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SPS Chapter Research Award Proposal

Project Proposal Title	Enhancing Cellular Uptake of Magnetic Nanoparticles for Cancer Therapy via Nanoparticle Engineering and Sonoporation
Name of School	Kettering University (B)
SPS Chapter Number	3451
Total Amount Requested	$2,000

Abstract

Magnetic induction heating of iron oxide nanoparticles has been proposed as a method for noninvasive cancer treatment without the side effects of chemotherapy and ionizing radiation. At Kettering University we propose to improve the uptake of nanoparticles by cells through the use of nanoparticle engineering and ultrasonic fields.

Proposal Statement

Overview of Proposed Project

Research Question

This project proposes to answer the following research question: What properties of iron oxide nanoparticles and what ultrasound conditions will best enhance nanoparticle uptake by cancer cells?

Motivation

Answering this question has the potential to impact the development of improved cancer therapy. Because iron oxide nanoparticles will heat up when placed in an alternating magnetic field, they can be used as a method to (1) cause drug-loaded nanoparticles to release their payload to cancer cells or even (2) heat up the cells enough to directly kill them. Because the heating only takes place where the nanoparticles are located, the cancer treatment can be targeted to specific areas. The challenge is to achieve enough uptake of nanoparticles to the cells for the treatment to be effective.

Brief Description & Research Goals

Our proposed solution is to guide the iron oxide nanoparticles to targeted cells with the use of magnetic fields, where they will then be forced into the cells by ultrasound via sonoporation. At our university, faculty have been working on methods to make and characterize nanoparticles and also use ultrasound and microbubbles to induce delivery of molecules to cells that would otherwise be impermeable. Our research project will contain two parallel approaches. In one path, we will conduct experiments to synthesize nanoparticles with different physical properties. We will create particles with a range of sizes, surface charges, and coatings and test their uptake in normal and cancer cells in vitro. In the other path, we will investigate different ultrasound conditions to improve MNP uptake in cells, both healthy and cancerous. Ideally, this research will tell how to optimize nanoparticle uptake and provide some insight into why these approaches are the best.

Student Benefits & SPS Connection

This project will give us the opportunity to do hands-on research in the biophysics/biomedical field, allowing us to use scientific equipment, learn to culture cells, and learn proper research methods. The work may also be used by one or more students to complete Kettering University's requirement for an undergraduate thesis. We hope that it will allow us to present in professional meetings and journals and give us experience as scholars in the physics community. Finally, it will give our SPS chapter at Kettering a chance to work together on an organized research project.

Background for Proposed Project

Iron oxide nanoparticles possess properties that offer new opportunities of improving the quality of magnetic resonance imaging (MRI), hyperthermia treatment for malignant tumors, and site-specific drug delivery. One major hurdle to the widespread clinical use of nanoparticle therapy is the problem of getting the particles inside cancer cells. In general, natural uptake can depend on the nanoparticle parameters, which include particle size, surface characteristics, volume and strength of drug particle binding, as well as access to the affected organ and the nature of the infusion process.

Studies of cellular uptake have attracted much attention because efficient localization of nanoparticles into the cytosol is important for a variety of applications including cancer treatment, intracellular imaging, and drug and gene delivery. Three major factors affect natural uptake of nanoparticles:

(1) Nanoparticle size. Even though nanoparticles have a certain size after synthesis, during studies *in vitro* they might aggregate into vastly different shapes and sizes that may dictate the outcome and interpretation of results.
(2) Surface charge. Positively charged nanoparticles are adsorbed more efficiently on the negatively- charged cell surface and consequently show greater internalization than neutral and negatively charged nanoparticles.[1,2] However, uptake of negatively charged particles can also occur.[3] Cellular uptake of nanoparticles is also dependent on the incubation time and nanoparticle concentration.[4]
(3) Surface properties. These properties include hydrophilicity/hydrophobicity, chemical group, and molecular composition and are determined by the biomolecules used to modify or functionalize nanoparticles. For example, the use of cell-penetrating ligands on the surface of the nanoparticles can help in the fusion of magnetic nanoparticles with cell membranes and their uptake into the cytoplasm.[5]
To keep the project with reasonable scope, we will primarily focus on nanoparticle size and surface coating in our experiments.

While it would be desirable for sufficient nanoparticle uptake to occur naturally, this process may be too slow for practical use. Thus it is also important to explore artificially enhancing uptake by the noninvasive application of ultrasound. For example, the pressure oscillations induced by the ultrasound can cause radiation pressure in the tissue. In the presence of microbubbles, ultrasound can induce bubble oscillation and collapse resulting in localized fluid jetting, streaming, shear, which has been shown in some cases to enhance transport of agents into cells (*sonoporation*).[6] For example, some studies with short pulses have clearly demonstrated that ultrasound exposure of cells in the presence of microbubbles induces uptake via transport through pores in the plasma membrane,[7,8] Some evidence also exists to show ultrasound may also cause larger molecules to be taken up via endocytosis (process where cell encapsulates object to bring it inside),[9] and this may also occur for nanoparticles.

The proposed research would add to previous work in this field by determining the properties of the nanoparticle that will best increase uptake for iron oxide nanoparticles. It will also determine the parameters that should be used to increase delivery of the iron oxide nanoparticles to cells using ultrasound.

Expected Results

We expect that our research will show how to optimize the uptake of iron oxide nanoparticles into cells. Our specific project aims and expected results are:

(1) For the particles, we expect to determine which particle sizes and surface coating types will be most effective. Based on guidance from existing literature, we hypothesize that iron oxide nanoparticles with size 30–40 nm, electrokinetic surface charge (zeta) potential of 50–60 mV, and lauric acid coating will likely be most suitable for optimizing cellular uptake, but a variety of particle conditions will be explored.
(2) For ultrasound exposure, we expect to determine which pulse repetition frequencies and pulse durations are most effective. We will investigate the use of 1 MHz pulsed ultrasound without and with microbubbles. Based on published work, we hypothesize that a series of relatively short pulses (microseconds) will be more effective than fewer long pulses (milliseconds).

If time permits, we would like to investigate also expect that our research will give us a measurement of how the uptake of cancer cells differs from the uptake of normal cells.

Description of Proposed Research - Methods, Design, and Procedures

Synthesis of coated iron oxide nanoparticles

We will use a precipitation method[10] to synthesize iron oxide (magnetite) nanoparticles with size of 5−100 nm. Briefly, the nanoparticles will be prepared by mixing solutions of $FeCl_2 \cdot 4H_2O$ and ~7 M ammonium hydroxide at 80−90°C. The precipitate thus obtained will be filtered and left for drying. The concentration percentage of ferrous chloride will be varied from 0.025 to 3.00 to obtain particle sizes between 6 and 90 nm.[11] The particles will then be coated with lauric acid. To investigate the effect of different coatings, we will fix the range of particle size by using a concentration percentage of ferrous chloride of 1.00 and then cover the nanoparticles with different short- and long-chain fatty acids, such as lauric acid, myristic acid, and oleic acid. The nanoparticles will be added to a buffered saline solution that is osmotically balanced with the cells.

Chararacterization of coated nanoparticles

We will attach fluorescein isothiocyanate (FITC) dye to the coated nanoparticles so that the amount of uptake can be quantitatively determined by the brightness of the cells when viewed in a fluorescence microscope. The composition of the nanoparticles will be characterized by X-ray diffraction using facilities at Kettering. As needed, cells with nanoparticle uptake will be fixed and imaged using a scanning electron microscope or a transmission electron microscope. Nanoparticle concentration measurements will be performed via an existing faculty research collaboration with another nearby university.

Cell culturing

We will culture cancer cells in vitro in the new biology laboratories at Kettering using standard methods. We plan to primarily focus on cancer cells (e.g., MDA-MB-231 breast adenocarcinoma[12] or SK-OV-3 ovarian adenocarcinoma[13]). If time permits, we may also study the uptake in related normal cells from the same area of the body (e.g., MCF 10 breast epithelial cells[12] or human ovarian microvascular endothelial cells [HOMEC][13]).We will culture the cells in dishes until they reach 70% confluency (coverage) prior to the uptake experiments.

Testing of uptake changes with different particle sizes and coatings

To test uptake by the cells with different particle sizes or coatings, the nanoparticles will be added to the cells in culture media, and an external magnet will be used to concentrate the nanoparticles near the cells during a fixed time. The cells will then be placed back into an incubator for 24 hours. At that time, the cells will rinsed to remove the culture medium and any extracellular nanoparticles and then be examined by flourescence microscopy to determine the extent and distribution of the uptake. The cells treated with different types nanoparticles will be compared with each other and against a control with no nanoparticles added.

Testing uptake changes with ultrasound

To test uptake by the cells with ultrasound, we will again add the nanoparticles to the cells in culture media, and concentrate them nanoparticles near the cells for a fixed time. We will then expose the cells an ultrasound field from a transducer with a 1 MHz center frequency available. We will vary the pulse duration, pulse repetition frequency, and possibly the intensity of the signal to try to optimize uptake. Uptake will be assessed immediately after ultrasound exposure and after 24 hours in the incubator using fluorescence microscopy. Uptake will also be assessed without and with commercially-available microbubbles (e.g., Definity or Targestar). Research will be disseminated by giving presentations to our local SPS chapter, poster presentations at Kettering recruitment and alumni events, presentations at other SPS chapters or zone meetings, posters or presentations at professional conference, and hopefully a peer-reviewed journal article.

Plan for Carrying Out Proposed Project

Personnel

Spring/Fall quarters: Alexis Siegel, Sally Dagher, and other interested students

[Note: Kettering runs on a quarter system, and students typically perform co-op work every other academic quarter as a required part of their degree program.]

Special Expertise

Nathaniel Mosher and Emily Perkins-Harbin have been working on their undergraduate research theses regarding nanoparticle synthesis and characterization during the past year. They will help to train us about the basic techniques for making iron oxide nanoparticles. Denis Volobuev, a student from the Applied Biology, will assist in training us in cell culturing techniques. Finally, Robert Cunningham, a senior technician in the Physics Department, will assist us in learning to use the x-ray diffractometer.

Research Space

The research will be primarily carried out in the Materials Science & Nanotechnology Laboratory in the Physics Department at Kettering. The chemical synthesis procedures will primarily be done in the Inorganic Chemistry Laboratory in the Chemistry Department. Cell culturing will take place in the Cell Biology Laboratory in the Applied Biology Department. As needed, we may access a transmission electron microscope at Michigan State University and a physical properties measurement system and magnetometer at Wayne State University.

Faculty Contributions

1. Prof. Ronald Kumon (Physics) will provide training on ultrasound, sonoporation, and fluorescence microscopy, including access a probe sonicator for nanoparticle dispersion.
2. Prof. Prem Vaishnava and Prof. Ronald Tackett (Physics) will provide training on magnetite nanoparticle synthesis and characterization, including access to an x-ray diffractometer and magnetic measurements in the Materials Science and Nanotechnology Laboratory.
3. Prof. Lihua Wang (Chemistry and Biochemistry) will provide training on chemical synthesis procedures, including access to glasware and hoods in the Inorganic Chemistry Laboratory.
4. Prof. Cheryl Samaniego (Biology) will provide training in cell culturing and biology, including access to incubators and microscopes in Cell Biology Laboratory.

Project Timeline

Completion of the project will mainly occur while we are on campus during our spring and fall quarters:

Sub-project: Uptake with different nanoparticle sizes and coatings

Spring Quarter (April to June 2015)	*Fall Quarter (October to December 2015)*
Weeks 1–3: Synthesis of particles of different sizes	Synthesis of particles of different coatings
Weeks 4–7: Nanoparticle characterization*	Nanoparticle & coating characterization
Weeks 8–11: Cell culturing training & uptake studies	Cell culturing and uptake studies*

Sub-project: Uptake with ultrasound exposure

Spring Quarter (April to June 2015)	*Fall Quarter (October to December 2015)*
Weeks 1–3: Setup of ultrasound system	Data analysis of PRF studies
Weeks 4–5: Cell culturing training	Cell culturing and system setup
Weeks 6–11: Uptake studies of pulse repetition freq.*	Uptake studies of pulse duration*
*Week 7: Interim report preparation by 5/31/15	*Week 11: Final report prep. by 12/31/15

Budget Justification

Supplies and equipment will be necessary to complete the project:

1. Chemicals for Magnetite Nanoparticle Synthesis
   (1) Iron(II) Chloride
   (2) Iron(III) Chloride
   (3) Ammonium Hydroxide
   (4) Lauric Acid
   (5) Myristic Acid
   (6) Oleic Acid
   (7) Phosphate Buffered Saline
2. Microbubbles
   e.g., DefinityTM (Lantheus Medical Imaging) or Targestar (Targeson)
3. Cancer Cell Culturing
   (1) Cancer Cells (ATCC)
   (2) Cell Culture Medium
   (3) Trypsin (for detaching cells)
   (4) Disposable pipets
   (5) Pipette tips
   (6) Cell culturing (Petri) dishes
   (7) OptiCell cell culturing chambers (for microbubble studies)
4. Fluorescein isothiocyanate (FITC) for uptake measurements
5. Conference Poster Printing
   We will need funding to print posters about our work so that we can present our results at local and national conferences.

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

1. Glassware, pipetters, mass balance, hot plate, chemical fume hood
2. Optical fluorescence microscope
3. X-ray diffractometer
4. Scanning electron microscope
5. Magnetometry system
6. Ultrasound transducer, amplifier, pulser, waveform generator
7. Cell incubator and biosafety cabinet

The Physics Department and previously mentioned faculty will supply other miscellaneous materials, as needed for successful completion of the project.

Bibliography

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