01/30/2009 Final Report for the Project:

Quantitative magnetic resonance imaging (MRI) tracking of labeled stem and cancer cells

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Project goal: The objective of this project was to synthesize novel magnetic materials for quantitative cell labeling, incorporate these materials either attached or internalized into cells, and quantitatively track these cells both in-vivo and ex-vivo. The broad impact of this work is intended to be visualizing and tracking cancer and stem cells in a quantitative nature through MRI (Magnetic Resonance Imaging).

Overview: We are using a layered approach of three subprojects. These subprojects are run by graduate students helped by undergraduates. Sub-project one is a bottom-up approach that synthesizes Fe based single molecule magnets, SMM's, for cell loading and develops the nuclear magnetic resonance, NMR, protocols for measurements of loaded cells. Sub-project two is a top-down approach that synthesizes larger Fe_3O_4 (magnetite) clusters with smart site-directed chemical modifications. Sub-project three develops novel instrumentation for magnetic characterization of the synthesized materials and loaded cells. Our collaborator is Dr. Jialing Xiang and her graduate student Brad Godfrey who are providing the cell cultures and expertise in cell loading.

Results: The three sub-projects had great success. In the first sub-project Kim Davis worked with Brad Godfrey to develop cell loading protocols. These included toxicity studies. Here in Fig. 1 we show the toxicity study of the SMM Fe_8 on HeLa Cells. HeLa Cells are an immortal cervical cancer cell line used in medical research. The cells that were incubated in 1 mM Fe_8 died off (halo shapes Fig. 1 right) over the course of 24 hrs while the cells loaded with 0.5 mM Fe_8 were relatively healthy.

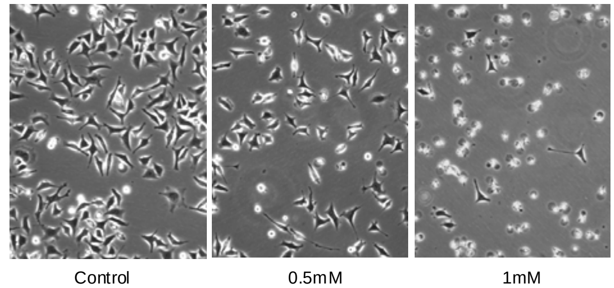


Fig. 1 Toxicity of Fe_8 as a function of loading concentration over 24 hour. Control, (left) shows very healthy cells, the 0.5 mM sample (middle) contains viable cells, however the cell death for 1 mM (halo shapes) is very high.

The next phase was developing NMR protocols for cell studies at 60 MHz. NMR lifetime experiments directly correlate with expected results in MRI. A major problem she faced is the small microlitre sample size of approximately one million HeLa cells. The protocols required: exact sample placement; development of the pulse sequences required for the experiment; calibration of these sequences with known standards; determination of detection limits; and consistency in results. Fig. 2 demonstrated the end results

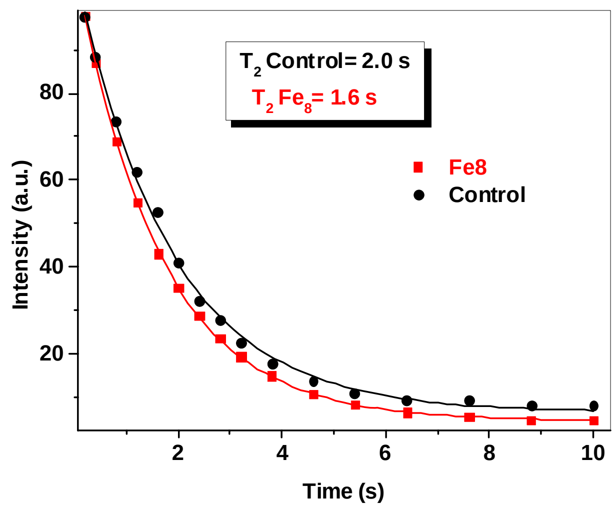


Fig 2 Decrease of the NMR signal intensity with time of cells loaded with Fe_8 vs. a control. Notice that the signal is less for loaded cells, leading to a darker or negative contrast.

of these efforts. The figure shows the NMR intensity as a function of the echo time for both a control and a sample of cells loaded with Fe_8. The NMR peak or signal intensity for cells loaded with Fe_8 drops off faster than the control. The exponential decay fit shows T_2 = 2.0 s for the control vs T_2 =1.6 s for loaded cells. These data directly correlate to contrast in the MRI, i.e., cancer cells loaded with Fe_8 will be darker than surrounding cells that have no Fe_8. These results indicate that we can synthesize SMMs, load them into cells, and detect them in-vivo.

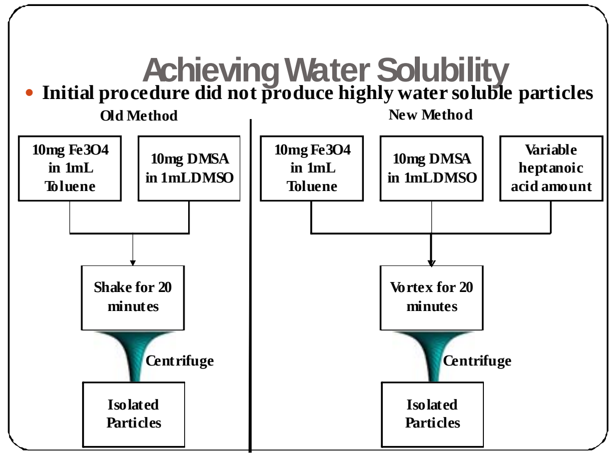


Fig. 3 Aqueous solubility synthesis

The second sub-project is one that is mainly composed of a series of undergraduate students working with limited supervision. This project had its infancy in my first semester of Fall 2007 by Paven Patel and Vivek Patel (no relation). In the spring 2008 they developed the synthesis of the Fe clusters and characterized them by use of dynamic light scattering (with help from Dr. S. Bishnoi, IIT) and NMR lineshift experiments (with Kim Davis).

Josh Morell an undergraduate who joined the group for summer 2008 grabbed a hold of this project and ran with it. A major problem with bioapplications of Fe particles is limited aqueous solubility. I gave Josh a literature example to replicate[i] (Fig. 3 left) and he had limited success. Josh scanned the literature and found that acidic conditions tended to favor the ligand exchange and formulated the process on Fig. 3 right, with more success. He is still working to increase the solubility.

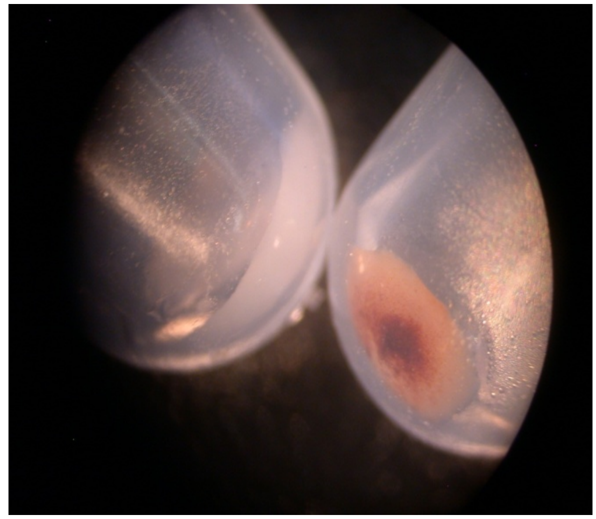


Fig. 4 Picture of control cells (left) and cells loaded with Fe_3O_4 (right). Notice the bright red color for loaded cells indicating iron content

Next Josh worked with Brad Godfrey to load the HeLa cells with his nanoparticles. Fig 4 gives a nice example. On the left is a control cell pellet and on the right is a pellet loaded with Fe clusters. The pellet on the right has a clear red color indicative of Fe content.

With the help of Kim Davis, Josh obtained the NMR results shown in Fig. 5. The control sample on the left shows a long T_2 time. The cells loaded with Fe_3O_4 particles demonstrate a much shorter T_2 time. This translates into high contrast in the MRI. We are working out the protocols to determine exact Fe loading concentrations. These results indicate that phase transfer is effective and the cells are well loaded with Fe_3O_4 particles.

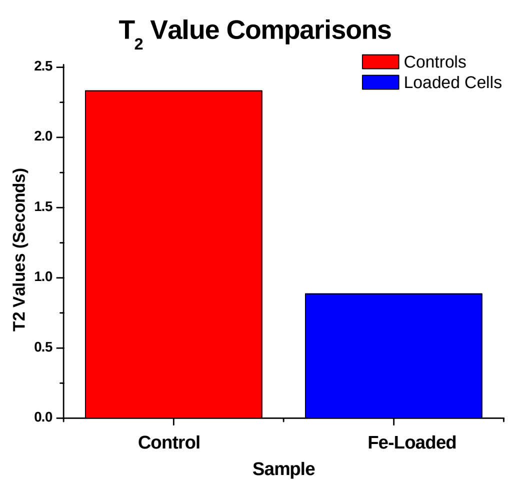


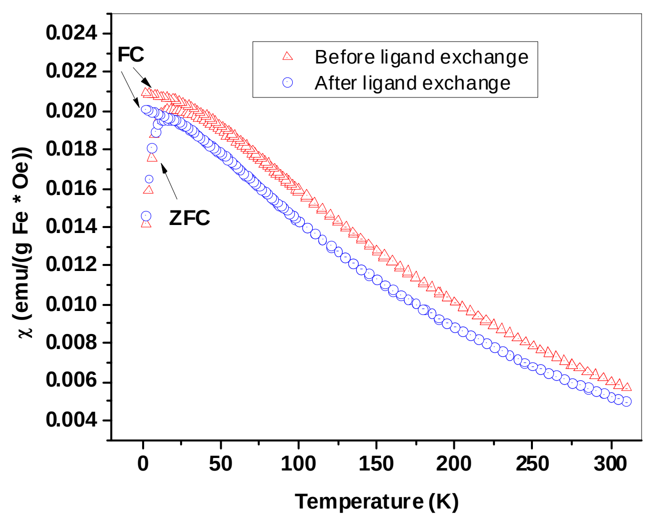
Fig. 5 T_2 of cells loaded with Fe_3O_4 (right) vs control cells (left). The cells loaded with Fe_3O_4 show a dramatic decrease in T2

The third sub-project is the development of novel instrumentation to characterize both our materials and cells that have been loaded with these materials. This is led by the graduate student James McNeely. James is a first year graduate student who joined the group early last summer. His contributions have included setting up the magnetometer for magnetic susceptibility measurements and building a prototype EPR.

Fig. 6 PPMS

For instance, in Fig. 7 James has measured the magnetic moment of J. Morell's nanoparticles before and after ligand exchange. These data show that if the system is cooled in zero magnetic field the behavior is different than if it is cooled in the presence of a field. The point at which the deviation occurs is the superparamagnetic blocking temperature. These data also show that Josh's particles are superparamagnetic after the ligand exchange synthesis.

A second major accomplishment of James is developing the prototype EPR probe. EPR interrogates magnetic materials in the presence of microwave irradiation.



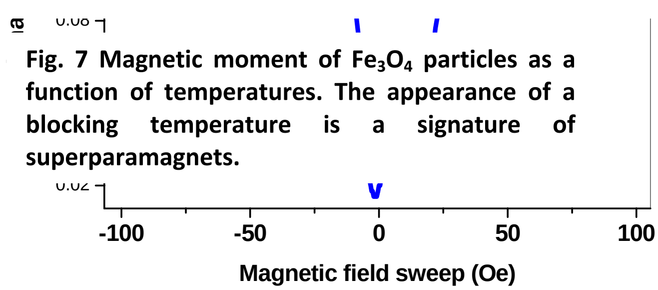


Fig. 7 Magnetic moment of Fe_3O_4 particles as a function of temperatures. The appearance of a blocking temperature is a signature of superparamagnets.

When a magnetic field of the energy strength equal to the microwave energy is applied, a resonant absorption of energy takes place (similar to NMR). One of James' current projects involves the carbon based radical called the Finland trityl. The trityl is an important material for increasing MRI signals by orders of magnitude. The interaction with resonant radiation is a factor. For example, James has obtained EPR spectra at 95 GHz and 1.8 K as shown in Fig. 8. When the magnetic field is swept, microwave energy is absorbed (the signal is driven towards zero). The linewidth, as shown by the double headed arrow, is a parameter for potential applications of the trityl in MRI and quantum computing. This linewidth data as a function of temperature has been accepted for publication in the Journal of Applied Physics.[ii]

Fig. 8 EPR of the trityl radical at 1.8 K and 95 GHz. The linewidth is an important parameter for MRI applications.

Current:

Subproject 1: Investigate NMR frequency dependence of loaded cells. Make contacts at UIC to begin MRI studies

Subproject 2: Attach proteins and antibodies to the ligand-exchanged Fe_3O_4 particles, determine particle size.

Subproject 3: Magnotometry and EPR studies of loaded cells.

PI: Amassing data for publication, using results for a proposal to NIH for magnetic site-directed drug delivery.

Synopsis:

Subproject 1: "Method Development and Analysis of Fe_8 on HeLa Cells"

Leader: Masters candidate Kim Davis

Undergraduates: Ademola Adekola, Riju Konwar, Josh Morell

Major Results: NMR protocols for cell studies at 60 MHz, loading of Fe_8 molecules into HeLa cells.

Subproject 2: "Fe_3O_4 Synthesis, Analysis, and Cell Studies"

Leader: Masters candidate Kim Davis

Undergraduates: Josh Morell, Paven Patel, Vivek Patel, Ademola Adekola, Riju Konwar

Major Results: Synthesis of Fe_3O_4 particles, phase transfer of Fe_3O_4 particles to aqueous solution, ligand exchange of Fe_3O_4 particles, loading ligand exchanged particles into cells.

Sub-project 3: "Synthesis and Characterization of Fe_{17}, development of EPR Probe, and Troubleshooting PPMS"

Leader: Ph.D candidate James Halley McNeeley

Undergraduates: Sonia Goyal, Ademola Adekola, Anthony Mihlosovitch,

Major results: Synthesis and characterization of molecular magnets, magnetic characterization of Fe_3O_4 particles, bringing the magnetometer on-line, development of a high frequency EPR probe prototype. Publication of the trityl EPR results in the Journal of Applied Physics.[ii]

[i] Jun, Y., Huh, Y., Choi, J., et. al., *J. Am. Chem. Soc.* **2005**, 127, 5732

[ii] B. Cage, J. McNeely, S. Russek, H. Halpern, J. Applied Phys. **105**, 1 (2009).