

Detoxification of Humans with Magnetic Nanoparticles

BioMagnetICS 2004 Review

Carol Mertz, Argonne National Laboratory

*A Collaboration Between Argonne National Laboratory and
The University of Chicago*

Michael D. Kaminski and Axel J. Rosengart, Co-PIs

Argonne National Laboratory

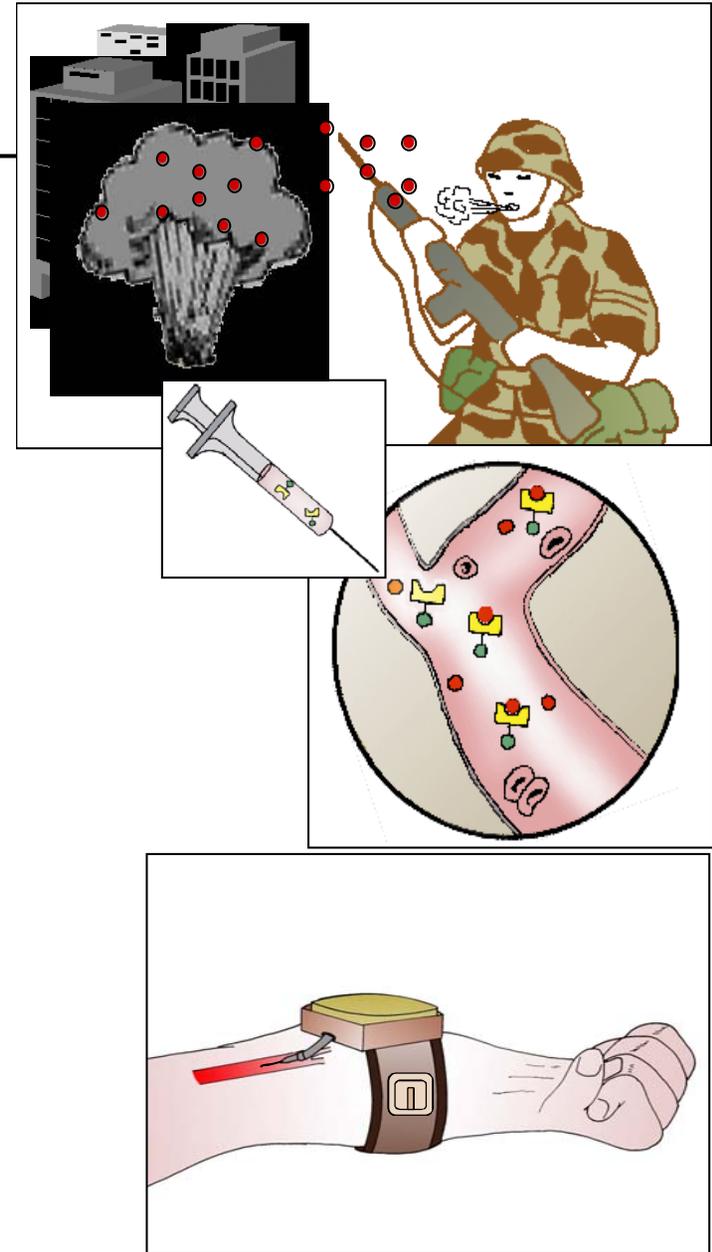


*A U.S. Department of Energy
Office of Science Laboratory
Operated by The University of Chicago*



Technology Description

- Develop a portable, rapid detoxification system for blood-borne biological toxins.
 - Self-injection of magnetic drug
 - Free circulation of magnetic drug
 - Specific binding of toxins due to selective surface receptors
 - Physical removal of toxin-drug complexes in hand-held magnetic filter



Team

- **Argonne National Laboratory**
 - M. Kaminski, C. Mertz, M. Finck, V. Sullivan, F. Stevens, K. Kasza, P. Fischer
- **The University of Chicago**
 - A. Rosengart, S. Guy
- **Illinois Institute of Technology**
 - Profs. Vincent Turrilo, Viji Balasubramanian, Kristine Stainszewski
- **Univ. South Carolina**
 - Prof. James Ritter, Dr. Armin Ebner
- **Graduate students**
 - Y. Xie, H. Chen, P. Caviness
- **Important consultations**
 - Prof. Ali Salem (University of Iowa)
 - DTRA, FBI, AFRRRI, DOE, NORTHCOM, SBCCOM, DOD
- **Virginia Polytechnic Institute and State University**
 - Prof. Judy Riffle
- **Collaboration started**

COLLABORATIVE INVESTIGATORS FOR APPLIED NANOTECHNOLOGY IN MEDICINE



Approach

- **Design, synthesize and test biodegradable magnetic nanospheres that have surface receptors with high affinity for the target biological toxin**
 - Start with model, functionalized, magnetic spheres to show potential for toxin removal
 - Synthesize copolymers of poly(lactic acid)-poly(ethylene glycol)-biotin
 - Synthesize microspheres and nanospheres to understand process parameters
 - Test receptor selectivity and affinity
- **Design, fabricate, and test a prototype magnetic filter to separate the magnetic nanospheres from the blood**
 - Start with simple permanent magnet designs to understand capability
 - Use computational models to improve designs
 - Experimentally test models and show feasibility



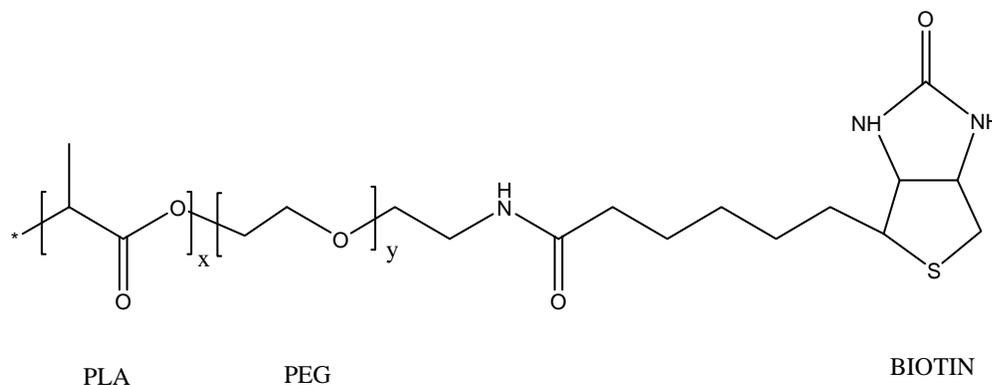
Milestones

- 1) Demonstrate in vitro separation of biotinylated enzyme from whole blood to better than 50% using model polystyrene-based magnetic spheres
 - 1) *In vitro* tests show **72% removal** of biotinylated enzyme in saline and whole blood
- 2) Demonstrate magnetic filtering of model polystyrene-based magnetic spheres in prototype unit to better than 95%
 - 1) *Laboratory tests show **94% removal** of particles in a single cycle; **100% removal** in third cycle; Simple designs*
 - 2) *Advanced designs being tested now based on computational design improvement*



Progress--Nanosphere Development (cont.)

PLA-PEG Biotin polymer synthesis



Polymer Composition: Monomer Units per PLA-PEG-biotin

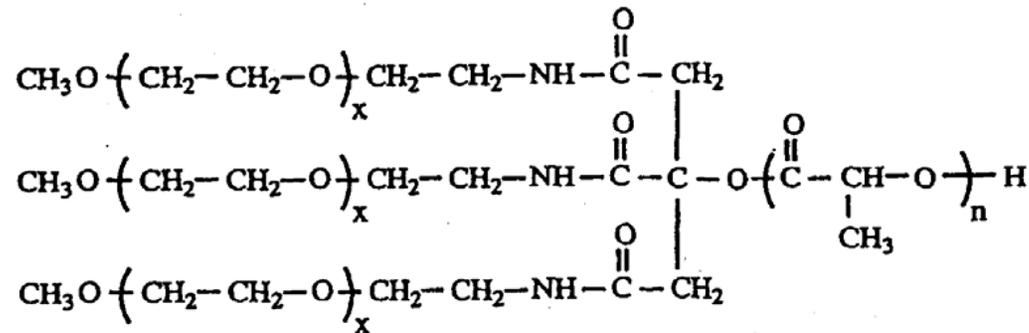
	Theoretical	Actual (Proton NMR)
PLA x=	~300	~250
PEG y=	~70	~60
Biotin	1	1



Progress--Nanosphere Development (cont.)

Enrichment of particle surface in PEG

(Methoxy-PEG)₃ - citric acid - PLA



- Branched multi block PLA-(PEG)₃ polymer will be synthesized according to Langer (MIT) via citric acid branched link.
- Further biotinylation of the branched PEG polymer will provide particle surface enrichment in biotin for reaction with streptavidin or biotin directed antibodies.



Progress--Nanosphere Development (cont.)

Tagging for detection of intact particles

- **Fluorescent tagging**

- Synthesis of PLA-PEG particles containing fluorescent dye
 - *Encapsulated dye fluoresces at 633 nm for intact particle detection via flow cytometry without interference by blood fluorescence*

- **Radioactive tagging**

- Synthesis of hydrophobic chelate for Indium-111
- *Indium-111 (radioactive gamma emitter) chelated by DTPA-SA and encapsulated in PLA-MPEG particles.*

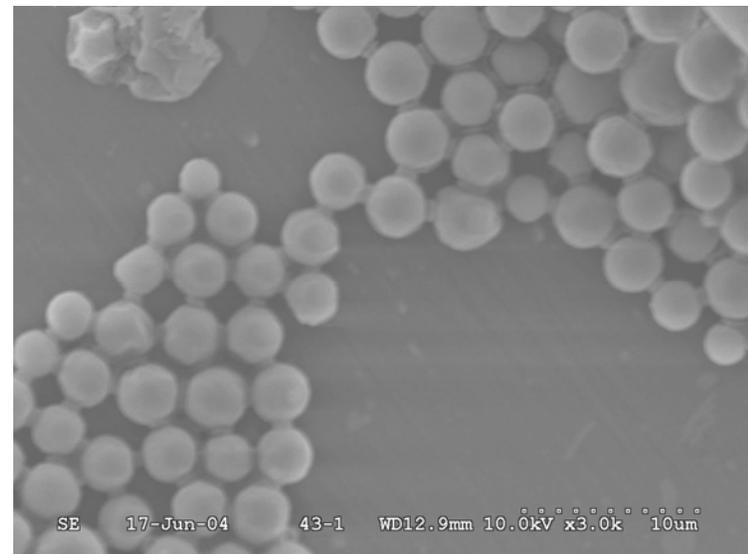
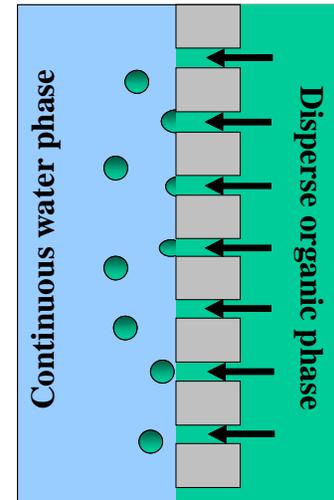
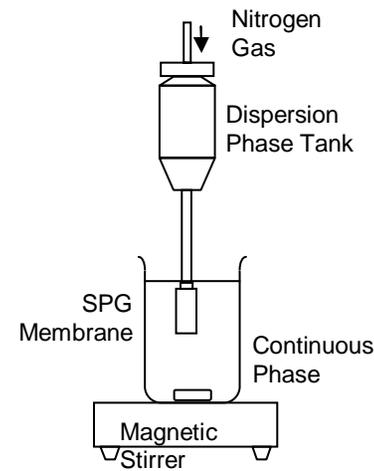
- *DTPA-SA = Diethylene Triamine Pentacetic Acid -Stearyl Amide*



Progress--Nanosphere Development (cont.)

Production of Uniform Microspheres

- Membrane emulsification produces monodisperse microspheres by controlling emulsification
- Best results so far achieved with PLA (poly (L-lactide)) in dichloromethane emulsified through a hydrophilic 3.0 μm pore size membrane into an aqueous solution of 1.2 wt% SDS (sodium dodecyl sulfate)
- Membrane pore size of 3.0 μm produced particles with a 3.5 μm average particle diameter

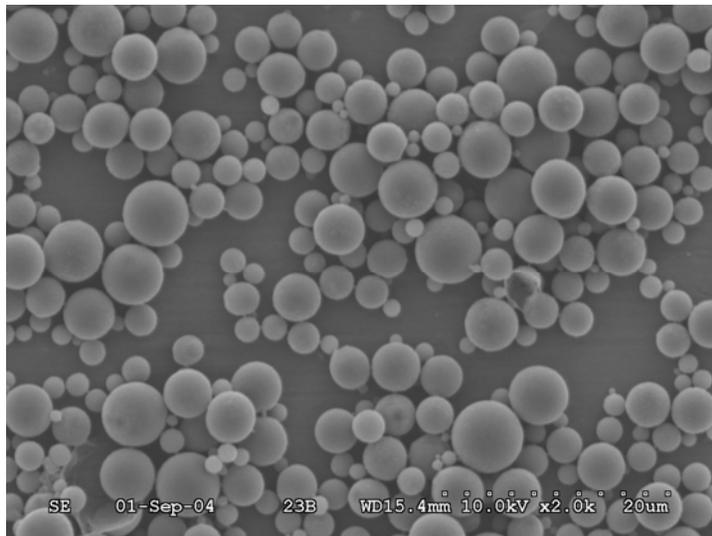


9

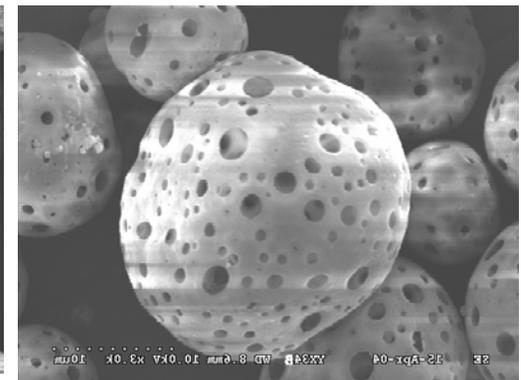


Progress--Nanosphere Development (cont.)

- **Successful synthesis of biodegradable polymer microspheres incorporating magnetic phases**
 - Encapsulation of both polymer (PLLA)-coated and non-coated magnetite (with different magnetizations)
 - Consistent size and narrow distribution of particles (<5 micron)
 - Current data set
 - *f(emulsifier concentration, magnetite concentration)*



PLA-PEG

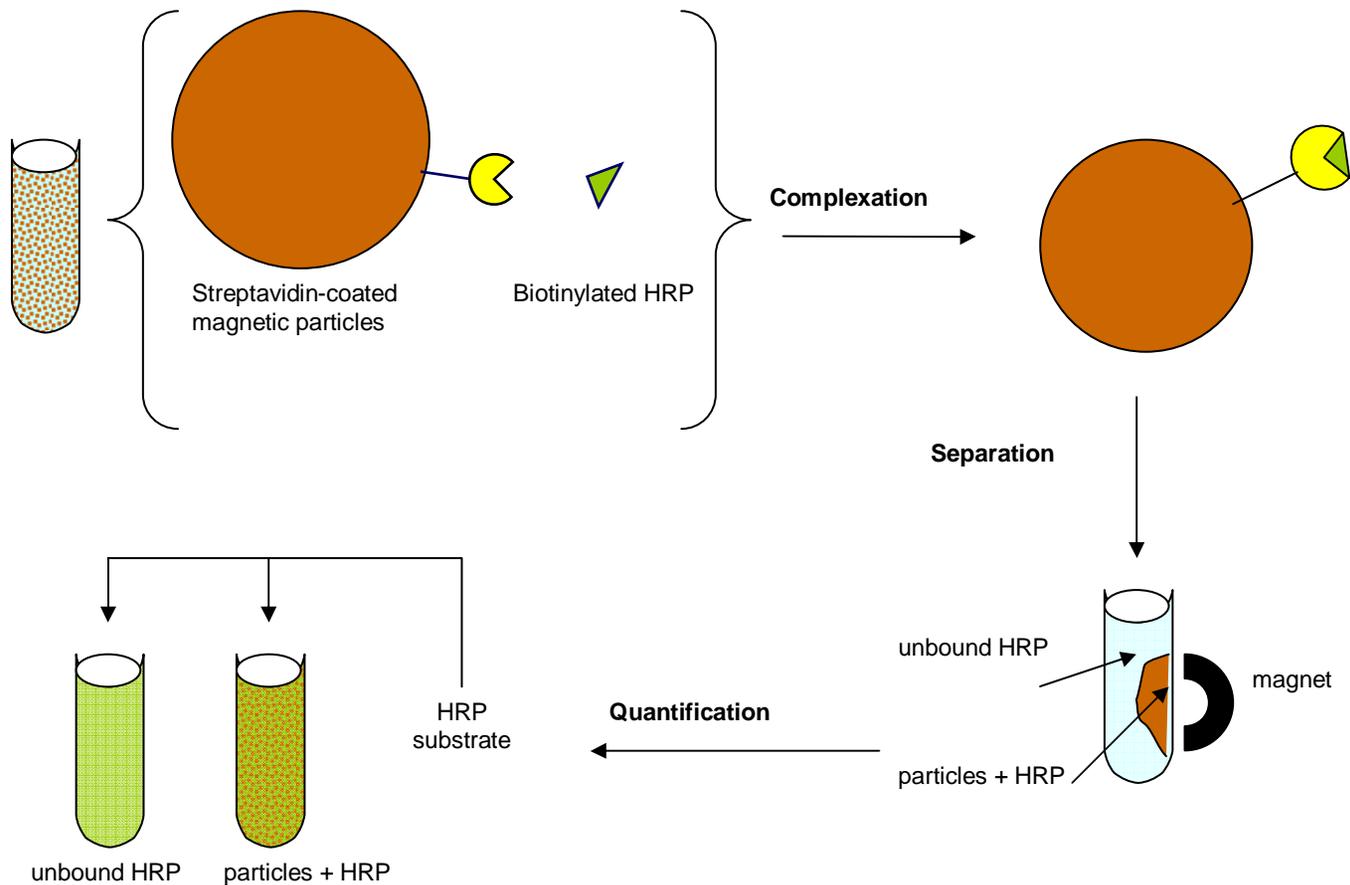


50 W sonicat



Progress--In Vitro Experiments

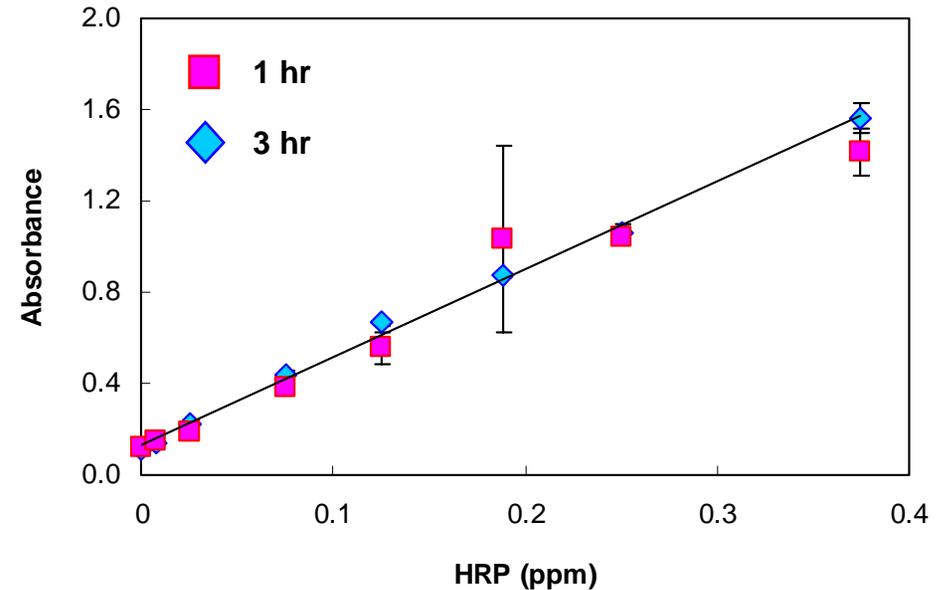
Procedure development



Progress--In Vitro Experiments (cont.)

Procedure Development

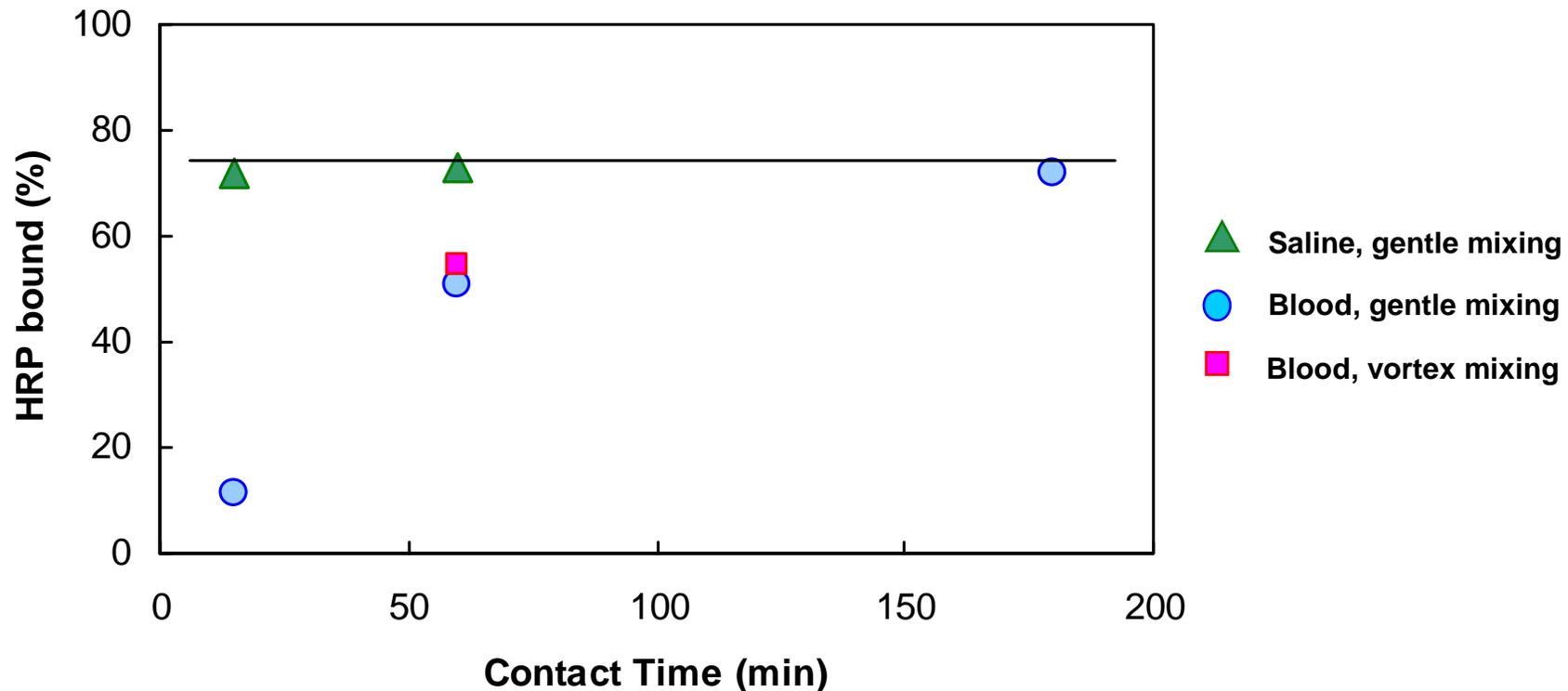
- Rapid kinetics for binding biotinylated horseradish peroxidase (bHRP) to streptavidin-coated microtiter wells (over a range of bHRP concentrations) based on incubation time
- High background due to presence of blood in detection measurements eliminated by sufficient rinsings of wells or dilution of blood samples



Sample Description	Absorbance @ 405 nm	# Well Rinses
Blood (undiluted)	0.84	3
Blood (diluted 1:2)	0.78	3
Blood (diluted 1:4)	0.42	3
Blood (diluted 1:6)	0.11	3
Saline	0.09	3
Water	0.09	3
Blood (undiluted)	0.10	10
Blood (undiluted)	0.09	15

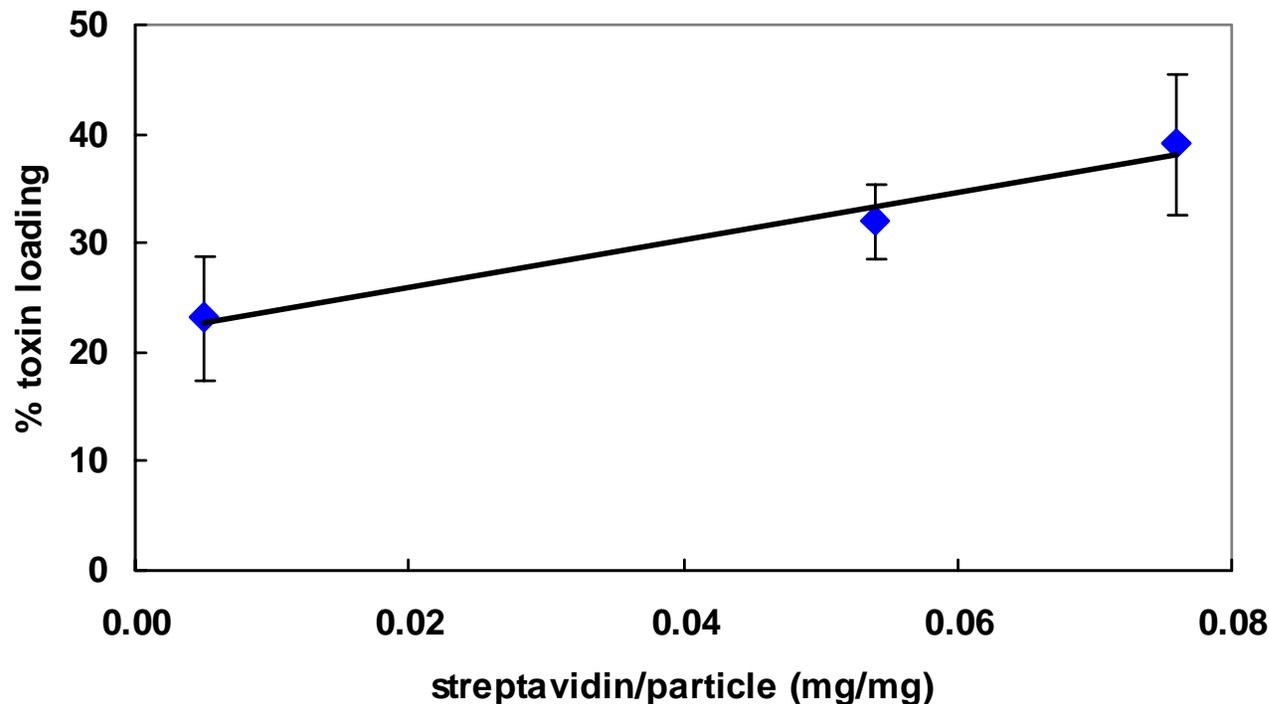
Progress--In Vitro Experiments (cont.)

- Model toxin (biotinylated HRP, bHRP) removal of 72% onto streptavidin-coated magnetic, latex nanoparticles (400 nm)
- Rapid kinetics/efficient removal for single contact promises greater removal efficiencies with optimization



Progress--In Vitro Experiments (cont.)

- Successful toxin (bHRP) removal using microparticles synthesized in our lab (streptavidin-coated biotinylated, poly(ethylene glycol)-poly(lactic acid))
- Up to **40% removal** of toxin with single particle contacts in saline media and 1 hr incubation; optimization promises greater capture efficiencies



Progress--In Vivo Experiments (cont.)

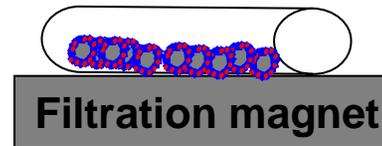
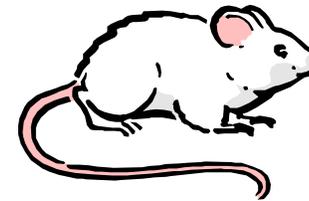
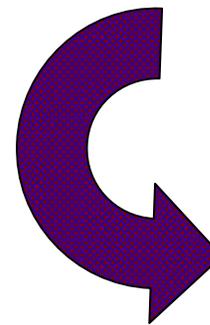
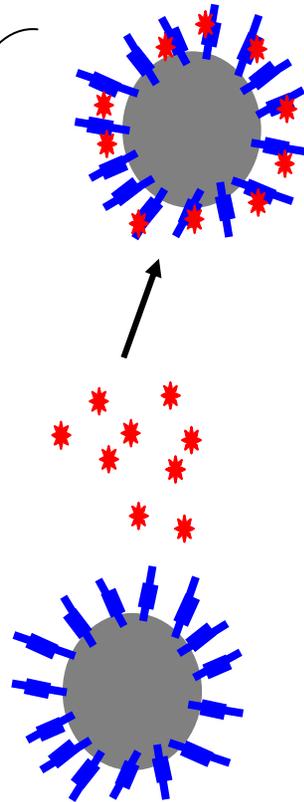
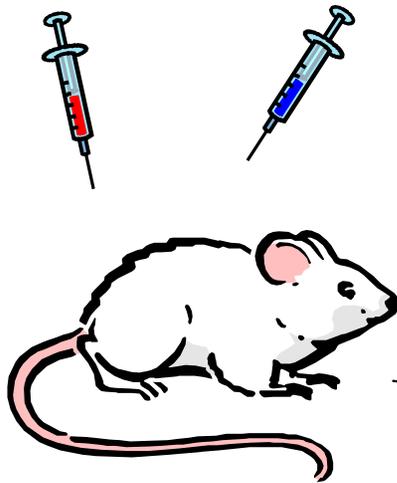
Design

Inject model toxin

Inject chelating particles

In vivo binding

Perform separation

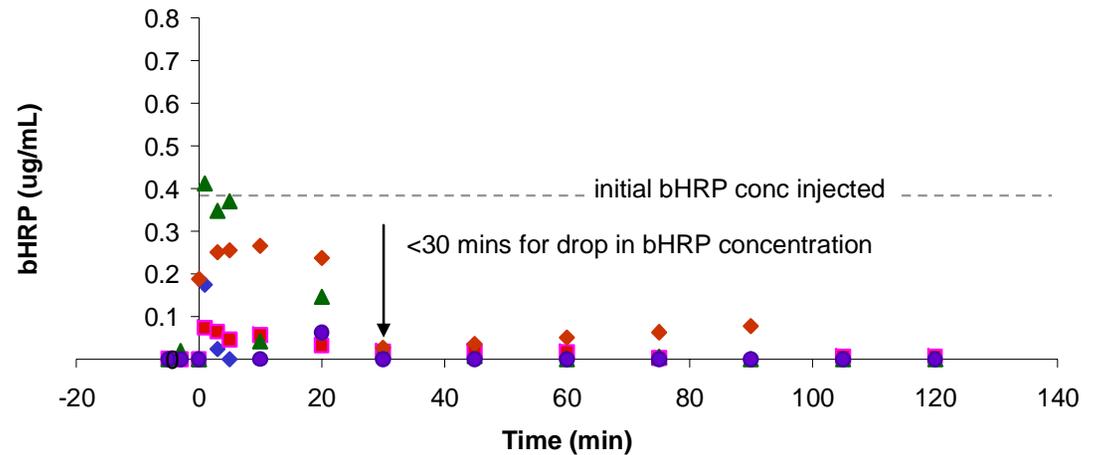


Progress--In Vivo Experiments (cont.)

Model toxin

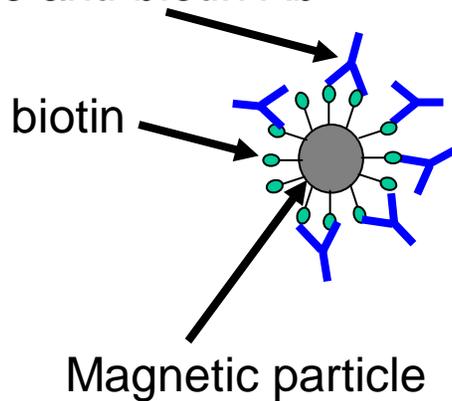
Initial – biotinylated HRP

removed naturally from circulation within 30 min



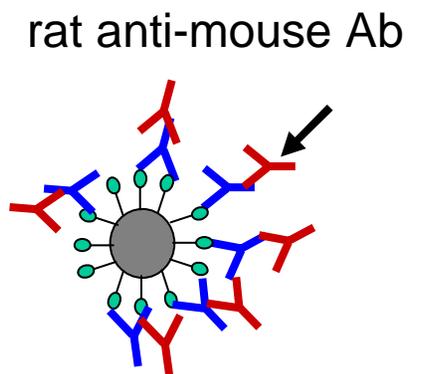
Future -

mouse anti-biotin Ab



Inject free rat anti-mouse Ab
(model toxin/prey)

Inject complex
(model particle/bait)

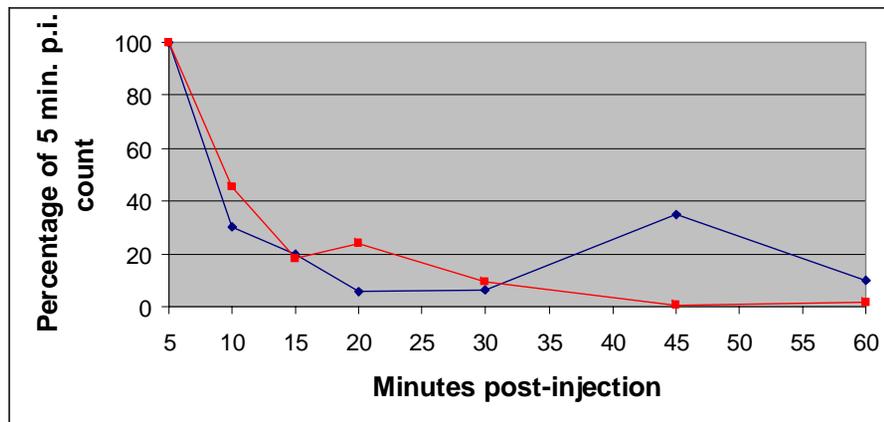


Progress--In Vivo Experiments (cont.)

Model particle half-life

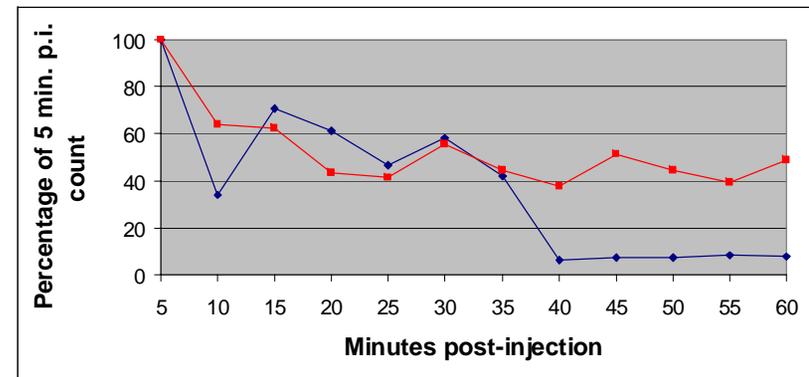
Commercially available 3 μ m polystyrene particles incubated with PEG 6000.

Less than 50% remaining after 10 minutes



Our PLA-PEG-biotin particles loaded with 10% PLA-coated magnetite and 0.5% DiD oil (dye)

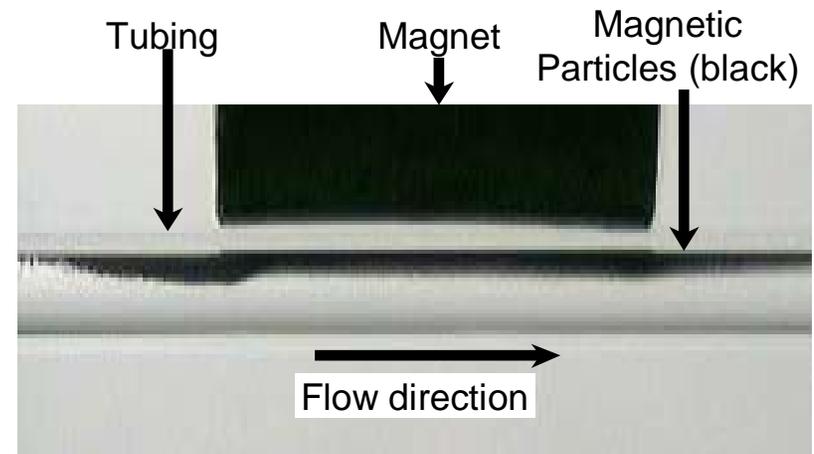
Over 50% remaining at 30 minutes



Progress--Magnetic Filter Development

- **Specifications:**

- Compact (hand-held, <1 lb)
- Zero power, no moving parts (permanent magnets)
- Self-applied (dual lumen catheter)
- High throughput (entire blood volume in < 30min)
- Minimal blood contact (sterilized, non-thrombotic tubing)
- Useful for bioassay or bioforensics (removable collection chamber)



Design Parameters for Magnetic Filter

- **Magnetic field (Magnet)**
 - Geometry
 - Local magnetic field strength & gradient
- **Magnetic nanosphere properties**
 - Type of magnetic materials
 - Content of magnetic materials
 - Size of spheres
- **Tubing system**
 - Size
 - Chemical / physical properties
 - Multiple passes
 - Parallel / capillary system



Femlab Simulations

Capture Efficiency (CE)

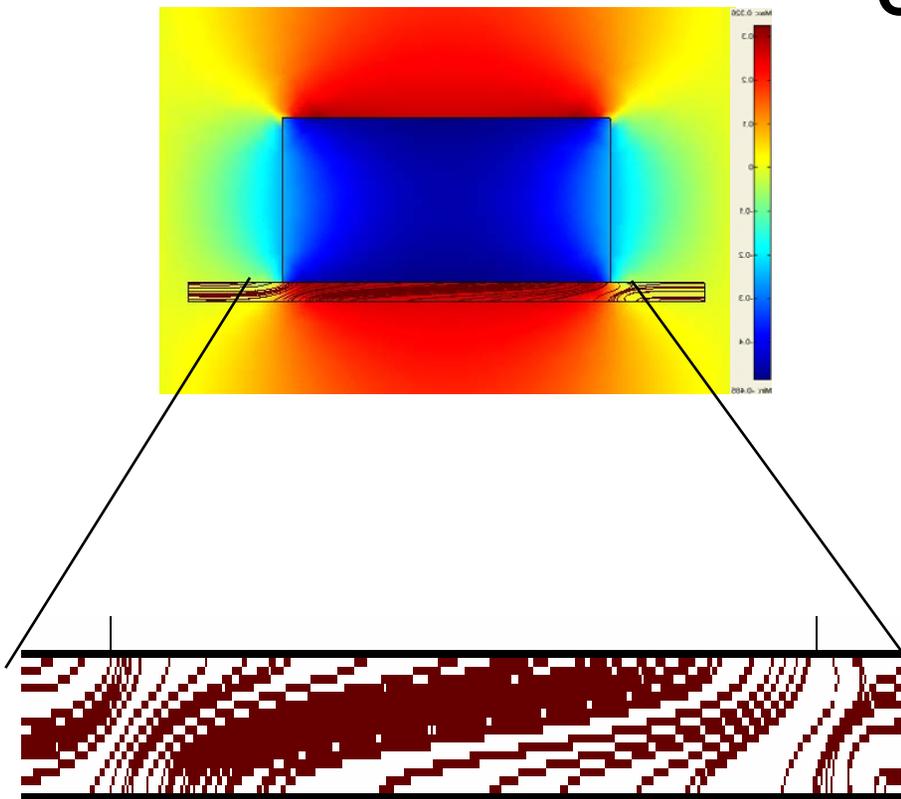
2D:

$$CE = \frac{Y - y_1}{D} \times 100\%$$

where Y is the y-coordinate of the upper inner wall of tubing, y_1 is the y-coordinate of the last streamline at the inlet that becomes attracted by the magnetic field, and D is the inner diameter of the tubing

3D:

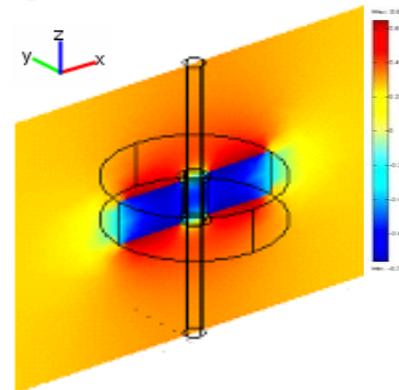
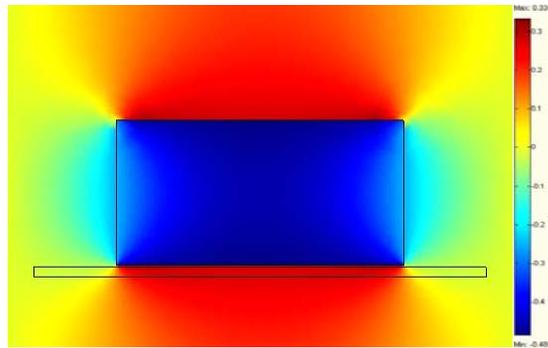
Femlab + Matlab Code



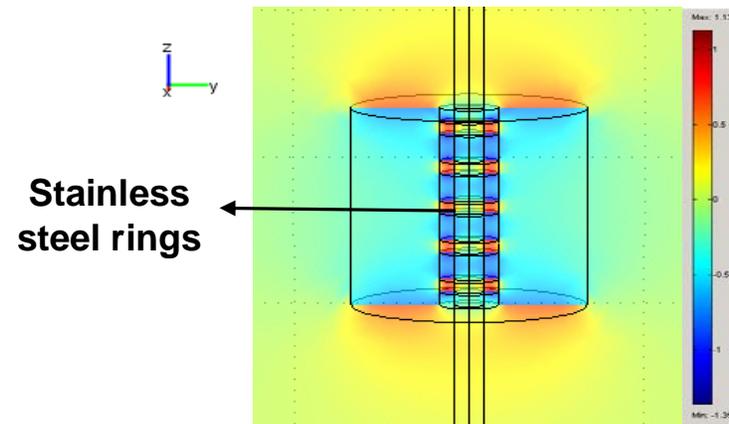
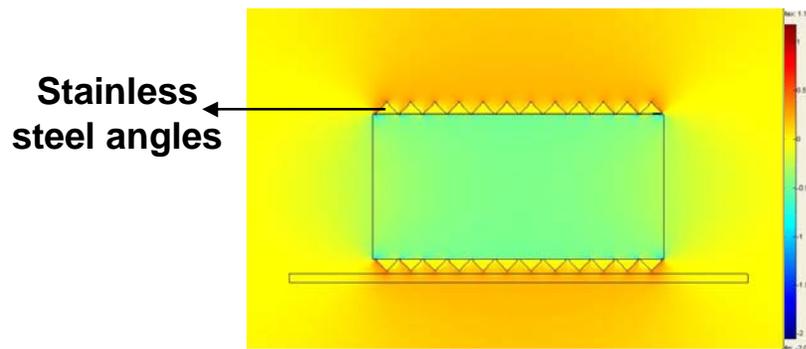
CE = 100% for single pass

Designs

Basic Designs (square and donut magnets, multiple passes)



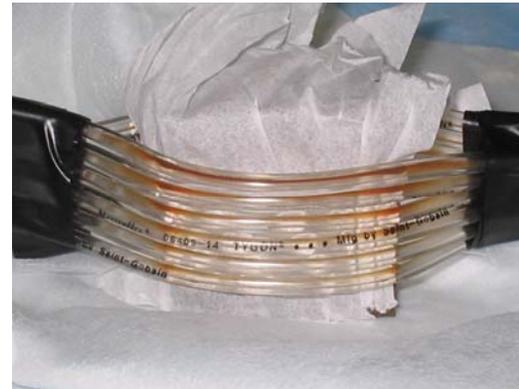
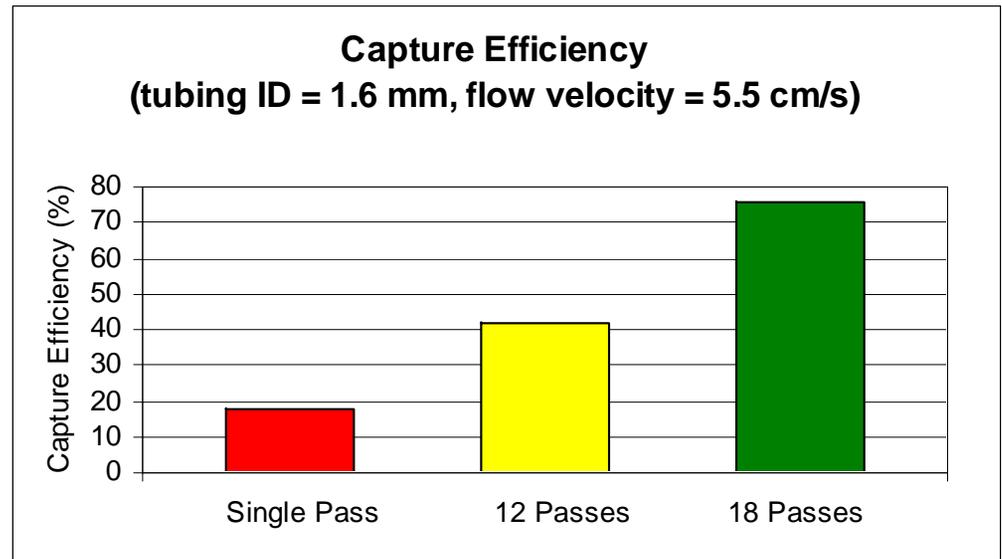
Hybrid Magnets (magnet + stainless steel elements)



Preliminary Experiments

- **Current Method**

- Multiple-pass design applied
- Square NdFeB magnet, 0.4T, 2"(L) x 2"(W) x 1"(H)
- 400 nm magnetic spheres were labeled with ^{59}Fe
- Gamma counter (2.5% - 3.5% error) measured initial and final solutions for each run



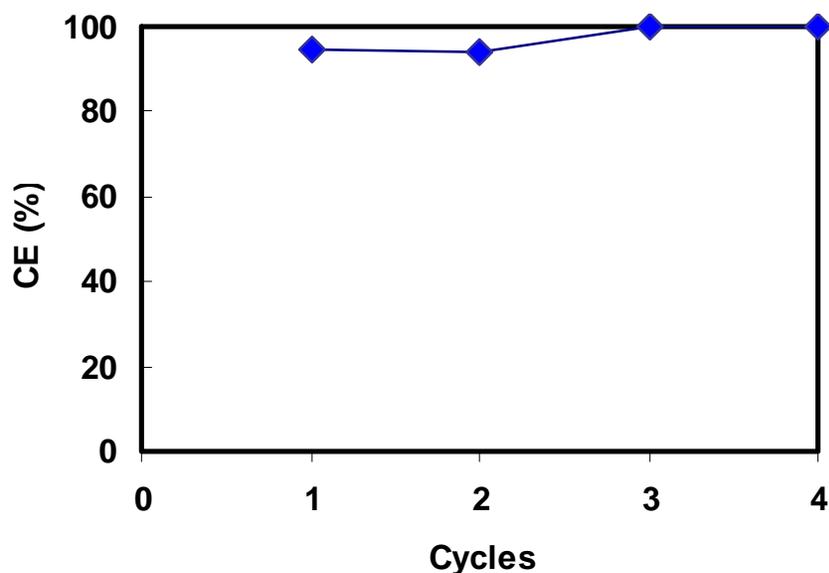
Capture of 400 nm magnetic nanospheres, 12 passes

22



Preliminary Experiments (cont.)

- Achieved 94% capture efficiency of 400 nm magnetic particles in first cycle using prototype magnetic filter; 100% capture in third cycle



Multiple-pass design applied (40 passes)

Flow rate of 6.6 mL/min

Two Square NdFeB magnet

Tubing ID = 1.6 mm and OD = 4.8 mm

400 nm magnetic spheres were labeled with ^{59}Fe

Gamma counter (2.5% - 3.5% error) measured initial and final solutions for each run

Future Goals--Magnetic Filter Development

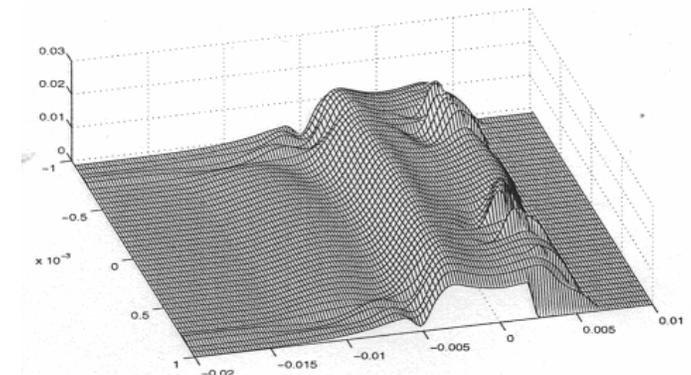
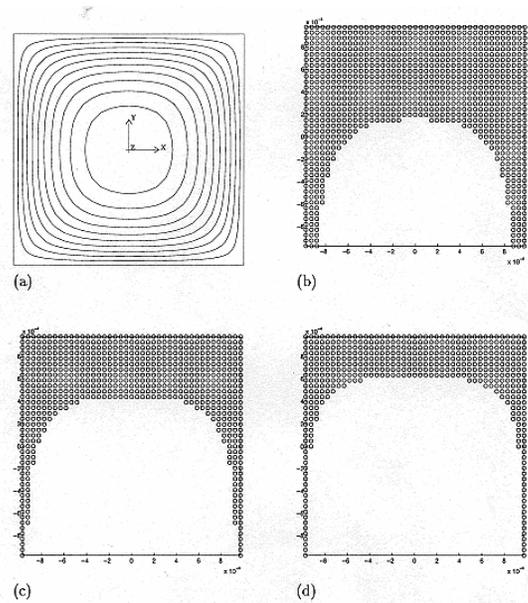
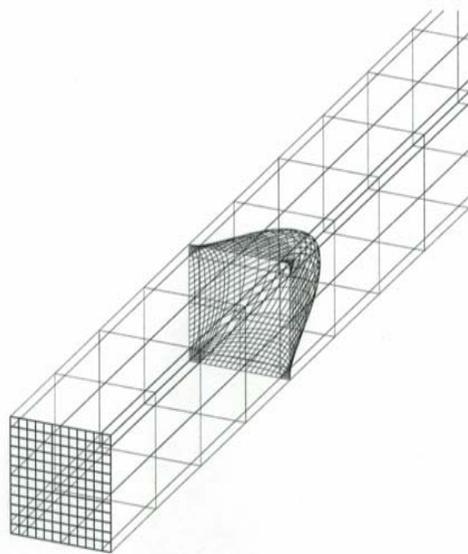
- **Improvement on magnet design**
 - Hybrid magnets (NdFeB magnet + 400 series stainless steel elements)
 - Magnets with different geometries (donut magnet and strip magnet, etc)
- **Improvement on tubing system**
 - Tubing with optimized inner diameter and wall thickness
 - More tubing passes through magnetic field
 - Capillary bed (single piece of tubing branching into multiple smaller tubes)



Progress--Magnetic Filter Development

- **DOE-leveraged model**

- Coupled fluid dynamics-magnetic field computational model tracks individual nanospheres in the filter
 - model effects of the full 3-dimensional B-field, including the effects of magnetization strength (i.e., not just the saturated magnetization level)
 - model influence of the trapped particle pile shape on the flow conditions and magnetic field strength (due to increased distance from the magnet)



Progress--Magnetic Filter Development (cont.)

- **Goal: Develop a pharmacokinetic model to help establish expectations of technology**
 - Therapeutic efficacy (how much can we bind and remove per treatment)
 - Dosage (mg of nanoparticles needed to impart effect)
 - Duration (circulation time before applying extracorporeal filter)
- **Model Construction**
 - Takes into account competitive adsorption, non-specific adsorption, organ filtration, kinetics
 - Couples model with experiment

Publications, Presentations, Publicity

- **8 papers submitted, accepted, or published**
- **3 reports or book chapters**
- **29 oral presentations/posters**
 - Areas include conceptual technology description, nanoparticle synthesis, biological separations, and magnetic filter design
- **General Press**
 - Small Times, Daily Herald, The Engineer, Wired News, Nanobiotech News, Materials Today

COLLABORATIVE INVESTIGATORS FOR APPLIED NANOTECHNOLOGY IN MEDICINE



- Mission: movement of nanotechnology into engineering technology and deployment for medical applications.
 - www.cmt.anl.gov/science-technology/nanomedicine/default.shtml

27



Key Challenges

- **Magnetic nanoparticles of uniform size**
- **Magnetic nanoparticles with high saturation magnetization**
 - Materials with $dB/dH > \mu_0$ at less than saturation
- **Efficient, industrially robust synthesis method for polymeric-based nanospheres**
- **Imaging tool for assaying surface properties of individual spheres**

Summary

- Made great strides in nanosphere synthesis (functionalization of biodegradable nanospheres, reproducible production procedure, increased magnetization)
- Demonstrated successful removal of simulant biotoxin with *in vitro* testing using model, functionalized nanospheres (up to 70% with a single contact)
- Developed enzyme linked immunosorbant assay (ELISA) for the quantitation of the toxin-bound magnetic nanoparticles (*in vitro* work being extended to *in vivo* studies)
- Demonstrated >95% removal of model nanoparticles using prototype magnetic filter (modeling work on design instrumental in evaluation and future development)
- Results from these studies support successful concept feasibility for detoxification of human subjects