

REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-03-

0456

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, gathering existing data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), Washington, DC 20540-6001. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to provide information if it does not have a valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

| | | | | | |
|--|--|---------------------------------------|--|---|--|
| 1. REPORT DATE (DD-MM-YYYY) 09/14/03 | | 2. REPORT TYPE Final Report | | 3. DATES COVERED (from - to) 6/1/01 - 5/31/03 | |
| 4. TITLE AND SUBTITLE SEEDLING Proposal to Establish Pilot Data for a Consortium on Magnetic Nanoparticle Assemblies: A New Tool for Drug Delivery, Sensors and Electronic Devices | | | | 5a. CONTRACT NUMBER F49620-01-1-0407 | |
| | | | | 5b. GRANT NUMBER | |
| | | | | 5c. PROGRAM ELEMENT NUMBER | |
| 6. AUTHOR(S) Dr. Judy S. Riffle, Dr. Richard O. Claus, and Dr. Urs Hafeli | | | | 5d. PROJECT NUMBER | |
| | | | | 5e. TASK NUMBER | |
| | | | | 5f. WORK UNIT NUMBER | |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Virginia Tech Dept. of Chemistry (0212) Blacksburg, VA 24061 | | | | 8. PERFORMING ORGANIZATION REPORT NUMBER 4-30917 | |
| The Cleveland Clinic Dept. of Radiation Oncology 9500 Euclid Avenue Cleveland, OH | | | | 10. SPONSOR/MONITOR'S ACRONYM(S) | |
| | | | | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) F49620-01-1-0407 | |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) AFOSR, Attn: Hugh DeLong Directorate of Chemistry & LS 801 Randolph St, Room 732 4015 Wilson Blvd, Room 715 Arlington, VA 22203-1977 1954 | | | | | |

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approve for Public Release: Distribution Unlimited.

13. SUPPLEMENTARY NOTES

20031104 015

14. ABSTRACT

This project has entailed the development of magnetic materials with potential suitability as vehicles for magnetic field-directed drug delivery, and the design and implementation of a laboratory-scale instrument for measuring the magnetophoretic behavior of new materials in flowing media. Triblock copolymers comprised of hydrophilic poly(ethylene oxide) tail blocks and a polyurethane anchor block containing carboxylic acid binding groups were designed and synthesized. A method for preparing stabilized dispersions of magnetite nanoparticles coated with the stabilizers was developed, and these coated nanoparticles could be dispersed in water. Dispersions of the particle-copolymer complexes remained stable (did not separate) when these materials were dialyzed rigorously and subjected to repeated centrifugation steps to eliminate potentially toxic extractables. An in-vitro assay of toxicity was developed to assess cell survival in the presence of the magnetite nanoparticle-copolymer complexes. A first generation magnet system was designed in collaboration with our colleagues at Stereotaxis (St. Louis, MO). This research scale magnetic guidance system is now in place at VA Tech.

15. SUBJECT TERMS

| | | | | | |
|--|--------------------------|---------------------------|---|----------------------------------|--|
| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT None | 18. NUMBER OF PAGES 12 | 19a. NAME OF RESPONSIBLE PERSON Judy Riffle |
| a. REPORT NC | b. ABSTRACT NC | c. THIS PAGE NC | | | 19b. TELEPHONE NUMBER (include area code) 540-231-8214 |

Final Report for F49620-01-1-0407 for the period 6/1/01-5/31/03

SEEDling Grant to Establish Pilot Data for a Consortium on Magnetic Nanoparticle Assemblies: A New Tool for Drug Delivery, Sensors and Electronic Devices

Principal Investigators

Judy S. Riffle, Dept. of Chemistry
Richard O. Claus, Dept. of Electrical and Computer Engineering
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

and

Urs O. Hafeli
Dept. of Radiation Oncology
Cleveland Clinic Foundation
Cleveland, OH

Collaborators

Rogers Ritter, Stereotaxis, Inc.
St. Louis, MO

Project Period: 6/1/01-5/31/02
No-cost extension: 6/1/02-5/31/03

October, 2003

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

Abstract

Magnetic Nanoparticle Assemblies: A New Tool for Drug Delivery, Sensors and Electronic Devices

This project has entailed the development of magnetic materials with potential suitability as vehicles for magnetic field-directed drug delivery, and the design and implementation of a laboratory-scale instrument for measuring the magnetophoretic behavior of new materials in flowing media. Triblock copolymers comprised of hydrophilic poly(ethylene oxide) tail blocks and a polyurethane anchor block containing carboxylic acid binding groups were designed and synthesized. A method for preparing stabilized dispersions of magnetite nanoparticles coated with the stabilizers was developed, and these coated nanoparticles could be dispersed in water. Dispersions of the particle-copolymer complexes remained stable (did not separate) when these materials were dialyzed rigorously and subjected to repeated centrifugation steps to eliminate potentially toxic extractables. An in-vitro assay of toxicity was developed to assess cell survival in the presence of the magnetite nanoparticle-copolymer complexes. A first generation magnet system was designed in collaboration with our colleagues at Stereotaxis (St. Louis, MO). This research scale magnetic guidance system is now in place at VA Tech.

I. Objectives

The objectives of this SEED project have been to demonstrate the feasibility of magnetic field directed drug delivery. This included 1) the synthesis of stable dispersions of magnetite nanoparticles enclosed in synthetic polymer substrates which enable their dispersion in blood, and incorporation of these materials into controlled size biodegradable microspheres, 2) development of 1st generation instrumentation for guiding magnetic microspheres through the arterial system, and 3) (if deemed feasible) a demonstration of magnetic microsphere control *in-vitro* and *in-vivo* in the presence of external fields.

II. Status of Effort

This project was initiated on 6/1/01 and the first and second annual reports were submitted in September of 2001 and 2002 respectively. A no-cost extension was granted in 6/02 to continue the toxicity and magnetic guidance portions of the work, and the materials work was continued under a new project (F49620-02-1-0308). This final report will summarize the materials work conducted from 6/1/01-5/31/02 and the toxicity and magnetic guidance research conducted from 6/1/01-5/31/03. The focus has been in 3 areas: (1) Synthesis of triblock copolymers with appropriate anchor and tail structures for adsorption onto magnetite and preparation of biocompatible magnetite dispersions, (2) Development of a research-scale magnet system for controlling the navigation of magnetic particles, and (3) In-vitro cell survival assays in tandem with confocal microscopy studies of cell growth in the presence of the nanomagnetite-polymer complexes as a first test for toxicity of the new materials. Triblock copolymers comprised of hydrophilic poly(ethylene oxide) tail blocks and a polyurethane anchor block containing carboxylic acid binding groups were designed and synthesized. A method for preparing stabilized dispersions of magnetite nanoparticles coated with the stabilizers was developed, and it these coated nanoparticles could be dispersed in water. Dispersions of the particle-copolymer complexes remained stable (did not separate) when these materials were dialyzed rigorously and subjected to repeated centrifugation steps to eliminate potentially toxic extractables. An in-vitro assay of toxicity was developed to assess cell survival in the presence of the magnetite

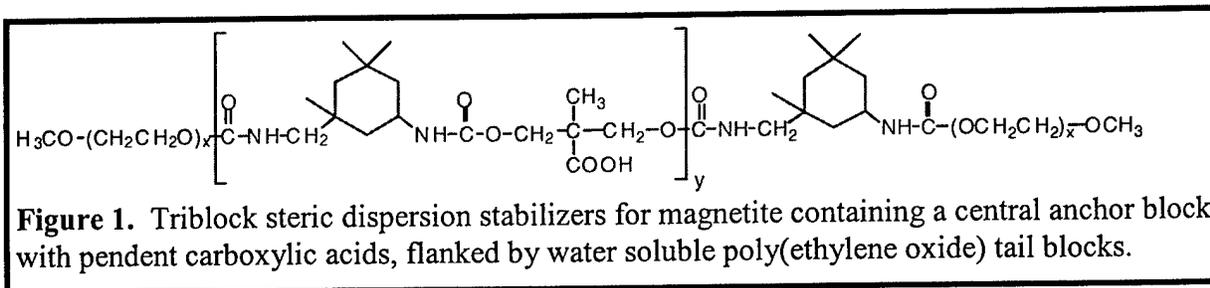
nanoparticle-copolymer complexes. A first generation magnet system was designed in collaboration with our colleagues at Stereotaxis (St. Louis, MO). This research scale magnetic guidance system is now in place at VA Tech.

III. Accomplishments

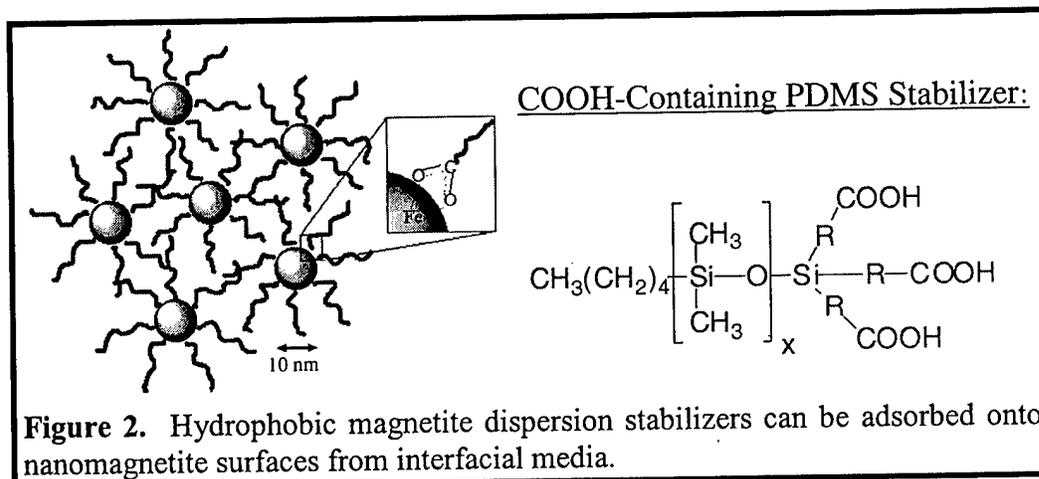
A. Synthesis of Components of Magnetic Drug Delivery Vehicles

Synthesis of Water-Dispersible, Magnetite Nanoparticle-Copolymer Complexes. Our approach has been to prepare triblock copolymers with controlled concentrations of carboxylic acid binding groups as steric stabilizers for dispersions of magnetite nanoparticles. The objective was to define conditions whereby nanoparticle dispersions in controlled ($\approx 5\text{-}20$ nm diameter) sizes could be prepared and to (ultimately) understand relationships between particle size, copolymer chemical structure and any toxicity. The materials are comprised of hydrophilic tail blocks to enable dispersion in vascular and extravascular fluids, and central segments containing carboxylic acid groups for anchoring to the nanomagnetite surfaces (figure 1). A range of compositions and molecular weights were investigated having averages of 3 - 10 carboxylic acid-containing repeat units in a central, water-insoluble, polyurethane anchor segment. Tail block lengths in this series ranged from $770\text{-}16,470$ g mol⁻¹ poly(ethylene oxide) oligomers. Related work (not directly funded by AFOSR/DARPA but valuable to the discussion) has afforded polydimethylsiloxane hydrophobic tail blocks with three carboxylic acids at one end of the chains, and these have been studied as a means for preparing biocompatible hydrophobic dispersions. The most promising structures are those depicted in figure 2 where a living anionic polymerization of the D₃ monomer was terminated with trivinylchlorosilane, then thioacids were added across the double bonds (figure 2). It is important that the carboxylic acid binding groups are closely located in any of these two types of copolymers as opposed to being randomly distributed along the chains (or on both ends) to afford the desired particle-polymer dispersions. It is also clear that three carboxylic acid groups per chain are sufficient to achieve irreversible binding (with both types of polymers), even upon rigorous centrifugation and dialysis (which are necessary to avoid any cell toxicity caused by ill-defined extractables). Optimization of the magnetite dispersions in terms of the concentration of magnetite as a function of stabilizer block length will be discussed in a report on the accompanying project (F49620-02-1-0308).

Magnetite nanoparticles were synthesized by co-precipitating aqueous FeCl₂ and FeCl₃ salt solutions at room temperature under N₂ with a hydroxide base. The stoichiometric molar ratio of Fe²⁺/Fe³⁺ was 0.5 to achieve quantitative conversion. All solutions were deoxygenated prior to use and reacted immediately to minimize oxidation of the Fe²⁺ species [1-2]. The two iron salts were dissolved separately and combined with vigorous mixing just prior to base addition, and the base was syringed into the flask with rapid stirring to a pH of 9.5. This produced a



nanomagnetite dispersion in water, which was only partially electrostatically stabilized with ammonium ion double layers. It should be noted, however, that these particles settled out of the dispersion at this stage if stirring was ceased.



Two slightly different methods for coating the nanomagnetite with the polymer stabilizers were used, depending on whether the polymers were dispersible in water. Numerous groups have previously reported that if carboxylic acid functional groups were present in the iron oxide solution during magnetite synthesis, the crystallization process to form the cubic inverse spinel crystalline structure of magnetite was greatly inhibited, and magnetite did not form [3-4].

The polymers were introduced to the aqueous magnetite particle dispersions immediately after magnetite formation as solutions in dichloromethane, a step which produced two-phase mixtures. The hydrophilic polymers were adsorbed onto the magnetite surfaces for 30 minutes in the two-phase mixture, then the dichloromethane was removed with a strong N₂ purge to transfer all of the polyethylene oxide based stabilizer-nanomagnetite complexes into the water phase. Again, it should be noted that if stirring was stopped at this stage, the polymer-magnetite complexed particles settled out of the dispersions. This suggested that the carboxylate groups were not yet irreversibly bound to the surface of the magnetite. The pH of the reaction mixture was subsequently lowered to pH 6.5 – 7 (to the physiological pH range) by titrating with acid, whereupon stable dispersions were obtained. The hydrophobic polysiloxane-magnetite complexes were also formed in dichloromethane-water two-phase mixtures. The polysiloxanes containing the carboxylic acid binding groups were introduced in dichloromethane to the aqueous magnetite dispersion at pH 9.5 and agitated for 30 minutes. The two-phase mixture was subsequently neutralized to yield a stable emulsion of polysiloxane-nanomagnetite in dichloromethane-water. The dichloromethane was removed to destabilize the emulsion, whereupon the polysiloxane-nanomagnetite complexes separated from the water (floated). With selected stabilizer block lengths (e.g., 3500 g mol⁻¹ polysiloxane), this transferred all of the polymer-particle complexes into the upper polysiloxane copolymer phase.

The interfacial adsorption process was clearly efficient in producing excellent dispersions with both hydrophilic and hydrophobic materials. A discussion of the mechanism of adsorption has been included in the accompanying materials report on contract #F49620-02-1-0308. This was studied under the accompanying materials grant.

Aqueous dispersion stabilities of the magnetite nanoparticles coated with the hydrophilic poly(ethylene oxide) containing block copolymers were investigated as a function of pH.

Results suggested that the magnetite coating process should be conducted at pH of $\approx 8-10$, then the dispersions should be neutralized. Stable dispersions were observed only at pH 7 and below. At neutral and lower pH, the carboxylate group chemisorbed onto the surface of magnetite, and sterically stabilized the dispersions. This provided stable dispersions at physiological pH's which are not sensitive to ionic strength or pH changes.

The concentration of magnetite in these materials has been maximized, and this study was conducted and discussed under the accompanying materials grant (F49620-02-1-0308).

B. Development of 1st Generation Magnetic Guidance Instrumentation

Essential elements in the source instrument for controlling superparamagnetic microspheres in a fluid stream are 1) the appropriate magnetizing field strength and direction, 2) a strong gradient to deal with the dynamic forces, and 3) appropriate access within the working space for visualizing particle control in the stream. The objectives of this part of the work have been to provide a controllable, but flexible, stage-one design to reduce the initial uncertainties in knowledge of particle agglomeration and the concomitant uncertainties in the required magnetic field and gradient properties. The need for a flexible design in initial studies to better understand particle agglomeration can be viewed as follows. Higher fields lead to greater interparticle interactions, which yield aggregates of particles acting as stronger dipoles than individual particles. These may require a lower gradient-to-field ratio for control, since in general, the fluid force increases as the square of the aggregate size, but the magnetic moment increases as its cube. Local field interactions are a complex many body issue, and cannot be predicted or modeled well at this time. An object of these initial studies has been to evaluate the needed fields and gradients for more advanced particle investigations.

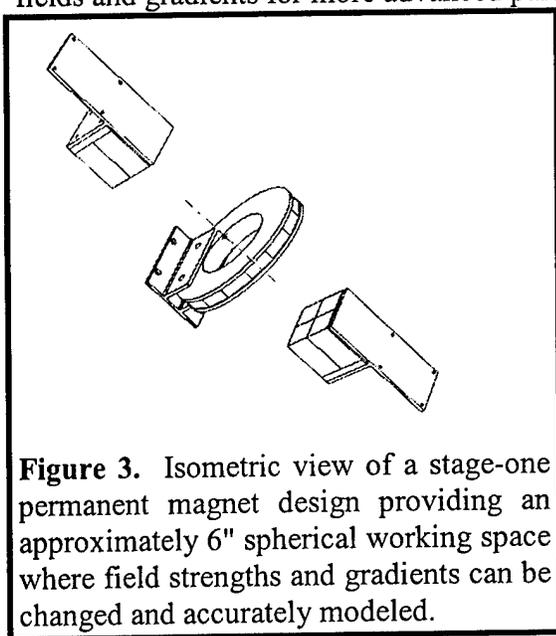


Figure 3. Isometric view of a stage-one permanent magnet design providing an approximately 6" spherical working space where field strengths and gradients can be changed and accurately modeled.

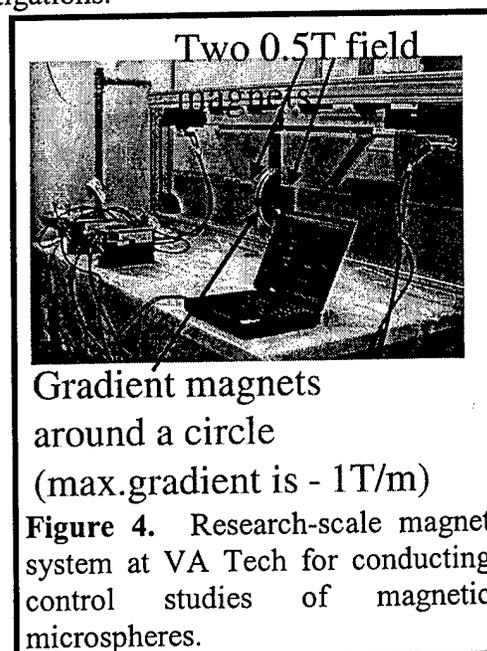


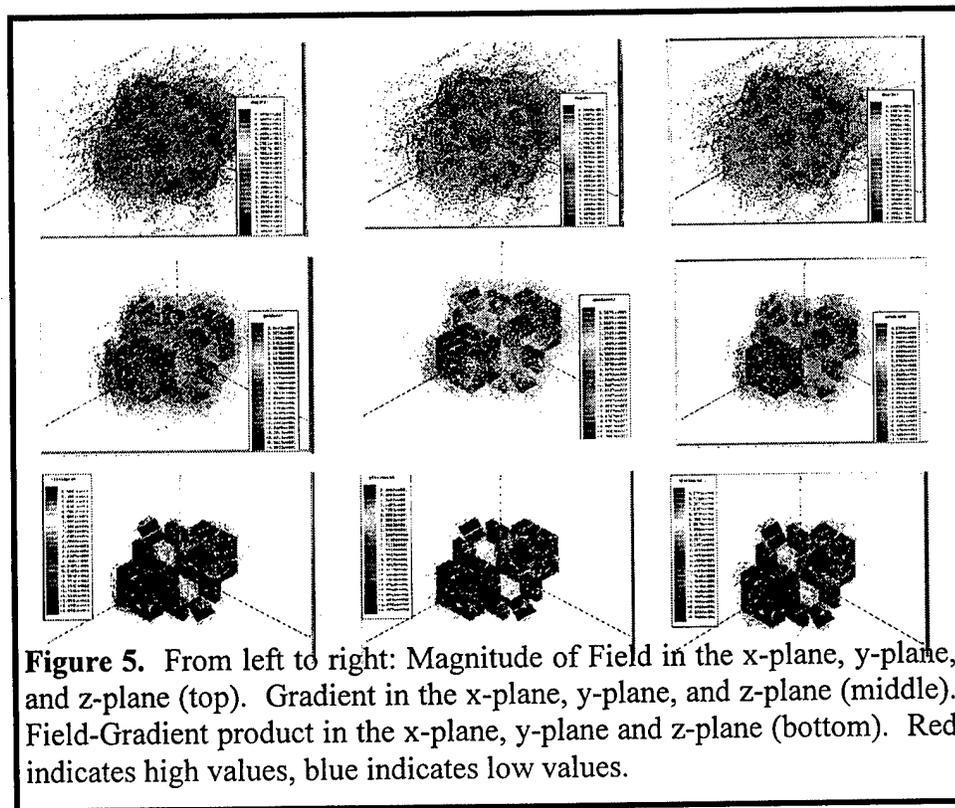
Figure 4. Research-scale magnet system at VA Tech for conducting control studies of magnetic microspheres.

Figures 3 and 4 depict the stage-one magnet system, which was designed by our colleagues at Stereotaxis. Variations on this initial design are now in place at both VA Tech and at the Stereotaxis St. Louis location. The system provides fields and gradients along a single axis. An approximately six-inch diameter spherical working space is available for the flow capillary and visualization equipment (in the center between the two end magnets). Figure 3 shows two, like,

field magnets on a common axis, one on each end, and a roughly circular, gradient set of magnets between them. The field magnets are each composed of four adhesively-bonded rectangular blocks of NdFeB, magnetized along the long axes. Each such four-block unit can be moved along its axis. The computer-controlled motors mounted on the upper slide (figure 4) move these two blocks apart, or together, in an antisymmetrical paired motion. When further apart their central field is weaker. At any fixed position they simulate a Helmholtz magnet pair having additive fields. This arrangement provides a relatively large and uniform region of constant field at the center. The strength of the field at that location is inversely related to the separation between the two magnets, and they contribute zero gradient at the center point. The gradient magnet is composed of eight segments magnetized parallel to the common axis, but in an opposite direction to that of the field magnets. This is a hollow octagon, having a magnetic quadrupole field as its dominant component. At full field (0.1 T) the field magnets will be 7.45" apart. The gradient can be removed by sliding the gradient magnets along the axis so that the center plane is at the midpoint between the field magnets. A (non-magnetic) visualization system which can resolve $\approx 1\mu\text{m}$ diameter particles, and which fits within the working space

remains under construction. We have obtained 10 μm and 600 μm i.d. hollow glass tubing clad with a protective polyimide overlayer.

The stage-one magnet system was characterized by comparing predicted and experimental results. Theoretical field strengths were predicted using ANSOFT's 3D Maxwell Equation Finite Element Analysis Software (figure 5). All



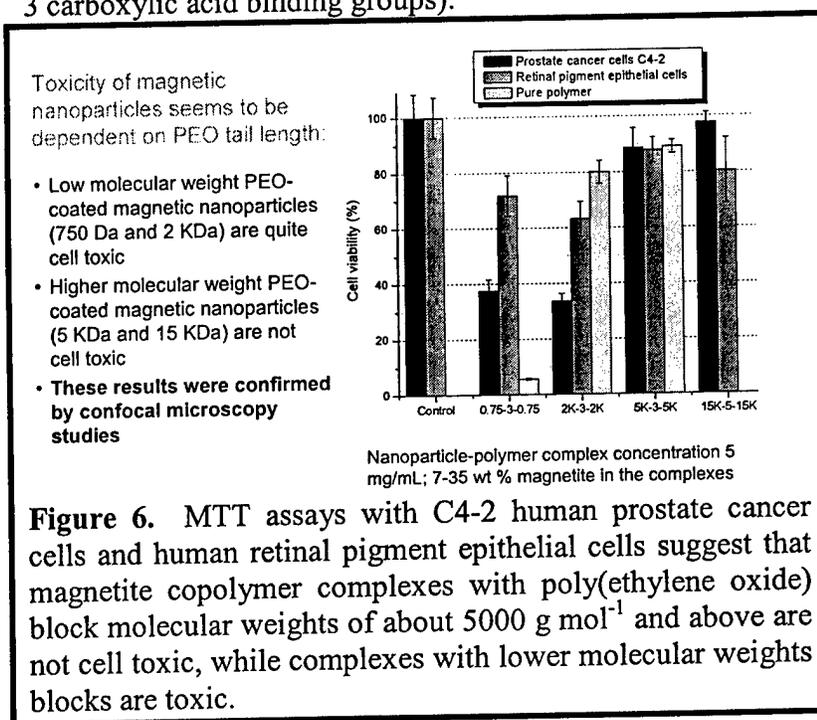
measurements were taken starting at the right side, every 12 mm with a 1.25 inch separation of each field magnet from the center gradient magnet.

C. In-Vitro Cell Survival Assays of Macromolecule-Magnetic Nanoparticle Complexes

Novel magnetic compounds such as the magnetite based nanoparticles, and the composite microparticles thereof, were tested for biological compatibility, inertness, extractability and non-toxicity. Cell proliferation assays (MTT) of control cultures without added complexes vs. those containing the complexes have been promising. The MTT experiment is a colorimetric assay

that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase [5-6]. MTT enters the cells and passes into the mitochondria where it is reduced to a blue, insoluble formazan product. The cells are then solubilized with DMSO and the color is quantified spectrophotometrically at 540 nm. Since reduction of MTT can only occur in metabolically active cells, the intensity is a measure of the viability of the cells.

An extensive study was conducted with complexes of approximately 7-nm diameter magnetite nanoparticles encased in hydrophilic copolymers. The triblock copolymers had terminal poly(ethylene oxide) (PEO) blocks with systematically varied molecular weights and a central anchor block containing carboxylic acid groups. The nomenclature utilized to designate these materials was to define the number average molecular weights of the tail blocks in kilodaltons (e.g., 0.75k = 750 g mol⁻¹), and to designate the number of repeat units in the anchor block (equals the average number of carboxylic acids per chain) as an integer (e.g., 0.75k-3-0.75k means the tail blocks were 750 g mol⁻¹ and the anchor block contained 3 repeat units with 3 carboxylic acid binding groups).



These magnetite-copolymer complexes were purified by repeated centrifugations to remove any magnetite that may not have been tightly encased in the copolymers, then by dialysis with Millipore water for a minimum of a week. MTT cell survival assays were conducted with each complex utilizing two cell lines: (1) Human prostate cancer cells (C4-2) and (2) Human retinal pigment epithelial cells. Cell proliferation and cell survival were compared to control populations (figure 6). A series of materials were studied wherein the average lengths of the PEO tail blocks were varied

from 750 to 15,000 g mol⁻¹. Cell proliferation was found to be strongly dependent on the tail block length. It should be noted that the central polyurethane blocks of these materials were insoluble in water even though they contained carboxylic acids on each repeat unit, and this indicates that the central blocks had significant hydrophobic character. The weight fractions of the hydrophilic blocks in the four block copolymers described in the MTT assays in figure 6 were 0.55 (for the 0.75-3-0.75 copolymer), 0.77 (2k-3-2k), 0.89 (5k-3-5k) and 0.94 (15k-5-15k). Thus, the copolymers in the complexes which do not exhibit toxicity in the MTT assays were significantly more hydrophilic relative to the materials which resulted in toxicity. Thus, the reasons for the differences in toxic responses of the more hydrophobic, lower molecular weight materials to this assay are not well understood as yet.

(Cambridge Technologies, Watertown, Massachusetts) and the cell viabilities were quantified. The average of the experimental 8 wells was subtracted from the average of 8 wells containing no cells (background) and divided by the background-corrected average of the control tumor cells. For each MTT assay, the control tumor cell viability was thus by definition 100%. As a separate control, 100 μ l aliquots of FeCl_3 -solutions of 10.0 mg/mL, adjusted to pH 7.4 with PBS, were tested for their toxic effects on the same cell line in exactly the same way.

IV. Personnel Supported

Three graduate students have been supported by the project: Linda Harris, Michael Zalich (summer only) and Jeffrey Leach. Linda Harris earned a Ph.D. in Chemistry under the direction of Prof. Riffle in May, 2002. She was awarded a National Science Foundation post-doctoral fellowship to conduct further study on these materials in the Dept. of Physics at the University of Western Australia. Linda joined Prof. Tim St. Pierre's biomagnetic physics group in July, 2002 and is contributing to the project with extensive characterization of the nanomagnetic particles and dispersions. Michael Zalich is a Ph.D. candidate in Chemistry under Prof. Riffle and Jeffrey Leach earned an M.S. in Electrical Engineering with this project under the direction of Prof. Richard Claus. Michael Zalich earned a Fulbright Fellowship to work on magnetic properties of particle-copolymer complexes in Perth (UWA) under the direction of Prof. Tim St. Pierre. He left for Perth in July, 2003. Linda Harris worked on (and is still working on) the synthesis of non-toxic macromolecular stabilizers and the methodology for magnetite synthesis. Mike Zalich began work on determining methods for preparing controlled size microspheres, and this work also continues. Jeffrey Leach has constructed the stage-one magnetic navigation instrument at VA Tech. The other people who have had input to the project thus far have been another M.S. student in Electrical Engineering, Keith Huie, who has helped with the VSM instrumentation and Jon Goff who is an undergraduate researcher in the Dept. of Chemistry.

V. Publications

Two short manuscripts have been published as a direct result of this portion of the project [7-8], and two additional short and one major longer paper were published during the year on the related hydrophobic polymer-magnetic complex work [9-11].

VI. Interactions and Transitions

Strong collaborations have begun with Dr. Rogers Ritter (senior technical leader, Stereotaxis) who is an expert at the physics of large magnet systems design, and with Prof. Tim St. Pierre (Physics, Univ. Western Australia) who has expertise in nanomagnetic particle physics. These collaborations have been, and continue to be, invaluable as we progress in this multi-disciplinary project. One of the PI's, Dr. Hafeli, organized and hosted a major symposium, *4th International Symposium on Scientific and Clinical Applications of Magnetic Carriers*, which was held in Tallahassee in May, 2002 [12]. This conference brought together 160 scientists from 20 countries for a week and provided an excellent forum for discussion and dissemination of knowledge in this field.

VII. New Discoveries

A new triblock stabilizer was identified for preparing non-toxic nanomagnetite aqueous dispersions.

Polysiloxane-magnetite dispersions were designed and prepared (U.S. Patent 6,464,968 entitled Magnetic dispersions stabilized by block copolymers).

IX. Honors and Awards

R. Claus was named the Lewis A. Hester Chair of Engineering, July 2001.

R. Claus was named Virginia Scientist of the Year for 2001.

K. Huie received the award from Virginia Tech's Graduate School for the best graduate research project, May 2001.

L. Harris received the Gencorp award for research excellence, June, 2001.

L. Harris was awarded an NSF post-doctoral international fellowship to work under Prof. St. Pierre at the University of Western Australia.

J. Goff won the Wightman undergraduate prize in chemistry for excellence in research, the Lou Sharpe award for undergraduate research in August, 2003, and third place (for best paper) at the National Polymer Conference for Undergraduates in October, 2003.

Mike Zalich was awarded a Fulbright Fellowship to study magnetic nanoparticle-copolymer properties with Prof. St. Pierre at the University of Western Australia.

X. References

1. R. M. Cornell, U. Schertmann, *Iron Oxides in the Laboratory: Preparation and Characterization*, VCH, Weinheim, 1991.
2. N. M. Gribov, E. E. Bibik, O. V. Buzunov, V. N. Naumov, *Journal of Magnetism and Magnetic Materials*, **85**, 7 (1990).
3. G. S. R. Krishnamurti, P. M. Huang, *Clays and Clay Minerals*, **39**, 28 (1991).
4. P. M. Huang, M. K. Wang, in *Advances in Geoecology*, Vol. 30, K. Auerswald, H. Stanjek, J. M. Bigham, Eds., International Development Centre, Ottawa, 241 (1997).
5. R. Pieters, D. R. Huismans, A. Leyva et al., Comparison of the rapid automated MTT-assay with a dye exclusion assay for chemosensitivity testing in childhood leukaemia, *Br. J. Cancer*, **59**, 217-220 (1989).
6. U. O. Hafeli and G. J. Pauer, In-vitro and in-vivo toxicity of magnetic microspheres, *J. Magn. Mag. Mater.*, **194**, 76-82 (1999).
7. K. S. Wilson, L. A. Harris, J. D. Goff, J. S. Riffle, and J. P. Dailey, A Generalized Method for Magnetite Nanoparticle Steric Stabilization utilizing Block Copolymers Containing Carboxylic Acids, *European Cells and Materials*, **3**, Suppl. 2, www.eurocellmat.org.uk, 206-209 (2002).
8. U. O. Hafeli, R. Ciocan and J. P. Dailey, Characterization of magnetic carriers and their magnetophoretic mobility using a digital microscopy method, *European Cells and Materials*, **3**, Suppl. 2, www.eurocellmat.org.uk, 24-27 (2002).
9. M. Rutnakornpituk, V. V. Baranauskas, J. S. Riffle, J. Connolly, T. G. St. Pierre and J. P. Dailey, Polysiloxane Fluid Dispersions of Cobalt Nanoparticles in Silica Spheres for use in Ophthalmic Applications, *European Cells and Materials*, **3**, Suppl. 2, www.eurocellmat.org.uk, 102-105 (2002).
10. J. Connolly, T. G. St. Pierre, M. Rutnakornpituk and J. S. Riffle, "Silica Coating of Cobalt Nanoparticles Increases their Magnetite and Chemical Stability for Biomedical Applications," *European Cells and Materials*, **3**, Suppl. 2, www.eurocellmat.org.uk, 106-109 (2002).

11. M. Rutnakornpituk, M. S. Thompson, L. A. Harris, K. E. Farmer, A. R Esker, J. S. Riffle, J. Connolly and T. G. St. Pierre, "Formation of cobalt nanoparticle dispersions in the presence of polysiloxane block copolymers," *Polymer*, **43**, 2337-2348 (2002).
12. <http://www.magneticmicrosphere.com>