Dual Antibody Conjugated Silk Nanoparticles as a Targeted Delivery System for GBM Therapies

Names: Maddie Yost, Sabrina Zhang, Olivia Zeiden, Elysia Chang
Faculty Advisor: Dr. David Kaplan, **Mentor:** Sunny Shaidani

PROJECT PLANS

- ✓ Confirm Efficacy of Single Conjugation
- Confirm Efficacy of Dual Conjugation
- Repeat with Antibodies of Interest
- Adjust Antibody Ratios for Better Binding

Specific Aim 1:
Two Separate Successful Single Antibody Conjugations on Silk Nanoparticles

Conjugate a single antibody (EGFRviii) onto loaded silk nanoparticle surface using EDC/NHS crosslinking protocol

Characterize silk nanoparticles:
1. Measure increase in molecular weight (130 kDa) via SDS-PAGE gel to confirm successful single antibody conjugation
2. Ensure antibody binding ability via fluorescent imaging

Conjugate another single antibody (IL13Ra2) onto loaded silk nanoparticle surface using EDC/NHS crosslinking protocol

Characterize silk nanoparticles:
1. Measure increase in molecular weight (50 kDa) via SDS-PAGE gel to confirm successful single antibody conjugation
2. Ensure antibody binding ability via fluorescent imaging

Specific Aim 2:
Successful Dual Antibody Conjugation on Silk Nanoparticles

Conjugate both EGFRviii and IL13Ra2 onto loaded silk nanoparticle surface using EDC/NHS crosslinking protocol

Characterize silk nanoparticles:
1. Measure increase in molecular weight via SDS-PAGE gel to confirm successful dual antibody conjugation
2. Quantify ratio of antibodies on silk nanoparticles via fluorescent imaging

Data and Analysis

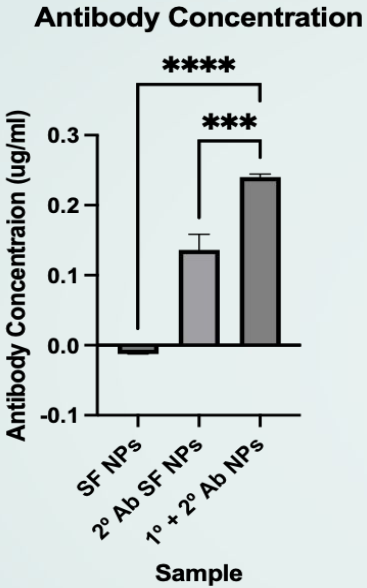

Figure 4. Antibody concentrations of blank nanoparticles, blank nanoparticles incubated with secondary antibody, and IL4 conjugated nanoparticles incubated with secondary antibody. (n=3; **** $p<0.0001$; *** $p = 0.0002$).

Figure 5. Fluorescent images of nanoparticles using the Keyence machine. (A) IL4 conjugated NPs tagged with Goat anti-Rat IgG Alexa Fluor™ 594 secondary antibody; (B) blank NPs incubated with secondary antibody; (C) blank NPs with no secondary antibody. (1-6 exposure, 362 μm scale).

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
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
Functional antibody expression on nanoparticles (single and dual conjugation)	Significant statistical difference in antibody concentration	All antibody used will not conjugate to the nanoparticle, but we will be able to determine through fluorescence microscopy and plate reading what concentration is present and binded	Keyence fluorescence microscopy and plate reading

What Has Been Done so Far?

01

LITERATURE REVIEW

Glioblastoma Multiforme (GBM) vs Breast Cancer vs HCC

02

ANTIBODY RESEARCH

IL-13Ra2 vs EphA2 vs EphA3 vs EGFRviii

03

LAB WORK AND RESEARCH

Silk processing, cell culture, and research on purchasing antibodies

04

SILK NANOPARTICLES

Created silk nanoparticles with diameter of 96.68 nm

05

SINGLE ANTIBODY CONJUGATION

Successfully conjugated IL-4 antibody to silk NPs using EDC-NHS protocol
Imaged using secondary antibody and fluorescence microscope

06

DUAL ANTIBODY CONJUGATION

Currently performing dual conjugation of PSTAT3 and anti-VEGF antibodies

Project Next Steps

1 Image Dual Conjugation

Image PSTAT3 and anti-VEGF conjugation
Wait for anti-IL13Ra2 and anti-EGFRviii

AB RECEIVED

2 Repeat Dual Conjugation with Chosen Antibodies

Confirm dual conjugation with delivered antibodies

3 Confirm Dual Conjugation and Adjust Ratios

Once dual conjugation has been confirmed, adjust antibody ratios for better expression.

NOT RECEIVED

2 Continue Project with Different Antibodies

Continue dual conjugation experiments using PSTAT3 and anti-VEGF