ΣΠΣ Undergraduate Research Award Proposal

Project Proposal Title	Magnetic Microbubbles for Targeted Drug Release
Name of School	Kettering University(B)
SPS Chapter Number	3541
Total Amount Requested	$1997.00

Student Research Team:
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Abstract

We propose to synthesize and characterize magnetic microbubbles for examining the use of magnetic microbubbles for targeted drug delivery. Using magnetic microbubbles a carriers, we will test how well drugs can be dispersed by means of ultrasound and hyperthermia.

Proposal Statement

Overview of Proposed Project

Ultrasound microbubbles (UMB) are tiny gas-filled lipid shells that have a variety of medical uses. One use is the delivery of anti-cancer drugs. UMB carrying an anti-cancer drug can be exposed to ultrasound to release their payload. Magnetic Microbubbles (MMB) are UMB with a shell of embedded magnetic nanoparticles (MNP). Magnetic nanoparticles that go into the UMB can also have anti-cancer drugs attached.

Faculty at our university have been working with UMB, MMB and MNP in various research projects. Currently, there is research into the use of MMB for targeted drug delivery (1). This involves using the magnetic properties of MMB to guide them to and concentrate them in a specific region, which may result in better drug delivery over UMB.

We wish to test if MMB have the same capacity for drug release as UMB. Determining that MMB and UMB have equivalent dispersal will show the feasibility of MMB for drug delivery. We also want to determine if magnetically-induced hyperthermia (the heating that occurs when MNP are exposed to alternating magnetic fields) has an effect on the drug's release.

To answer these questions in the first phase of the project we will be conducting experiments to compare the drug release of UMB and MMB exposed to varying combinations of ultrasound and magnetically-induced hyperthermia. To do this we must first synthesize MNP, synthesize MMB and either purchase or synthesize UMB. After these materials have been characterized we will perform three experiments (with appropriate controls):

1. Measure the release of drugs from MMB and UMB exposed to ultrasound (MMB and UMB will be measured separately for all cases).
2. Measure the release of drugs from MMB and UMB exposed to magnetically-induced hyperthermia.
3. Measure the release of drugs from MMB and UMB exposed to both ultrasound and hyperthermia.

In the second phase of the project, we will perform studies to determine the optimal parameters for maximizing drug absorption into the MNP and MMB and for maximizing drug delivery by ultrasound and/or magnetic field.

This project will also allow students to participate in a rich and active research field with medical relevance. Participants will have the opportunity to work with scientific equipment and gain valuable knowledge about performing research. Some students may also use this research to satisfy Kettering's requirement to complete a senior thesis. The project will also provide members of our SPS chapter a unifying opportunity to work together on a single project. We plan to present our research at undergraduate poster sessions at Kettering, and at national conferences and possibly even publish the results in a peer-reviewed journal. We also hope to promote our work at local events like recruiting days for high school students on campus, local science fairs, and/or our neighborhood children's museum.

Background for Proposed Project

Magnetic nanoparticles (MNP) have been used in a wide variety of application. Their small size allows them to penetrate many tissues. When inside of a body a magnetic field can be used to guide the particles to specific areas. Once in place, an alternating magnetic field can be used to heat the MNP via mechanisms of Brownian relaxation (frictional interaction) and Néel relaxation (magnetic viscosity), which then causes heat transfer to the surrounding tissue. These properties have been shown to be useful for cancer treatment via hyperthermia and drug delivery (2, 3). Magnetically-induced hyperthermia is the heating that occurs when MNP are exposed to alternating magnetic fields.

Ultrasound microbubbles (UMB) are also being examined for their use in drug delivery (4). Exposing UMB near a tumor region to ultrasound will cause them to oscillate or collapse. If anti-cancer drugs are either attached to the bubbles or in the vicinity of the tumor, enhanced delivery of the drug can be achieved via transiently-increased diffusion or transient creation of pores.

MMB have the properties of both UMB and MNP. MMB show promise for enhancing and targeting the delivery of drugs. The magnetic properties of MMB may allow them to be guided to and concentrated in a specific region of the body. The concentration of the drug would mean better delivery and less negative effect on cells that are not targeted.

Expected Results

We expect that our research will show that MMB release more drugs when exposed to ultrasound and magnetically-induced hyperthermia than MMB or UMB exposed to ultrasound alone. We also expect MMB exposed to ultrasound to release equivalent amounts of a drug as UMB exposed to ultrasound. If this is the case, then MMB will have been shown to be a feasible replacement for UMB. This will allow for further testing on delivery of MMB via their magnetic properties.

Description of Proposed Research - Methods, Design, and Procedures

SYNTHESIS

Nanoparticles. We will be using iron(II) oxide as our MNP. Synthesis will be done via a standard co-precipitation method (5, 6). We expect that this method will create nanoparticles of approximately 8 to 20 microns in diameter. To prevent clumping of the MNP, we will be coating the MNP in dextran by mixing the MNP with an alkaline dextran solution via a known method.

MNP functionalization. The dextran MNP will be functionalized by coating the particle with a rhodamine dye as a model drug. Rhodamine is a dye that changes its absorption properties when released from the MNP into solution. The MNP will be incubated for a fixed time (e.g., 2 hours) to allow the rhodamine to bond with the dextran. The resulting functionalized MNP will be loaded into the microbubble shell via sonication (described below).

Microbubbles. Synthesis of the MMB will be done via a published method (3). In brief, a phospholipid molecule (L-α-phosphatidylcholine) will be dissolved in an aqueous solution with the functionalized MNP. The

solution will then be exposed to a sonicator (a.k.a., a sonic cell dismembrator), which creates an high-intensity ultrasound signal at 20 kHz. This process results in vigorous bubble formation in the solution and results in spontaneous creation of the MMB, which have a gas core surrounded by a stabilizing phospholipid shell with the MNP embedded in the shell. We will also attempt to embed the MNP in a commercially-available and clinically-approved microbubble (Definity, Lantheus Medical Imaging) with a lipid shell and perfluorocarbon (C_3F_8) gas core.

TESTING

Characterization of MNP. Characterization of our MNP size and composition will be done using x-ray diffraction (XRD) and scanning electron microscopy (SEM) at Kettering University. We will also measure particle size distribution using transmission electron microscopy (TEM) and measure saturation magnetization via SQUID magnetometry at Wayne State University in Detroit, MI, through Prof. Vaishnava's collaboration with faculty there.

Characterization of microbubbles. We will measure the size distribution of our microbubbles via an optical microscope at Kettering. The microbubbles will be placed into a small chamber (or hemocytometer) to image the bubbles and measure the size distribution via microscopic imaging techniques.

Hyperthermia system. The MNP will heat up when exposed to an alternating magnetic field. To accomplish this task, we will use an magnetic induction system available at Kettering (Ambrell system in Prof. Vaishnava's lab). This system will allow us to vary the magnetic field frequency from 150 to 450 kHz and field strength from 50 to 150 gauss.

Ultrasound system. Ultrasound will be applied using a focused ultrasound transducer, amplifier, and pulser or function generator (from Prof. Kumon's lab). The transducer has a center frequency of 1 MHz and has the ability to apply pressure pulses that can oscillate and break the microbubbles.

Model drug release: Initial delivery testing. Testing the drug release properties will be done in three sections during the first phase of the project. The MMB will be placed into solution. The solution will then be treated under various conditions and then analyzed for release of the model drug (rhodamine) by spectrophotometry. The test conditions will include:

1. Control condition (no ultrasound or hyperthermia)
2. Ultrasound exposure.
3. Magnetically-induced hyperthermia.
4. Both ultrasound and magnetically-induced hyperthermia.

These tests will determine if the synthesized MMB are effective for enhancing release of the model drug, as suggested by some studies.

Model drug release: Optimization of delivery parameters. Once we demonstrate the possibility of reliable synthesis of MMB and delivery, the second phase of the project will determine the optimal properties of the drug absorption into the MNP and MMB and for maximizing drug delivery by ultrasound and/or magnetic field. Drug absorption into the MNP may be optimized by changing incubation time and possibly by changing particle size or shape. Delivery may be optimized by changing magnetic field parameters (e.g., frequency, field

strength) and/or ultrasound parameters (e.g., pulse center frequency, pulse repetition frequency, pulse duration, pressure amplitude).

PROJECT DELIVERABLES

Written reports. We will prepare interim and final reports of our research. We also hope to write and submit an article to a peer-reviewed journal.

Posters. We will prepare one or more posters for presentation at undergraduate research fairs at Kettering as well as for local or national conferences

Oral presentations. We will prepare one or more posters for presentation at undergraduate research fairs at Kettering as well as for local or national conferences. We may also consider preparing a short video summary of our results for web distribution.

Plan for Carrying Out Proposed Project

Personnel

Spring quarter: Nicolaas Winter, Nathaniel Mosher, Emily Perkins-Harbin,

Fall quarter: Douglas Blaisdell, Natalie Gerdung, Paul Kizer, and other interested students

(Note: Kettering students typically go to co-op jobs every other term and hence this set of students will be primarily working on this project during the spring and fall quarters).

Expertise

Several student bring particular skills to this project that will be useful for its success:

- Nicolaas Winter – experience with material synthesis and characterization. He has previously performed measurements on nanoparticles using x-ray diffraction and magnetic measurements using SQUID magnetometry.
- Nathaniel Mosher – experience with acoustical measurement and modeling.

Other members of the research team have technical experience in applied physics, engineering physics, and chemical engineering based on their majors.

Research space

The research will be carried out primarily in the Physics Department's laboratory facilities under the supervision of Prof. Prem Vaishnava and Prof. Ronald Kumon and the Chemistry Department's laboratory facilities under the supervision of Lihua Wang.

Contributions of faculty

1. Prof. Lihua Wang will provide supervision regarding the synthesis and chemical characterization and modification of the magnetic nanoparticles.
2. Prof. Ronald Kumon will provide supervision regarding the synthesis and characterization of the microbubbles.
3. Prof. Prem Vaishnava will provide supervision regarding the synthesis and characterization of the magnetic nanoparticles as well as the generation of hyperthermia using magnetic induction.

4. Staff: Dr. Robert Cunningham and Dr. Gary Thieme will assist with the use of the scanning electron microscope and other equipment used for characterization.

Project Timeline

Completion of the project will occur primarily during our Spring and Fall quarters based on the Kettering academic calendar:

Spring Quarter (April to June 2013)

Week	Milestones
1	Synthesis and coating of MNP
2	Characterize MNP
3	Synthesis of UMB and MMB
4	Characterize microbubbles
5	Ultrasound testing
6	Hyperthermia testing
7	Hyperthermia and ultrasound testing
8	Data analysis and report preparation
9	Data analysis and report preparation
10	Presentation of interim report

Fall Quarter (October to December 2013)

Week	Milestones
1	Synthesis of MNP and MMB
2	Initial delivery testing
3	Magnetic field parametric studies
4	Magnetic field parametric studies
5	Ultrasound parametric studies
6	Ultrasound parametric studies
7	Particle property parametric studies
8	Data analysis and report preparation
9	Data analysis and report preparation
10	Presentation of final report

The interim report will be prepared and submitted by May 31, 2013, while the final report will be prepared and submitted by December 31, 2013.

Budget Justification

Supplies and equipment will be necessary to complete the project:

1. Magnetic Nanoparticles Synthesis
   (1) Iron(II) Chloride
   (2) Iron(III) Chloride
   (3) Dextran
2. Microbubble Synthesis
   (1) Custom microbubbles: Hydrogenated L-alpha-phosphatidylcholine
   (2) Commercial microbubbles: Definity™ (Lantheus Medical Imaging, North Billerca, MA)
   (1 case of 4 bottles is the minimum order)
   (3) Aerated Phosphate buffered saline
3. Project Promotion

The small promotion allowance is necessary for advertising our project to current students and involving the school in our project. Our presentation will educate students who did not participate in the research to about physics and the research possibilities at Kettering.

4. Conference Poster Printing

We will need funding to print a large poster summarizing our studies so that we can present our results at local and national conferences.

5. Sonicator

We will need a sonicator to create both the magnetic nanoparticles as well as the microbubbles. We will look to purchase a used device. If the requested amount is not sufficient, then Prof. Kumon and Prof. Vaishnava have committed to provide the balance of the funding to purchase a new sonicator (~$2100 new for a Fisher Scientific Model 50 Sonic Dismembrator).

Borrowed equipment and in-kind support will include access to:

1. Miscellaneous glassware, pipettors, and hemacytometer.
2. Ambrell magnetic induction system
3. Ultrasound transducer, amplifier, and pulser/function generator
4. Optical microscope
5. Environmental scanning electron microscope (ESEM)
6. Transmission electron microscopye (TEM)
5. X-ray diffraction (XRD) system
6. SQUID magnetometry system
7. Spectrophotometry system

Bibliography

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(3) Regmi R, Black C, Sudakar C, Keyes PH, Naik R, Lawes G, Vaishnava P, Rablau C, Kahn D, Lavoie M, Garg VK, Oliveira AC. Effects of fatty acid surfactants on the magnetic and magnetohydrodynamic properties of ferrofluids. Journal of Applied Physics 2009;106,113902.

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(5) Berger P, Adelman NB, Beckman KJ, Campbell DJ, Ellis AB, Lisensky GC. Preparation and properties of an aqueous ferrofluid. Journal of Chemical Education. 1999;76:943–948.

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