UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

SESSION 1O

CANCER BIOLOGY

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 389
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Optimization of Chlorotoxin Conjugation to Nanoparticle: Targeting Chemotherapeutics to Glioblastomas
Dua Naveed Khan, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Miqin Zhang, Materials Science & Engineering

Glioblastomas (GBMs) are malignant brain tumors associated with lethal cancers that have a great tendency to rapidly invade healthy brain tissue, leaving patients with short survival times and decreased quality of life. The median survival time for patients treated with the chemotherapeutic, temozolomide (TMZ), and radiation therapy is around 12 months, and many patients develop resistance to TMZ due to high activity of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repairing protein. However, the inclusion of an MGMT inhibitor, such as O6-benzylguanosine (BGS), could work to combat this problem. Currently, the Zhang lab is working on creating nanoparticles (NP) with drug-delivery capabilities, which can cross the blood-brain barrier and deliver chemotherapeutics to targeted glioma cells. This highly drug loaded NP model (IOPH-pBGS) efficiently inhibits MGMT and sensitizes GBM cells to TMZ. However, we wanted to know to what extent a targeting agent would improve this model, and how much efficacy can be increased through optimization of the targeting agent conjugation process. We hypothesized that we can significantly increase drug delivery to tumors by avoiding nonspecific uptake of NPs in healthy cells. My research project was to optimize chlorotoxin (CTX) conjugation to the NPs for use against GBMs. CTX is a small amino-acid peptide sequence that has the ability to target GBM cells. We optimized: the point in the NP synthesis process to add CTX, the length of polyethylene glycol linker to attach CTX to the NP surface, and the reaction ratio of CTX to NP in order to optimize both drug loading and targeting efficacy. In order to evaluate the effectiveness of the various CTX conjugated NPs, SDS-PAGE peptide quantitation assays, cell targeting assays, MGMT quantitation assays and clonogenic cell survival assays were utilized.

POSTER SESSION 2

MGH 241, Easel 137
1:00 PM to 2:30 PM

A Study of Red Blood Cell Derived Exosomes from Blood Plasma
Zhiying (Sabrina) Xie, Sophomore, Pre-Sciences
Mentor: Jing Zhang, Pathology
Mentor: Tessandra Stewart, Pathology, Pathology

The presence of protein biomarkers in specific human tissues can be indicative of diseases such as Parkinson's Disease (PD). One such protein, α-synuclein (α-syn) commonly exists in brain and blood and can be transmitted between different cell types in secreted membrane vesicles called exosomes. The blood levels of α-syn are very high, and α-syn originally from red blood cells (RBCs) could be transported in exosomes. In order to study α-syn in RBC exosomes, we have collected α-syn-containing exosomes from RBCs by culturing fresh blood samples. However, RBCs degrade quickly, meaning that in the past, RBC exosomes could only be collected from fresh samples on the same day as collection. So the purpose of this work is to save time and resources from culturing exosomes by determining if they can be pulled down from previously frozen blood plasma. RBC derived exosomes could be targeted by the RBC membrane specific cell surface protein CD235a and using antibody coated superparamagnetic beads to pull down the desired exosome from samples. We hypothesize that the technique of immunoprecipitation is able to make a pure preparation of RBC exosomes which can be use for studying exosome α-syn from RBCs.

POSTER SESSION 2

MGH 241, Easel 136
1:00 PM to 2:30 PM

Inhibitory Effect of *Acer Saccharum* Extracts on aSyn Fibrillation in Dementia with Lewy Bodies
Veenasravani (Veena) Pillalamarri, Senior, Biology (General)
Mentor: Jing Zhang, Pathology
Mentor: Tessandra Stewart, Pathology, Pathology

Aggregation of alpha-synuclein is known to be a critical step in the development of Parkinson's disease and Dementia with Lewy bodies . Dementia with Lewy bodies and Parkinson's disease are both chronic, neurodegenerative diseases that affect motor movements and cognitive development. The aggregation of alpha-synuclein induces neurotoxicity, which contributes to disruptive synaptic behavior which results in the neurodegenerative disease. Misfolded protein structures are a result of the accumulation of aggregated aSyn that form fibrils. Recent studies have found that plant extracts from plants with longer lives have inhibitors that could decrease fibrillation of alpha-synuclein. Previous research from our lab included screening of extracts from different plants. We found that some of the plant extracts that contain molecules smaller than 3kDA had anti-aggregation activity. Acer Saccharum extracts had results that showed a similar inhibitory effect. In the present study, we used this extract to treat brain tissue of Dementia with Lewy bodies and a healthy control on tissue slides. Treatments included testing with Phosphate Buffered Saline as a control as well as using multiple concentrations of the Acer Saccharum extract. We applied Thioflavin T Fluorescence assay to quantify and analyze the relative amount of alpha-synuclein to the experimental conditions. Based on previous research, we expect the fluorescence intensity to be lower for tissue treated with the extract, especially for the higher concentration. Elucidating the inhibitory effect the plant extract has on fibrillation of aSyn can potentially lead to pharmacological developments for future therapies for Parkinson's disease and Dementia with Lewy bodies Disease patients.

POSTER SESSION 2

Balcony, Easel 116
1:00 PM to 2:30 PM

Characterization of Quantum Dot Toxicity for Potential Use as a Biomarker in Brain Injury
Kate Hildahl, Senior, Chemical Engineering
Mary Gates Scholar
Mentor: Elizabeth Nance, Chemical Engineering
Mentor: Mengying Zhang, Molecular Engineering and Science

Quantum dots (QDs), fluorescent semiconductor nanocrystals, can be used as a biomarker and diagnostic tool because of their unique optical and electrical properties, including high luminescence, resistance to photobleaching, and broad excitation and emission spectrums. But in order to use QDs as biomedical imaging devices, particularly in sensitive organs such as the developing brain, toxicity must be adequately characterized. Little is known about the toxic effect of QDs on the brain, especially on the neonatal brain. QDs consist of a metal core, typically cadmium selenide, and a shell to protect the core. Cadmium is known to be highly toxic to many of the body's systems, including the central nervous system. However, expanding the shell and attaching functional biomolecules prevents cadmium release and changes the characteristics of the particles. In our study, several surface ligands were tested at variable concentrations and periods of exposure to measure the toxic potential of QDs on an *ex vivo* organotypic brain slice model. Three methods of characterization were used: a lactate dehydrogenase (LDH) assay, a propidium iodide stain, and a fluoro-jade C stain. The cellular stains were evaluated using confocal microscopy. Results showed that toxicity is a function of QD exposure time, surface chemistry, and concentration. Several QD conjugations displayed minimal or no toxic effect after 24 h of exposure. The results of this study will be useful in identifying a QD conjugate as nontoxic in a biological setting. QDs can then be tailored to site and cell-specific uptake in the brain, thereby improving the selectivity of current imaging techniques and providing a powerful diagnostic with regards to diseased cell fate.

SESSION 2B

CHEMISTRY, BIOCHEMISTRY, AND MATERIALS SCIENCE

Session Moderator: Sharona Gordon, Physiology and Biophysics
MGH 228
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Hexanoyl-Chitosan-PEG Copolymer Coated Iron Oxide Nanoparticles for Hydrophobic Drug Delivery
Guanyou Lin, Senior, Bioen: Nanoscience & Molecular Engr
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Miqin Zhang, Materials Science & Engineering

Paclitaxel (PTX) is one of the most commonly applied chemotherapeutic drugs to treat cancers. PTX inhibits mitotic spindle assembly and chromosome segregation so that cancer cells cannot proliferate normally. While highly effective in arresting cancer development, PTX is extremely hydrophobic and insoluble in physiological environment. Hence loading PTX onto a bio-compatible and water soluble drug carrier is important. Here we report a nanoparticle formulation that can effectively load and stabilize PTX, and trans-

port it into cells. To synthesize the nanoparticle drug delivery systems, iron oxide nanoparticles were first nucleated, grew and capped with oleic acid to form oleic acid coated iron oxide nanoparticles (IONP-OA). Amphiphilic triblock copolymer hexanoyl-chitosan-PEG (CP6C) was made by attaching methoxy polyethylene glycol and hexanoic anhydride to chitosan backbone. CP6C was coated onto IONP-OA through hydrophobic interaction between oleic acid and hexanoyl groups. PTX was loaded in the hydrophobic region between the two groups. Then, chlorotoxin (CTX) as the targeting ligand for brain tumor was conjugated onto nanoparticle through a heterobifunctional linker. The desired product structure (CTX-PTX-NP) was confirmed by TEM, FT-IR and 1H NMR. Characteristic FT-IR and 1H NMR peaks of hexanoyl and mPEG were observed. The product nanoparticles were stable in size (˜50 nm) after a 4-week incubation. Importantly, the PTX loading efficiency was 31.3% PTX/Fe weight to weight ratio determined by HPLC and ferrozine assay. The product showed only minimum drug leak in aqueous solution, indicating that the hydrophobic drug was successfully encapsulated and stabilized in nanoparticle. Furthermore, when applied to glioma cells U118MG, the nanoparticles showed robust cellular uptake and were able to inhibit 80% cell growth in vitro. In summary, our nanoparticle can successfully load hydrophobic drug PTX and delivery it into glioma cells effectively.

SESSION 2Q

CENTRAL NERVOUS SYSTEM DISEASE MODELS

Session Moderator: Gwenn Garden, Neurology

JHN 026

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Quantum Dot Localization in Glia and Neuronal Cells in the Developing Brain

Binh Dang, Senior, Chemical Engineering
Mary Gates Scholar
Mentor: Elizabeth Nance, Chemical Engineering
Mentor: Mengying Zhang, Molecular Engineering and Science

Quantum dot (QD) semiconductor nanocrystals offer significant advantages over conventional fluorescent markers due to broad excitation spectrum, narrow emission spectrum, and very high photo stability, which enables long term visualization. Because of this, there is great interest in using QDs as biomarkers, where cellular uptake of QDs play an important role. In this study, we aim to characterize QD uptake in neuronal and glial populations in the developing brain as a function of QD surface functionality. Previous research has shown that the behavior of QDs in biological systems can be dictated by surface functionality; however, this has not been systematically studied, particularly in the developing brain. Here, we used cadmium-selenide (CdSe) QDs with either cadmium-sulfide (CdS) or zinc-sulfide (ZnS) shell. These QDs are coated with a layer of surface ligands to protect the core-shell and minimize their hydrophobicity. We set out to examine five different surface coatings, including: mercaptoundecanoic acid (MUA), mercaptopropionic acid (MPA), polyethylene glycol-amine (PEG-NH2), polyethylene glycol-methoxy (mPEG) and carboxylic acid (COOH). We incubated organotypic neonatal rat brain slices in 0.1 and 0.01 μM of QDs over a 24 h period. Using immunohistochemistry and co-localization analysis, we observed QDs with PEG-NH2 functionality localize in neurons, whereas QDs with MUA or MPA surface functionality remain in the brain extracellular space. High resolution confocal imaging is used to visually assess the stained brain slices, and the degree of colocalization with cell stains can be quantified using ImageJ. Furthermore, we investigated the region-dependent of QD-PEG-NH2 localization in neurons. This provides insight into the mechanism of uptake for future studies, which include using QDs as biomarkers of inflammatory processes, mediated by glia, in the developing brain.

POSTER SESSION 4

Commons West, Easel 28

4:00 PM to 6:00 PM

Hyaluronic Acid Coated Polyblend Nanofibers for Promotion of Gliobastoma Migration

Jialu Sun, Senior, Mat Sci & Engr: Nanosci & Moleculr Engr
Mary Gates Scholar, UW Honors Program
Mentor: Miqin Zhang, Materials Science & Engineering
Mentor: Ariane Erickson, Material Science and Engineering

Complications and mortality from glioblastoma multiforme (GBM) are often the result of tumor metastasis and thus, understanding the underlying mechanisms in glioma cell migration to help minimize tumor diffusion is of vital importance. Previously in our lab, aligned co-polymer blend chitosan-polycaprolactone (C-PCL) nanofibers have been shown to promote a migratory phenotype and an upregulation of invasion-related genes in GBM cells after 24 hours of culture. Here, we aim to further investigate the effects of nanofiber chemistry on the migratory and invasive character of GBM cells by coating C-PCL nanofibers with hyaluronic acid (HA), a major component of the brain extracellular matrix (ECM). Human U-87 MG GBM cells, a model glioma cell line, were used to study the GBM behavior on the nanofibers. Proliferation assays to determine the biocompatibility of the nanofiber platforms with and without HA were evaluated followed by cell morphology analysis to evaluate elongation and

alignment. Overnight migration studies were conducted to determine if migration profiles on the nanofiber platforms were similar to those reported *in vivo*. Drug resistance and invasion-related genes were assessed to determine if culture on HA-coated C-PCL nanofibers could increase malignancy in GBM. Finally, flow cytometry was used to pinpoint the population of cells expressing a more invasive phenotype and thus provide an idea if HA-coated C-PCL nanofibers were a suitable *in vitro* platform for developing high-throughput anti-invasion cancer therapies.