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Comparison of Free versus Encapsulated Drugs on 3T3 Differentiation

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Comparison of free versus nanoparticle encapsulated drugs on 3T3 cell differentiation

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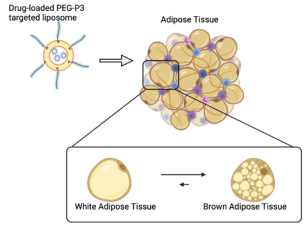
Introduction

The scope of this project was to design, synthesize and test targeted nanoparticles containing hydrophobic and hydrophilic drugs that promote browning in adipose tissue. For hydrophilic drugs the use of liposomes and their hydrophilic core is more useful than the PLGA nanoparticles which have hydrophobic cores. The inhibition of the FOXO1 pathway and modulation of autophagy in adipose tissue can promote browning of white adipose tissue, or an energy burning state where excess energy is burned as heat instead of stored in the cell. If successful, these drugs would offer an alternative treatment for obesity where changes to the patient's lifestyle, such as dieting and frequent exercise, have had little desired effect. The targeted nature of this treatment offers several potential benefits over free drug doses. The results will demonstrate whether encapsulation and targeted encapsulation improves the response and/or allows for a lower drug dose as compared to the free drug.

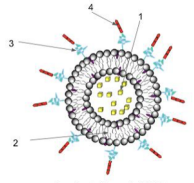
Theoretical



The FOXO1 pathway interacts with the insulin signal in cells and the inhibition of this pathway in many cell types throughout the body may have various unintended side-effects. Targeted drug delivery using nanoparticles may result in a more efficient transfer of the drug to the adipose tissue and may allow for a lower active drug-load for treatment.

Figure 1. Proposed Drug-Interaction Mechanism

Nanoparticle Structure (Liposomes Only)



1. Drugs : Bafilomycin A1 or CL 316,243
2. Lipid: Skeleton of the particle, structural integrity is key since we don't want premature release of the drug.
3. PEG: Provides protection from surroundings in vivo
4. Marker (P3): Necessary for cell identification. Specific to the cell we are trying to target

Figure 2. Structure of Liposomal Nanoparticles **(1)**

Nanoparticle Synthesis

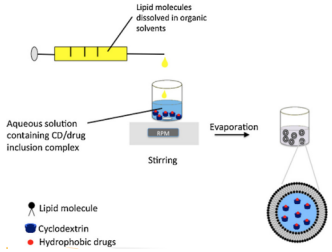


Figure 3. Process for synthesizing Nanoparticles **(2)**

We have successfully synthesized PLGA nanoparticles containing AS1842856 and DMPC/DPPC liposomes containing Balfilomycin-A1 or CL316243 using a turbulent jet mixing approach. A targeting peptide, P3 which binds to prohibitin in white adipose tissue vasculature, was conjugated to the PLGA nanoparticles. The particle size, as measured by dynamic light scattering, was found to range between 140-210 nm for the PLGA nanoparticles and 90-220 nm for the liposomes. Particles produced this way have shown stability at 4℃ for 4 weeks.

Cell Differentiation

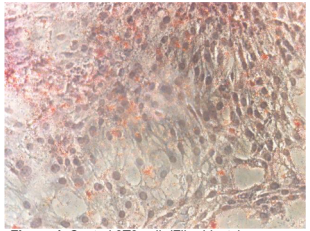


Figure 4. Control 3T3 cells(Fibroblasts)

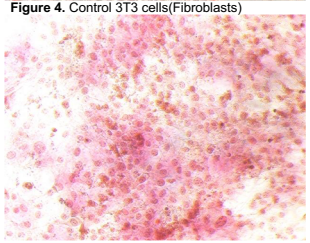


Figure 5. Differentiated 3T3 cells(Pre-adipocytes)

We are currently testing the free drugs and nanoparticle encapsulated drugs using the 3T3 cell line. FOXO1 and autophagy inhibitors can prevent differentiation of 3T3 cells into preadipocytes. The 3T3 cells have been successfully differentiated into preadipocytes as measured using Oil Red O staining and dose response testing is ongoing. The large blue areas stained are the cell nuclei, this helps with identification of individual cells. The smaller red areas are the areas of interest for our treatment

Differentiation Procedure
We differentiate the 3T3 fibroblasts by exposing them to a rosiglitazone solution followed by insulin exposure. After exposure, there are 8 more days of continued growth in basal media, to replicate in vivo conditions, the cell differentiation procedure's effects are visible. The total procedure last 10-12 days.

Experimental Design

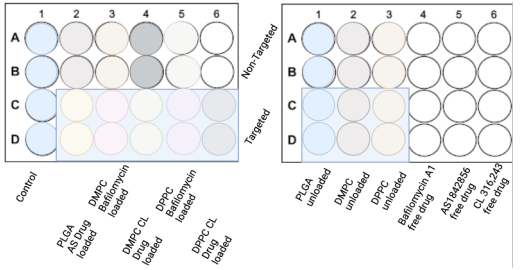


Figure 6. Well-Plate Schematic for Drug and Nanoparticle Exposure

Expected Results

During the standard differentiation of 3T3 cells, the cells develop lipid dense areas within each cell that can be visibly identified using Oil Red O staining. The exposure of cells to FoxO1 inhibitors reduces the formation of these lipid dense regions. The exposure of cells to nanoparticle encapsulated drugs should significantly reduce the required drug-load or increase the efficacy of similar drug loads to free drug concentrations; an amplification of the drugs effects should be observed when encapsulated and targeted.

Current Work

We are currently working on exposing the cells to the drug in the manner displayed under the Experimental Design section. We are designing the nanoparticles to carry a drug concentration of 6 mg/ml which requires further experimentation, the current drug concentration is at 2 mg/ml which has proven unresponsive in *in vivo* tests carried out by our collaborator.

Acknowledgements/References

We want to thank our collaborator Dr. Cheng for his contributions to the understanding of the effects of the FoxO1 inhibitors. Without the advice and continued support of Dr. Lewinski this project would not have been possible, we want to greatly thank her for everything she has done.

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