sensitivity, outperforming conventional techniques. Because previous work demonstrated that coated iron oxide nanoparticles display superparamagnetic properties, the theory was that if medicines could be immobilized onto the particles and then into the system, the coated nanoparticles could be guided directly to a tumor using an external magnetic force. Also, unlike conventional chemical therapy, which requires higher doses to overcome the dilutional effects that occur when the chemical migrates randomly to the tumor site, a controlled magnetic particle could, in theory, be used in a much lesser dosage.

The first step in this research project was to vary the environment in which the iron oxide nanoparticles are coated with the coupler of choice. To test the coupler's efficiency, it was important to observe how both temperature and pH would affect the amount of organic coupler coating on the nanoparticles.

Stocks of iron oxide (molecular formula Fe_3O_4) nanoparticles were prepared using the procedure outlined in work by Fu.[4] Chosen as suitable biological couplers were ethylenediaminetetraacetate ion (EDTA) (Figure 1), a sodium salt used in commercial products like soap and mayonnaise; and Dextran (Figure 2), a polysaccharide composed primarily of 1-6 linked α-D-glucopyranose units with the medicinal value of increasing blood volume after trauma and vaccine production. The functional groups present on each coupler seemed likely links for binding both to the iron oxide nanoparticles and directly to the biological molecules.

To test the effects of pH, iron oxide particles were coprecipitated with organic coupler at neutral pH and a slightly acidic pH of 5. Nanoparticles were also prepared in advance, and then predissolved coupler and nonpredissolved coupler were added to disperse sonicated nanoparticles.

To test the effects of temperature, experimental solutions were heated to 25°C, 50°C, and 80°C.

After preparation of the coated nanoparticles with the chosen coupler, Bovine Serum Albumin (BSA), a biological protein associated with lipid-binding properties and prevention of diseases like insulin-dependent diabetes and autoimmune disease, was added at room temperature and neutral pH. Following the method published by Koneracka et al.,[3] the mass ratio (mg) of mixed iron oxide to organic coupler to biological molecule was 3:1:1. Finally, Streptokinase was added directly to nanoparticles to test the potential for direct enzyme immobilization. Experimental setup and results are listed in Tables 1 through 6.

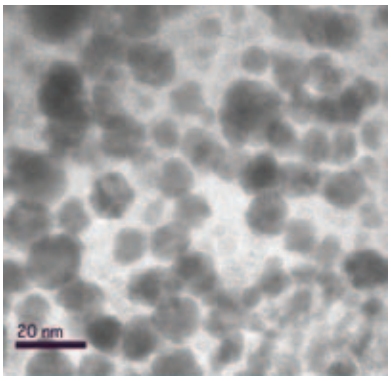
Results

TEM images confirmed an average particle size of 10 nm, regardless of the pH or temperature environment in which the nanoparticles were prepared. While the images show very little contrast (because organic coating offers little electron density to render contrast to particle layers), any distinguishable circle present on the image measures roughly 10 nm. Figures 3 and 4 show images taken from two of the experimental setups.

Because transmission electron microscopy (TEM) would not allow determination of the amount of coupler coating on the particle, a thermal gravity analyzer (TGA) was used to test the change in mass of nanoparticles plotted against high temperatures (C). Figure 5 shows an image of a typical TGA readout. The change in slope is proportional to the total weight loss from the iron oxide nanoparticles and can be used as a method for determining the amount of immobilization onto the nanoparticles.

Discussion

The experiment confirmed that both EDTA and Dextran are capable of coating the nanoparticle surface. TGA data showed that Dextran caused the stronger interaction, but EDTA was better able to immobilize BSA onto the particles, immobilizing nearly three times as much as Dextran. This information promotes



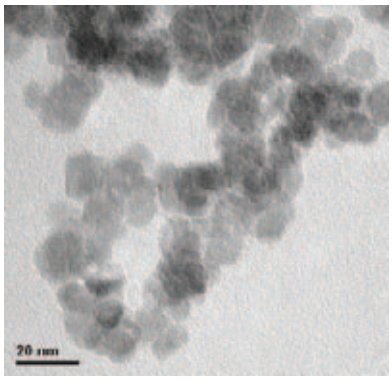


Figure 3: (Left) BSA-coated nanoparticles with no coupler coating.

Figure 4: (Right) pH 5, 80°C, coprecipitation of Dextran and nanoparticle.

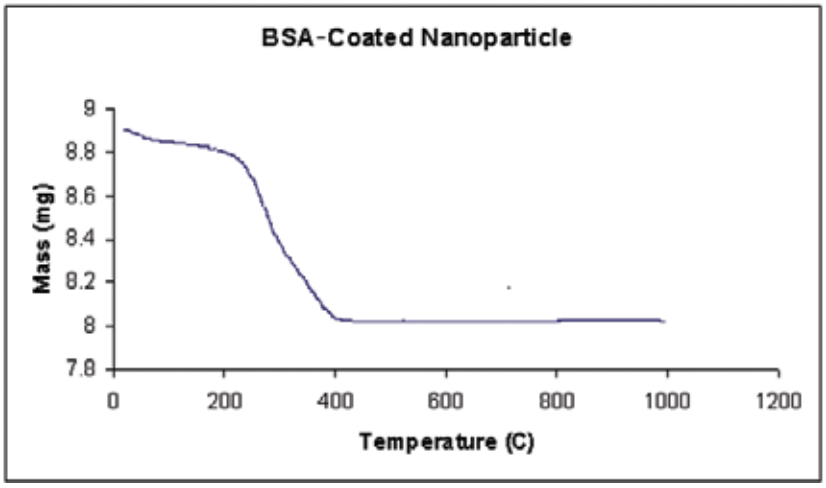


Figure 5: TGA Graph Fe_3O_4 (mg) vs. temperature (C).

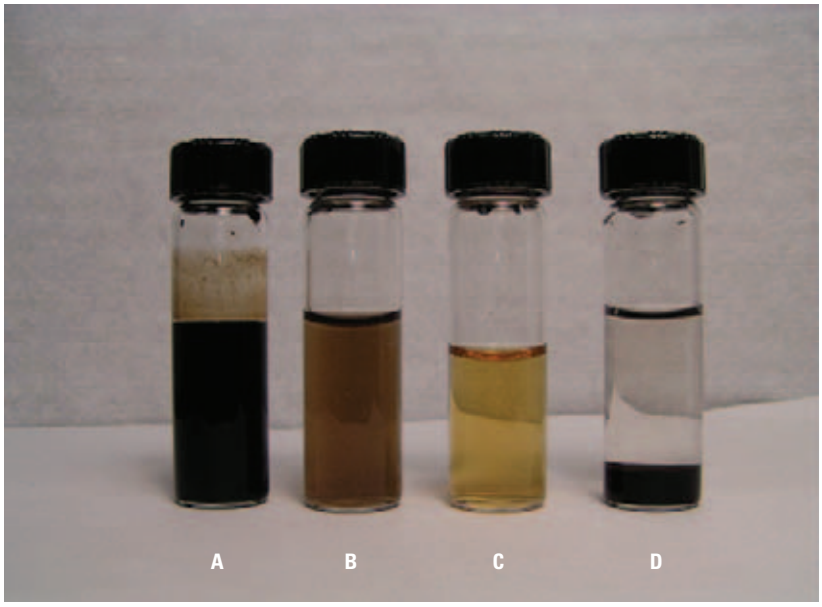


Figure 6: Iron oxide magnetic fluids. A, B, and C represent differing concentrations of suspended-coated nanoparticles. D shows a solution of uncoated nanoparticles that has agglomerated and settled to the bottom of the vial.

understanding of the functional groups necessary for each of these couplers to facilitate binding to biological proteins and medicines. It is important because nanoparticles potentially used in drug delivery must be as small as possible to allow diffusion and migration throughout the body.

While TGA helped determine the amount of organic coupler and biological immobilization, questions remain as to how much of the unbound reagents may have remained in solution. Even after several washes, it was impossible to definitively determine if trace amounts of organic coupler (unbound to the particles) might still be trapped in nanoparticles settled at the bottom of the vial. However, the total weight of unbound coupler was most likely insignificant to the total immobilized coupler, even if some remained mixed with the nanoparticles. Ideally, quantifying the possible residual coupler could create standards. Implications for small-scale applications are not significant, but for bulk manufacturing, this quantitative information would be critical to design the most effective product at the optimal cost.

Coated nanoparticles remain colloidal in solution (Figure 6) compared with uncoated nanoparticles, which agglomerate and settle down. A stable colloidal suspension of nanoparticles adds to the versatility of possible treatments. Currently, brain-stem cancer is considered untreatable because many surgeons consider an operation too risky. Because cerebrospinal fluid flows freely along the spinal cord and around the brain stem, one could imagine that medicine-coated nanoparticles injected into cerebrospinal fluid could migrate to the tumor site and administer the toxic response with

increased target specificity and sensitivity. Research by Professor Vinayak Dravid at Northwestern University has shown that coated nanoparticles display superparamagnetic properties[5] that allow them to be guided under the force of an externally applied magnetic field.

Conclusions

This research brings the medical community one step closer to employing nanoparticle treatments in the fight against cancer and other deadly diseases. It begins to establish procedures for characterizing and quantifying stable biological attachment to iron oxide nanoparticles. Professor Tatjana Pauneska of Northwestern University's Feinberg School of Medicine described the potential of titanium oxide (TiO_2) nanoparticles to act with the precision of a "Swiss Army knife," targeting even genes with extreme specificity.[6]

Table 1

Fe_3O_4 : Dextran		
Mass ratio (mg)	3 : 1	
pH	5	7
Temperature (C)	50	80
Average nanoparticle size (nm)	10	10
Total amount of coating (mg)	.9	.9

Table 2

Fe_3O_4 : EDTA		
Mass ratio (mg)	3 : 1	
pH	5	7
Temperature (C)	50	80
Average nanoparticle size (nm)	10	10
Total amount of coating (mg)	1	1

Table 3

Fe_3O_4 : Dextran : BSA		
Mass ratio (mg)	3 : 1 : 1	
pH (nanoparticles prepared at this pH)	5	7
Total amount of coating (mg)	2	2

Table 4

Fe_3O_4 : EDTA : BSA		
Mass ratio (mg)	3 : 1 : 1	
pH (nanoparticles prepared at this pH)	5	7
Total amount of coating (mg)	6	6

Table 5

Fe_3O_4 : Streptokinase	
Mass ratio (mg)	3 : 1
pH	7
Total amount of coating (mg)	.28

References

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(6) "Nanotechnology May Help Overcome Current Limitations of Gene Therapy." Northwestern University News Release. April 18, 2003. Evanston, IL.