#1 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 if EGFRv3 is the best receptor to target for GBM, induce successful antibody conjugation to the silk nanoparticle surface, and determine the proper antibody quantity required to have the nanoparticle be attracted to EGFRv3 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).
- What system, component, or process is to be designed?
    - Our target of interest is an antibody for EGFRv3, a receptor expressed on the surface of many mutated GBM cells and not expressed in healthy brain tissue. Currently, most GBM target delivery uses transferrin (TF) receptors; however, TF receptors are expressed on healthy cells, like red blood cells, in addition to GBM cells. 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. For our project we will have to design a protocol to conjugate EGFRv3 antibodies to the silk nanoparticle.
- 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, and cell culture.
- 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 build 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. Finally, none of us have ever performed viral transfection before, so this is a new area requiring training and certificates from the Biosafety Office.

- 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, MRP3 and EPHA3 offer alternative targets for GBM if EGFRv3 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 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.
- Competition: 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 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. 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. Furthermore, past studies have noted an abundance of EGFR v3 protein on the surface of GBM cells that healthy cells lack. The antibodies we will conjugate to the surface of the silk nanoparticles will bind to the EGFRv3.

- How we chose GBM
- 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
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, organization of group to meet with Sunny, lead silk processing 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
- 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 to learn silk processing, met with Sunny to conduct silk nanoparticle training, added to/edited Biweekly report

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