NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2021 Project Summary

1. Student name & home university:

Alexandra Alvarado, University of Notre Dame

2. ND faculty name & department:

Ryan K. Roeder, Aerospace and Mechanical Engineering Department

3. Summer project title:

Nanoparticle theranostic agents for breast cancers

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

I learned how to use the mass spectrometer, the spectrophotometer, the ICP machine, how to conduct MTT assays, and learned how to use a microCT machine for a side project. I also learned how to make BP-Peg-AuNPs.

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

Use gold nanoparticles in cancer treatment therapeutically and as contrast agents in CT imaging.

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

The application of gold nanoparticles in breast cancer treatment can be used as a tool for early detection of breast cancer and as a therapeutic treatment by inhibiting mitogenesis. Bisphosphonate pegylated gold nanoparticles (BP-Peg-AuNPs) have a high binding affinity to hydroxyapatite microcalcifications commonly found in breast cancer because of their high retention effect, permeability, and affinity for calcium. My project focused on measuring the cytotoxicity of spherical BP-Peg-AuNPs within the body through *in vitro* testing using an MTT assay on HS578T cells, which measures cell metabolic activity.

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

According to research complied by Sung, Ferlay, and Siegal in *A Cancer Journal for Clinicians*, there were 2,261,419 new cases of female breast cancer and 684,996 of these cases resulted in the death of the patient as seen in **Table 1** below (2021, p. 6).

Table 1. New Cases and Deaths for 36 Cancers and All Cancers Combined in 2020

CANCER SITE	NO. OF NEW CASES (% OF ALL SITES)		NO. OF NEW DEATHS (% OF ALL SITES)	
Female breast	2,261,419	(11.7)	684,996	(6.9)
Lung	2,206,771	(11.4)	1,796,144	(18.0)
Prostate	1,414,259	(7.3)	375,304	(3.8)
Nonmelanoma of skin [a]	1,198,073	(6.2)	63,731	(0.6)
Colon	1,148,515	(6.0)	576,858	(5.8)
Stomach	1,089,103	(5.6)	768,793	(7.7)
Liver	905,677	(4.7)	830,180	(8.3)
Rectum	732,210	(3.8)	339,022	(3.4)
Cervix uteri	604,127	(3.1)	341,831	(3.4)
Esophagus	604,100	(3.1)	544,076	(5.5)
Thyroid	586,202	(3.0)	43,646	(0.4)
Bladder	573,278	(3.0)	212,536	(2.1)
Non-Hodgkin lymphoma	544,352	(2.8)	259,793	(2.6)
Pancreas	495,773	(2.6)	466,003	(4.7)

One area of significant scientific investigation in the detection of breast cancer is the use of gold nanoparticles (Au NPs) to improve the contrast and detection during radiographic imaging. Hydroxyapatite (HA) microcalcifications (µcals) in breast tissue indicate the presence of breast cancer. We know this because the survival rate of breast cancer patients that have HA microcalcifications vs. those without microcalcifications is lower. An experiment done by the Department of Breast Surgery at the Fudan University Shanghai Cancer Center showed that the 8-year overall survival for patients with calcifications was 82.2% compared with 91.9% for those without (Ling et al., 2012). The use of BP-PEG-Au NPs would solve the shortcomings of radiographic detection which include the following: µcals in the breast are sometimes too small

or have too low of a concentration and do not show up in the image; and μcals are often too big or highly concentrated so that they cannot be distinguished from normal, dense breast tissue. The usage of BP-PEG-Au NPs would also be able to help the patient therapeutically, such as inhibiting mitogenesis, or cell proliferation. This is a beneficial task that can be done by the prevention of mitogen-activated protein kinase signaling (Arvizo et al., 2013). Thus, the necessity of proving AuNPs to be nontoxic is key to the implementation of their usage.

To measure the effects of BP-Peg-AuNPs on HS578T cells, I used an MTT assay, which is a colorimetric assay for assessing cell metabolic activity. The NAD(P)H-dependent oxidoreductase enzymes in the cells that have mitochondrial activity occurring bind to the MTT and reduce it down to formazan, whose chemical structures are seen in **Figure 1.** below (Millipore Sigma, p. 1).

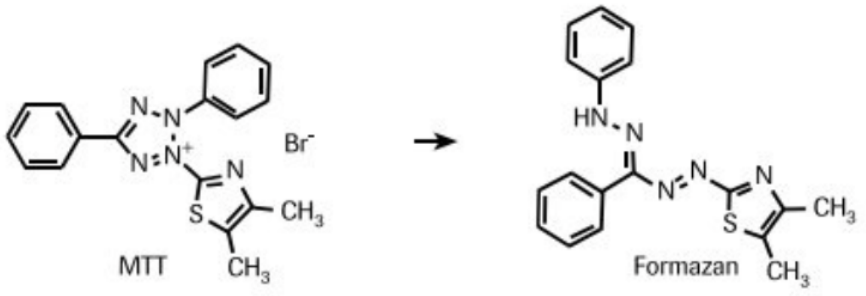

Fig 1. Chemical structure of MTT assay before and after binding with enzymes in viable cells

The MTT assay was used to compare cell viability between cells who contained a certain concentration of BP-Peg-AuNPs and cells containing no level of BP-Peg-AuNPs. The cells were seeded in a 96 well plate at 50,000 cells, using the procedure of cell counting to make sure the proper amount of cells were seeded each time. There were four rows of replicates and varying concentrations of nanoparticles were tested in each column, most commonly three at a time. There were two controls added, one column of just media and one column of media with whatever concentration of nanoparticles was being tested. The reason for these controls was that to test the viability of the cells, their absorbance was measured in a spectrophotometer at 560nm, and there still might be a level of absorbance detected for the media alone. As a result, the

controls were subtracted from whatever absorbance was measured for the cells with and without nanoparticles. In **Figure 2.** below, a depiction of what the crystals that are formed as a result of the MTT being reduced down to formazan looks like (AJMB p. 9).

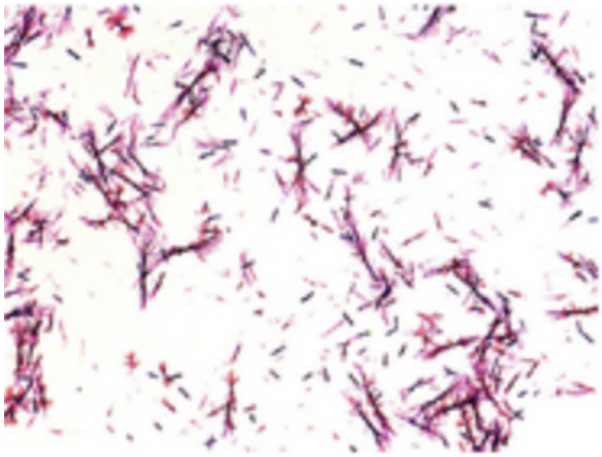

Fig 2. MTT crystals in well containing cells under a light microscope

In order to formulate the BP-Peg-AuNPs I use for this experiment, we first make the AuNP's and add the Peg, and then to create an acidic environment for the bisphosphonate to bind to the Peg-AuNP, 2-(N-morpholino)ethanesulfonic acid (MES buffer) is added to bring the pH down. The chemicals used in the lab to create the bisphosphonate variation we use in this experiment, which is alendronate, was ethylene dichloride (EDC) and N-Hydroxysuccinimide (NHS). After the two are added to the solution, a phosphate buffered saline (PBS buffer) is added to bring the pH back up to neutral. Appropriate washing steps occur in between the steps and after the solution is made, and then we are able to use the BP-Peg-AuNPs for our experiments. I tested concentrations of BP-Peg-AuNPs 5μg/mL to 160μg/mL with the purpose of continuing to increase the concentration until I reach LD50, which is the cutoff for deeming a substance toxic as 50% of the cells have died. The concentration was varied by using the same single stock of AuNPs and adding differing levels of media to change the concentration in order to increase efficiency and reduce variability between results. The value of LD50 I found for the HS578T cells this summer was around 120μg/mL.

Our confidence with moving forward in this project is due to past success with increased X-ray attenuation and high surface binding affinity for BP-AuNPs specifically, as seen in **Figure 3.** below (Cole, p.4).

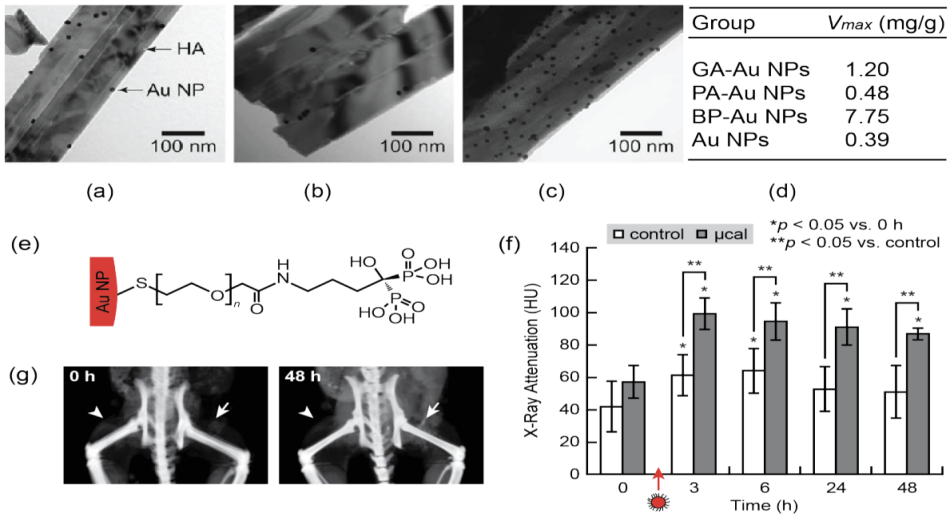

Fig 3. TEM micrographs showing binding of (a) glutamic acid (GA), (b) phosphonic acid (PA), and (c) bisphosphonate (BP) functionalized Au NPs to HA crystals. (d) BP-Au NPs exhibited the greatest maximum surface binding (V_{max}) measured by a Langmuir adsorption isotherm [13]. (e) The molecular structure of BP-Au NPs prepared with a polyethylene glycol (PEG) spacer. (f) X-ray attenuation (HU) measured *in vivo* from 3D CT reconstructions for model HA µcals compared with negative, contralateral controls in a murine model mimicking dense breast tissue. (g) Representative 2D grayscale image projections showing targeted HA µcals (arrows) compared with negative contralateral controls before (0 h) and 48 h after delivery of BP-Au NPs [11,13].

Other labs have tested the effects of varying groups of AuNPs, with results that exhibit less surface binding and lower attenuation than BP-Peg-AuNPs. As a result, moving forward, the desire is to verify these results and then test the desired concentration of nanoparticles *in vivo* in order to measure the effects or possibility of accumulation of these nanoparticles in a patient's organs.

7. References

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