2014

Magnetic Microbubbles for Targeted Drug Release

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SOCIETY OF PHYSICS STUDENTS
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SPS Chapter Research Award Final Report

Project Title	Magnetic Microbubbles for Targeted Drug Release
Name of School	Kettering University (B)
SPS Chapter Number	3541
Total Amount Awarded	$1997.00
Total Amount Expended	$1997.00
Project Leader	Nicolaas Winter

Abstract

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

Statement of Activity

Overview of Award Activity

In this project we proposed to answer the research question: Can magnetic microbubbles (MMB) be used to enhance drug delivery? To do this, we have successfully synthesized magnetic nanoparticles (MNP), coated them with surfactant, characterized them with respect to material and size distribution, integrated the MNP into MMB, and characterized the MMB.

We found that MNP synthesis was more challenging than the literature suggested. We used a standard co-precipitation method, but were getting inconsistent results and often failures. After a few months of refining our technique we determined that performing our synthesis under a non-oxidizing environment was a key factor in successfully synthesizing Fe_3O_4 MNP, which was the first key goal for the project.

Having made the particles, we then needed to coat them with a surfactant to prevent their agglomeration and enable them to be made into a biocompatible solution that can be integrated into microbubbles. We chose dextran first because of its excellent biocompatibility, wide availability, and water-solubility. Coating with dextran also proved to be a challenge at first and particles would fall out of solution. Eventually we determined that using a higher powered probe sonicator enabled much better coating compared with the original bath sonicator. However, we were still unable to form MMB with dextran-coated MNP.

We then investigated the use of oleic acid to coat the particles. Our hypothesis was that oleic acid would be better for MMB integration than dextran because it was hydrophobic. We found that coating the MNP with oleic acid was simpler than the dextran coating and we carried it out successfully. We are still investigating the comparative properties of MMB with dextran-coated MNP versus MMB with oleic acid-coated MNP.

Given the multiple difficulties associated with the synthesis of the MNP and thus MMB, we have not yet functionalized the MNP so that a model drug can be attached. The original proposal proposed functionalizing dextran-coated particles, but the chemical functionalization of oleic acid is different. We are currently investigating how this might be performed.

Nicolaas Winter proposed the original project. Prior to his graduation in the Spring 2014, Nathaniel Mosher and Emily Perkins-Harbin began working as the primary student researchers with additional participation by Brandon Aho (Physics), Natalie Gerdung (Chem. Eng.), and Denis Volobuev (Biology). The three primary student researchers were members of SPS National and four of the students were participants in our local Physics Club. Several more students will be continuing work on magnetic nanoparticles and microbubbles in the coming year. A side project to build an enclosure unit for the ultrasonic probe sonicator involved an additional three students, one of whom was a member of SPS National and our local Physics Club.

Description of Research - Methods, Design, and Procedures

Nanoparticles. We have, after many trials and refinements, successfully synthesized dextran-coated iron oxide (Fe_3O_4) magnetic nanoparticles via a chemical co-precipitation method.[1,2] This method, briefly, involves the addition of a strong base in excess to a solution containing a 1:2 molar ratio of Fe^{2+}:Fe^{3+} ions under a non-oxidizing atmosphere. The overall chemical reaction is described by:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$$

The addition of the base causes bare Fe_3O_4 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 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. We characterize the resulting MNP using x-ray diffraction and transmission electron microscopy (TEM) to determine phase and size distribution. We also prepare samples for magnetic characterization via SQUID magnetometry.

When a MNP fluid is placed in an alternating magnetic field, the fluid will heat up as a result of energy dissipated from physical rotation of the particles (Brownian relaxation) and changes in the directions of the

particles' internal magnetic domains (Néel relaxation), a phenomenon called magnetic fluid hyperthermia (MFH). To characterize the hyperthermic response of the MNP, we use an Ambrell induction system, which runs an alternating current through a coil to create an alternating magnetic field, thereby heating the MNP sample placed within the coil. The system can be adjusted to output different frequencies and magnetic field strengths. An optical thermometer placed in the MNP sample is used to measure the heat generated from MFH. The sample is insulated to minimize heat transfer to the environment, which affects the apparent heating rate. Any heat transfer not eliminated is accounted for in the heating rate calculation. We also investigated the physical mechanisms responsible for MFH by building an apparatus to enable the ferrofluid to be frozen into a "ferrosolid" by submerging the ferrofluid in liquid nitrogen until it froze completely. We then apply an alternating magnetic field to the frozen sample and compare the heating rates to those of liquid samples.

Microbubbles. We created control (non-magnetic) microbubbles by mixing magnetic nanoparticles and L-α-phosphatidylcholine into a phosphate-buffered saline solution. The microbubbles formed via exposure to the probe sonicator for 30 s, following by manual shaking for 30 s. For the creation of magnetic microbubbles,[3] we repeat the process with the addition of the magnetic nanoparticles and another sonication step.

We characterize the MMB primarily by viewing under a microscope. By placing the MMB in a hemocytometer with known scale markings, we are able to determine the size distribution. To test whether the MMB are actually magnetic, a 30 mT bar magnet is placed by the microscope slide containing the MMB, and they are viewed to see if they respond to the field.

Discussion of Results

Nanoparticles. Through x-ray diffraction, we found that the MNP were composed of Fe_3O_4 and Fe_2O_3, as expected. Figure 1 shows TEM imaging of a typical MNP sample, revealing that the particle size ranges from 5–20 nm, which is well within the range of a superparamagnetic single domain material. Magnetic characterization experiments are planned at an off-campus location during early 2015.

We measured the hyperthermic ability of the MNP by exposing them to alternating magnetic fields of various frequencies. The heating rates of the MNPs were then calculated for each frequency to determine their efficacy, as described in the midyear report. The effectiveness of the heating suggests that thermally-induced drug release from the MNP is at least plausible. We also investigated heating mechanisms of the MNP by comparing the heating rate between liquid (Brownian and Néel relaxation) and frozen states (only Néel relaxation). Figure 2 shows the temperature of the sample as a function of time for a frozen sample. We find that frozen MNP typically heat at a reduced rate as compared to the liquid MNP, which makes sense because the Brownian motion is reduced or even completely suppressed by the immobilization of the MNP due to the freezing. A liquid sample heated under the same conditions as those seen in Fig. 2 would therefore have a steeper slope. Analysis of data and calculation of specific absorption rates (SAR in J/g) for multiple frequencies between 150 kHz and 350 kHz is ongoing and will be reported at the 2015 APS March Meeting.

To determine the bioeffects of the MNP, a proof-of-concept experiment was run in cooperation with Dr. Hakan Demerci of the University of Michigan Kellogg Eye Center for retinoblastoma cells cultured *in vitro* to determine cytotoxicity levels and the ability of MFH to induce cell death. We found that a majority of the cells were killed after exposure to MFH up to 50℃. Future work is planned to test the MNP in an animal model to get results more representative of what we could expect in the human body.

Microbubbles. The microbubbles created with the MNP were visibly darker than the plain microbubbles, both macro- and microscopically. The color difference was taken as an indication that the MNP were incorporated with the bubbles. When placed in a field, as in Fig. 3, the MMB can be seen forming chains parallel to the field, suggesting that the bubbles are magnetic. These bubbles averaged 5 μm across. Microbubbles that did not contain MNP did not respond to the field.

We found that we had better incorporation of the MNP into the microbubbles with oleic-acid-coated MNP than with dextran-coated MNP. We believe that this could be because the phosphatidylcholine forms a lipid monolayer with the hydrophobic tails directed in towards the air at the center of the bubble. Consequently the hydrophobic oleic-acid-coated nanoparticles are likely traversing the monolayer to congregate near the lipid

tails to minimize contact with the water. It is also possible that the bubbles are formed of lipid bilayers, and that the oleic acid nanoparticles will congregate between the layers for the same reason.

Dissemination of Results

- Each spring the Kettering University sponsors a "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. We presented two posters related to the current project on 17 May 2014:
  - E. Perkins-Harbin, N. Mosher, R. Cunningham, L. Wang, P. Vaishnava, R. E. Kumon, "Magnetic Microbubbles for Biomedical Applications"
  - N. Mosher, N. Winter, E. Perkins-Harbin, N. Gerdung, R. Cunningham, C. Rablau, L. Wang, R. E. Kumon, P. Vaishnava, "Synthesis and Characterization of Biocompatible Magnetic Nanoparticles"
- We also presented these posters at the Wayne State University 8th Annual Undergraduate Research Conference on 7 November 2014 and Discover Kettering, a recruiting day for high school students, on 8 November 2014. The posters have been on display in the hallway outside of our lab since May 2014.
- We were involved in the production of a video entitled "Magnetic Nanoparticles and Microbubbles" as part of Kettering University's "Making a Better World" series. The video features Prof. Ronald Kumon, Prof. Prem Vaishnava, Nathaniel Mosher, and Emily Perkins-Harbin and was distributed via the Kettering University YouTube channel (https://www.youtube.com/watch?v=IJ2qi15jOaQ). It had 402 views as of 31 December 2014.
- Emily Perkins-Harbin also made a presentation to her Medical Physics class on 16 December 2014, entitled "Enhancing Cancer Treatment with Magnetic Fluid Hyperthermia," which was open to the public.
- We co-authored an abstract that has been accepted for an oral presentation to the 2015 APS March Meeting: N. Mosher, E. Perkins-Harbin, B. Aho, L. Wang, R. Kumon, P. Vaishnava; R. Tackett, "Determination of the magnetocrystalline anisotropy constant from the frequency dependence of the specific absorption rate in a frozen ferrofluid"
- Two senior theses are currently in preparation, with anticipated completion by April 2015:
  - Emily Perkins-Harbin, "Synthesis and Characterization of Magnetic Microbubbles"
  - Nathaniel Mosher, "Drug Delivery using Magnetically-Induced Hyperthermia and Ultrasound"

Examples of disseminated results are included in Appendix A.

Bibliography

[1]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.

[2]Massart R. Preparation of aqueous magnetic liquids in alkaline and acidic media. IEEE Transactions on Magnetics 1981;MAG-17(2):1247–1248.

[3]Stride E, Porter C, Prieto AG, and Pankhurst Q. Enhancement of microbubble mediated gene delivery by simultaneous exposure to ultrasonic and magnetic fields. Ultrasound in Medicine and Biology. 2009;35:861–868.

Acknowledgments

We would like to thank Prof. L. Wang and J. Rivet in Chemistry, Prof. M. Ammerman, Prof. C. Samaniego, and M. Muxlow in Biology, Profs. S. Farhat M. Gilliam, S. Nartker in Chemical Engineering for guidance and access to equipment at various times to synthesize the MNP, and Profs. R. Kumon, C. Rablau, R. Tackett, and P. Vaishnava in Physics for help in synthesizing and characterizing the MNP and MMB and performing MFH experiments. We would also like to thank H. Nemala, M. Paliahawadana-Aracchchige, and R. Regmi, for guidance in MNP synthesis and coating, R. Cunningham for technical assistance with the x-ray diffractometer, and A. Withrow and Prof. X. Zhou, for help with TEM imaging. Finally, we would like to thank the national Society of Physics Students and Kettering University for funding this work.

Impact Assessment:

How the Project Influenced your Chapter

This experience was very much worthwhile and exposed many members of our SPS chapter to many valuable experiences and created many opportunities. One of the biggest lessons we learned was that learning to work with an interdisciplinary group is invaluable. Our project extends beyond physics to chemistry and even biology. As such, we have had the pleasure to work with people and subject matter that we are not normally exposed to in physics education and came out better for it.

This work involved work from many members from our SPS chapter and that meant working closely with each other for a good length of time. We came to know each other a lot better as a result. Additionally, because of our close work with faculty from the entire university, the SPS chapter's ties with faculty also became much stronger.

Additionally, simply being exposed to research in a university setting has given us a real taste of what academic research is like. Also, we are now familiar with the processes vital to academic research such as publishing papers, data management, presenting scientific results, etc. As a result, several members are actively pursuing graduate school where before they may not have.

Our advice to other SPS chapters: don't be afraid to work with subject matters other than physics; work as an interdisciplinary team and everybody will be better for it.

Key Metrics and Reflection

How many students from your SPS chapter were involved in the research, and in what capacity?	Six students were involved in the main research project, four of which were active members of the local club. These students wrote the proposal, synthesized nanoparticles and microbubbles, and characterized the nanoparticles and microbubbles, and performed experiments on nanoparticle heating. An additional three students were involved in a side project.
Was the amount of money you received from SPS sufficient to carry out the activities outlined in your proposal? Could you have used additional funding? If yes, how much would you have liked? How would the additional funding have augmented your activity?	The amount of funding was sufficient for some of the basic supplies and equipment, but additional funding was needed to cover the costs of some of the more expensive equipment and basic laboratory supplies, as noted in the Expenditures section.
Do you anticipate continuing or expanding on this research project in the future? If yes, please explain.	Yes, this project is still ongoing and we anticipate it will continue for some time. More research can be done exploring the structure as well as the effect that different nanoparticle coatings and different lipid chain lengths have on the characteristics of the bubbles.
If you were to do your project again, what would you do differently?	Having a better idea of the time and complexity of the initial steps would have helped planning the next steps.

Press Coverage (if applicable)

One of the projects that we were involved in was testing of the use of magnetic nanoparticles for killing ocular cancer cells in vitro in collaboration with Kellogg Eye Center at the University of Michigan in Ann Arbor. This work received coverage via local media and social media:

- Kettering University News, "Kettering, University of Michigan researchers partner to transform ocular cancer treatment" by Pardeep Toor, 29 October 2014, http://www.kettering.edu/news/kettering-university-michigan-researchers-partner-transform-ocular-cancer-treatment
- Google Plus posting, Kellogg Eye Center, 30 October 2014, https://plus.google.com/+kelloggeyecenter/posts/fmoTgpd3TyT
- DBusiness Daily News, "Kettering, U-M researchers treating ocular cancer without radiation," 07 November 2014, http://www.dbusiness.com/daily-news/Annual-2014/Kettering-U-M-Researchers-Treating-Ocular-Cancer-without-Radiation/
- MLive.com, "Kettering University professor helps develop new technique to fight ocular cancer" by Sarah Schuch, 12 November 2014, http://www.mlive.com/news/flint/index.ssf/2014/11/kettering_university_professor_14.html
- University of Michigan Kellogg Eye Center 2014–15 Annual Report, "Collaborations in ocular oncology: Using thermal energy to eliminate side effects in eye cancer therapies," p. 15, available at http://issuu.com/u-mkelloggeyecenter/docs/ar_2014_055b4d66b24bfc?e=6943966/10813102

Examples of this press coverage are included in Appendix B.

Expenditures

Expenditure Table

Item	Please explain how this expense relates to your project as outlined in your proposal.	Cost
Mesh basket for ultrasonic bath	The mesh basket is used to hold the magnetic nanoparticle samples during the coating process.	$81.08
Hemacytometer (phase-contrast, bright-line)	The hemacytometer are used to size and count the magnetic microbubbles.	$179.93
Adjustable Pipettor (0.5 to 10 µL), Adjustable Pipettor (10 to 100 µL), Adjustable Pipettor (100 to 1000 µL)	The adjustable pipetters are used to dispense liquids during magnetic nanoparticle and microbubble synthesis and characterization.	$559.80
Iron (II) Chloride Tetrahydrate, Iron (II) Chloride Tetrahydrate, Sodium hydroxide concentrate, Ammonium hydroxide solution, Hydrochloric acide solution, Oleic acid	These chemical supplies are used to synthesize magnetic nanoparticles, except for the oleic acid. The oleic acid is used to create a hydrophobic coating for the magnetic nanoparticles.	$410.69
Dextran (low fraction)	Dextran is used to create a hydrophilic coating for the magnetic nanoparticles to make them stable in an aqueous solution.	$132.46
Fluorescein isothiocyanate (FITC), Epichlorohydrin	These supplies are used to functionalize the coatings on the magnetic nanoparticles (i.e., to cross-link and florescently label them).	$107.68
Separatory funnel	The funnel is custom-made glassware used to synthesize the magnetic nanoparticles.	$258.86
Dialysis device	Dialysis devices are used to remove excess sodium hydroxide introduced in the synthesis procedure of the magnetic nanoparticles.	$108.90
Phosphatidylcholine	This phospholipid is used to create the shell around the magnetic microbubbles.	$205.85
	Slight overage covered by external support	($48.25)
Total of Expenses Covered by SPS Funding		$1997.00

Note: The figures shown in this final expense report may differ slightly from the midyear report, as these final figures account for the total costs including shipping and handling.

Significant support for this project was covered by other sources of funding from Kettering University to faculty advisors (Prof. Ronald Kumon, Prof. Prem Vaishnava):

Item	Please explain how this expense relates to your project as outlined in your proposal.	Cost
Ultrasonic bath sonicator	The bath sonicator is used to more gently coat magnetic nanoparticles than the	$828.00
Probe sonicator	The probe sonicator is used to disperse magnetic nanoparticles and create magnetic microbubbles.	$2250.00
Sound enclosure	This hand-made enclosure is used to reduce the loud, high-pitched sound generated by the probe sonicator for occupational health & safety.	$90.88
Microscope slides & cover slips	Microscope slides and cover slips are used to view magnetic microbubbles.	$5.25
Benchtop protectors	These protectors are used to protect the benchtops from chemical spills.	$37.22
Immersion oil for microscope	This oil is used to view magnetic microbubbles under a high magnification oil-immersion microscope objective.	$8.50
Phosphatidylcholine (hydrogenated & from egg yolk)	This phospholipid is used to create the shell around the magnetic microbubbles.	$126.50
Phosphate-buffered saline (PBS) tablet and 10X solution	These supplies are used to create an aqueous solution that is osmotically balanced for use with cells.	$86.55
Refrigerator/freezer	This small refrigerator/freezer is used to store chemical supplies.	$119.00
Nitrile gloves (small, medium, & large)	These gloves are used during the synthesis of the magnetic nanoparticles and microbubbles for occupational health & safety.	$114.04
Sharps container	The sharps containers are used during synthesis of the magnetic nanoparticles and microbubbles to collect broken glass or other sharp metal tips.	$4.86
Digital thermometer	The thermometer is used to monitor temperature of the solution during nanoparticle synthesis.	$18.38
Beakers & graduated cylinders	This glassware is used during the synthesis of magnetic nanoparticles.	$183.97
Pasteur pipettes & dropper bulbs	These pipettes are used during the synthesis of magnetic nanoparticles.	$62.38
Wash bottles	These bottles are used to clean glassware and provide water during experiments.	$42.42
Microcentrifuge tube rack	These racks are used to hold the microcentrifuge tubes used to hold the magnetic nanoparticles samples.	$26.81

Paraffin wrapping film	This film is used to seal containers as part of the magnetic nanoparticle synthesis	$20.83
Wipes	The wipes are used to clean up minor spills during experiments	$4.46
pH paper	This paper was used during the early magnetic nanoparticle synthesis procedures to intermittently estimate pH of the solution.	$10.09
pH sensor & storage solution	The pH meter was used during the later magnetic nanoparticle synthesis procedures to continuously measure pH of the solution.	$98.58
Labelling tape	This tape is used to label samples of magnetic nanoparticles & microbubbles.	$5.06
Magnetic retreiver	The retreiver is used to retrieve the magnetic stir bar used during parts of the magnetic nanoparticle synthesis.	$8.88
Weighing dishes (small & large)	The dishes are used to weigh out powdered chemical supplies during the magnetic nanoparticle synthesis & characterization.	$110.53
Chemical waste containers	The containers are used to dispose of waste products from the magnetic nanoparticle and microbubble synthesis.	$5.36
Transmission electron microscope user fees	These fees were paid to a transmission electron microscope user facility at Michigan State University to characterize the size and shape of the magnetic nanoparticles.	$150.00
Poster printing	The university covered the cost of printing two posters to be presented at the annual Homecoming poster session.	$60.00
	Slight overage covered by external support	$48.25
Total of Expenses Covered by Other Support		$4607.88

Activity Photos

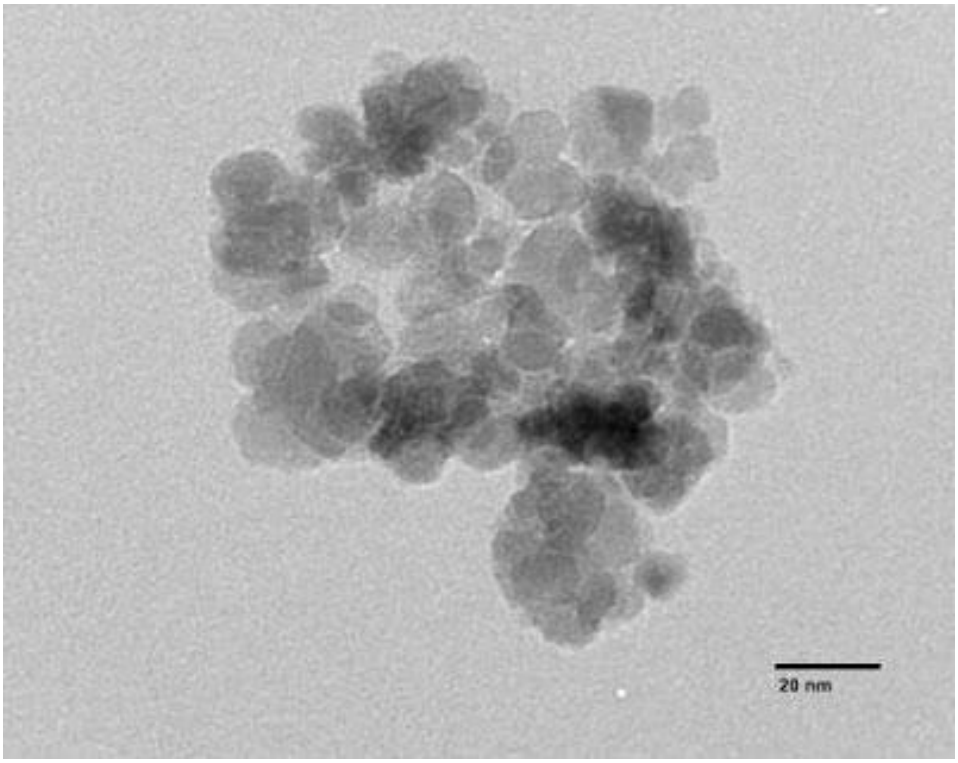


Figure 1. TEM image of MNP coated with dextran. [Image by X. Zhou]

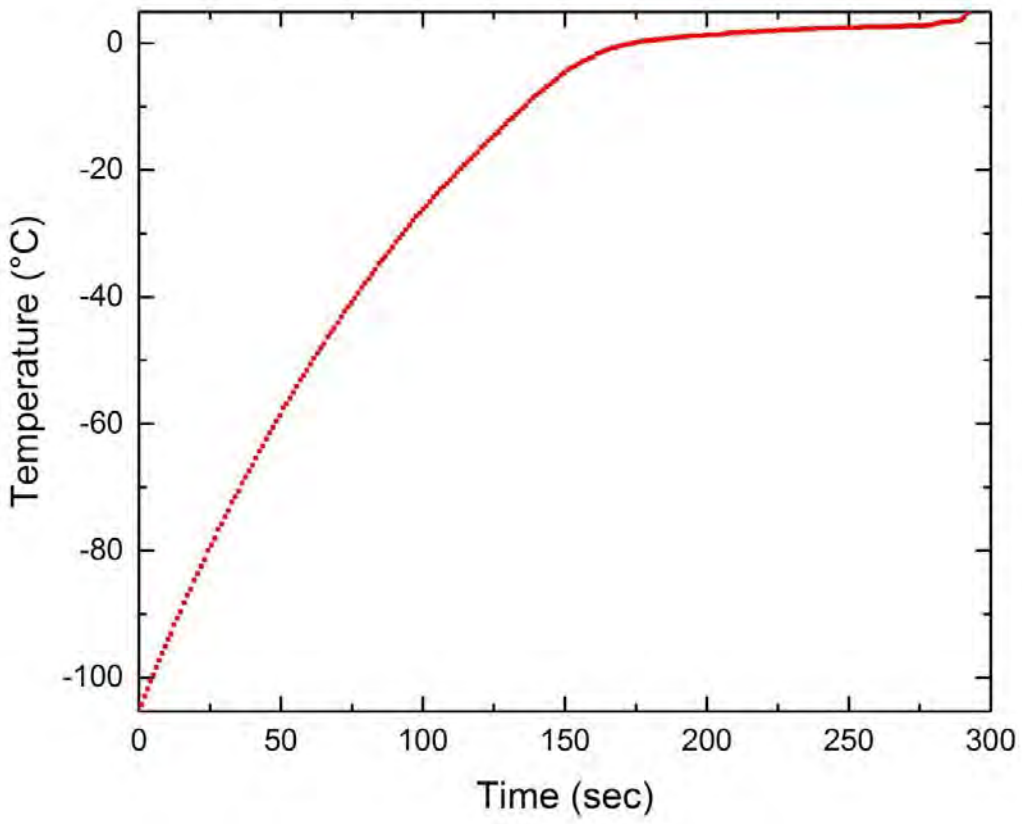


Figure 2. Heating curve of frozen ferrofluid undergoing magnetic fluid hyperthermia with magnetic field oscillating at 150 kHz. [Figure by Nathaniel Mosher]

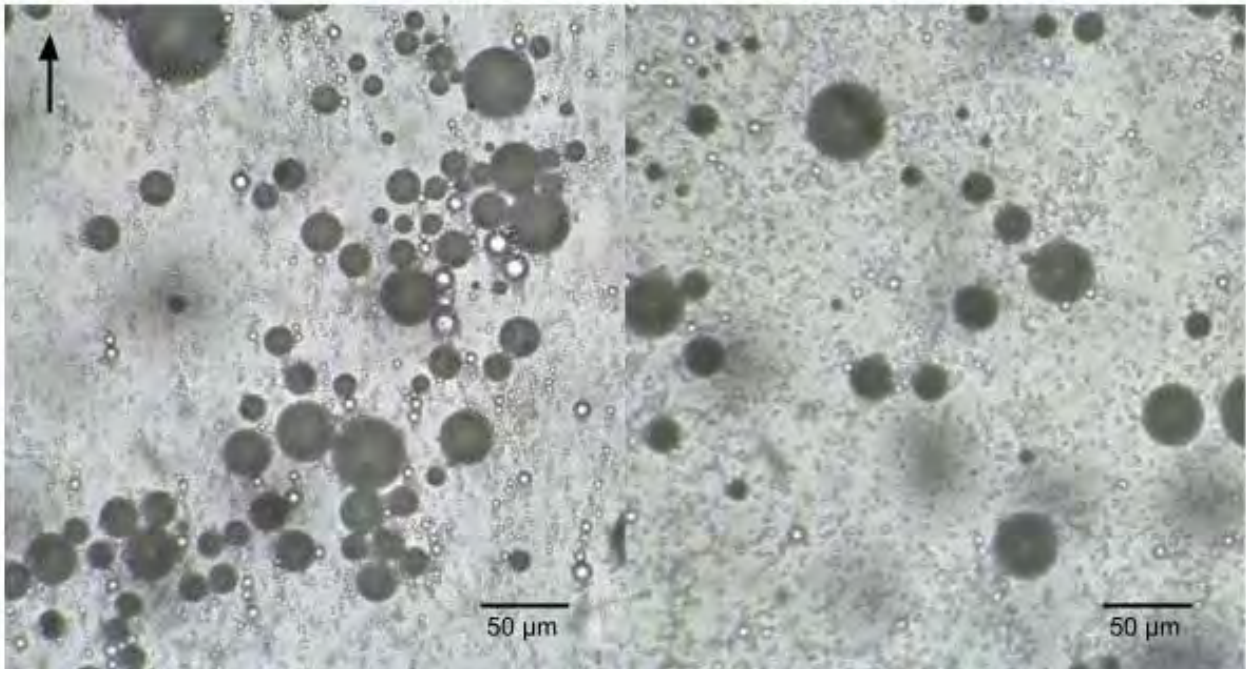


Figure 3. The left image shows magnetic microbubbles forming chains as they align with the magnetic field, indicated by the arrow. The right image shows the same bubbles without a magnetic field. The large, dark spots are clusters of excess nanoparticles that did not join with the bubbles. The images were taken at 20X magnification. [Images by Emily Perkins-Harbin]

Appendix A: Examples of Disseminated Products

Magnetic Microbubbles for Biomedical Applications

[1]E. Perkins-Harbin, [1]N. Mosher, [1]R. Cunningham, [2]L. Wang, [1]P. Vaishnava, [1]R. E. Kumon

[1]Department of Physics [2]Department of Chemistry/Biochemistry Kettering University, Flint MI

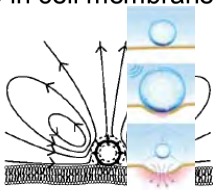
Abstract

Both microbubbles and magnetic nanoparticles have been used in medical applications. Combining them creates additional benefits and new applications. This project explores the creation and use of magnetic microbubbles with a goal of targeted cancer treatment.

Potential Applications

Targeted Drug & Gene Delivery

- Microbubbles exposed to ultra-sound can cause *sonoporation*, or transient increase in cell membrane permeability.
- Pores can be opened by shear stresses from oscillation and collapse of microbubbles.



[Image from A. Delalande et al., Bubble Sci. Engin. Technol. (2011)]

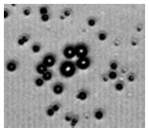
- Ultrasound allows for spatial & temporal control of delivery.
- Side effects of treatment may be reduced and more healthy tissue spared.

Hyperthermia

- Magnetic nanoparticles in an oscillating magnetic field will heat up surrounding medium.
- Heat can induced drug release & increase drug efficacy or even directly kill cancer cells.
- Heat produce by the nanoparticles can be controlled to reduce damage to healthy cells.

Background

Microbubbles



- Bubbles typically between 1 μm and 1 mm in diameter.
- Clinically they are used primarily as an ultrasound contrast agent.
- They are hard to direct in the body.
- We coated them with a surfactant (phospholipid) to increase lifespan.

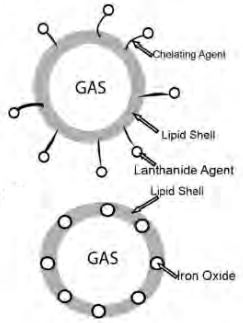
Nanoparticles

- Particles typically between 1–100 nm.
- They are used for drug delivery and cancer treatment.
- Because of their small size, they can penetrate biological barriers well.
- We used iron oxide nanoparticles for magnetic properties.

Suspension of iron oxide nanoparticles

Magnetic Microbubbles (MMB)

- MMB are microbubbles with magnetic nanoparticles embedded in the coating.
- Combines useful acoustic and magnetic properties of both agents.



[Image from X. Cai et al. Theranostics (2012)]

Methods

Fe_3O_4 Nanoparticles

- $Fe(II)Cl_2$ and $Fe(III)Cl_3$ are coprecipitated

Microbubbles

- L-α-phosphatidylcholine added to phosphate-buffered saline
- Sonicated for 30 seconds
- Shaken for 30 seconds

Magnetic Microbubbles

- Fe_3O_4 nanoparticles added to microbubbles
- Sonicated for 30 seconds
- Shaken for 30 seconds

Figure 1: Sonication of microbubble-nanoparticle mixture with 3 mm probe driven at 20 kHz.

Results

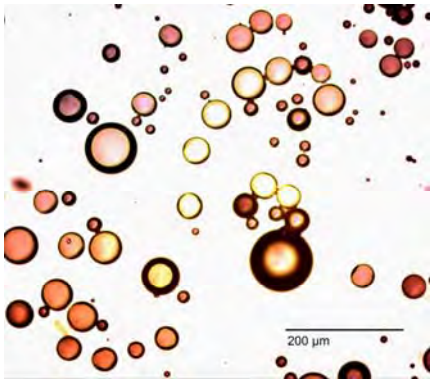


Figure 2: Magnetic microbubbles

Future Work

Characterization

- Sizing
- Lifespan
- Density of nanoparticle coating
- Ultrasonic properties

Drug Delivery

- Attach model drug to MMBs.
- Repeat characterization.
- Testing drug-loaded MMBs *in vitro*.

Acknowledgments

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- Society of Physics Students Sigma Pi Sigma Undergraduate Research Award
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Synthesis and Characterization of Biocompatible Magnetic Nanoparticles

[1]N. Mosher, [1]N. Winter, [1]E. Perkins-Harbin, [2]N. Gerdung, [1]R. Cunningham, [1]C. Rablau, [3]L. Wang, [1]R. E. Kumon, [1]P. Vaishnava

[1]Department of Physics [2]Department of Chemical Engineering [3]Department of Chemistry/Biochemistry Kettering University, Flint, Michigan

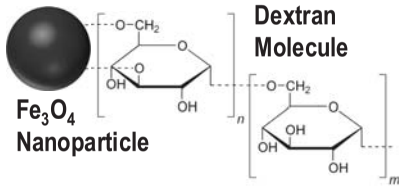
Motivation

- Traditional chemotherapy has undesirable systemic side effects.
- Magnetic nanoparticles (MNPs) can be remotely-activated to kill cells using magnetically-induced heating and/or drug release only where treatment is needed.
- **Project Goal:** Create & characterize MNPs for targeted treatment of cancer

Introduction

- For biological use, MNPs should:
  - Be non-toxic and biocompatible
  - Have a modifiable surface
  - Have high magnetization values
  - Have narrow size distributions
  - Be able to cross biological barriers

- Fe_3O_4 (iron oxide) is one of the most promising and popular MNP choices
  - Superparamagnetic (1–30nm)
  - High saturation magnetization
  - Able to be functionalized and coated in many ways
  - Surfactant required for stability



Synthesis: Fe_3O_4 + Dextran

Overall chemical reaction:

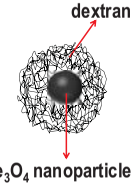
$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$$

Create aqueous mixture of Fe^{2+} and Fe^{3+} chloride at 1:2 molar ratio.

Drip NH_3OH into Fe solution until pH ~11.

Black precipitate will settle.

Decant and rinse until pH 7.



Mix Fe_3O_4 particles with NaOH and dextran

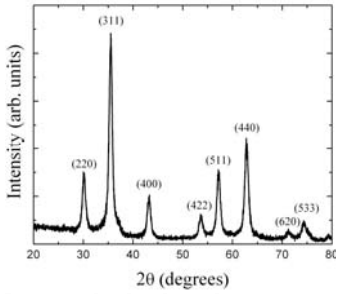
Sonicate until colloid becomes stable

Biocompatible, superparamagnetic Fe_3O_4 MNPs are obtained.

Characterization

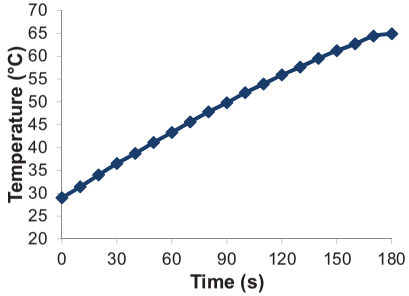
X-ray Diffraction (XRD)

- Method to determine crystalline structure by scattering of x-rays from nanoparticle powder
- Diffraction peaks are consistent with Fe_3O_4:



AC Magnetic Induction

- When MNPs are placed in an alternating magnetic field, they try to align with their field by rotating their internal magnetic moment or physically rotating in space.
- Both processes result in heating of the surrounding medium.



Induction heating results (100.8 A at 186 kHz for 180 s)

Results & Conclusions

- Fe_3O_4 nanoparticles have been synthesized by a co-precipitation method.
- XRD shows MNPs are crystalline & in the size range expected.
- AC induction shows that Fe_3O_4 is viable for hyperthermia.

Future Work

- Synthesize Fe_3O_4 with oleic acid coating & compare with dextran.
- Characterize MNPs further:
  - Saturation magnetization
  - Size distribution by Transmission Electron Microscopy (TEM)
- Cross link and functionalize dextran.
- Optimize particle size and concentration for hyperthermia.
- Attach anti-cancer drug doxorubicin.
- Develop drug releasing mechanisms.
- Test magnetic nanoparticles in cancer cell cultures *in vitro*

Acknowledgments

Funding was provided by:

- Society of Physics Students Sigma Pi Sigma Undergraduate Research Award
- Kettering University Faculty Research Fellowship (R.E.K. & P.V.)

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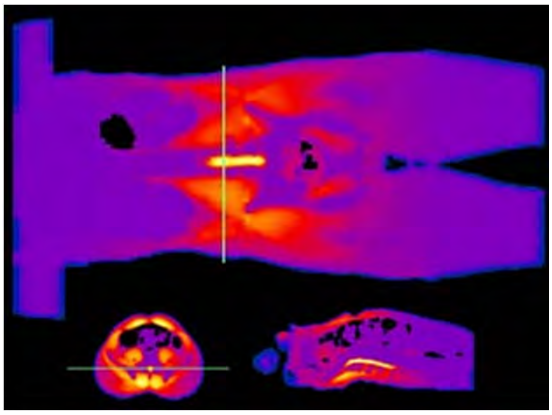
Enhancing Cancer Treatment with Magnetic Fluid Hyperthermia (MFH)

Emily Perkins-Harbin
Phys 354 - Medical Physics

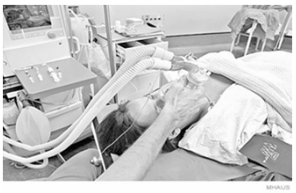
16 December 2014



(Lockwood, 2011)

Kettering UNIVERSITY

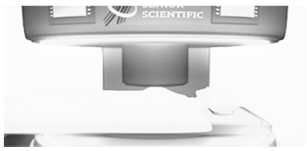
MFH as a cancer treatment option may have significant advantages over current treatments



Current treatments can be improved upon

(Nanoprobes.com, 2014)

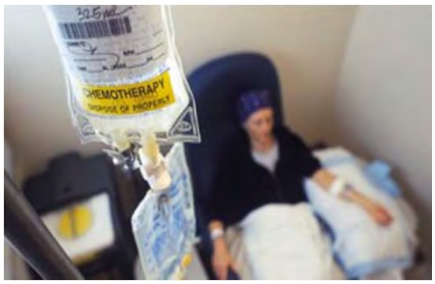
How does MFH work?



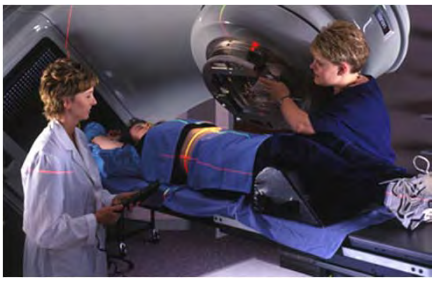
(Seniorscientific.com, 2014)

Applications/Research

The problem: Chemo and radiation therapy kill healthy cells which can lead to serious side effects



Chemotherapy

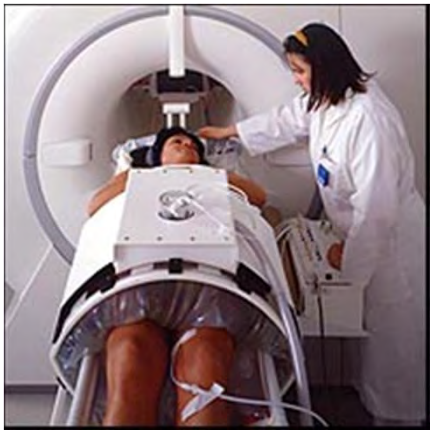


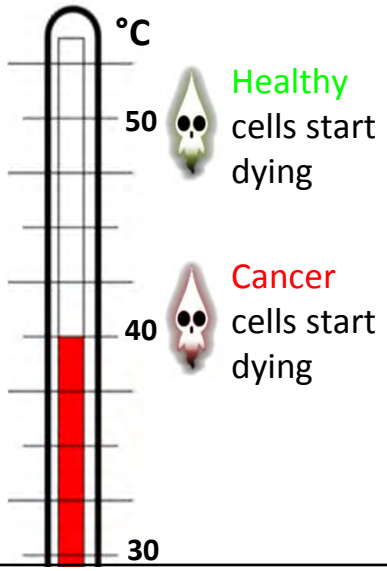
Radiation therapy

(Martino et al., 2013)

(Online Degrees, 2013)

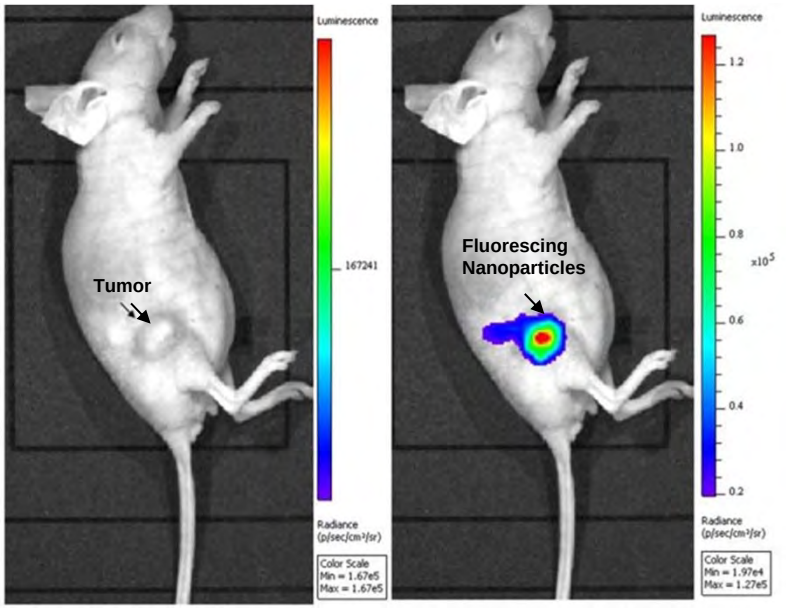
Hyperthermia uses heat to kill cells





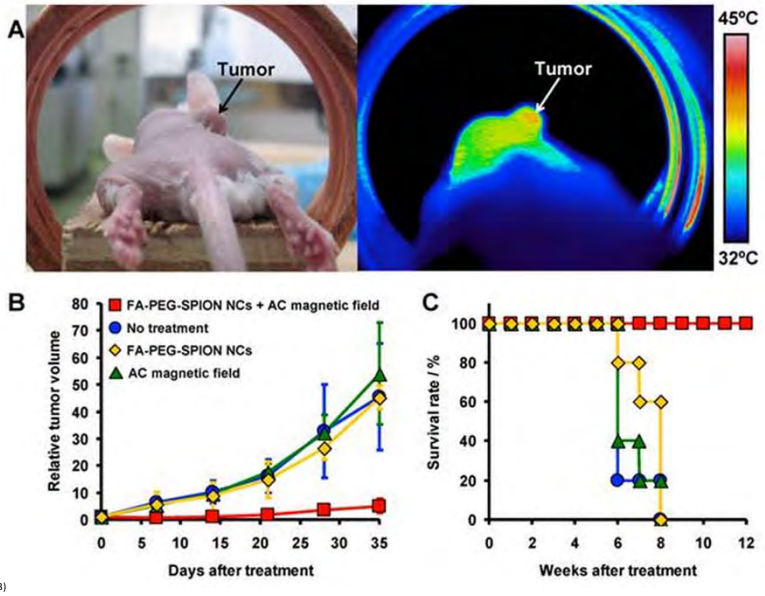
(Autoren, 2014)

We can use magnetic nanoparticles to provide targeted heating, limiting side effects

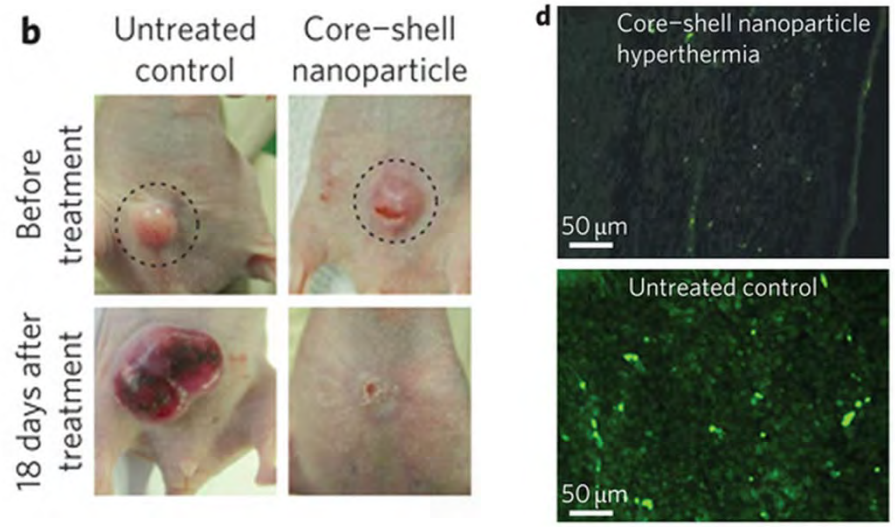


(Yuan et al., 2014)

The heat generated is used to kill cancer

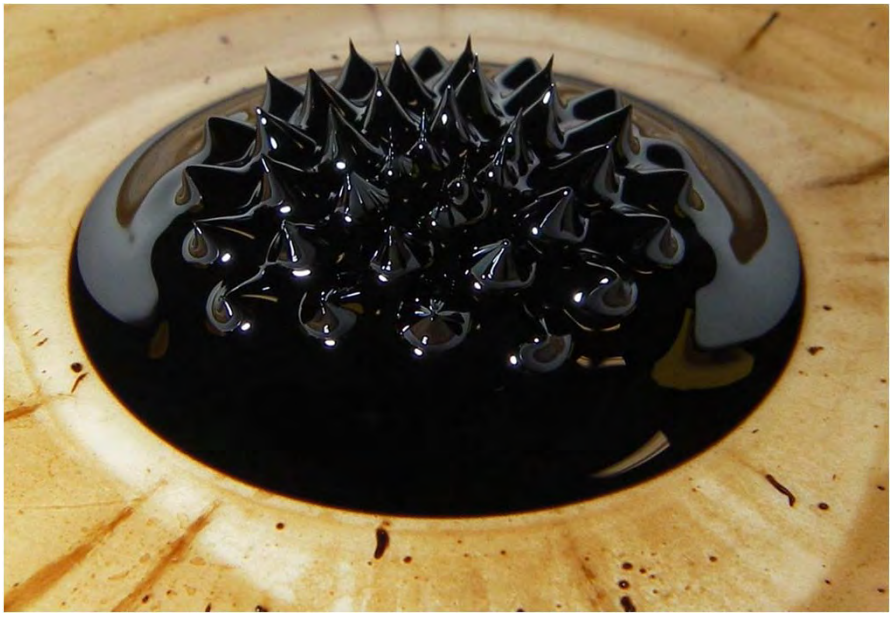


(Hayashi et al., 2013)

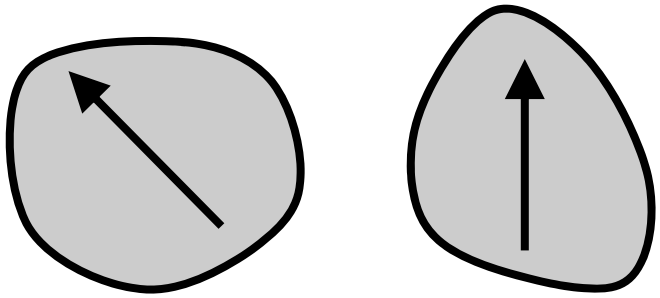


(Lee et al., 2011)

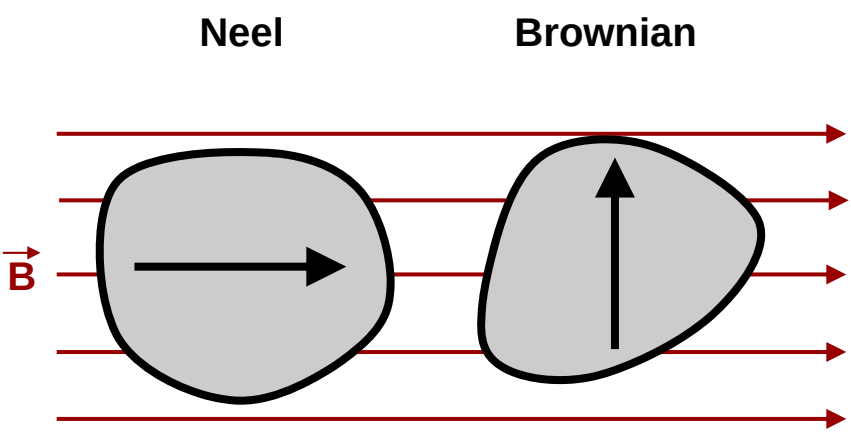
The magnetic fluid used in MFH is comprised of magnetic nanoparticles



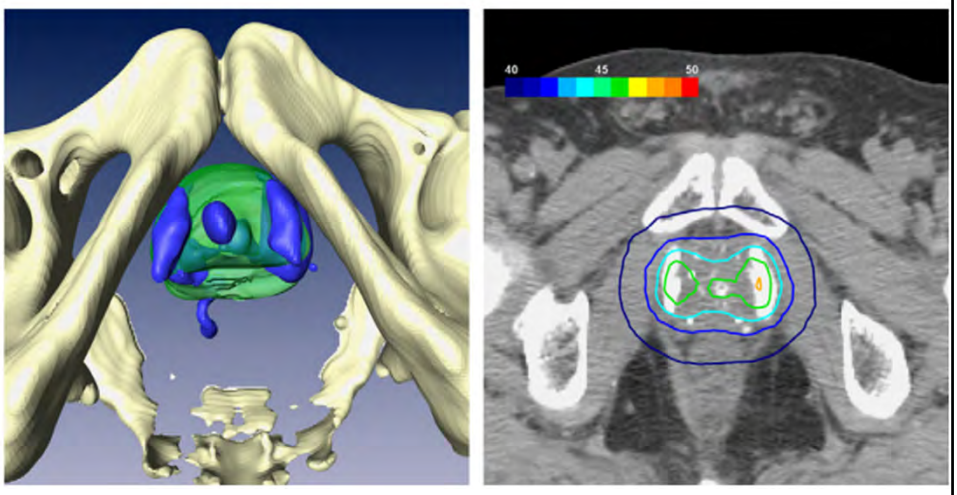
Nanoparticles are so small they only have one domain



Nanoparticles align with a magnetic field in two different ways

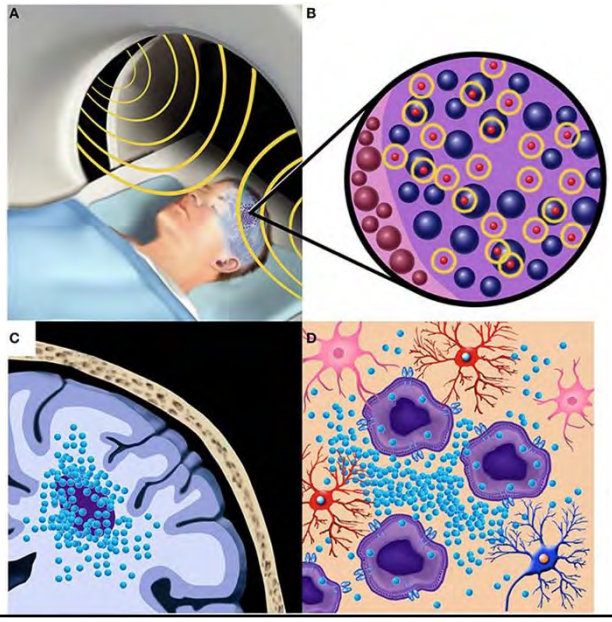


Treatment of prostate with MFH is currently in human trials in Germany



(Johannsen et al., 2006)

There is research being done on using MFH to treat GBM, a very deadly tumor with no other form of treatment



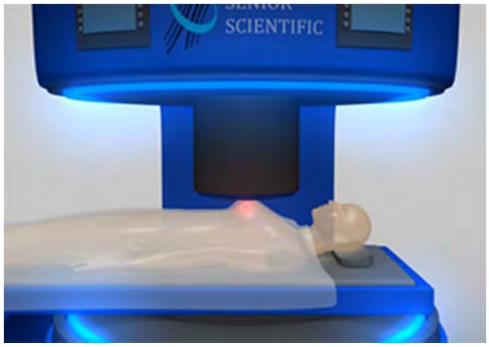
(Mahmoudi and Hadjipanayis, 2014)

In conclusion, MHF has the potential to replace or enhance the current methods of treating cancer

Targeted means that only cancer cells get killed

Fewer side effects

Enormous number of research paths to explore



(Seniorscientific.com, 2014)

Questions?

Kettering University

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MAR15-2014-001338

Abstract Submitted
for the MAR15 Meeting of
The American Physical Society

Sorting Category: 10.1.1 (E)

Determination of the magnetocrystalline anisotropy constant from the frequency dependence of the specific absorption rate in a frozen ferrofluid[1] NATHANIEL MOSHER, EMILY PERKINS-HARBIN, BRANDON AHO, Kettering University Department of Physics, LIHUA WANG, Kettering University Department of Chemistry and Biochemistry, RONALD KUMON, CORNELIU RABLAU, PREM VAISHNAVA, RONALD TACKETT, Kettering University Department of Physics, THERAPEUTIC BIOMATERIALS GROUP TEAM — Colloidal suspensions of superparamagnetic nanoparticles, known as ferrofluids, are promising candidates for the mediation of magnetic fluid hyperthermia (MFH). In such materials, the dissipation of heat occurs as a result of the relaxation of the particles in an applied ac magnetic field via the Brownian and Neel mechanisms. In order to isolate and study the role of the Neel mechanism in this process, the sample can be frozen, using liquid nitrogen, in order to suppress the Brownian relaxation. In this experiment, dextran-coated Fe_3O_4 nanoparticles synthesized via co-precipitation and characterized via transmission electron microscopy and dc magnetization are used as MFH mediators over the temperature range between -70 °C to -10 °C (Brownian-suppressed state). Heating the nanoparticles using ac magnetic field (amplitude ∼300 Oe), the frequency dependence of the specific absorption rate (SAR) is calculated between 150 kHz and 350 kHz and used to determine the magnetocrystalline anisotropy of the sample.

[1]We would like to thank Fluxtrol, Inc. for their help with this project

Ronald Tackett
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Kettering Univ

[X] Prefer Oral Session
[ ] Prefer Poster Session

Date submitted: 11 Nov 2014 Electronic form version 1.4

Appendix B: Examples of Press Coverage

From http://www.kettering.edu/news/kettering-university-michigan-researchers-partner-transform-ocular-cancer-treatment

Kettering, University of Michigan researchers partner to transform ocular cancer treatment

Oct 29, 2014

"We have documented proof that our procedure would kill cancer cells without using chemotherapy and/or radiation."

Dr. Prem Vaishnava is working on a new technique to treat ocular cancer.

A newfound partnership between Dr. Prem Vaishnava, professor of Physics at Kettering University, and Dr. Hakan Demirci at the University of Michigan Kellogg Eye Center is attempting to forever change the treatment of ocular cancer in the United States through a targeted and precise technique that has proven to mitigate the disease without the side effects of chemotherapy and radiation.

“We are confident our technique would work because we have results of preliminary testing in a petri dish,” Vaishnava said. “We have documented proof that our procedure would kill cancer cells without using chemotherapy and/or radiation.”

The treatment uses magnetic nanoparticles and magnetic microbubbles to specifically target Chorodial Melanoma and Retinoblastoma – both cancers of the eye. This technique is currently being used to treat cancer in humans in Europe but has not yet been approved in the United States.

“The science is there and there’s proof that it works. Phase I and Phase II trials have been conducted successfully using magnetic nanoparticles in animals in the United States,” Vaishnava said.

How It Works

Demirci is an oncology specialist at the Kellogg Eye Center in Ann Arbor. He reached out to Vaishnava to express his interest in using magnetic nanoparticles and hyperthermia to treat eye cancer. In a simple experiment, retinoblastoma cancer cells were mixed with magnetic nanoparticles in a petri dish and the mixture was exposed to an alternating magnetic field of frequency 350 kHz. The magnetic nanoparticles produced heat which is sufficient to kill cancer cells and the healthy cells remain unharmed.

Related:
Using nano-technology to treat cancer
NSF grant will help Kettering acquire X-Ray diffractometer

In several instances heat is also provided by microware. Vaishnava explains that this treatment can be a bit “nasty” because an antenna is needed to be placed underneath the skin near the eye ball to heat the tumor. The procedure burns the skin and tissues during the installation of the antenna. Magnetic nanoparticles do not have such side effects.

Magnetic nanoparticles are iron oxide molecules that are suspended in a solution and can be injected into the body or directly into tumor without any harm. Once injected, particles disperse randomly, however, what Vaishnava has determined that if a magnet is positioned around the region where the cancer is located in the eye, the magnetic nanoparticles will be attracted to that site. Once they are concentrated around the the cancer site, a magnetic field can be applied which will cause the nanoparticles to oscillate or “dance” giving off heat and killing cancer cells.

Using this exact principle, Vaishnava and his research team (Professor Ron Kumon and students Nathaniel Mosher and Emily Perkins) have expanded on this technique by attaching anti-cancer drugs to the magnetic nanoparticles. Once the magnetic nanoparticles reach the cancer site, the drugs are released right on to the cancer site in the eye.

“There are so many aspects to it,” Vaishnava said. “Although the principle looks very simple, when you start using it, there are so many challenges.”

Going one step further, Vaishnava has plans to use “microbubbles” that are 1,000 times bigger than magnetic nanoparticles. Each “microbubble” is surrounded by magnetic nanoparticles and can burst with ultrasound to unleash a large concentrated amount of medication and nanoparticles at the cancer site.

“We took retinoblastoma cancer cells and mixed them with magnetic nanoparticles in a petri dish, not even a microbubble, and we heated them at 42 degrees Celsius for three minutes and were surprised with the results - we killed 90 percent of the cancer cells.”

Next Steps

Vaishnava in partnership with Demirci at the Kellogg Eye Center is seeking funding for Phase II trial which will involve treating mice with ocular cancers. In the future, Phase III trial will involve humans.

“We are really excited because not only can we heat and kill cancer cells but we can deliver the drugs,” Vaishnava said. “Our procedure is a combination of limited chemotherapy and hyperthermia.”

What is the greatest benefit of magnetic nanoparticle treatment? There are no side effects.

“Because this is just heat, for human beings, they might feel some sensation of warmth at the site of the tumor,” Vaishnava said.

Despite the concentrated and precise nature of the treatment, Vaishnava suggests that magnetic nanoparticle treatment will be most effective when combined with traditional forms of therapies such as radiation and chemotherapy in order to ensure that cancer cells aren’t lingering in other parts of the body. A tandem approach to treatment can also have complementary results.

“There are some tumors which are resistant to radiation and chemotherapy. This is because of their hypoxic nature.” Vaishnava said. “By heating the tumor, we can increase the profusion rate increasing the amount of oxygen in the tumor. Both radiation and chemotherapy have a greater impact when there’s rich oxygenated blood flow to the cancer site.”

For now, Vaishnava and his colleagues will continue to perfect the science and techniques as they proceed through the various phases before this technique receives widespread approval for use in the United States.

“There’s a great demand for this kind of work. This is a step forward in melanoma and retinoblastoma treatment,” Vaishnava said.

Written By Pardeep Toor | Contact: Patrick Hayes - phayes@kettering.edu - (810) 762-9639

DETROIT'S PREMIER BUSINESS JOURNAL
dbusiness

Kettering, U-M Researchers Treating Ocular Cancer without Radiation

DBUSINESS DAILY NEWS 3

Published: Friday, November 7, 2014

Researchers at Kettering University and the University of Michigan Kellogg Eye Center are finding early success in developing a treatment that kills ocular cancer cells without using chemotherapy or radiation.

"The science is there and there's proof that it works," says Prem Vaishnava, professor of physics at Kettering University in Flint, who is working on the project with Dr. Hakan Demirci at U-M in Ann Arbor.

Vaishnava says the technique is now being used in Europe, but has not been approved in the United States.

The treatment uses magnetic nanoparticles — iron oxide molecules that are suspended in a solution and can be injected into the body or directly into a tumor — to target Chorodial Melanoma and Retinoblastoma, both cancers of the eye.

“Phase I and Phase II trials have been conducted successfully using magnetic nanoparticles in animals in the United States," Vaishnava says.

Once injected, magnetic nanoparticles can be guided to the cancer in the eye by using a magnet positioned around the given area. Once they are concentrated around the cancer site, a magnetic field can be applied, causing the nanoparticles to oscillate and give off heat, thereby killing cancer cells.

The researchers have expanded the technique by attaching anti-cancer drugs to the magnetic nanoparticles. Once the magnetic nanoparticles reach the cancer site, the drugs are released right on to the cancer site in the eye.

Vaishnava says future plans are to use microbubbles, which are 1,000 times bigger than magnetic nanoparticles, in the tests. Each microbubble is surrounded by magnetic nanoparticles and can burst with ultrasound to release a large, concentrated amount of medication and nanoparticles at the cancer site.

“We took retinoblastoma cancer cells and mixed them with magnetic nanoparticles in a petri dish, not even a microbubble, and we heated them at 42 degrees Celsius for three minutes and were surprised with the results — we killed 90 percent of the cancer cells,” he says.

The researchers are seeking funding for Phase II trials, which will involve treating mice with ocular cancers. In the future, Phase III trial will involve humans.

Kettering University professor helps develop new technique to fight ocular cancer

Sarah Schuch | sschuch@mlive.com By Sarah Schuch | sschuch@mlive.com

on November 12, 2014 at 1:00 PM, updated November 12, 2014 at 2:07 PM

Kettering professor Prem Vaishnava is working on a new technique to treat ocular cancer that doesn't require radiation or chemotherapy.
Courtesy of Kettering University

FLINT, MI -- One Kettering University professor hopes to change the way the health community looks at treatment for ocular cancer.

Prem Vaishnava, Kettering physics professor, partnered with Hakan Demirci at the University of Michigan Kellogg Eye Center to develop a new technique to fight ocular cancer without the use of radiation or chemotherapy.

The treatment uses magnetic nanoparticles and magnetic microbubbles to specifically target Chorodial Melanoma and Retinoblastoma – both cancers of the eye.

So far in 2014 there have been 2,730 new cases of cancers of the eye and orbit in the United States -- 1,440 in men and 1,290 in women -- according to the American Cancer Society.

There has been 310 deaths from cancers of the eye and orbit so far this year, according to the American Cancer Society's website.

This technique being tested by Vaishnava and Demirci is currently being used to treat cancer in humans in Europe but has not yet been approved in the United States, according to a release.

"The science is there and there's proof that it works. Phase I and Phase II trials have been conducted successfully using magnetic nanoparticles in animals in the United States," Vaishnava said in a written statement.

How does it work?

In a simple experiment, retinoblastoma cancer cells were mixed with magnetic nanoparticles in a petri dish and the mixture was exposed to an alternating magnetic field of frequency 350 kHz. The magnetic nanoparticles produced heat which was sufficient to kill cancer cells and the healthy cells remain unharmed.

Using that same principle, Vaishnava and his research team have expanded on the technique by attaching drugs to a magnet and then guiding that magnet to another magnet underneath the eye.

The drugs are released right on to the cancer site in the eye.

Now the team hopes to move forward in the research.

Vaishnava in partnership with Demirci at the Kellogg Eye Center is seeking funding for Phase II trial which will involve treating mice with ocular cancers. In the future, Phase III trial will involve humans.

"There's a great demand for this kind of work. This is a step forward in melanoma and retinoblastoma treatment," Vaishnava said in a written statement.

Excerpt from University of Michigan Kellogg Eye Center 2014–15 Annual Report, "Collaborations in ocular oncology: Using thermal energy to eliminate side effects in eye cancer therapies," p. 15, available at http://issuu.com/u-mkelloggeyecenter/docs/ar_2014_055b4d66b24bfc?e=6943966/10813102

Prem Vaishnava, Ph.D.

Using thermal energy to eliminate side effects in eye cancer therapies

Dr. Demirci and colleagues are working with Prem Vaishnava, Ph.D., professor of physics, Kettering University, Flint, Michigan, to find new treatment modalities for cancers inside the eye. "Our collaborative and innovative research involves treatment of ocular malignancies using thermal energy. Unlike chemotherapy and radiation, our approach has no side effects," says Dr. Vaishnava. "It has long been known that tumor cells are more sensitive to heat than normal human cells. Raising temperatures to 42°C to 45°C causes death of cancer cells, while normal cells can survive these temperatures. Our preliminary results have shown that after being exposed to three minutes of thermal energy, 90 percent of the cancer cells were killed."