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Engineered PLGA Nanoparticles for Delivery of siRNA in MCF-7 Breast Cancer Cells

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Introduction

Breast cancer is the second most common type of cancer and the second leading cause of cancer-related deaths (1). RNA interference (RNAi) is a eukaryotic cellular pathway in which a short strand of RNA is able to induce the degradation of its mRNA complementary sequence and decrease the expression of that gene (1,3,5). RNA interference can be applied to therapeutics in which siRNA can be synthetically produced which are capable of cancer gene-specific knockdown (1,5).

Naked siRNA is subject to degradation and removal by enzymes (3,5,6). It is also too large and too negatively charged to cross cellular membranes due to its strong anionic charge of the phosphate repelling the anionic charge of a cells membrane surface(3-6). Therefore a safe and effective system of delivery must be developed in order to facilitate transfection and artificially induce RNAi. Nanocarriers synthesized from polymers such as poly lactic co-glycolic acid (PLGA) have been shown to control release, protect its contents, remain stable, and can be modified for targeted delivery (2,4).

If administered systemically, the particles must be able to target the cells or tissue while avoiding uptake by non-target tissues, kidney filtration, and degradation by phagocytes and enzymes (3,4). Active targeting of the tissue may be incorporated into the mode of delivery which can assist in in vivo biodistribution and delivery (3). Some tumors allow the entry of larger molecules, up to 200nm in diameter, which can accommodate drug delivery nanoparticles through passive targeting (3). Therefore, appropriate and efficient delivery remains one of the biggest challenges in the development of siRNA as an anti-cancer treatment

Hypothesis

It was hypothesized that the tumor targeting peptides would effectively penetrate the cell and transfect our particles. Subsequently, gene silencing was expected to be observed in the transfected cells.

Experimental Methods

Cell Culture

MCF7 cells were grown in RPMI media containing L-Glu, non-essential amino acids, penicillin/streptomycin, and 10% FBS at 37°C. Cells were plated (10,000 to 20,000 cells/well) on a 96-well plate in 200ul of media with serum. Cells were plated 20-24 hours in advance.

Nanoparticle Preparation/Peptide Conjugation

Nanoparticles were composed of 1:10 PLGA-PEG:PLGA-PEG-Mal in 2% PVA solution. Double emulsion was done using DCM. The particles were sized and the PVA supernatant was used to measure entrapment efficiency. Peptides were conjugated to the nanoparticles at 1.3x the malemide concentration The conjugation solution was left for 4 hours and then collected in RNase-free water.

Experimental Methods cont.

Transfection/Visualization

The conjugated nanoparticles were left to transfect for 6 hours at 37°C. Solution was replaced with RPMI media for 48 hours. Darmafect 4 was used as a control reagent. Fluorescent and raw images were taken using a fluorescence microscope.

Functional Assay

GAPDH activity was measured in the cells 42 hours after the transfection according to the procedure outlined in the Life technologies GAPDH KDalert Kit. Absorption and fluorescence were taken on a black plate.

Results

Nanoparticle Characterization

Nanoparticles	Z-average size (nm)	Encapsulation efficiency
siRNA containing NP	164.5 SD ± 5.53	8.11% SD ± 0.62
siRNA x2 NP with BSA	173.7 SD ± 1.21	44.35% SD ± 0.20
siRNA x2 NP with BSA, CQ, ENX	163.5 SD ± 4.77	N/A

Table 1. Nanoparticle sizes and encapsulation efficiency

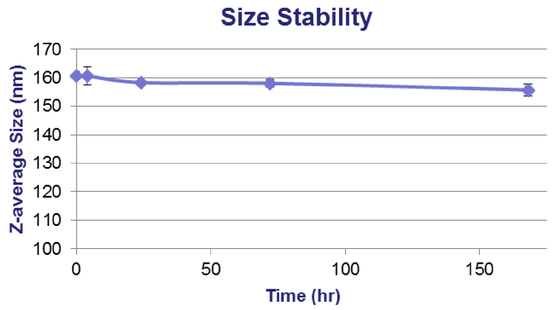

Figure 1. Size stability of siRNA-containing nanoparticles over 7 days in 1x PBS

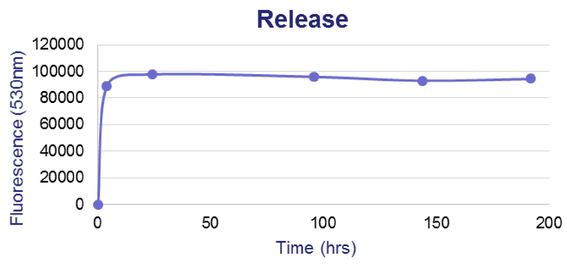

Figure 2. Release rate of siRNA-containing nanoparticles over 8 days in 1x PBS at 125RPM at 37°C

As indicated by Figure 1, the size of the nanoparticles stay relatively constant over a period of 7 days and can sustain the time required to release the nanoparticle's contents. PLGA and PLGA-PEG are sufficient materials that can be used for nanoparticle formulation and sustain the conditions required for extended drug release without degradation of the polymer. As shown by Figure 2, the siRNA contained in the nanoparticles is completely released by 24 hours. This rate of release is faster than anticipated and bovine serum albumin was later added to increase the entrapment efficiency as well as to possibly decrease the rate of release. A fast release rate could contribute to a significant loss in the available siRNA for gene silencing and despite the nanoparticles remain stable.

Transfection

Peptide conjugates contained a fluorescent tag to help visualize the cells. Fluorescence can be observed in the cells that were transfected using the conjugated nanoparticles. Since fluorescence was not observed in the controls, this indicates that a penetrating peptide is required for cellular uptake of PLGA nanocarriers. The fluorescent images do not indicate which of the tested peptides is the most effective at cellular penetration.

Functional Assay

The functional assay was conducted using the same nanoparticle design. Silencing was observed with commercially available lipoamine transfection reagents using the same siRNA strands. There were, however, no observable changes in the GAPDH activity using the PLGA nanoparticles as the delivery vehicle. Further modifications also failed to produce any gene silencing effects.

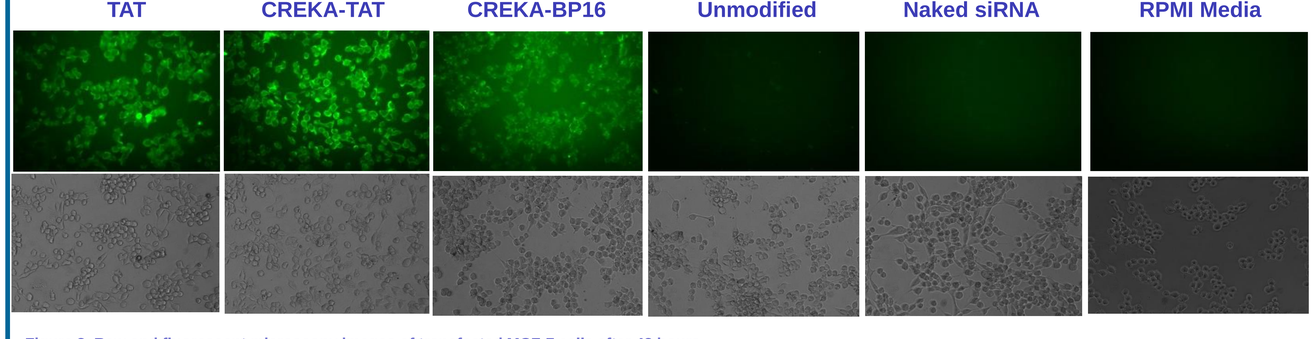

Figure 3. Raw and fluorescent microscopy images of transfected MCF-7 cells after 42 hours

Conclusion

The nanoparticles have shown the ability to maintain physical integrity under in vitro conditions for an extended period of time in order to release the entire contents. This allows for modifications to increase the entrapment efficiency and extend the rate of release for prolonged drug effects. However, the rapid release rate observed in this experiment could have potentially contributed to the lack of gene silencing. A significant amount of the entrapped siRNA may have been released during the transfection process outside of the cell adhering to the outside to produce the fluorescent images. This would lead to an insufficient amount of siRNA in the cytoplasm to interact with the RNAi machinery.

Although fluorescence was observed, this does not provide any information regarding the release of the nanoparticle from the endosome, the release of the siRNA from the nanoparticle, or the RNA interference activity levels. Nanoparticles may be taken up by natural endocytosis, however the presence of a peptide significantly affects the active targeting of the cancer cells.

The ultimate goal of this project was to optimize nanoparticle formulation and to test the effectiveness of these nanoparticles as siRNA delivery vehicles in order to decrease gene activity. Silencing, however, was not observed with any of the various nanoparticle formulations or their deviations.

Future Research

Extensions on this project are currently underway to optimize the GAPDH functional assay. Further variations of nanoparticles are being tested with different cell-penetrating conjugates and formulas in order to increase entrapment efficiency, improve intercellular release, and improve potency to produce gene silencing effects. Additional tests will be conducted to discover if the nanoparticles are released from the endosomes and the siRNA strands are released from the nanoparticles.

Acknowledgements

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