#2 Biweekly Engineering Design Report
Project Title: EGFRv3 Antibody Conjugated Silk Nanoparticles for Targeted Doxorubicin Delivery in GBM
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Project Description:
Glioblastoma Multiforme (GBM) is an aggressive tumor initiated by mutated astrocytes that can be found in the brain and spinal cord. As of now, the current treatment options for GBM are mainly surgery, radiation, and chemotherapy. These are all invasive or have severe side effects, so a targeted delivery system for chemotherapy using antibody-conjugated silk nanoparticles would be an important avenue to explore. A current antibody of interest would target EGFRv3, a receptor expressed on the surface of many mutated GBM cells, and not expressed in healthy brain tissue, but this could be subject to change. The goals of this project are to determine the best receptor to target for GBM, determine the appropriate nanoparticle size for dosing and crossing the BBB, induce successful antibody conjugation to the silk nanoparticle surface, and determine the proper antibody quantity required to have the nanoparticle be attracted to targeted receptors expressed by U87 cells.

Engineering Design Elements:
- What are the objectives of the project and the criteria for selecting them?
    - The objective of the project is to use antibody-conjugated silk nanoparticles as a potential method of targeted delivery to treatment of glioblastoma multiforme (also known as GBM for short). We aim to develop a 3D GBM model by seeding silk sponges with U87 cells that are transfected with a mutation of choice to carry the receptor we plan to target. We also plan to formulate silk nanoparticles of an appropriate size, loaded with doxorubicin, and conjugated with our antibody of choice. We will add these nanoparticles to the 3D model and conduct live dead assays and imaging to quantify and analyze the efficacy of the drug delivery system. We also plan on validating antibody attachment to the particle surface using western blots, ELISAs, and fluorescent tagging. These objectives were chosen as they are crucial steps for developing and testing the nanoparticles to evaluate their potential and efficacy as a future treatment.
- What system, component, or process is to be designed?
    - Our target of interest is an antibody for a receptor expressed on the surface of many mutated GBM cells and not expressed in healthy brain tissue. Currently, a lot of research focuses on using transferrin (Tf) receptors for GBM target delivery because of its ability to assist nanoparticles in crossing the blood brain barrier. This is due to the fact that high levels of Tf receptors are expressed in brain capillary endothelial cells and glioma cells involved in receptor-mediated transcytosis across the BBB. However, this means Tf receptors are expressed on healthy cells, in addition to GBM cells, so this does not give precise delivery to the tumor site, it just allows for NPs to cross into the brain. We will determine what is the best antigen to target in this situation based on expression levels in GBM vs. expression levels in surrounding healthy brain tissue. Currently, we are focusing on EPHA3, MGMT, EGFRviii, and IL-13Rα2f for potential targets. For

our project, we will have to design a protocol to conjugate antibodies to the silk nanoparticle, but it will likely be done using EDC/NHS, a technique we have learned about through literature review. Additionally, we will conduct a literature search to determine the appropriate nanoparticle size for proper dosing while also crossing the BBB.

- What need does it fulfill (clinical, research, etc)?
    - As of right now, the current treatment options for GBM are mainly surgery, radiation, and chemotherapy. These are all invasive or have severe side effects, so a targeted delivery system for chemotherapy would be an important avenue and unmet need to explore slowing down disease progression/relapse while decreasing side effects.
- What scientific, math, and/or engineering methods will be applied?
    - Some of the scientific and engineering methods that need to be applied are silk processing, nanoparticle formation, antibody conjugation, doxorubicin loading of the nanoparticles, silk sponge formation for 3D brain model testing, and cell culture. We will also likely employ western blots, ELISAs, fluorescent tagging, or mass spectrometry to be used in detecting the presence of desired proteins and antibodies.
- What realistic constraints (cost, safety, reliability, aesthetics, ethics, and social impact, etc) are to be considered?
    - One of the realistic constraints we are considering is whether we need to find a blood brain barrier model in order to prove that our nanoparticles are able to cross it, and if this could be explored if time permits. Another constraint is understanding how/if EGFRv3 is the right receptor to be targeting, or if we should explore another option. Known limitations of targeted antibody therapies include off-target interactions. By researching and choosing antibodies with low-levels of expression outside of GBM tumors, we can decrease unnecessary exposures to chemotherapy. Finally, none of us have ever performed viral transfection before, so this is a new area requiring training and certificates from the Biosafety Office. If this is needed for our project this year, we will need GFP/RFP expressing cell lines, for example.
- What alternative solutions or changes to the plan will be considered?
    - At a first glance, one alternative solution to the plan that we were considering in the beginning was to focus on using antibody-conjugated silk nanoparticles as a potential method of targeted delivery to explore for the treatment of hepatocellular carcinoma (or HCC for short) since nanoparticles commonly cluster in the liver due to its leaky vasculature being similar to that of tumors. Ultimately, we determined that HCC was not a great target for these nanoparticles as common therapies for the disease are not things that can be transported. As a result, our group circled back and decided that GBM was the best way to proceed onwards for now. In addition, IL-13Ra and EPHA3/2 offer alternative targets for GBM if EGFRviii does not work in our model.
- What are the planned tests and what are the quantitative milestones that will demonstrate achievement of the objectives?
    - Some of the milestones that we have achieved so far include learning how to process silk and culture U87 cells in the Kaplan Lab (led by one of our group

members Maddie) as well as learning how to make nanoparticles from Sunny (our lab mentor). A future quantitative milestone would include a protocol that has reproducible significant efficacy in producing EGFRv3 antibody conjugated silk nanoparticles.
    - **Project Timeline**: https://docs.google.com/spreadsheets/d/1_7PQyerNKIqvtC1rLekw4sH9gVnIw__k4sOVCxkgSeg/edit#gid=0
    - Specific aims:
        1. Defining best antibody and receptor for targeting GBM through literature reviews
        2. Produce U87 culture in a way where we can test if antibody-receptor binding is efficacious. This will be tested using the methods below: western blots, ELISAs, and fluorescent tagging of receptor to antibody to secondary fluorescently tagged antibody
        3. Nanoparticle conjugation method will be determined using literature review (How much do we need? How much will stick? How to document this well?) EDC/NHS lit review
- What else is going on in the field that would compete with the project plans?
    - Something interesting going on in the field that could compete with the project plans is that some researchers were able to test silk fibroin nanoparticles coated with Tween-80 in GBM cell lines and found that they were able to release doxorubicin for up to 72 hours. Being able to cross the blood brain barrier is not necessarily something we must target in this capstone project, but it could be a future consideration to take into account if time permits. Our project also differs from this since ours would be more targeted due to antibody conjugation. https://www.sciencedirect.com/science/article/pii/S014181302034085X

Introduction: Background and set the stage for the work - why, how, when

Glioblastoma Multiforme (GBM) is the most common tumor in the central nervous system (CNS) and accounts for 65% of all CNS malignancies. GBM is one of the most deadly forms of cancer, with a median survival rate of just 12.6 months after diagnosis[1]. Attributing to this severe prognosis are the tumor's location in the brain or spinal cord, severely limiting the success of traditional chemotherapies, radiation therapies, and surgical removal. The blood-brain barrier (BBB) is formed by tight junctions between microvascular endothelial cells which regulate and protect the brain environment. While this restricts blood-borne materials it also can inhibit therapies from accessing tumors present in the CNS. Alone, mRNA, monoclonal antibodies and antibody-drug conjugates are unable to bypass the BBB and require additional assistance to reach desired targets. GBM can disrupt the BBB, however patients with GBM still can have an intact barrier around their tumor rim that inhibits access to therapies. Nanoparticles, however, are able to mitigate many of the obstacles that currently available therapies cannot overcome. Their advantages include "biocompatibility, reduced toxicity, more excellent stability, enhanced permeability and retention effect, and precise targeting[2]." Especially due to the challenge of drug availability past the blood brain barrier, nanoparticles present a unique opportunity to pass through it and deliver appropriate doses of chemotherapy. The targeting ability of these nanoparticles can be further enhanced with antibodies that bind to proteins on the surface of the selected cancer cells.

While nanoparticles can be composed of various materials, silk was selected as the appropriate material due to its biocompatibility, availability, and ease of size optimization and loading. Further research will be done to determine the suitable size and loading dosage for the doxorubicin loaded silk nanoparticles, for which we will follow an established protocol. Furthermore, past studies have noted an abundance of EGFRviii expressed on the surface of GBM cells due to a mutation commonly associated with the cancer, leading to continued expression of tyrosine kinases, activating uncontrolled cell proliferation, growth, etc. The antibodies we will conjugate to the surface of the silk nanoparticles will bind to the EGFRviii by soaking them in an antibody solution using the EDC/NHS coupling technique. U87 cells will be seeded on 3D silk scaffolds and used for metabolic assays for nanoparticle efficacy testing.

Questions

- How we chose GBM
- How will we be able to test for antibody specificity?
- How we ruled out other cancers
- Silk processing
- U87 cell culture
    - Side Note: What we learnt in the two weeks

Methods: Experimental tools, what they are, how they work, how they fit your goals

Preparation of Silk Solution

B.mori silkworm cocoon fibers

Preparation of Silk Nanoparticles from silk solution

Determine Concentration:

- Calibration curve
- Plot absorbance versus concentration

Loading Doxorubicin into Silk Nanoparticles

Doxorubicin Loaded Silk Nanoparticle Characterization

- SEM

Results: Data, figures, tables, what they tell you relative to your project goals; iterations based on the findings, etc

Discussion: What have you learned, place this in the context of the field, the literature, patents

Future Work: What should students do next year to pick up on your project from this year, were it to continue. Or let us know it should not continue and why, etc

Participation: List individual contributions of each group member to the project

- Maddie: GBM lit review research, antibody (EGFRviii) lit review research, lead silk processing and cell culture training for group, added to/edited Biweekly report
- Olivia: GBM lit review research, Breast cancer lit review (ultimately ruled out), met with maddie to learn silk processing, met with Sunny for silk nano particle training, added and edited Biweekly report, updated project timeline with relevant dates and aims, Antibody lit review

- Sabrina: GBM lit review research, hepatocellular carcinoma (ruled out target) lit review, met with Maddie for silk processing, met with Sunny for silk nanoparticle training, edited project schedule, wrote brief blurb for Sunny on the need for our proposed GBM treatment, added to/edited Biweekly report
- Elysia: GBM lit review research, met with Maddie and the group to learn silk processing and cell culture, met with Sunny to conduct silk nanoparticle training, added to/edited Biweekly report, created the project timeline, HCC initial research (ruled out target), EGFRv3 research to see if it is a good target

Scoring Metrics

Project Description: 2 points

Engineering Design Elements: 8 points (1 point per question)

Advice - Structure the design reports so they evolve into your mid semester and final technical reports, as a living document to make your writing and reporting easier

Appendix 1 Project Schedule

Aims	Sub-Aims	Completion	September	October	November	December	January	February	March	April	May
Define Project		100									
1st Biweekly Report		100									
Antibody Lit Review	Choose antibody for	100									
2nd Biweekly Report		100									
Cell Culture Training		0									
Culturing U87s		0									
Technical proposal report draft		0									
Project Presentations		0									
Preparing Nanoparticles	Load Doxorubicin	0									
Start website	Add home page, people sections, References, and project update	0									
3rd Biweekly Report		0									
Risk assessment analysis		0									
Update Website	update project section and references	0									
Order Materials necessary for antibody conjugation		0									
4th Biweekly Report		0									
Update Website	update project section and references	0									
5th Biweekly Report		0									
Project presentations		0									
Finalized website		0									

Technical report		0									