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Center for Nano Science and Technology

NDnano Summer Undergraduate Research 2018 Project Summary

1. Student name & home university:
Margo Waters, University of Notre Dame

2. ND faculty name & department:
Dr. Prakash Nallathamby, Department of Aerospace and Mechanical Engineering
Dr. Paul Helquist, Department of Chemistry and Biochemistry

3. Summer project title:
Magneto-Electric Silica Nanoparticles (MagSiNs) for Combinatorial Chemotherapeutics Against Metastatic Cancers

4. Briefly describe new skills you acquired during your summer research:

During my research this summer, I learned how to synthesize the magneto-electric silica nanoparticles that have a magnetic core and piezoelectric shell so that chemotherapeutics could be conjugated to them. I also learned cell culture techniques in order to be able to bring up and test the drugs on different cancer and control cell lines. I was taught how to operate a Nikon TE-2000U Epifluorescence Microscope, which I used to image cells after performing a live/dead assay. One particularly important thing I had to do was to be flexible and develop a plan to carry out my research project. About a week in to bringing up the cell lines to create and freeze a stock to use throughout the summer, the CO_2 tank of the cell culture incubator went out over a weekend, and all of my cells died before I had a chance to freeze any of them. Fortunately, I had planned in two extra weeks into my research schedule, so I was able to use that time to readjust my plan, and order and bring up new cells. Sometimes, anything that can go wrong will go wrong, and you just have to be prepared to handle those situations to the best of your ability.

5. Briefly share a practical application/end use of your research:

Presently cancer treatment drug formulations involve only one type of drug being administered at a time. However, we studied if two or more anticancer drugs encapsulated within a PEG scaffold-capped, magneto-electric silica nanoparticle could be co-administered as a single dose in an *in vitro* model system. Our results showed not only that when the drugs were released from the PEG scaffold-capped MagSiNs, they were twice as toxic to cancer cells than normal control HUVEC cells; they also showed increased cytotoxicity to metastatic cancer cells while making traditionally toxic doses of chemotherapeutics seven times more biocompatible with normal non-cancerous cells. The end use of this research would allow for the potential of the administration of multiple drug dosages over time with fewer side effects than current chemotherapeutic treatments.

6. 50- to 75-word abstract of your project:

This project sought to identify whether chemotherapeutics could be combined and conjugated to magneto-electric silica nanoparticles (MagSiNs) for a more effective and targeted delivery to metastatic cancers. MagSiNs are a drug carrier system, so the chemotherapeutics can be magnetically guided directly to the cancer cells using permanent magnets. The drugs can then be triggered for instantaneous release

when exposed to an external electromagnetic field. We compared the effects of this targeted drug delivery to normal free drug addition, as well as the biocompatibility to normal tissue.

7. References for papers, posters, or presentations of your research:

1. Chen Q1, Schweitzer D, Kane J, Davisson VJ, Helquist P. "Total synthesis of iejimalide B" J Org Chem. 2011 Jul 1;76(13):5157-69.
2. Guduru, R.; Liang, P.; Runowicz, C.; Nair, M.; Atluri, V.; Khizroev, S., Magneto-electric Nanoparticles to Enable Field-controlled High-Specificity Drug Delivery to Eradicate Ovarian Cancer Cells. Scientific Reports 2013, 3, 2953.
3. Nallathamby, P. D.; Hopf, J.; Irimata, L. E.; McGinnity, T. L.; Roeder, R. K., Preparation of fluorescent Au-SiO2 core-shell nanoparticles and nanorods with tunable silica shell thickness and surface modification for immunotargeting. Journal of Materials Chemistry B 2016, 4 (32), 5418-5428.
4. Nallathamby, P.D. & Xu, X.H.N. Study of cytotoxic and therapeutic effects of stable and purified silver nanoparticles on tumor cells. Nanoscale 2, 942-952 (2010).
5. Singh M, Chowdhury S, Koley S. Advances of azide-alkyne cycloaddition-click chemistry over the recent decade. Tetrahedron, 2016. 72(35):5257-5283.

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One-page project summary that describes problem, project goal and your activities / results:

Presently cancer treatment drug formulations involve only one type of drug being administered at a time due to the physical toll on the body. However, treatments have the potential to be much more successful if multiple chemotherapeutics were administered at one time. Additionally, treatments currently kill many non-cancerous cells along with the tumor. The Nallathamby lab has developed Magnetoelectric silica nanoparticles (MagSiNs) loaded with drugs that can be spatially directed to specifically penetrate malignant cells while sparing normal tissues.[2] Combination therapy is very important in achieving more effective results as many cancers are mutating or becoming drug resistant. Additionally, using targeted MagSiNs mitigates the debilitating side-effects of current chemotherapeutic regimens by using the MagSiNs as a nanocarrier for delivering low doses of therapeutics with increased accumulation of the therapeutics at the tumor site through magnetic field guidance.

In this study, I first synthesized the MagSiNs to which the drugs were conjugated. The nanoparticles had a magnetic core and a piezoelectric shell. The cores were created through pH-induced co-precipitation. The shell was then added by condensation on a polymer primed surface. The particles were spun down in the centrifuge, washed, and sonicated twice. The drugs were then conjugated to the surface by copper-catalyzed azide-alkyne CLICK chemistry.[5] These would be tested on the cells in Lo and Hi concentrations which were determined from current data in peer-reviewed publications.

Next, I used cell culture techniques to bring up three cancer cells lines and one control cell line. We chose to look at triple-negative breast cancer, metastatic prostate cancer, and chemoresistant ovarian cancer. The control cell line was human endothelial cells. We first exposed the cells to free drug forms of doxorubicin, V-ATPase inhibitors, and a 50:50 mixture of doxorubicin and V-ATPase inhibitors to mimic current chemotherapy treatments.

Following that, we tested doxorubicin and V-ATPase inhibitors (separately and in a 50:50 mixture) conjugated to the MagSiNs against the cell lines. The MagSiNs are a drug carrier system[2], so the chemotherapeutics were magnetically guided directly to the cancer cells. Instantaneous release of the encapsulated drug molecules was triggered at the cancer cells site by exploiting the rapid expansion and contraction of MagSiNs in an external electromagnetic field. Once the drugs were released, we observed if they successfully inhibited cancer cell growth and cancer cell migration while avoiding damage to healthy control cells through a LIVE/DEAD assay. Total incubation time before LIVE/DEAD assay was at least 24 hours for all formulations. Additionally, we tested some slides without exposure to the magnetic fields as a control for the experiment. As one final experiment, a PEG scaffold was capped onto the MagSiNs to see if biocompatibility could be improved.

Initial results have indicated very effective drug delivery to the cancer cells. Specifically, when doxorubicin or V-ATPase inhibitors were released from the PEG scaffold-conjugated MagSiNs, they were two times more toxic to cancer cells than normal control HUVEC cells. PEG scaffold -conjugated MagSiNs loaded with drugs were also three times more cytotoxic to cancer cells than formulations that lacked a PEG scaffold tag. The biggest advantage of the MagSiNs formulation was increased cytotoxicity to metastatic cancer cells while making traditionally toxic doses of chemotherapeutics seven times more biocompatible with normal non-cancerous cells. This shows potential for the future administration of multiple drug dosages over time with less side-effects. These results also demonstrate that it is possible to selectively deliver chemotherapeutics to cancer cells and release them on demand at dosages lower than current treatments.

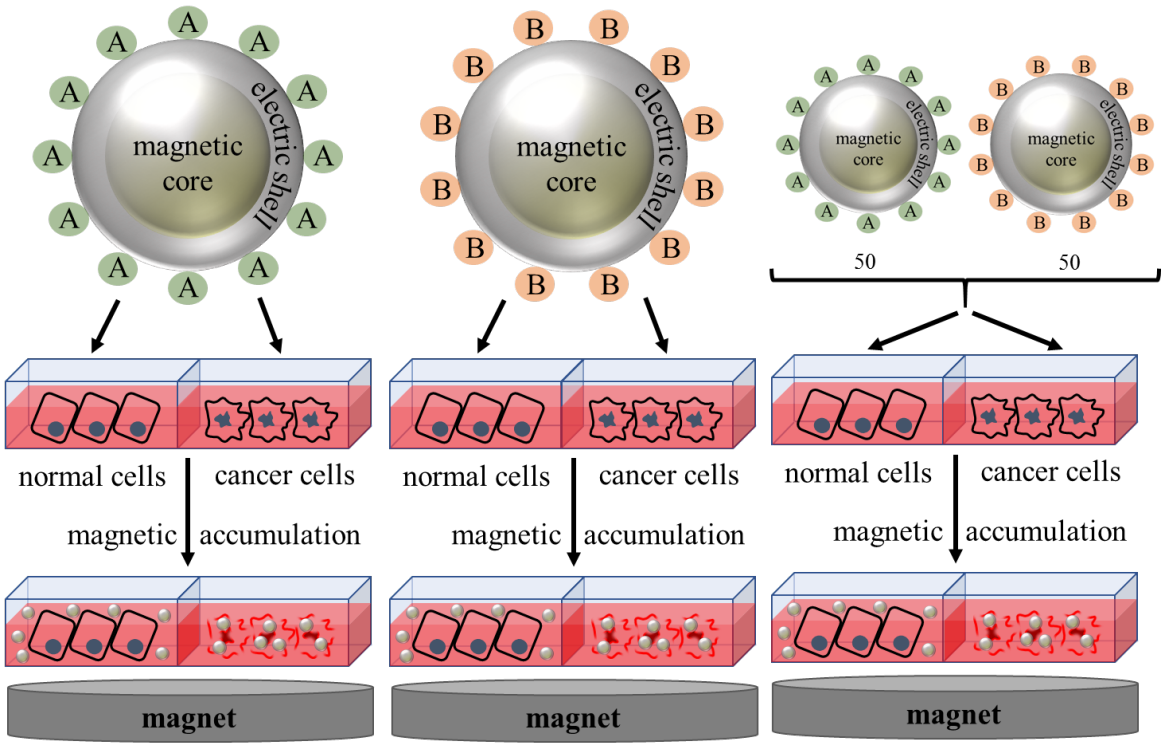

Figure 1. Schematic illustration of the addition of drugs conjugated to magneto-silica nanoparticles (MagSiNs) to normal and cancer cells. "A" represents doxorubicin, and "B" represents V-ATPase inhibitors, which are both chemotherapeutic drugs. These drugs were also added as a 50:50 nanoparticle mixture. The drugs on the MagSiNs were incubated with the cells while exposed to a permanent magnet, which allowed them to interact with and permeate the cell membrane.