Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.
This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Nancy Luo

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 2018-2019
1. Title	Electrostatic Targeting of Feraheme Using Doxorubicin Conjugates for Prostate Cancer	Utilizing Iron Oxide Nanoparticles for Prostate Tumor Treatment
2. Change in goal/ purpose/objective	To load Doxorubicin conjugated with a PSMA targeting agent onto Feraheme in order to increase uptake by prostate cancer cells	To load various prostate cancer treatment drugs into Feraheme, a type of iron oxide nanoparticles and to detect cell death in comparison with free drug treatment
3. Changes in methodology	Calculated amounts of reagents to use in a chemical reaction for the bioconjugation of Doxorubicin with a PSMA targeting agent. Treated 4 different prostate cancer cell lines: Du145, 22Rv1, LNCaP, and PC3. Used microscopy, flow cytometry, and fluorescence assays in order to measure percent uptake by the cancer cells.	Performed dropwise loading of Erastin and SIldenafil citrate into Feraheme and treated 22Rv1 prostate cancer cells for 24 hours and 48 hours. Used MTS cell viability assay to analyze change in cell viability. Used protein assay and western blot to determine changes in protein levels after treatment with the Sildenafil citrate drug.
4. Variable studied	Wanted to see if modified Doxorubicin can load onto Feraheme and if the Feraheme particles would be internalized differently from free drug treatment.	Wanted to see if the drugs Erastin and Sildenafil citrate could load onto Feraheme and deliver successful cell death
5. Additional changes	Doxorubicin does not have a therapeutic effect to the cells, but it can be modified for treatment purposes. Rather than measuring cell viability, fluorescence will be used to determine uptake by the cells after treatment.	Performed western blots to determine the presence of specific proteins after treatment to the prostate cancer cells. The drugs loaded induced cell death through ferroptosis, a programmed cell death dependent on iron accumulation inside the cells.

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 2018-2019

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Nancy Luo	Nancy Luo	07/13/19
Student's Printed Name(s)	Signature	Date of Signature (mm/dd/yy)

Nancy Luo
2018-2019
research plan

a. **RATIONALE**: Most of the current prostate cancer treatment methods involves diagnosis with imaging and treatment by removing the prostate or using chemotherapeutics. These methods can be very expensive. (Ruggiero, 2011) In addition, free drug that is not targeted can harm healthy cells instead of cancerous ones. Attaching targeting ligands to the drugs is a chemical modification that can affect the efficacy of the chemotherapeutics. Some drugs are also susceptible to pre-mature breakdown in the body. (Thorek, 2014) Past research has shown that iron oxide nanoparticles are capable of being imaged under various modalities such as magnetic resonance imaging and optical imaging. Also, cancer treatment drugs has been shown to be successfully loaded into nanoparticles and delivered to the tumor site. (Daldrup-Link, 2017) When drugs are delivered with iron oxide nanoparticles, it is more likely that they will have an effect since these nanoparticles can be targeted and can protect the chemotherapeutics from breakdown. (Kaittanis, 2014) More cost-effective methods are needed for prostate cancer treatment. If iron oxide nanoparticles provide non-invasive diagnosis and treatment simultaneously, then a cost-effective method is provided. (Santra, 2009)

b. **RESEARCH QUESTION(S):**

- Will iron oxide nanoparticles be able to deliver cell death to prostate cancer cells?
- Will loaded iron oxide nanoparticles perform better than free drug?
- What drug combination will work best with iron oxide nanoparticles?

HYPOTHESES:

- It is hypothesized that multiple prostate cancer treatment drugs can be successfully loaded into iron oxide nanoparticles because dropwise loading increases the chances of successful loading.
- It is hypothesized that there will be a higher chance for cell death when multiple drugs are loaded into iron oxide nanoparticles because combinational therapy increases efficacy.

ENGINEERING GOAL(S):

- Develop an iron oxide nanoparticle that retains various drugs for prostate cancer treatment.
- Determine a combination of drugs that can be loaded into iron oxide nanoparticles for effective cell death.
- Determine whether free drug or drug-loaded iron oxide nanoparticles are more effective.

EXPECTED OUTCOMES:

- Drug should be loaded without precipitation.

- Loaded iron oxide nanoparticles should be more effective than free drug.

c. Procedures:

1. To prepare drug-loaded iron oxide nanoparticles, 30 microliters of stock Feraheme will be mixed with 370 microliters of MES buffer (pH 8)
2. Different concentrations of drugs will be prepared and labeled:
3. Drug will be diluted with DMSO (different amounts according to concentration that will load into iron oxide nanoparticles)
4. All drugs should be loaded as 1mM if possible. If not, a more diluted concentration will be used for loading
5. 100uM Erastin will be loaded to Feraheme mixture
6. 1mM Sildenafil citrate will be loaded to Feraheme mixture
7. 100 microliters of drug will be added dropwise (1 drop per second) to the Feraheme mixture while the mixture spins on the vortex
8. Let the loaded particles spin for 30 seconds on the vortex after all drug has been added
9. A centrifuge will be used (14000 rcf for 2:30 minutes) to separate out unloaded drug
10. The loaded particles will be washed twice with PBS using the centrifuge
11. The free drug in this experiment will be 10mM at most concentrated
12. A series of 1 to 3 dilutions of free drug, Feraheme, and loaded particles will be made with PBS
13. The dilutions will be made in a 96-well plate where the first row contains the concentrated treatments, and the following 5 rows contain 100uL of PBS each
14. 50uL of each concentrated treatment will be added to the next row, and 50uL from that row will be added to the next and so on
15. 10uL of the diluted treatment will be added into 96-well plates with LNCaP and 22Rv1 cells
16. Non-diluted free drug, Feraheme, and loaded particles will be added to 6 well plates of LNCaP and 22Rv1 cells
17. Data will be measured and recorded 24 or 48 hours after treatment
18. Data of 96-well plates will be collected with MTS assay to measure cell viability
19. Cells will be washed with RPMI media, and 20uL of BCA dye will be added to the cells
20. 96-well plates will be read 4h after preparing the MTS assay
21. Cells from 6-well plates will be collected with cell scrapers into separate eppendorfs
22. They will be spun in the centrifuge in order to separate cell debris from protein
23. Data of 6-well plates will be collected with protein assay and iron digest to determine amount of protein and iron
24. Western blot of protein will be performed after protein assay
25. 10 uL of different protein will be added to the gel
26. A ladder will be used so the data does not become confusing
27. The gel will run for about 3 hours and then set up for transfer
28. The gel will transfer overnight at a low voltage
29. The membrane will be retrieved and cut into 3 sections (section 1 has largest protein and section 3 the smallest)

30. For the primary antibody incubation, IREB2, FPN, and GPX4 antibodies will be used
31. The primary antibody incubation will occur overnight and the secondary antibody incubation will be done for about 2 hours
32. The membranes will be read and intensities will be graphed

Mentor's Role: All work in the lab will be conducted when the mentor is present and with instructions from the mentor. My mentor will teach me everything that I need to know in order to conduct the experiments such as using different equipment, performing data analysis, and cleaning up afterwards. My mentor will answer questions that I may have with my project and help me when I am having trouble with my experiment.

Risk and Safety: There is minimal risk in the procedures for this project. Hoods, lab coats, and gloves are provided to prevent accidental spills of chemicals or contamination. All wastes will be disposed of appropriately depending on the category of waste. The amount of chemotherapeutics used will not be enough to cause harm because of accidental ingestion.

Data analysis: Data of cell culture plates will be collected approximately 24 or 48 hours after treatment. Data of 96-well plates will be collected at 2 different time points (3 and 4 hour). After data is collected, the collected data will be normalized. The data of MTS assays, protein assays, and iron digests will be put into Prism and interpolated to create different charts of viability or absorbance for each test sample. 3 tests of each sample are performed for viability tests. The procedure will be repeated 3 times for both 22Rv1 and LNCaP cells.

d. WORKS CITED:

I. Daldrup-Link, H. (2017, September). Ten Things You Might Not Know about Iron Oxide Nanoparticles. Retrieved July 13, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/28825888
II. G. (n.d.). Targeting the Internal Epitope of Prostate-Specific Membrane Antigen ... Retrieved July 13, 2018, from https://mafiadoc.com/targeting-the-internal-epitope-of-prostate-specific-membrane-antigen-_5a37968d1723dd53981ac630.html
III. Kaittanis, C., Shaffer, T., Ogirala, A., Santra, S., Perez, J., Chiosis, G., . . . Grimm, J. (2014, March 04). Environment-responsive nanophores for therapy and treatment monitoring via molecular MRI quenching. Retrieved July 13, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/24594970
IV. Santra, S., Kaittanis, C., Grimm, J., & Perez, J. (2009, August 17). Drug/dye-loaded, multifunctional iron oxide nanoparticles for combined targeted cancer therapy and dual optical/magnetic resonance imaging. Retrieved July 13, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/19384879
V. Thorek, D., Ulmert, D., Diop, N., Lupu, M., Doran, M., Huang, R., . . . Grimm, J. (2014, January 20). Non-invasive mapping of deep-tissue lymph nodes in live animals using a multimodal PET/MRI nanoparticle.

Retrieved July 13, 2018, from
https://www.nature.com/articles/ncomms4097

3. Potentially hazardous biological agents research:

a. The prostate cancer cell lines are accepted human cell lines and used in cell cultures. Prostate cancer cell lines are BSL 2. They are obtained from ATCC. LNCaP: CRL-1740 and 22Rv1: CRL-2505

b. All biological materials are autoclaved and destroyed off site by a professional waste cleanup company. All materials used during cell culture are sent to this waste stream.

4. Hazardous chemicals, activities & devices:

Laboratory work will be performed with supervision of the mentor or other members of the lab. The iron oxide nanoparticles, Feraheme, are FDA approved. Both Feraheme and chemotherapeutics are used at safe levels. Chemicals wastes are combined with other compatible wastes and disposed of according to their solvent nature and hazard type.

2018-2019 Abstract

OFFICIAL ABSTRACT and CERTIFICATION

2018-19

Utilizing Iron Oxide Nanoparticles for Prostate Tumor Treatment

Nancy Luo

Prostate cancer is a deadly disease that lacks effective and cost-efficient treatment. Studies by Kaittanis in 2014 and Santra in 2009 have shown the use of Feraheme (FH), a type of iron oxide nanoparticle, to deliver chemotherapeutics. The loading of more combinations of drugs in FH and their effects on cell viability need to be tested on prostate cancer cells in order to find the most effective treatment. Erastin and Sildenafil citrate were loaded in FH to find their effects on the cell viability of 22Rv1 human prostate cancer cells, and Fluvastatin and Erastin combined with FH were used induce ferroptosis, which is a type of programmed cell death dependent on iron accumulation, to cancer cells. The effect of Sildenafil citrate on ferroportin (FPN) levels, which shows how Sildenafil citrate works to induce cell death, in 22Rv1 cells was shown. The results show that Sildenafil citrate and Erastin loaded into FH successfully induced cell death in 22Rv1 cells, and FH alone did not cause cell death afters 24 hours of treatment. These decreases in viability could not be compared to the decreases in viability shown with Sildenafil citrate and Erastin alone, showing drug-loaded FH did not perform better than free drug in inducing cell death. FPN levels were not significantly affected by Sildenafil citrate treatment, meaning cell death after Sildenafil citrate treatment was not caused by a decrease in FPN levels. These findings suggest that more research should be done to determine and boost the amount of treatment loaded into FH.

Category
Pick one only—
mark an "X" in
box at right

- Animal Sciences ☐
- Behavioral and Social Science ☐
- Biochemistry ☐
- Cellular & Molecular Biology ☐
- Chemistry ☐
- Computational Bio/ Bioinformatics ☐
- Computer Science ☐
- Earth Science ☐
- Engineering ☐
- Environmental Science ☐
- Mathematical Sciences ☐
- Medicine and Health ☑
- Microbiology ☐
- Neuroscience ☐
- Physics and Astronomy ☐
- Plant Sciences

1. As a part of this research project, the student directly handled, manipulated, or interacted with (check ALL that apply):

   ☐ human subjects ☒ potentially hazardous biological agents
   ☐ vertebrate animals ☒ microorganisms ☐ rDNA ☒ tissue

2. This abstract describes only procedures performed by me/us, reflects my/our own independent research, and represents one year's work only ☒ Yes ☐ No

3. I/we worked or used equipment in a regulated research institution or industrial setting: ☒ Yes ☐ No

4. This project is a continuation of previous research. ☐ Yes ☒ No

5. My display board includes non-published photographs/visual depictions of humans (other than myself): ☐ Yes ☒ No

6. I/we hereby certify that the abstract and responses to the above statements are correct and properly reflect my/our own work. ☐ Yes ☐ No

This stamp or embossed seal attests that this project is in compliance with all federal and state laws and regulations and that all appropriate reviews and approvals have been obtained including the final clearance by the Scientific Review Committee.