2014 SPS/ΣΠΣ Undergraduate Research Award: Interim Report (31 May 2014)

Project Title: Magnetic Microbubbles for Targeted Drug Release
Name of School: Kettering University (B)
SPS Chapter Number: 3541
Total Amount Awarded: $1,997.00

Primary Student Research Team:
Nathaniel Mosher, Department of Physics
Emily Perkins-Harbin, Department of Physics
Nicolaas Winter, Department of Physics
Natalie Gerdung, Department of Chemical Engineering

Faculty Advisers:
Prof. Ronald E. Kumon, Assistant Professor, Department of Physics
Prof. Prem Vaishnava, Professor, Department of Physics

SPS Chapter Presidents:
Nathaniel Mosher (Fall 2013)
Rebekah Gowman (Spring 2014)

SPS Chapter Adviser:
Prof. Ronald E. Kumon

Original Abstract
We propose to synthesize and characterize magnetic microbubbles for investigating their potential for application to targeted drug delivery. Using magnetic microbubbles as carriers, we will test how well drugs can be dispersed by means of ultrasound and hyperthermia.
Progress Report

Summary

To begin, we acquired the necessary materials and equipment to perform the synthesis procedures. We focused first on the synthesis of the magnetic nanoparticles as they are needed to create the magnetic microbubbles. We next characterized the magnetic nanoparticles to determine their chemical composition and ability to cause heating via magnetic induction. We then synthesized the magnetic microbubbles and imaged them using optical microscopy. As a side project, we created and tested a sound enclosure for the probe sonicator in cooperation with an acoustics class at the university. Finally, we discuss our planned directions for future work.

Equipment and Materials

For the synthesis of the magnetic nanoparticles, we acquired FeCl₂, FeCl₃, NH₃OH, NaOH, and HCl (Sigma Aldrich, St. Louis, MO). We also acquired biochemical-grade dextran (Acros Organics, Fair Lawn, NJ) and oleic acid (Sigma Aldrich, St. Louis, MO) for coating and stabilizing the magnetic nanoparticles. In support of our efforts, our faculty advisers acquired an ultrasonic bath (CXP3800H, Branson, Danbury, CT) for use in dispersing and coating the magnetic nanoparticles and a small refrigerator/freezer to store chemical supplies. We have also used existing glassware, scales, stirrers, and hotplates in the Chemistry Department during the chemical synthesis procedure.

For the synthesis of the magnetic microbubbles, we have acquired a set of three adjustable pipettes (Research Plus, Eppendorf N.A., Hauppauge, NY) to enable liquid handling volumes in the range from 0.5 to 1000 μL, a hemacytometer (Model 3200, Hauser Scientific, Horsham, PA) to enable sizing and counting of the microbubbles. In support of our efforts, our faculty advisors acquired L-α-phosphatidylcholine to use as a stabilizing surfactant for the bubble shell, phosphate-buffered saline solution, and a probe sonicator (Q125, Qsonica, Newtown, CT) with a 1/8” probe capable of 125 W of output power to create the microbubbles. They also cleaned and made available an upright optical microscope (BX50F4, Olympus, Center Valley, PA) with 4X–100X objectives and digital camera to image the microbubbles.

A detailed list of expenditures from the award along with additional support from Kettering University is provided in the Appendix.

Synthesis

Nanoparticles. We have, after many trials and refinements, successfully synthesized dextran-coated iron oxide (Fe₃O₄) magnetic nanoparticles via a chemical co-precipitation method.¹² This method, briefly, involves the addition of a strong base in excess to a solution containing a 1:2 molar ratio of Fe²⁺:Fe³⁺ ions. The overall chemical reaction is described by 

$$\text{Fe}^{2+} + 2\text{Fe}^{3+} + 8\text{OH}^- \rightarrow \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O}.$$  

The addition of the base causes bare Fe₃O₄ nanoparticles to precipitate out of solution, at which point they are
washed and decanted. We then add the bare nanoparticles to a basic solution with the surfactant (dextran) and expose it to ultrasonic radiation (via an ultrasonic bath and/or an ultrasonic probe sonicator) until the dextran is fully adsorbed onto the surface of the nanoparticles. Dextran allows the nanoparticles to remain stable in aqueous solution because it provides steric repulsion between each nanoparticle, thereby preventing agglomeration and precipitation. It also enhances the nanoparticles’ biocompatibility, which is crucial for biological applications. Figure 1 shows several steps in the synthesis procedure along with the resulting Fe₃O₄ ferrofluid.

Figure 1. Steps in the synthesis of magnetic nanoparticles. (a) Solutions of FeCl₂ and FeCl₃. (b) Gradual dripping of NH₃OH into Fe solution. (c) Sonication of ferrofluid with dextran solution to stabilize nanoparticles. (d) Ferrofluid of Fe₃O₄ magnetic nanoparticles.
Microbubbles. Creation of control (non-magnetic) microbubbles was accomplished by mixing magnetic nanoparticles and L-\(\alpha\)-phosphatidylcholine into a phosphate buffered saline solution. The microbubbles were formed via exposure to the probe sonicator for 30 s, following by manual shaking for 30 s. For the creation of magnetic microbubbles, the process was repeated with the addition of the magnetic nanoparticles and another sonication step, as shown in Fig. 2.

![Figure 2. Sonication of magnetic nanoparticle solution with phospholipid surfactant to synthesize magnetic microbubbles.](image)
Characterization of Nanoparticles

There are many factors that determine how well-suited any one sample of magnetic nanoparticles will be for targeted cancer treatment. For example, magnetic core size, core size distribution, concentration of iron in solution, and the morphology of the iron oxide can all affect the efficiency of hyperthermia via magnetic induction. Going forward, it is also important to know that we can produce magnetic nanoparticles with consistent properties. To that end, we have begun to characterize the nanoparticles. Thus far, we have obtained initial data on chemical composition and crystal morphology and rate of hyperthermia (specific absorption rate).

Chemical composition and crystal morphology. We determined the chemical composition by x-ray diffraction (Miniflex 600, Rigaku, The Woodlands, TX) of a powdered sample of bare magnetic nanoparticles. Although there is a moderate amount of noise in the spectrum, Figure 3 still clearly shows six peaks whose location and relative amplitude clearly indicated that our sample consists primarily of crystalline iron oxide, as expected.

Figure 3. Raw x-ray diffraction spectrum of uncoated magnetic nanoparticles indicating peaks characteristic of Fe$_3$O$_4$. 

Hyperthermia. When magnetic particles are placed in an alternating magnetic field, they can generate heat in the surrounding medium via the mechanisms of Brownian and Néel relaxation. We put our coated magnetic nanoparticles in a magnetic induction system (Easyheat 0112, Ambrell, Scottsville, NY) to measure how much heat they produced (Fig. 4). In one test we measured the heat produced by the same sample run twice, changing the AC frequency, but keeping power and magnetic field strength the same. Figure 5 shows data taken with frequencies of 186 kHz and 342 kHz at 101.6 A for 180 s. Using data like this, we can calculate the specific absorption rate (SAR) of a sample.

Figure 4. Magnetic induction system consisting of optical thermometer (left), power supply and control unit (center), and magnetic coil & impedance matching network (right).

Figure 5. Heating of a suspension of dextran-coated magnetic nanoparticles at multiple frequencies.
Characterization of Microbubbles

As successful synthesis of the magnetic nanoparticles was only recently completed, our microbubble data is limited. Preliminary examination of the microbubbles shows a size range of approximately 10–100 μm (Fig. 6).

Side Project: Sound Enclosure for Sonicator

We found that in the course of its normal operation the probe sonicator makes a loud, unpleasant screeching sound due to its oscillation at 20 kHz and the resulting cavitation (bubble formation) in the surrounding fluid. The manufacturer of the sonicator sells a sound enclosure which reduces the sound pressure levels by at least 20 dB, but its purchase was precluded by its high cost ($695). Instead, we worked together with students in the senior-level acoustics laboratory course (Engineering Physics 485) to design, assemble and test an enclosure with equivalent or better performance for under $100. Participants in this project were Emily Perkins-Harbin, Sean Commet, Frederick Garcia, Mike Howell, and Nathaniel Mosher. We made transmission loss estimates using basic 1D acoustical theory followed by more realistic 2D finite-element simulations using COMSOL Multiphysics (v. 4.3, COMSOL, Burlington, MA). Figure 7 shows the geometry of the simulation, while Fig. 8 shows a comparison of the simulated sound field with and without the enclosure, indicating a significant sound pressure level reduction.

We constructed the actual sound enclosure using 1.5”x1.5” pine wood pieces. We built the inner and outer shells of the housing with 3/16” thick Masonite, a very dense wood material. We filled the space between the inner and outer shells with fiberglass to absorb the sound coming from the sonicator. The enclosure was also fitted with a door containing an acrylic plastic window for viewing samples during treatment. We used a foam strip to prevent sound loss from the door and latched the door shut. We performed transmission loss measurements in an anechoic chamber in the Kettering Acoustics Laboratory with a handheld sound pressure level meter using A-weighting (33-2055,
Radio Shack, Fort Worth, TX) or a small omnidirectional electret microphone (33-2055, Radio Shack, Fort Worth, TX) connected to a spectrum analyzer (SR785, Stanford Research Systems, Sunnyvale, CA), as shown in Fig. 9.

Figure 7: Diagram of COMSOL model of enclosure and source. The 2D model represents a vertical slice through the center of the enclosure, back to front. Smaller arrows indicate edges of window. The vertical and horizontal scales are in units of meters.

Figure 8: 2D Finite-element simulations for sonicator-like sound source at 20 kHz (a) without and (b) with the sound enclosure. The colors show sound pressure level as a function of position. The sonicator was modeled as rectangular source with oscillating pressure boundary conditions to simulate its operation while immersed in a beaker. The outer boundary was set to cylindrical wave radiation boundary conditions. The small circles represent areas where the average SPL was computed for comparison with experimental measurements.
Figure 9. Sound pressure level measurements of probe sonicator (a) without and (b) with sound enclosure. The sonicator and chamber were placed on a turntable for purposes of directivity measurements (not shown).

We found that measured spectrum had peaks at 20 kHz, as expected, but also at 5.76 kHz, 9.86 kHz, and 14.2 kHz. Table 1 shows that the enclosure achieved the design goal of at least 20 dB(A) reduction in sound pressure level at distances that would be typical of an operator under normal conditions. While the experimental results did not match the simulations in terms of predicted transmission loss, our choice of design parameters was still sufficient to achieve the desired design goal.

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>SPL: Without Enclosure (dBA)</th>
<th>SPL: With Enclosure (dBA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>99</td>
<td>66</td>
</tr>
<tr>
<td>0.5</td>
<td>89</td>
<td>64</td>
</tr>
<tr>
<td>1</td>
<td>82</td>
<td>61</td>
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</table>

**Student Involvement**

At Kettering University, students are required to engage in co-op work experiences, which typically occur during every other academic quarter. This situation gives rise to largely separate A-section (Winter & Summer) and B-section (Spring & Fall) student groups. Our original research proposal was made by B-section physics students led by Nicolaas Winter. Therefore most of the work will be done during the Spring 2014 and Fall 2014 quarters, but A-section students have also expressed an interest in participating. Figure 10 shows some of the students and faculty that have been involved with the project. Nicolaas Winter will be graduating during June 2014, but Nathaniel Mosher and Emily Perkins-Harbin will be carrying the project forward into the fall. As mentioned above, several B-section Engineering Physics and Mechanical Engineering students were also involved in the construction and testing of the sound enclosure. Natalie Gerdung, a chemical engineering major, has also been involved with characterization of the magnetic nanoparticles.
All Kettering University students are required to submit a senior thesis prior to graduation. While many of these theses are performed with co-op employers, students have recently been allow to complete research theses under the supervision of faculty members on campus. Each thesis has a proposal which is reviewed by a faculty committee prior to the start of thesis work. Two research thesis projects have been approved for work related to the current project:

Nathaniel and Emily are planning to present the results of their work this term to the B-section Physics Club before the end of the current term and provide a tour of the Kettering Nanotechnology Laboratory where the research is being performed. We hope to continue to involve students from a variety of disciplines in the project.

Figure 10. Selected students and faculty involved in current research efforts. From left to right: Prof. Ronald Kumon, Nicolaas Winter, Nathaniel Mosher, Emily Perkins-Harbin, Cody Grant, Kiran Vekaria, Prof. Prem Vaishnava.

Dissemination of Results

Each spring the university sponsors a “Kettering Homecoming” event for alumni to come back to campus. As part of this event, the university organizes a poster session for students, faculty, and staff to present their activities. Three posters were presented related to the current project (Figs. 11–12):

The posters were well-received at the event.
Figure 11: Student posters at the 2014 Kettering Homecoming Poster Session: (a) Nathaniel Mosher and (b) Emily Perkins-Harbin and Prof. Ronald Kumon.

Figure 12: Physics Department posters at the 2014 Kettering Homecoming Poster Session. From left to right: Prof. Prem Vaishnava, Provost Robert Simpson, Nathaniel Mosher, Emily Perkins-Harbin, Rebecca Mikolajczyk, Physics Department Chair Prof. Kathryn Svinarich, Prof. Ronald Kumon, and Prof. Gillian Ryan.

We were also involved in the production of a video clip entitled “Magnetic Nanoparticles and Microbubbles” as part of the university’s “Making a Better World” series. The video describes the project and features Prof. Kumon, Prof. Vaishnava, Nathaniel Mosher, and Emily Perkins-Harbin. The video is available on YouTube at https://www.youtube.com/watch?v=IJJ2qi15jOaQ.
Future Work

We have plans for a variety of future studies:

1. Synthesis and characterization of magnetic nanoparticles. We plan to measure particle size distribution using transmission electron microscopy (TEM) as well as the magnetic properties of the nanoparticles. We may also try to coat the iron nanoparticles with a different biocompatible surfactant (e.g., oleic acid).

2. Functionalization of magnetic nanoparticles. To increase stability of the nanoparticles, we plan to cross-link the coating in our existing dextran-coated magnetic nanoparticles using epichlorohydrin and then label them with fluorescein isothiocyanate (FITC) for in vitro fluorescence imaging. We also plan to add a drug molecule to the nanoparticles.

3. Cytotoxicity of magnetic nanoparticles. Toxicity tests will be done in conjunction with a nearby academic medical center to determine the highest concentration of dextran-coated magnetic nanoparticles that can be used while minimizing cell death. The tests will be run on two lines of cancer cells.

4. Characterization of magnetic microbubbles. Further characterization of the bubbles is planned, as well as testing of their magnetic response and acoustic properties. Early examination of the magnetic properties of the bubbles suggests that development of techniques to fine tune the quantity of magnetic nanoparticles loaded onto each bubble would be worthwhile. Development of an optical sizing and counting technique is also planned. Briefly, microscopic photographs of the bubbles in a hemacytometer will be taken, and the images processed to determine size distribution and density of the bubbles.

5. Local cell culturing. Kettering University recently opened a new Applied Biology laboratory. We plan to use these new facilities to culture cells locally for future testing of model drug delivery using magnetically-induced hyperthermia and ultrasound.

6. Drug delivery studies. We plan to study the effects of drug delivery from functionalized magnetic nanoparticles and magnetic microbubbles without and with magnetically-induced heating and ultrasound.

7. Mechanisms of magnetic relaxation. The detailed mechanisms of the relaxation process of magnetic nanoparticles during induction are still a topic of current research. We plan to investigate the relative role of Brownian relaxation (friction) and Néel relaxation (magnetic viscosity). We plan to investigate these effects using several electromagnets available in the Physics Department (Fig. 13).
Figure 13. Electromagnets available in the Kettering University that may be used for future studies on the relaxation processes of magnetic nanoparticles: (a) Helmholtz coils with laser (far left) for optical monitoring of specimen and (b) large electromagnet capable of generating core magnetic fields of up to 1 T.

Acknowledgments
We would like to acknowledge contributions of Prof. Lihua Wang from the Chemistry Department in the chemical synthesis of the magnetic nanoparticles, Prof. Cornel Rablau from the Physics Department for advice regarding optical imaging of magnetic nanoparticles, and Robert Cunningham from Physics Department for assistance with the x-ray diffractometer, magnetic induction system, and large electromagnet. We would also like to acknowledge financial and in-kind support provided by the Department of Physics, Provost’s Office, and Office of Sponsored Projects at Kettering, particularly for acquisition of the magnetic induction system. The x-ray diffractometer used in this study was provided by Major Research Instrumentation grant from the National Science Foundation. Prof. Kumon and Prof. Vaishnava would also like to acknowledge support from a Faculty Research Fellowship at Kettering University. Finally, we would like to thank the Society of Physics Students and Sigma Pi Sigma for providing their support to make this project possible.

References

Appendix: Expenditure Report

1. Expenditures from SPS/ΣΠΣ Undergraduate Research Award

<table>
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<th>Item</th>
<th>Cost</th>
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<td>Hemacytometer (Phase Contrast, Bright-Line)</td>
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<td>Adjustable Pipette (0.5 to 10 µL)</td>
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<tr>
<td>Adjustable Pipette (10 to 100 µL)</td>
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<tr>
<td>Adjustable Pipette (100 to 1000 µL)</td>
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<tr>
<td>Iron (II) Chloride</td>
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<td>Iron (III) Chloride</td>
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<td>Dextran (for biochem)</td>
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TOTAL $1,166.09

2. Expenditures from Other Support (Kettering University)

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<td>Microscope Slides/Cover Slips</td>
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<td>Benchtop Protectors</td>
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<td>Immersion Oil for Microscope</td>
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<td>L-alpha-Phosphatidylcholine, hydrogenated</td>
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TOTAL $3,632.98