Magnetic Nanoparticles for Disease Detection and Therapy

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**Projects:**

Prostate Cancer
Breast Cancer
Transplant Rejection
Leukemia (Magnetic Biopsy Needle)
Ovarian Cancer
Alzheimer’s Disease
Cystic Fibrosis
What is a Superparamagnetic Iron Oxide Nanoparticle (SPIION)?

- iron oxide ($\text{Fe}_3\text{O}_4$ and/or $\text{Fe}_2\text{O}_3$) nanoparticles (5-50 nm diameter) embedded in an inert material (starch, plastic, glass…) to form beads 10 nm - 5 µm in diameter

- number of SPIIONs per bead can vary widely (from 1 to tens of thousands)

- “superparamagnetic” each SPIION acts like a massive electron spin which aligns with an externally applied magnetic field (no alignment in zero field) – these are “temporary” (as opposed to permanent) magnets
Why would anyone want to be injected with SPIONs?

- SPIONs are already clinically-approved as MRI contrast agents for liver and lymph imaging.
- SPIONs targeted to a particular disease site (e.g., by loading them into cells or attaching ligands to them) would enable specific, non-invasive detection by magnetic methods (think “beeping Tri-corder”…)
- SPIONs attached to therapeutic agents may be targeted (biochemically and/or magnetically) for the specific delivery of drugs (think “multi-functional” – therapy and detection…)
- SPIONs can be used as a local heat source by exposing them to oscillating magnetic fields, enabling hyperthermia of tumors and/or timed-release of drug.
- As metals go, the iron oxides are pretty innocuous.
Detection of SPIONs *In Vivo*

- Attaching recognition ligands to the nanoparticles (which are administered intravenously) enables specific targeting of disease *in vivo*
- Superconducting Quantum Interference Device (SQUID) Magnetometers are sensitive enough to directly detect the magnetic fields (~pT) due to magnetized SPIONs
- Magnetic Resonance Imaging (MRI) detects SPIONs indirectly – because the SPION magnetic field perturbs the MR signal of nearby water protons
MRI of Nude Mouse with C4-2 Prostate Xenograft Tumors, injected with SPIONs

Two adjacent axial slices (2.5 mm thick, 0.25 mm in plane resolution), before intratumoral injection of SPIONs

Same slices, after intratumoral injection of SPIONs

1 Tesla, T₁-w multi-slice spin echo image (TE=4 ms, TR=500 ms, NEX=10)

SPIONs are causing mainly negative contrast in this case
SQUID “Image” of Mouse with Human Xenograft Tumor Injected with SPIONs

-- nude mouse with MCF7 human breast tumor 1 hour after intratumoral injection with anti-Her2 labeled SPIONs

--contours depict the strength of the SQUID-detected magnetic fields (in picoTesla) as a function of position in a horizontal plane (the x and y axes are in centimeters)

--the anti-Her2-labeled SPIONs are retained in the tumor, which was centered under the sensor system

But where’s the mouse??
Why SQUID…

…when MRI is an established detection modality that provides so much spatial information?

Possibilities for SQUID detection of SPIONs:
--Easier Quantitation
--Higher Sensitivity and/or Specificity
--Faster, Cheaper (could be used as a pre-screening with MRI for more detailed follow-up)
Example: *In Vitro* MRI of Cells Labeled with SQUID-visible SPIONs

MR images (spin echo, TE = 10 ms, TR = 1500 ms, $B_0 = 1$ T)

test tubes contain agarose gel and human ovarian cancer cells (cell line TOV112D) labeled with SPIONs

Nanoparticles (SiMAG 1411, Chemicell, Berlin) are attached to antibodies to the CA125 antigen expressed on the surface of the cells
Comparison of SQUID and MR Detection

In general, MR contrast is not linear in the number of NPs present and depends on many factors (type of pulse sequence, repetition time, echo time, background relaxation rates…) – spatial resolution is great, but quantitation is more difficult.

SQUID signal is simply linear in the number of bound NPs – quantitation is much more straightforward.
7-channel SQUID system

Detector Electronics

1-He dewar

Array of 7 pick-up coils

Helmholtz Coils for magnetizing samples

No Magnetically Shielded Room!
7-channel SQUID System

Pick-up coils: convert time-varying fields into voltages ($V \sim dB/dt$), 2$^{nd}$ order gradiometer configuration rejects signals due to distant sources

FIG. 26. Various types of flux transformers: (a) magnetometer; (b) series planar gradiometer; (c) parallel planar gradiometer; (d) symmetric series axial gradiometer; (e) asymmetric series axial gradiometer; (f) symmetric parallel axial gradiometer; and (g) second-order series axial gradiometer.
SQUID Relaxometry Experiment

0.3 s pulse of dc magnetic field ($|B|=B_z=38$ G) to magnetize nanoparticles

50 ms “dead time” before SQUIDs are turned on

2 s acquisition (1 kHz sampling rate) of decaying nanoparticle magnetization

Direct detection of longitudinal relaxation!
SQUID Data and Analysis

--Magnitude and direction of the B-field detected at each sensor depends on where the location of the magnetic source and its total moment (i.e. how many SPIONs)

--Knowing the amplitude of $B_z$ at 7 sensor locations allows determination of $x$, $y$, $z$, and $m_z$ assuming one dipolar source (by fitting)

--Multiple sample positions allow multiple sources to be located

**Raw data:** 10 repetitions, 7 sensors
Source Localization by SQUID Method

<table>
<thead>
<tr>
<th></th>
<th>$\Delta x$ (cm)</th>
<th>$\Delta y$ (cm)</th>
<th>$\Delta z$ (cm)</th>
<th>$m_z$ ($10^{-18}$ J/T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQUID</td>
<td>2.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.36 0.42</td>
</tr>
<tr>
<td>MRI</td>
<td>2.2</td>
<td>--</td>
<td>0.2</td>
<td>--</td>
</tr>
</tbody>
</table>

SQUID Image

MRI

particles
cotton
sticks
Relaxation Mechanisms

- Brownian: rotation of the whole particle w.r.t to the medium (depends on particle size, viscosity…), dominant mechanism for unbound particles

- Neel: reorientation of the magnetic moment only (depends on magnetic properties only), dominant mechanism for cell-bound particles

For small enough particles, Brownian relaxation is very fast (< 1 ms) so that unbound particles are not detected

Signal is specific to bound particles!
SQUID so far...

- Only bound SPIONs give rise to a signal (in principle), which is great for specificity
- Signal is linear in the number of bound SPIONs, which is great for quantitation
- What about sensitivity?
  - Currently our detection limit is ~10 billion 100-nm multi-core particles (or ~200,000 fully-labeled 20-micron cells or 800,000 10-micron cells)
  - SQUID signal depends very strongly on SPION volume (size control could give us up to a 300-fold enhancement of signal from the same amount of Fe$_3$O$_4$)
But Neel Relaxation Time is wildly sensitive to nanoparticle size…

\[ \tau_N = \tau_0 \, e^{(KV/kT)} \]

\( \tau_0 \sim 1 \text{ ns} \)

\( K \) is anisotropy energy density (J/m\(^3\)), arises from crystalline anistropy

\( V \) is particle volume (proportional to diameter\(^3\))

We need very uniform SPIONs! (non-trivial)
Characterization of Ocean Nanotech SHP Nanoparticles by TEM

Nominal Particle Diameters:

<table>
<thead>
<tr>
<th>Nominal Diameter (nm)</th>
<th>Average Measured Diameter (nm)</th>
<th>Standard Deviation (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20.1</td>
<td>1.5</td>
</tr>
<tr>
<td>25</td>
<td>26.4</td>
<td>2.8</td>
</tr>
<tr>
<td>30</td>
<td>24.7</td>
<td>2.7</td>
</tr>
<tr>
<td>35</td>
<td>36.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Based on analysis of ~5000 particles from TEM images
Comparison of Size and Detected Magnetic Moment Ocean-SHP Nanoparticles

“20 nm” particles are too small

“35 nm” particles are too big

“25 nm” and “30 nm” particles are essentially the same size (26.4, 25.7 nm), but give very different SQUID signals
**SPION Characterization Status**

- Currently, the fraction of Fe₃O₄ that is “SQUID-visible” is of order 0.3% - 2%, depending on which commercial SPIONs we use.

- Great improvements in sensitivity will come from better size-control during nanoparticle synthesis! (work underway in Dale Huber’s lab at Sandia CINT)

- Meanwhile, we can’t theoretically predict which lot of SPIONs will give the highest signal, and batch-to-batch variations are huge – we have to experimentally characterize many batches to find the good ones!
Characterization of Magnetite Nanoparticles for SQUID-relaxometry and Magnetic Needle Biopsy

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Abstract Magnetite nanoparticles (Chemicell SiMAG-TCL) were characterized by SQUID-relaxometry, susceptometry, and TEM. The magnetization detected by SQUID-relaxometry was 0.33\% of that detected by susceptometry, indicating that the sensitivity of SQUID-relaxometry could be significantly increased through improved control of nanoparticle size. The relaxometry data were analyzed by the moment superposition model (MSM) to determine the distribution of nanoparticle moments. Analysis of the binding of CD34-conjugated nanoparticles to U937 leukemia cells revealed 60,000 nanoparticles per cell, which were collected from whole blood using a prototype magnetic biopsy needle, with a capture efficiency of >65\% from a 750 µl sample volume in 1 minute.
Detection of Leukemia by Magnetic Needle Biopsy (in vivo magnetic cell sorting)

Idea: Inject SPIONs targeted to leukemia cell surface antigens into bone marrow; capture labeled blast cells with magnetic needle

Goal: enhanced sensitivity to low-level disease

Polyimide Needle Sheath

“Needle”: Stainless Steel Rod + magnets in polyimide sleeve

Bands of SPIONs collected on sheath
In Vitro Magnetic Needle Experiment:

Goal: retrieval of CD34-expressing cells (U937 cell line) from spiked normal blood in vitro using anti-CD34-conjugated nanoparticles and the magnetic needle (for proof of principle)

Cells: 9 different concentrations of U937 cells in cell media mixed with normal blood to achieve 750 uL volume (control experiment performed using same ratios of blood to cell media with no cells)

SPIONs: Add anti-CD34 labeled SPIONs, incubate for 60 minutes (measurements of magnetic moment of whole vial at 1 min and 60 min)

Needle: Insertion of magnetic needle for 1 minute to retrieve magnetically-labeled cells

-- Measurement of magnetic moment of:
   1) material left behind in vial
   2) material collected on needle
SQUID Measurements: U937 Cells, spiked in blood, and labeled with SPIONs

Incubation of U937 cells and media with anti-CD34 labeled NPs in Normal Blood

Small aggregates move to needle slowly and are mostly left behind in vial

Large aggregates (i.e. cells) move to needle quickly and are preferentially collected by needle
SQUID Measurements: Control (no cells)
SQUID Measurement of Material Collected on Needle: U937 cells and control

Magnetic Moment Collected on Needle vs. Cell Number

- Magnetic moment (pA-m-m)
- # cells spiked (or equivalent dilution)

Graph showing magnetic moment collected on needle vs. cell number for U937 cells and control.
Specificity in capturing labeled cells is due to both biochemistry and physics...

- \( v_{\text{cell}} / v_{\text{nano}} = n \frac{r_{\text{nano}}}{R_{\text{cell}}} \), where \( n \) is number of nanoparticles on cell
  (of order 100 for realistic parameters)

- large, poorly labeled things (e.g., a neutrophil that ate a few SPIONs) also have smaller terminal \( v \)
Why is material collected on needle decreasing at high marrow fraction (when there clearly are more blasts in the sample tube)? Too few SPIONs to adequately label all those blasts!
The ultimate goal: enhancement of sensitivity to low blast counts for detection of Minimal Residual Disease

Patient bone marrow, diluted in normal donor blood, *in vitro* needle extraction

<table>
<thead>
<tr>
<th>Sample (ratio of patient marrow to donor blood)</th>
<th>Pre needle draw</th>
<th>Needle Extract</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (blood)</td>
<td>0.0%</td>
<td>0.0%</td>
<td>NA</td>
</tr>
<tr>
<td>1 (marrow)</td>
<td>96.0%</td>
<td>97.0%</td>
<td>1.01</td>
</tr>
<tr>
<td>1/2</td>
<td>84.5%</td>
<td>94.5%</td>
<td>1.12</td>
</tr>
<tr>
<td>1/4</td>
<td>74.0%</td>
<td>75.5%</td>
<td>1.02</td>
</tr>
<tr>
<td>1/8</td>
<td>61.5%</td>
<td>69.5%</td>
<td>1.13</td>
</tr>
<tr>
<td>1/16</td>
<td>48.0%</td>
<td>59.5%</td>
<td>1.24</td>
</tr>
<tr>
<td>1/32</td>
<td>39.5%</td>
<td>51.5%</td>
<td>1.30</td>
</tr>
<tr>
<td>1/64</td>
<td>32.0%</td>
<td>49.5%</td>
<td>1.55</td>
</tr>
<tr>
<td>1/128</td>
<td>27.0%</td>
<td>41.0%</td>
<td>1.52</td>
</tr>
<tr>
<td>1/256</td>
<td>19.5%</td>
<td>39.5%</td>
<td>2.03</td>
</tr>
<tr>
<td>1/512</td>
<td>13.0%</td>
<td>34.5%</td>
<td>2.65</td>
</tr>
<tr>
<td>1/1024</td>
<td>5.5%</td>
<td>29.5%</td>
<td>5.36</td>
</tr>
</tbody>
</table>

In the case of low blast counts (where enhanced sensitivity is most helpful), there are plenty of SPIONs to fully-label the blasts.

Their concentration (relative to the total white cell concentration) is greatly enhanced!
Conclusions

• SQUID relaxometry is a promising method for detecting SPIONs in biological systems, with many details to work out.
• Careful particle synthesis, conjugation, and characterization is essential.
• Magnetic Needle Biopsy is ready for first *in vitro* clinical trials.
Thank you for your attention!

SQUID-Mouse

courtesy of Helen Hathaway
SPION fairy tales...

• Ab-conjugated SPIONs enter the bloodstream or other media as single beads. They don’t stick to each other to form big nasty clumps.

• Ab-conjugated SPIONs only bind to those cells presenting the appropriate antigen. Otherwise, they don’t interact with cells.
Beware of SPION Aggregation

Aggregates are **bad** if they are large enough to act like cells (i.e., Brownian relaxation is too slow, so the Neel mechanism dominates) – we detect a SQUID signal, and it looks like it’s caused by binding to cells

Nominally the same SPIONs (same product code) from two different lots, suspended in same medium – tube on left gives a huge signal, even though there are no cells and no Abs (YIKES)
Some combinations of SPION/Ab/conjugation method are better than others...

CD3$^+$ T cells incubated with SPIONs (conjugated to CD3 antibody or no Ab)

CD3
No Ab
BAD

CD34$^+$ leukemia cells incubated with SPIONs (conjugated to CD34 antibody or no Ab)

CD34
No Ab
BETTER