Synthesis of Acid-Sensitive, Taxol-loaded, Enzyme-Responsive Nanoparticles for Targeted Drug Delivery

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Abstract

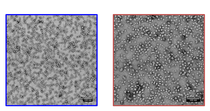
Nanoparticles have been extensively studied for the use of targeted drug delivery for cancer therapy; however, they have not yet been made to efficiently release the given drug at the location of the tumor. In this project, a paclitaxel-loaded monomer was synthesized with an acid-sensitive linker. A diblock copolymer was then prepared from the acid-sensitive drug monomer and an enzyme-responsive peptide monomer, which was then dialyzed to form nanoparticles. In future experiments, the nanoparticles will be exposed to the enzyme MMP, which is present in tumor tissue and has the ability to cleave off the peptides on the particles, which will cause aggregation of the material and exposure of the hydrophobic drug core. The acidic environment of the tumor should then trigger the release of the drug. The nanoparticles will ultimately be analyzed for the rate of drug release in comparison to previous models of drug-carrying nanoparticles with no acid-sensitive linkers. By synthesizing these nanoparticles, we ultimately hope to create a far more effective and harmless cure for cancer.

Nanoscale Drug Delivery

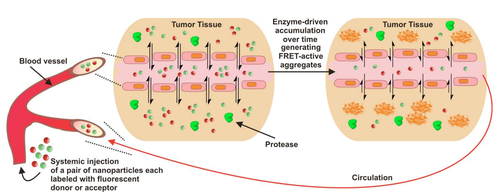
The conventional treatment of cancer involves the administration of chemotherapeutic small molecules. This often leads to serious toxic side effects, due to the low selectivity, high cytotoxicity, and widespread systemic distribution of these drugs. A commonly sought after solution to these issues is to attempt the use of a nanoscale drug delivery system.

In the advancing field of nanomedicine, synthetic nanomaterials are designed to carry cancer drugs, such as Taxol or doxorubicin, and release them at a tumor. The goal of these systems is to prevent the destruction of healthy cells while maintaining the drugs' activities towards their intended target.

Nanoparticles visualized through Transmission Electron Microscopy (TEM) (100nm)



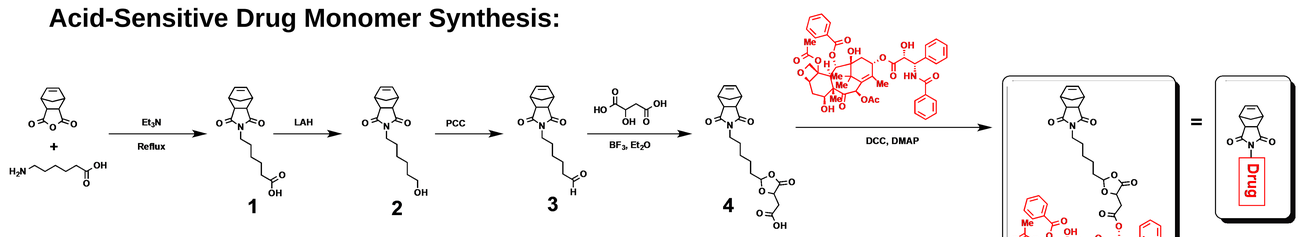
Enzyme-Responsive Systems



The Gianneschi group was the first to demonstrate the to use of an enzyme switch of nanoparticle shape and selectively accumulate material at tumor tissue. Coupling this technology with an acid-cleavable drug moiety could provide superior cancer treatment.

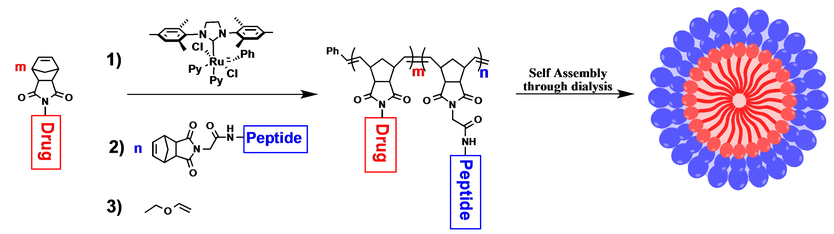
Synthetic Schemes: Monomer, Polymer, and Nanoparticle

Acid-Sensitive Drug Monomer Synthesis:



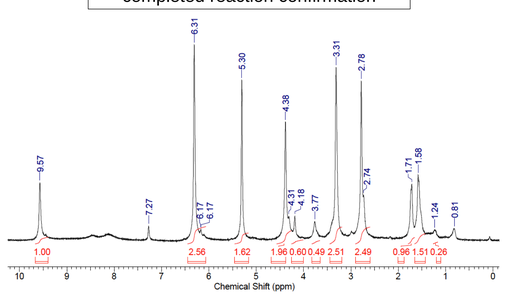
Cis-5-norbornene-exo-2,3-docarboxylic anhydride anhydride was condensed with 6-aminohexanoic acid to form compound **1**	The carboxylic acid moiety was reduced by LAH (Lithium Aluminum Hydride) to form an alcohol (**2**)	The alcohol moiety was oxidized by PCC (Pyridinium Chlorochromate) to form an aldehyde (**3**)	The acid-sensitive cycloacetal moiety was then installed through coupling of malic acid with compound **3**	Paclitaxel (red) was coupled to compound **4** to afford the completed acid-sensitve drug monomer (**5**)

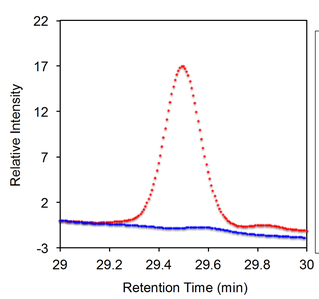
General Polymerization Scheme:



Experimental Analysis and Confirmation

Representative NMR spectrum of completed reaction confirmation



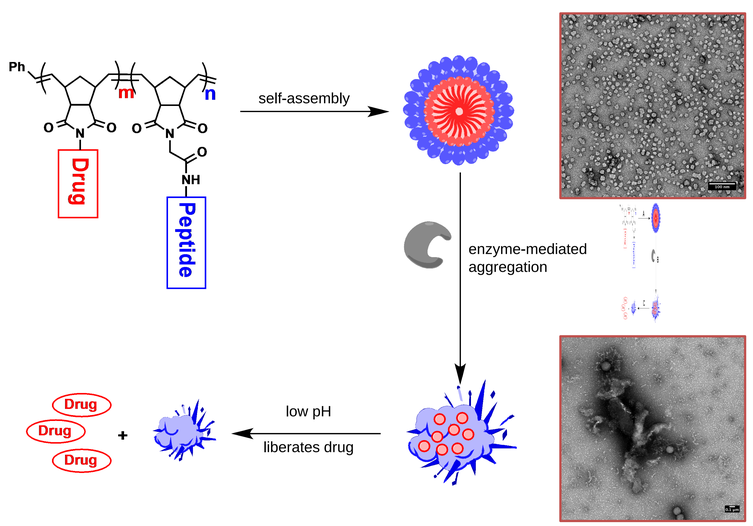


Hypothetical HPLC trace of drug release from acid-sensitive nanparticles.

Blue line = before acid exposure

Red line = after acid exposure.

Peak height is proportional to drug release.



Why pH?

- One specific characteristic of tumor tissue is its relatively low pH in comparison to other tissues in the body. As this differentiates cancer cells from healthy cells, nanoparticles that respond to pH variation may release cancer drugs *selectively* at the necessary location.
- Even though other nanoparticles have been shown to respond to other tumor characteristics, acid-sensitive particles, coupled with enzyme-mediated aggregation, are hypothesized to trigger the release of drug in a more efficient and selective manner.

Methods

- Organic Separation and Purification used to isolate product from reactants
- Column/Silica Gel Chromatography used to separate and purify molecules
- TLC (Thin Layer Chromatography) was used to determine if product was formed
- NMR (Nuclear Magnetic Resonance Spectroscopy) was used to confirm the molecule made by reaction

Conclusions, Future Work

- Generate a diblock copolymer that incorporates the drug monomer and an enzyme-cleavable peptide
- Generate nanoparticles from these polymers
- Test the ability to liberate drug before/after enzyme cleavage in comparison to non-acid-sensitive particles
- Test efficacy of the nanoparticle system *in vitro* and *in vivo*
- Use this solution to create a more efficient cure for cancer

Acknowledgements

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