#4 Biweekly Engineering Design Report
Project Title: Antibody Conjugated Silk Nanoparticles as a Targeted Treatment for Glioblastoma Multiforme (GBM)
Team Members: Maddie Yost, Sabrina Zhang, Elysia Chang, Olivia Zeiden
PI/Mentor: Sunny Shaidani

Things highlighted in this color are new!

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. The dual use of antibodies that target EGFRviii and IL-13Ra2 receptors is of interest. EGFRviii is a receptor expressed on the surface of around 30% of GBM cells, and not expressed in healthy brain tissue; IL-13Ra2 is expressed on 75% of GBM cells, but low-level expression is found in the brain. The goals of this project are to determine the best receptors to target for GBM, determine the appropriate nanoparticle size for tumor uptake, 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 for the treatment of Glioblastoma Multiforme (GBM). 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 antibodies of choice. We will add these nanoparticles to the 3D model and conduct imaging techniques such as live dead assays 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. 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 would be an important avenue to explore for this unmet need, slowing down disease progression or relapse while decreasing major side effects.
- What system, component, or process is to be designed?
    - We plan on formulating dual IL-13 and anti-EGFRviii conjugated nanoparticles to target GBM tumor cells. These antibodies bind to IL-13Ra2 and EGFRviii respectively, which are both expressed on the surface of many mutated GBM cells and have little to no expression in healthy brain tissue. Since different receptors

have been found to have varying levels of expression on healthy tissue and GBM cells, the dual targeting of two receptors is of interest. We determined the best antigen combination to target is IL-13Ra2 and EGFRviii. While IL-13Ra2 has one of the highest expression rates in patients, it is found in healthy brain tissues at low levels. EGFRviii, on the other hand, is only found on GBM cells, though it is only present in 20-30% of patients. The combined use of receptors will reduce off-target interactions and increase the GBM cells targeted in patients. The nanoparticles will be fabricated to be within a 100-120 nm size range, and they will be loaded at about 100-300 ng/ml concentration of doxorubicin[1]. Furthermore, another component that must be designed is a 3D GBM model. U87 cells, which are human-derived glioblastoma cells, will be transfected to express IL-13Ra2 and EGFRviii receptors. While many wild-type U87 cells already express these receptors, the population is so heterogenous that it will be most efficient for our project to ensure greater numbers of cells express our target receptors to test for efficacy. For our project, we will design a protocol to conjugate antibodies to the silk nanoparticle. It will likely be done using EDC/NHS, a crosslinking technique we have learned about through the literature review. EDC, in conjunction with NHS, allows for a 2-step coupling of two proteins without affecting the carboxyls of the second protein. An alternative to EDC/NHS is coating the NPs with the antibodies, a process that would involve incubating the NPs in the antibodies diluted with PBS to induce tagging to the particle surface. Through literature review, past studies have used flow cytometry to calculate and validate the success of antibody-NP surface conjugation[2].

- 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 culturing. We will also likely employ western blots, ELISAs, fluorescent tagging, or mass spectrometry in order to detect 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 is understanding the right receptor to target; currently, our group is leaning towards a dual-targeting nanoparticle drug delivery technique, combining IL-13Ra2 with EGFRviii. We will only be relying on literature reviews for this decision, as purchasing three different antibodies and their transfection agents would be overly time-consuming and expensive. Known limitations of targeted antibody therapies include off-target interactions. By researching and choosing antibodies with low-level expression outside of GBM tumors, we can decrease unnecessary exposure to chemotherapy. Finally, the U87

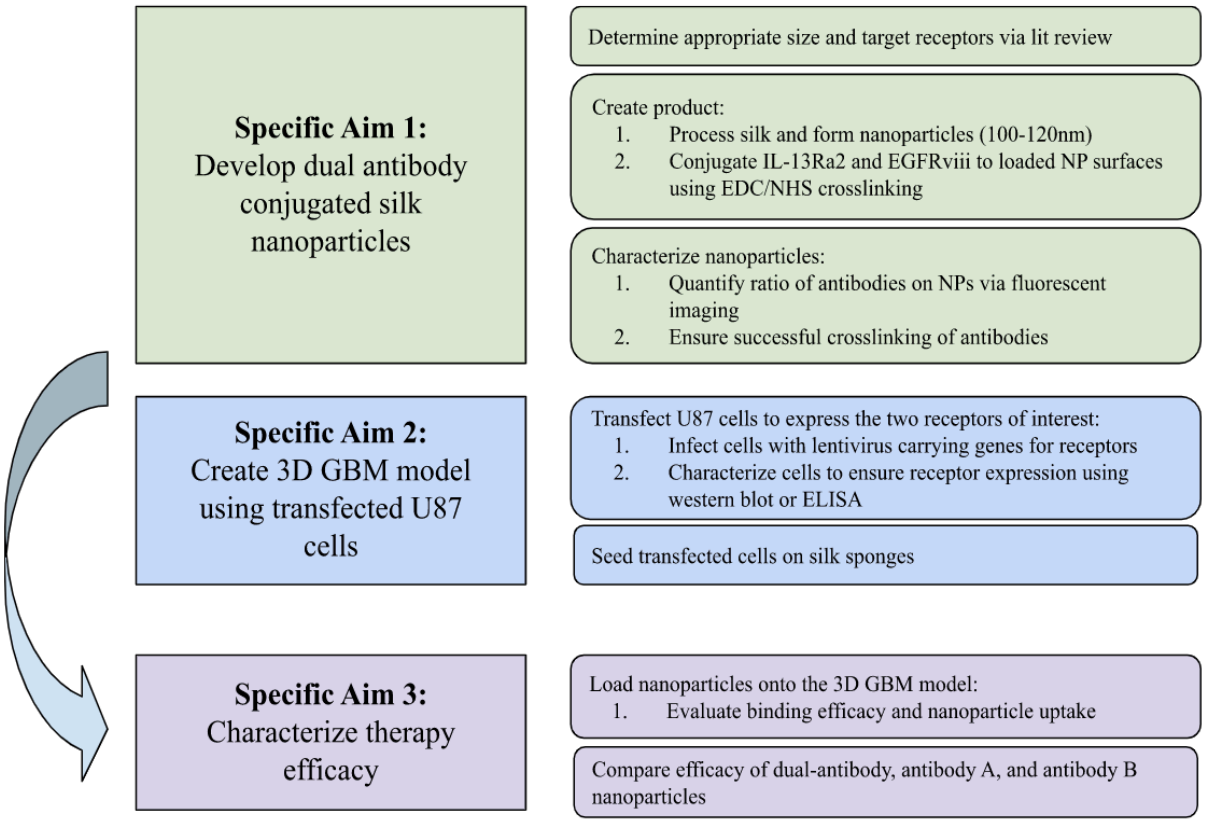
cells we are planning on using require transfection for the expression of biomarkers commonly found in patient tumors. None of us have ever performed viral transfection before, so this is a new area requiring training and certificates from the Biosafety Office. If this is needed for our project this year, we will need GFP/RFP expressing cell lines, for example. Furthermore, another constraint is time--there are few papers addressing dual-antibody conjugation, so the ability to successfully do so and develop techniques to evaluate our experiments may exceed our time limit of one school year.

- What alternative solutions or changes to the plan will be considered?
    - Our group decided that GBM was the best way to proceed onwards for now. Initially, we had only thought to conjugate just one antibody; our group's most recent alternative solution is to proceed with a dual targeting nanoparticle drug delivery approach and technique, combining IL-13Ra2 with EGFRviii. This will allow us to target a greater population of GBM cells while reducing off-target interactions. If we are unable to get lentivirus certification, instead of transfecting U87 cells to get higher expression levels of targeted receptors, we could use cell sorting to isolate cells with IL-13Ra2 and/or EGFRviii expression and culture them from there. We are also now in contact with Olivia's mentor from the City of Hope in potentially getting cells that express IL-13Ra2 (one of our targets of interest). This would then only require us to transfect the cells with EGFRviii, reducing the workload compared to double transfection. If EDC/NHS is unsuccessful, we will look into simply coating the nanoparticles in the antibodies.
- What are the planned tests and what are the quantitative milestones that will demonstrate achievement of the objectives?
    - Some of the milestones and results 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 silk nanoparticles from Sunny (our lab mentor). We recently learned the process of sonification of the nanoparticles by Sunny, and we've decided that we want to order our antibodies from Abcam (we will fill out an order form ASAP for Olivia to order this!). A future quantitative milestone would include a protocol that has reproducible significant efficacy in producing either IL-13Ra2 and EGFRviii OR IL-13Ra2 and EphA2 antibody-conjugated silk nanoparticles.

Project Design Chart

Characteristic	**Target Value**	**Why This Value**	**How We Will Test**
Nanoparticle size	100-120 nm	Appropriate size for entering tumors via leaky vasculature and for tumor cell uptake	DLS/SEM imaging
Nanoparticle antibody expression	**TBD →** enough to have efficient	Throughout various experiments, we will determine the target value for nanoparticle antibody expression based on	FTIR Analysis, Fluorescence microscopy with secondary antibody

	uptake in GBM cells	which values optimize cellular uptake	
Silk concentration	6%	6% silk has been determined by past studies to result in 100-120 nm particles	Concentration calculations by weighing 1000ul of silk solution, leaving overnight in 60°C oven, and weighing remaining silk
Uptake efficiency	**TBD →** enough to have efficient uptake in GBM cells	This value will be dependent on the various experiments we conduct to test nanoparticle antibody expression uptake efficiency (uptake is changed a lot by cell line & nanoparticle size[3])	FITC and lysosomal fluorescent microscopy or flow cytometry
Cell receptor expression	Cells express one of each receptor	This is important to test the efficacy of dual antibody conjugation, making sure both biomarkers are expressed whether we transfect cells with both, or receive IL-13Ra2 cells and transfect with EGFRviii	Flow cytometry and/or Western blot



- This flowchart describes the design of the proposed experiment. We will develop the dual antibody conjugated silk nanoparticles using established protocols used by the Kaplan Lab and EDC/NHS protocols published online, and we will characterize the nanoparticles produced. We will also transfect U87 cells to express our target receptors. These cells will be cultured, then later seeded onto silk sponge scaffolds to create a 3D GBM model. Together, specific aims 1 and 2 will be used in conducting specific aim 3. The antibody-tagged nanoparticles will be loaded onto the 3D GBM model and their performance will be evaluated. The efficacy of dual antibody and single antibody NPs will be compared to determine the best antibody coating for GBM targeting.

- **Project Timeline**: Click Here!
  - Specific Aims:
    1. Develop dual-antibody conjugated silk nanoparticles
       1. To test for the feasibility and efficacy of the project, the therapy must first be developed. Based on literature reviews and past studies, we aim to have a nanoparticle size of 100-120 nm and IL-13Ra2 and EGFRviii will be dually conjugated to particle surfaces. Established protocols are already in use at the Kaplan Lab for processing silk and measuring the size of the nanoparticles for characterization. Chosen antibodies will be conjugated onto the surface of the NPs using EDC/NHS protocols from past studies, and further testing will be conducted to determine the best way to conjugate two different types of antibodies at a constant,

optimizable ratio. EDC/NHS is the conjugation method of choice as it is the most prevalent in previous literature, but we are planning on reaching out to experts in the lab who have performed antibody conjugation before to see what method they suggest before determining what protocol to use. We hope to fabricate nanoparticles with an antibody IL-13Ra2:EGFRviii ratio in three groups--1:1, 2:1, and 1:2. High-resolution fluorescent imaging will likely be used to quantify antibody conjugation on individual NPs and quantified using ImageJ. This is necessary to ensure reproducible targeting effects and to better understand the formation of this therapy of interest. Successful completion of this aim will conclude in an established protocol for consistent dual-antibody conjugated nanoparticles.

2. Create a 3D GBM model using transfected U87 cells
    1. We hypothesize that a 3D model of GBM will allow us to get the best *in vitro* observations of treatment efficacy and behavior. U87 cells are malignant glioblastoma cells; however, because they are a heterogeneous population, it is best to transfect the cells to express the receptors of choice (biomarkers) so that they can be targeted by the antibodies. The U87 cells will be infected with a viral vector carrying the DNA sequence for either receptor and cultured. After cells are cultured and passaged, cells can be tested for receptor protein expression using western blots or flow cytometry. We hope to obtain a significant concentration of receptor protein when testing with western blots and find that at least 60% of the U87s express one or more of the transfected receptors. The binding affinity of receptors to the purchased antibodies can be evaluated using flow cytometry, which will suggest the ideal receptor-to-antibody (therapy) ratio for best binding and interaction. Once they proliferate to a certain number, the transfected U87s will be seeded onto a silk sponge scaffold to form a 3D model and used for further testing. The expected outcome of this aim is a 3D GBM model with U87 cells that express target receptors that can bind to the purchased antibodies. Completion of this aim will produce a U87 culture transfected with receptors of interest to use in later experiments. There is the possibility we will be receiving patient-derived cells that already express IL-13Ra2, so we will only need to transfect these cells with EGFRviii to represent both biomarkers of interest. This will be interesting to see how and if these patient-derived cells will be able to take up the foreign receptor.
3. Characterize nanoparticle therapy efficacy
    1. Characterization of nanoparticle efficacy is extremely important as it validates the feasibility of the product as a potential therapy for GBM. Nanoparticles of three groups would be loaded onto the 3D GBM models developed in Specific Aim 2: IL-13Ra2 + EGFRviii, IL-13Ra2, and EGFRviii. The efficacy of the three groups would

be compared in terms of nanoparticle uptake. Nanoparticle uptake will be evaluated based on fluorescently imaging nanoparticles (using FITC) and lysosomes, then superimposing the images for overlap. The uptake of the three groups will be compared to the uptake of nanoparticles with no antibody coating (control), and their success will be measured with an uptake efficiency of 50% or above. These are all important markers of nanoparticle success for targeting and drug delivery. Successful completion of this aim will establish the efficacy of the dual-conjugated antibodies and confirm successful binding.

- 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[4]. 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.
- **Project Website**: Click Here!

Introduction and Background

Glioblastoma Multiforme (GBM) is the most common tumor in the central nervous system (CNS) and accounts for 65% of all CNS malignancies[5]. GBM is one of the most deadly forms of cancer, with a median survival rate of just 12.6 months after diagnosis[6]. Attributing to this severe prognosis is 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, excellent stability, enhanced permeability and retention effect, and precise targeting[7]. The unique targeting ability of these nanoparticles can be enhanced with antibodies that bind to proteins on the surface of the selected cancer cells and deliver the drug of interest.

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[8]. Nanoparticles around 100 nm in the bloodstream are known to be too big to enter healthy tissue, but are able to enter tumors due to their leaky vasculature. Once they have entered the tumor and bound to the cell receptors, they can be endocytosed to deliver the drug. Larger nanoparticles have been found to have longer rates of internalization; therefore, it may be advantageous to a NP large enough to only target cancerous tissue, yet small enough to be engulfed at an appropriate rate [9].

Epidermal growth factor receptors (EGFR) are transmembrane receptor tyrosine kinases (RTK) and are overexpressed in 50% of glioblastomas[10]. Epidermal growth factor variant three (EGFRviii) is a mutated wildtype EGFR expressed on the surface of GBM cells and commonly associated with GBM. This mutation has been found to lead to continued expression of tyrosine kinases, and activate uncontrolled cell proliferation, growth, etc. EGFRviii is expressed in 25-33% of all GBM tumors in patients and it is not expressed in normal brain tissue [11, 12]. Some studies go so far as to claim that EGFRviii has never been detected in healthy tissue[12]. Its low

expression in normal tissue makes it a suitable target for GBM therapies. Gliomas with EGFRviii have increased Ras activity, Akt/PI3k signaling, and increased expression of VEGF and IL-8[13]. EGFRviii CAR T cells are in Phase I studies and have shown low off-target toxicity[14].

Eph receptors are a class of tyrosine kinase receptors, and they are divided into A and B categories to indicate their extracellular domains. Subclass A indicates they are anchored to the membrane through glycosylphosphatidylinositol (GPI) linkage[15]. EphaA2 receptor was first discovered in 1990 and has since become a highly relevant receptor due to its abundance in several solid tumors[15, 16]. EphA2 is over-expressed in ~60% of GBM tumors and is present in 98% of cells at moderate and or strong levels[17]. It is found to have a low-level expression in normal brain tissue. EphA2 is an attractive target since its associated with poor prognosis and survival due to its role in tumor cell proliferation, growth, and neovascularization [18]. Functionally, EphA2 is a transcriptional target of the Ras-MAPK pathway[19]. Additionally, it has been found that in cancer cells, EphA2 receptors have ligand-independent kinase activity[20].

Interleukin-13 receptor alpha2 (IL-13Rα2) was discovered as a glioma marker in 1995 by the Debinski laboratory[21], since then it has become one of the most studied tumor-specific antigens in glioblastoma research [22]. IL-13Rα2 is a high-affinity membrane receptor of IL-13 and is expressed in many tumors[23]. It has been found to be overexpressed in up to 75% of glioma patients[24]. Expression of IL-13Rα2 is high in the testis and placenta but has low expression in other organs[22]. A Phase III trial targeting IL-13Rα2 reported high levels of neurotoxicity due to off-target interactions with IL-13Rα1, a related receptor that is expressed in healthy brain tissue[25]. While this trial revealed the dangers of working with IL-13Rα2, it suggests that there is a promise if an antibody more specific to IL-Rα2 is found and used. Currently, CAR T-cell therapy targeting IL-13Rα2 is now in Phase I clinical trials [26]. Dual combinations of IL-13Rα2 and EphA2 have shown to be expressed in 90% of GBM patients} indicating promising data for better targeting specificity[27]. EGFRviii and IL-13Rα2 targeted therapy have both been associated with recurrent antigen loss variants after initial treatment[28].

The combination of two receptor targets would allow for a greater number of GBM cell targets among its heterogeneous population, while also maintaining selectivity and reducing off-target interactions. Silk nanoparticles offer a unique opportunity to customize the drug, target, and dose of interest. In this project, dual-antibody conjugated nanoparticles will allow for more direct targeting of GBM cells; compared to traditional therapies, a successful formulation will result in more efficacious treatment for better patient outcomes.

Methods

Silk processing and nanoparticles formation are protocols from previous studies, supplied by Nafis Hasan and Sunny Shaidani (Kaplan Lab)

Silk Processing

Cut cocoons and remove inside layers, weigh out 4.24g sodium carbonate and add to 2L of boiling distilled water. Add 5g of cocoons to the solution to degum silk fibers so that sericin is washed away and only fibrin protein remains. Wash degummed silk three times in 1.5L of distilled water, changing the water each time, for 20 minutes each. Remove silk, pull by hand and air dry inside a fume hood. Add silk into 9.3 M LiBr solution to remove beta sheets and let sit for at least 4 hours in a 60C oven. Pour dissolved silk into dialysis tubing and place tubing into a 2L beaker of distilled water and spin for 3 days. Change dialysis water 3 times on the first day, twice on the second day, and once on the third day to wash out LiBr solution. Collect silk

solution on day 3 and centrifuge solution twice for 20 minutes at 5-10C on 9000rpm, then store in the fridge for up to two weeks.

Concentration calculation:

1. Weigh an empty weighing boat (W1)
2. Add 1 mL silk solution (measured accurately with a 1000uM micropipette) and record the weigh (W2)
3. Leave the weighing boat into a 60 ºC oven overnight
4. Next day, weigh the weighing boat again (W3)
5. The concentration of the silk solution (w/v) is:
   % = (W3-W1/W2-W1) x 100

Silk Nanoparticles

6% silk, 500 rpm, 30 min boil

Day 1:

1. Add 15-20 ml acetone to graduated cylinder, add acetone to labeled small glass jar
2. Place correctly sized stir bar into jar (small one, but not smallest)
3. Secure jar onto center of stirrer with polymer clay, set to 500 rpm
4. Measure out 4 ml silk in separate beaker, pour into glass dropper
5. Open glass dropper carefully and slowly
    1. Drops should fall into vortex side

Day 2:

1. Add 2 ml of DI water

Day 3:

1. Liquid level should be at around 4 ml
2. Move solution to 15 ml tube, add DI water up to 10 ml
3. Clean sonicator tip with ethanol and Kim wipe
4. Hold tube to sonicator tip
5. 30% amplitude for 30s 2x, cool down in between move up and down, don't touch sides/bottom (press stop)
6. Check size with particle solutions app
7. On button in back in DLS/SEM
8. PLACE SAMPLE INTO LITTLE sample thing
9. Add 15 ul sample (inverted and then taken from the middle) to DI water in thing covering window from the drawer at least 200 ul water
10. Little Window facing right
11. Black rectangle thing must be inside for correct height
12. Hit new SOP load Craig silk NP, name sample, start!
13. Label leftover nanoparticle solution and store in 4C fridge

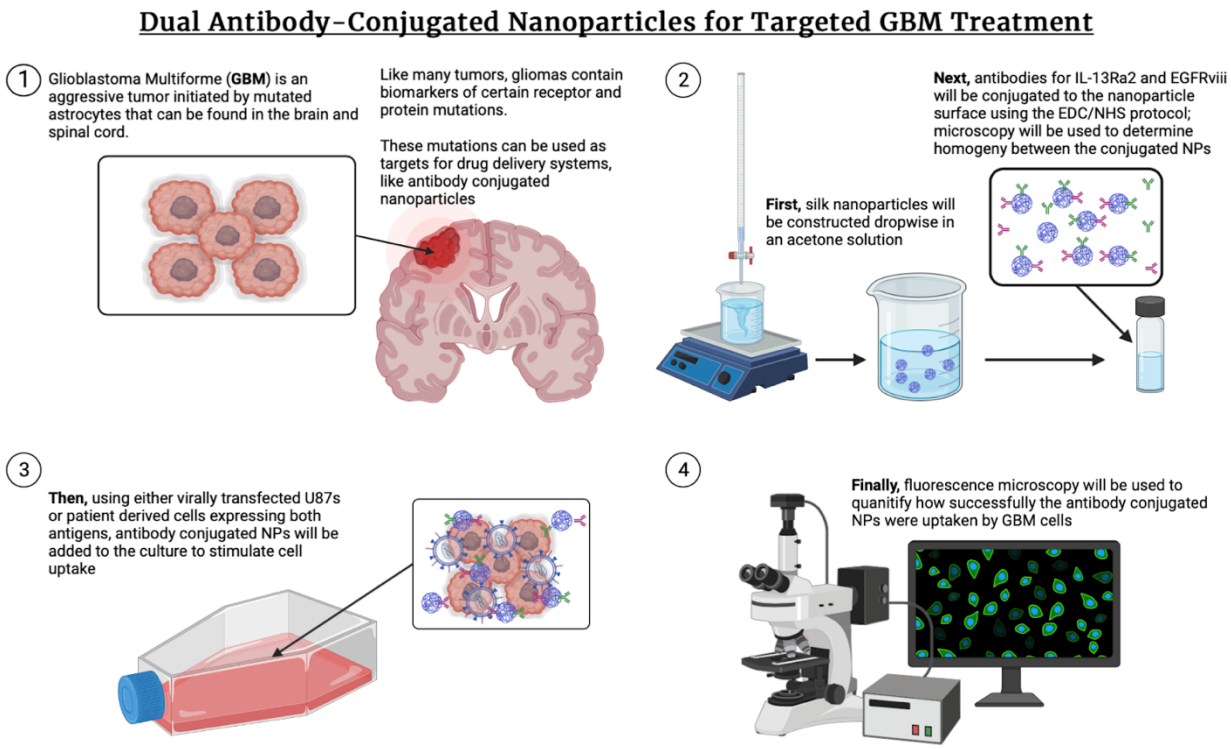
U87 Culturing

After taking 1mL U87 cell line out of the liquid nitrogen freezer and thawing in metal beads around 34C, 100 µL of that cell suspension can be added to 9.9mL of cell culture media in a T75 to grow for 2+ days until 80-90% confluency is reached, which is about 2-3 million cells in a T75. U87 cell culture media contains MEM, FBS, penicillin, and streptomycin. U87 cells are non-adherent and naturally like to grow in aggregates, making spheroids. To prevent this, cells are cultured on plates that have been coated with Poly-L-ornithine (PLO), which promotes cell

adherence and long term culture of neuronal cells. After a few days, the cells will need to be processed, which involves suctioning out the old culture media, adding 2mL of trypsin (a digestive enzyme) to the T75, and incubating for 5-10 minutes at 30 C until the cells have lifted from the PLO plate. After incubation, the flask is visualized under the microscope to determine if the cells have lifted. Once this has occurred, 8mL of cell culture media is added to the cell suspension, and the 10mL solution is transferred to a 15mL tube where it is centrifuged at a speed of 400G for 5 minutes. After centrifugation, the cells are left as a pellet at the bottom of the supernatant, which is removed to leave the pellet. The pellet is resuspended in 1mL of culture media using a P1000 pipette. A T75 flask with PLO seeded is taken from the incubator to plate the cells. To plate at a lower density, 100 µL of cell solution is added to 9.9mL of media for a 1:10 ratio, while a 1:5 ratio would be 200 µL of cell solution with 9.8mL of media. The new T75 is placed back into the incubator until processing.

Results

We were able to successfully obtain 13 mL of silk solution, which we then evaluated for concentration. We realized we had created a 10% silk solution, and we wanted a 6% silk solution to get the proper sized nanoparticles. To do this, we determined we wanted 30 mL of a 6% silk solution, so we took 18 mL of our 10% silk solution and added 12 mL of DI water to reduce the silk concentration to 6%. We used the 500 rpm, 6% silk, 30 min boil technique to formulate nanoparticles of 100-120 nm in diameter. Following the protocol listed above, we were able to formulate nanoparticles of a diameter of 76 nm, which was very small, almost too small to the point where we think the DLS machine is broken and in need of service. We are planning on making more nanoparticles with new silk to test any other issues that might be causing the discrepancies in size. The group also knows how to culture U87s, so transfected versions of U87 cells expressing biomarkers IL-13Ra2 and EGFRviii will be used to demonstrate cellular uptake of the silk NPs.



We were also able to create a unifying figure for our entire project, which is a simple figure that provides a visual of our project: the problem of interest, objectives, and end goals.

Discussion

We processed silk two times this semester. The first time, the pH of the water was around 5, which affected the silk and resulted in unexpected visual cues after dissolving in LiBr. This prompted us to discard that batch and process silk a second time, where the dissolving and dialysis of the silk was much more routine. As stated in the results, we were able to obtain a 13 mL 10% silk solution that we were able to change the silk concentration of to 6% to create nanoparticles with about 76 nm diameter. These nanoparticles that were created are not going to be used in our experiments, but it was good we were able to have a trial round surrounding creation and were able to test out certain bugs, like the DLS machine not working properly, so that next time we make nanoparticle we can be more certain in our approach.

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

- **Maddie Yost**: GBM lit review research, antibody (EGFRviii) lit review research, lead silk processing and cell culture training for group, added to/edited Biweekly report and Midterm Technical Report/Presentation, Zoom meeting with Dr. Saul Priceman (PhD from City of Hope) who is an expert in the field for this type of research, sonification training with Sunny
- **Olivia Zeiden**: 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, EphA2 Antibody lit review, added to the midterm report and presentation,added to

midterm report #3, Zoom meeting with Dr. Saul Priceman (PhD from City of Hope) who is an expert in the field for this type of research, sonification training with Sunny, continued contact with Saul Priceman about cell lines

- **Sabrina Zhang**: GBM lit review research, hepatocellular carcinoma (ruled out target) lit review, met with Maddie for silk processing and U87 cell culture training, 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 #1, lit review for IL-13Ra2 as potential target, added to Biweekly report #2 and Midterm Mid Semester Technical Report, Zoom meeting with Dr. Saul Priceman (PhD from City of Hope) who is an expert in the field for this type of research, sonification training with Sunny, submitted order form for antibodies (Abcam)
- **Elysia Chang**: 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, created Midterm Presentation file (because unable to present in-person/on Zoom due to the Society of Women Engineering 2022 Conference in Texas), created/added to Midterm Mid Semester Technical Report, Zoom meeting with Dr. Saul Priceman (PhD from City of Hope) who is an expert in the field for this type of research, sonification training with Sunny, created/edited website

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 Targeting Research	100									
Silk Processing		100									
Silk Nanoparticles		100									
2nd Biweekly Report		100									
Cell Culture Training		50									
Culturing U87s		25									

Technical Proposal Report Draft		100									
Project Presentations		100									
Preparing Nanoparticles		50									
Start Website	Add in Home Page, People Sections, References, and Project Update	100									
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									
Project Presentations		0									
Finalized Website		0									
Technical Report		0									

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