Dual Antibody Conjugated Silk Nanoparticles as a Targeted Drug Delivery System for Glioblastoma Multiforme

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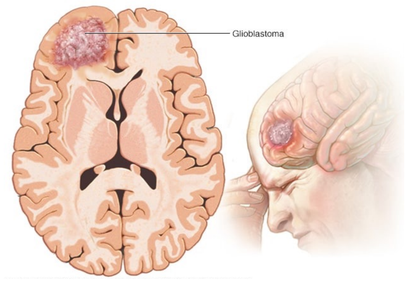
BACKGROUND

Glioblastoma multiforme (GBM) is an aggressive tumor initiated by mutated astrocytes found in the central nervous system (CNS). GBM accounts for 65% of all CNS malignancies.

Current treatment options include surgery, chemotherapy and radiation, which are invasive and have severe side effects.



Nanoparticles enhanced with antibodies on their surface can bind to specific biomarkers on cancer cells, stimulating endocytosis.

This would provide targeted drug delivery and reduce off-target interactions.

Two biomarkers found in GBM with little to no expression in healthy brain tissue are:

- EGFRviii: a mutated wildtype epidermal growth factor receptor overexpressed in ~50% of GBM tumors
- IL-13Ra2: a tumor-specific receptor overexpressed in ~75% of GBM tumors

The combination of two or more antibodies would increase the number of GBM targets among its heterogeneous population.

OBJECTIVES

The goal of this project was to develop a targeted drug delivery system using dual antibody conjugated nanoparticles to treat Glioblastoma Multiforme.

We aimed to successfully:

1. Perform two separate single antibody conjugations on silk nanoparticles
2. Perform dual antibody conjugation on silk nanoparticles

METHODS AND MATERIALS

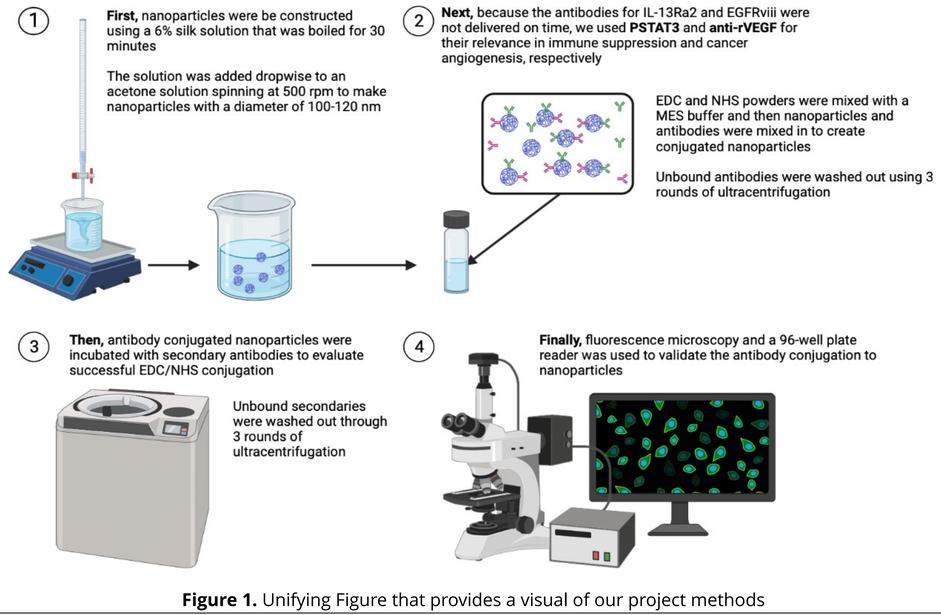
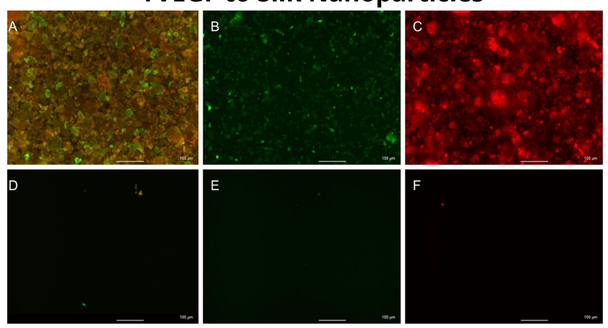


Figure 1. Unifying Figure that provides a visual of our project methods

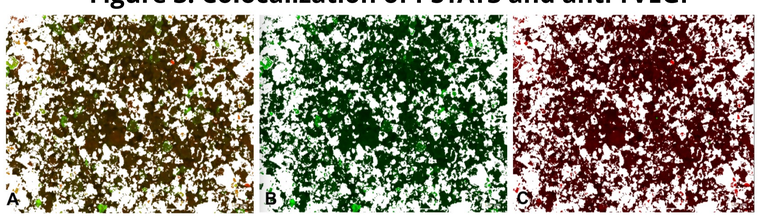
RESULTS

Figure 2. Visualization of Dual and Single Conjugation PSTAT3 and anti-rVEGF to Silk Nanoparticles



Fluorescence microscopy of conjugated antibodies confirms presence of (A) PSTAT3 and anti-rVEGF, (B) PSTAT3, (C) anti-rVEGF in respective sample groups. Blank nanoparticles incubated with respective secondary antibodies show results seen in A-C are not from off-target binding or autofluorescence.

Figure 3. Colocalization of PSTAT3 and anti-rVEGF



ImageJ analysis of dual conjugated nanoparticles and the overlap (seen in white) between PSTAT3 and anti-rVEGF show **80.969%** of PSTAT3 is colocalized with anti-rVEGF, and **82.012%** of anti-rVEGF is colocalized with PSTAT3.

RESULTS cont.

Figure 4. Significant Levels of Antibody Conjugated to Silk Nanoparticle Surface

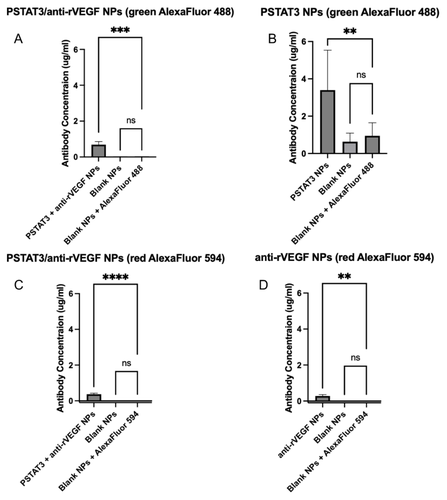


Plate reader data of dual PSTAT3/anti-rVEGF, single PSTAT3, and single anti-rVEGF conjugated nanoparticles quantify significant levels of antibody in conjugated samples. This is in comparison to samples of blank nanoparticles and blank nanoparticles incubated with respective secondary antibodies. Results suggest both PSTAT3 and anti-rVEGF were successfully conjugated individually and together on silk nanoparticles.

CONCLUSIONS

Our experiments have produced a novel proof of concept for dual antibody conjugated silk nanoparticles using EDC/NHS. PSTAT3 and anti-rVEGF were successfully conjugated to silk NPs individually and together. Based on high levels of colocalization, dual antibody conjugation on individual NPs surface is predicted.

FUTURE DIRECTIONS

- Replicate studies using PSTAT3 and anti-rVEGF in order to validate results
- Develop validation method to quantify antibody conjugation to surface of individual NPs
- Future studies focusing on conjugation using GBM specific antibodies (e.g. EGFRviii and IL-13Ra2)

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