Antibody-coated magnetic nanoparticles: Targeting and treating cancer

Philip Schlenoff
Maclay School (Tallahassee, Florida)

DOI:10.4103/0974-6102.68743

Used as far back as the ninth century to make pots glisten gold, nanoparticles have a rich history filled with a wide variety of applications. In fact, sol-gel synthesis (silica based nanoparticle creation) became so popular in the 1990s that over 35,000 papers were published on the process. Modern day applications include medicinal and food-based uses, even being synthesized in beer bottle glass to make it less breakable.[1] Once magnetic nanoparticles were introduced, the opportunities for nanotechnology skyrocketed. Their ability to be guided, tracked, and affected by various kinds of radiation, including Radio Frequency (RF) and laser, gives them certain advantages compared to particles made of materials like silica or clay.

Because magnetic nanoparticles can be heated by RF radiation, they have been proposed for the treatment of cancer. It is envisioned that these nanoparticles can be injected intravenously, circulate, and if coated with the proper antibodies,[2] selectively target cancer cells and be ingested by them. In the next step, RF irradiation of approximately 350 kHz may be used externally to irradiate the tumor saturated with the particles. Once the temperature rises above 45°C, the cancer cells are eradicated. However, these nanoparticles tend to aggregate in the bloodstream because of the presence of proteins such as serum albumin, limiting their effectiveness in reaching the brain or metastasized tumors.[2] This project aims to solve the aggregation problem, eventually leading to a cheap, effective, and safe nanoparticle-based cancer treatment.

Current literature on nanomedicine treatments for cancer typically involve using quantum dots or nanorods coated with gold and poly(ethylene) glycol and heating them *in vivo* using near infrared (NIR) laser treatment.[3-6] However, due to NIR's low penetration depth, the usefulness of this method is limited. For tumors deep inside the body or metastasized tumors, NIR would require invasive surgeries to reach the targeted area. Radio frequency, on the other hand, penetrates the entire body without losing field strength, and can be applied to the entire body at once. Iron oxide is also less toxic to organic systems.[7]

This research project had initially followed a different path. In 2007, my project entitled "What Size Nanoparticles Do Vertebrate Cells Preferentially Ingest During Endocytosis" was a study into the optimal ingestion size of lab-made sub-100nm silica "beads" for cancer cells [Figure 1]. Silica was used for its biocompatible properties[8] and each particle was coated with a zwitterion "mask," SBS, [Figure 2] to facilitate cell ingestion by stabilizing the nanoparticles in the cell medium [Figure 3].[9]

Particles were synthesized at five different sizes ranging from 12-87nm and then incubated with cells for two hours. Because the nanoparticles had been tagged with a fluorescent dye, confocal microscopy revealed the location of the ingested particles in the cells [Figure 4]. Results indicated that for the time frame used, all different sizes of nanoparticles were ingested equally and fully. The significance of this project was

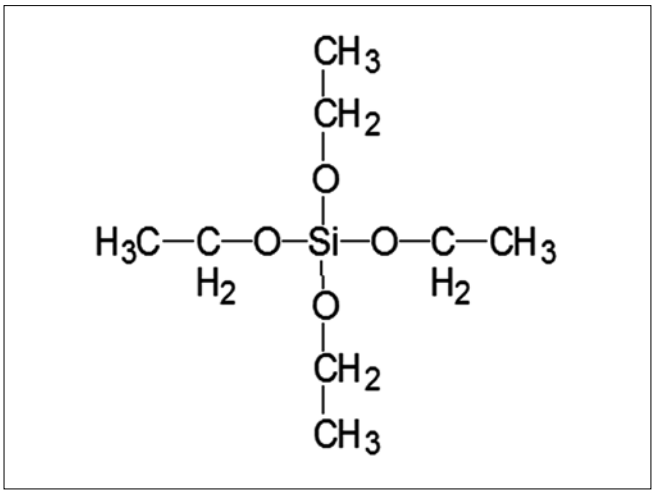

Figure 1: Structure of tetraethyl orthosilicate (TEOS)

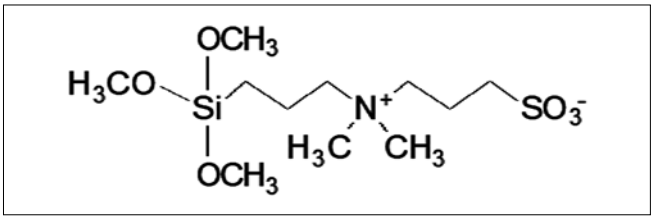

Figure 2: Structure of SBS. The close proximity of the + and – charges on the ends help stabilize particles in the blood

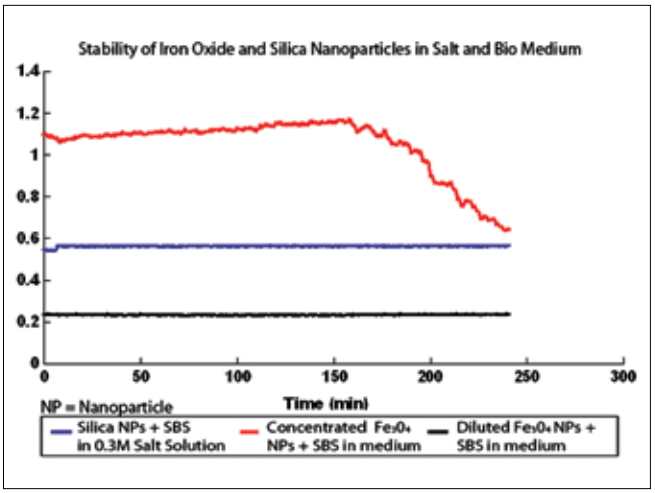

Figure 3: Stability of high and low concentrations of iron oxide nanoparticles in cell medium, and low concentrations in salt solution. This graph indicates that the zwitterion coated silica shell/iron oxide core particles are stable in cell medium, which mimics most destabilizing conditions of blood

not the rather uneventful results, but the experience it gave me working with nanoparticles. Furthermore, it gave me the idea of tagged nanoparticles, labeled with antibodies that could specifically target cancer tumors.

In 2008, this idea evolved into a project titled "Stealthy

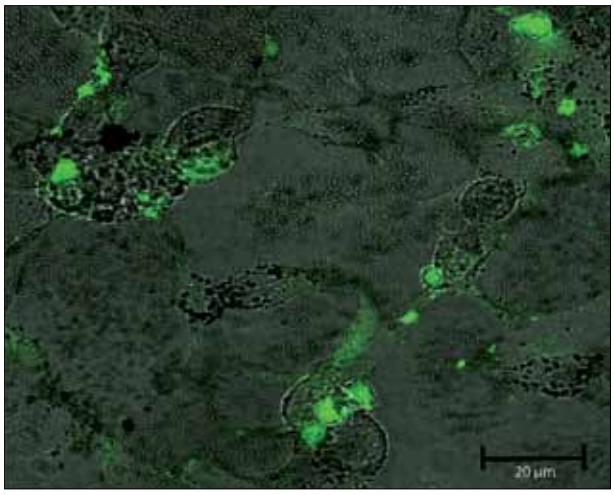

Figure 4: Confocal microscopy reveals the location of the fluorescent nanoparticles inside the cells; they aggregate to certain organelles

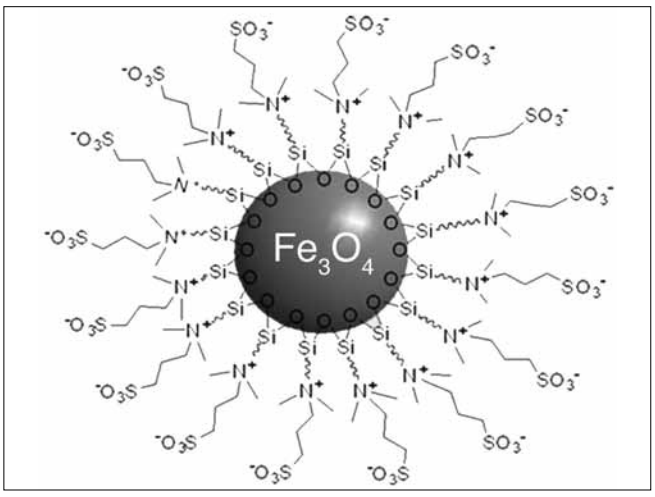

Figure 5: Structure of iron oxide and silica nanoparticles with SBS coating (all structures drawn using ChemDraw Pro). Particles used for 2007 experiments lacked the iron oxide core

Iron Oxide Nanoparticles: Towards the Identification and Eradication of Cancer Cells." After reading some literature about doping silica nanoparticles with metals, it was decided that an iron oxide (Fe_3O_4) core would fit the goal [Figure 5]. The magnetic material inside the particle allows control over the temperature of the nanoparticles from a distance, and has no effect on healthy cells [Figure 6].[10] A unique zwitterion coating (a material which carries 0 net charge), originally designed to hide stents from the body's immune system, provided these nanoparticles with an incredible advantage. The extremely close proximity of the positive and negative charges on the ends of SBS [Figure 2] mimics the phospholipid bilayer of the cell membrane, and masks the foreign particles from the body's immune system and from cell receptors.[9] This allows these "stealthy" iron

Q1

oxide nanoparticles great versatility *in vivo* (in the bloodstream).[11]

After synthesizing nanoparticles with an iron oxide core, fluorescent dye, a silica shell, and a zwitterion coating, the nanoparticles were fed to prostate cancer cells for two hours.[12,13] They were exposed to metal-heating radio frequency (RF) irradiation for thirty and forty minutes, then the cell cultures' viability was measured. For the control, nanoparticles without iron oxide at their core, the cell culture remained fully viable after incubation. For the cells that were fed magnetic nanoparticles, after RF treatment was applied, there was 16% cell death after thirty minutes. After forty minutes, 30% of the cells had died. This was a proof-of-concept that the treatment could work effectively inside the body, which heats even faster than a cell culture, promising an even higher overall cell death count for the duration the RF was applied. To verify that the nanoparticles were inside the cells and not on the surface, the cells were washed intensively, then imaged with confocal microscopy and subjected to a "magnet test," where cells were observed to move in a magnetic field [Figure 7]. Dynamic Light Scattering was performed to measure the hydrodynamic radius of the particles, which averages around 25.1nm [Figure 8].

The success of these trials led to my current research, entitled "Antibody-coated Magnetic Nanoparticles: Targeting and Treating Cancer" [Figure 9]. The goal of this project is to synthesize a viable cancer treatment for future *in vivo* animal trials. While initial steps for creating these finalized nanoparticles have remained the same, a final addition of antibodies allows them to target cancer cells [Figure 10]. These particles have been conjugated to the breast-cancer specific antibody, anti-HER2, enabling selective properties that will leave healthy cells untouched.[14] At this time, the zwitterionic nanoparticles have been shown to fluoresce under secondary antibody staining (which binds to the primary antibody) 800% more intensely than the control, indicating that the primary antibodies are present on the surface of the particles.

Current and future trials involve testing these antibody-coated nanoparticles in a cell culture, to ensure the antibodies can fulfill their selective abilities. It may also prove useful to test the treatment in a flowing system. If these steps are successful, they could help zwitterionic nanoparticles become a more viable option for cancer treatments. However,

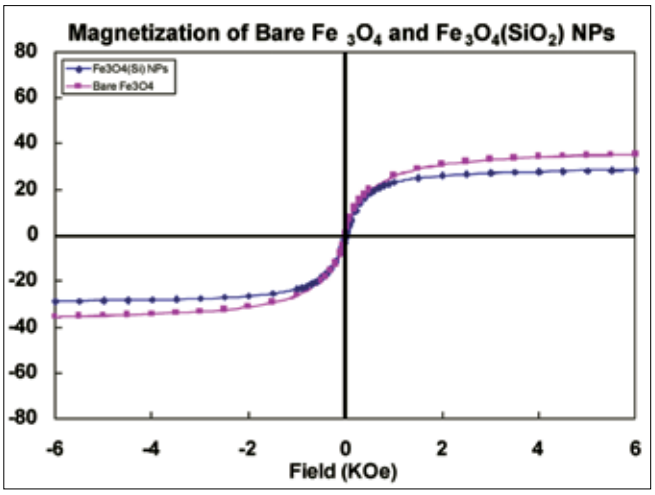

Figure 6: In order for the particles to react to Radio Frequency irradiation, they have to be superparamagnetic (zero net orientation at zero magnetic field). This graph shows that they are superparamagnetic before and after adding silica

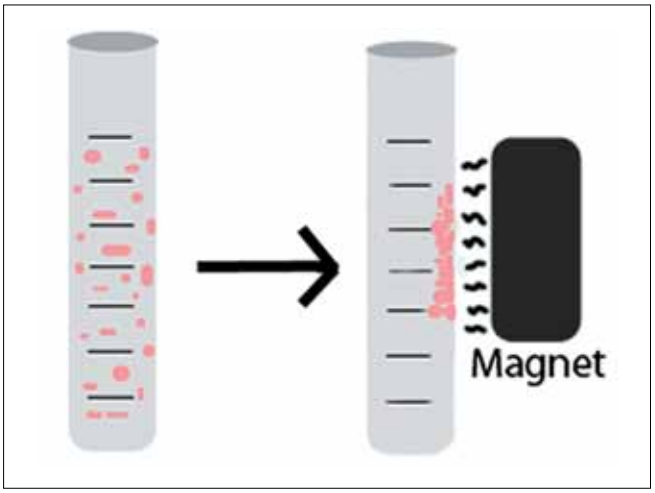

Figure 7: After feeding and multiple washings, the cells with nanoparticles were attracted to a magnet placed near the test tube. Also known as the "magnet test"

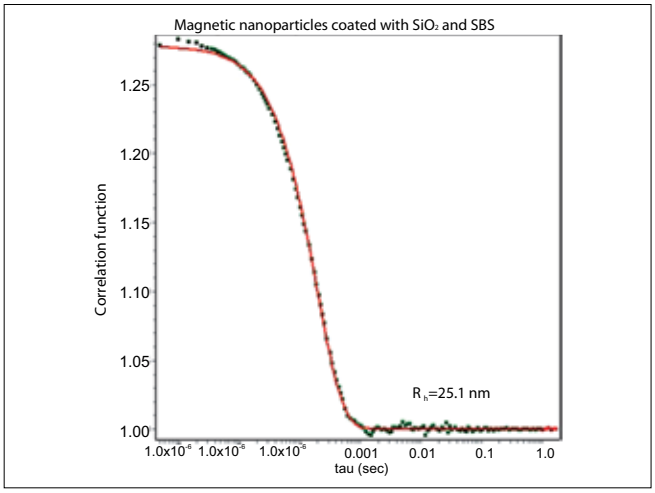

Figure 8: Dynamic light scattering is used to determine the hydrodynamic radius of the nanoparticles

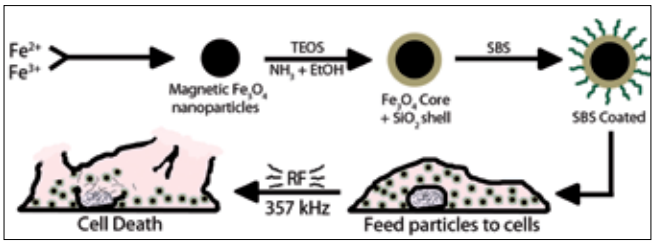

Figure 9: Procedure for synthesizing particles, feeding them to cancer cells, and killing them with RF irradiation

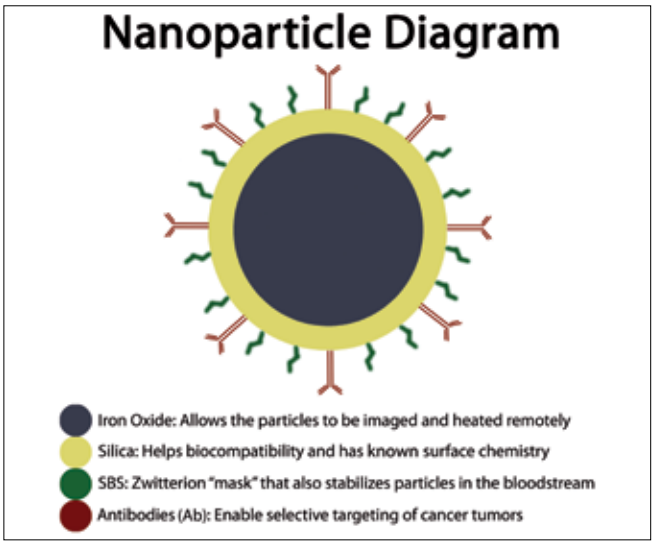

Figure 10: The nanoparticles are composed of Iron Oxide, Silica, Antibodies, and a Zwitterion molecule

other questions also need to be answered before a nanoparticle based cancer treatment can work its way into the mainstream. Even though these nanoparticles are biocompatible, it is important to know how they are filtered out by the body after treatment. In addition, detailed *in vivo* thermal diffusion studies will reveal the amount of time to heat cancer tumors to the required 45 degrees Celsius, without damaging the surrounding tissue. I hope to continue this research not only through college, but also as a career. Cancer kills over twenty thousand people a day[15] and putting even a small dent in that number could have a huge impact throughout the entire world.

References

1. Available from: http://en.wikipedia.org/wiki/Nanoparticle. [cited in 2009].
2. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev 2004;56:1649-59.
3. von Maltzahn G, Centrone A, Ji-Ho P, Ramanathan R, Sailor MJ, Hatton TA, *et al.* SERS-coded gold nanorods as a multifunctional platform for densely multiplexed near-infrared imaging and photothermal heating. Adv Mater 2009;21:3175-80.
4. von Maltzahn G, Park JH, Agrawal A, Bandaru NK, Das SK, Sailor MJ, *et al.* Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. Cancer Res 2009;69:3892-900.
5. Gao X, Cui Y, Levenson R, Chung L, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 2004;22:969-76.
6. Lo C, Xiao D, Choi M. Homocysteine-protected gold-coated magnetic nanoparticles: Synthesis and characterization. J Mat Chem 2007;:2418-27.
7. Casals E, Campos S, Bastus N, Puntes V. Distribution and potential toxicity of engineered inorganic nanoparticles and carbon nanostructures in biological systems. Trends Analyt Chem 2008;27:672-83.
8. Gerion D, Pinaud F, Williams SC, Parak WJ, Zanchet D, Weiss S, et al. Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. J Phys Chem B 2001;105:8861-71.
9. Schlenoff JB. Stabalized silica colloids. US Patent Appl 2009;20:2816.
10. Lee H, Lee E, Kim do K, Jang NK, Jeong YY, Jon S. Antibiofouling polymer-coated superparamagnetic iron oxide nanoparticles as potential magnetic resonance contrast agents for in vivo cancer imaging. J Am Chem Soc 2006;128:7383-9.
11. M. Racuciu1, D. E. Creanga, and A. Airinei. Citric-acid-coated magnetite nanoparticles for biological applications. J Eur Phys Jr E 2006;21:117-21.
12. Santra S, Tapec R, Theodoropoulou N, Dobson J, Hebard A, Tan W. Synthesis and characterization of silica-coated iron oxide nanoparticles in microemulsion: The effect of nonionic surfactants. Langmuir 2001;17:2900-6.
13. Lu Z, Dai J, Song X, Wang G, Yang W. Facile synthesis of Fe3O4/SiO2 composite nanoparticles from primary silica particles. Colloids Surfaces A Physicochem 2008;317:450-6.
14. Santra S, Zhang P, Wang K, Tapec R, Tan W. Conjugation of biomolecules with luminophore-doped silica nanoparticles for photostable biomarkers. Anal Chem 2001;73:4988-93.
15. Garcia M, Jemal A, Ward EM, Center MM, Hao Y, Siegel RL, *et al.* Global cancer facts & figures 2007. Atlanta, GA: American Cancer Society; 2007.

About the Author

Philip Schlenoff has recently graduated from Maclay School in Florida, and has just started at the University of Florida where he is majoring in biomedical engineering. Aside from research he enjoys playing piano and reading.

Author Query

Q1: Please check the figure contents, is it SO_3^- or ^-O_3S????