siRNA Targeted Delivery to Ovarian Cancer Cells via Folate Conjugated Triblock Co-polymer

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ABSTRACT SUMMARY
We have demonstrated that our tri-block co-polymers are effectively able to condense and deliver siRNA and achieve knockdown within ovarian cancer cells. This suggests that our polymers could be a good mechanism to deliver siRNA for oncogene knockdown.

Introduction
Folate Receptors are over expressed in several human cancers, but most noteworthy for this project in ovarian cancer. In 90-95% of ovarian cancers, there is an over expression of Folate-receptor-α (FR). Outside of a full oophorectomy, treatments for ovarian cancer include radiation and chemotherapy. A new approach to treating certain cancers is the delivery of siRNA to knockdown the expression of specific oncogenes. To side-step challenges in standard treatment; such as drug/chemo-resistance, relapse or toxicity, we plan on specifically targeting the FRα using a non-toxic, folate coupled polymer that will encapsulate both the siRNA and Paclitaxel (PTX). The siRNA will be used to down-regulate the expression of proteins, such as Toll Like Receptor 4 (TLR4) or Multi Drug Resistance Gene 1 (MDRG1) that have been cited to play a role in chemotherapy resistance.¹

In hopes to translate siRNA based therapy to the clinic, two major hurdles have to be overcome. First, siRNA is quickly degraded in circulation before it can exert its effects. Secondly, just delivery of siRNA does not give specificity to this treatment. Our approach of using a tri-block copolymer that consists of polyethyleneimine-graft-polycaprolactone-block-poly(ethylene glycol), or folate coupled PEI-g-PCL-b-PEG-Fol, overcomes both of these obstacles.² The PEI helps shield the siRNA from outside proteins, and the conjugated folate ligand on the particle surface gives specificity towards cells that over-express FR. In our work, we looked at these polymers with different molecular weights of PEG, as well as different grafting degrees of the (g-PCL-b-PEG-Fol) chains to PEI to find the optimal delivery mechanism for siRNA uptake into FR positive cancerous cells.

Experimental Methods
Several methods were used to both characterize the tri-block co-polymers as well as to assess their in vitro capabilities. Characterization techniques used to look at the physical structure of our tri-block polymers are NMR, DLS for size and zeta measurements at N/P 5, 6, and 7, as well as TEM.

To test the ability of our polymers to encapsulate and deliver siRNA, we utilized a different set of experiments. A SYBR Gold Encapsulation Assay was performed at N/P ratios ranging from 1-20. The condensation ability of the polymers was measured on a UV-vis spectrophotometer. Next, we had to see whether or not our polymers were able condense and shield the siRNA in the presence of competing polyanions (Heparin); to simulate in vivo conditions at pH 7.4. Furthermore, we changed the pH of the assay to 4.5 to mimic the release profiles of the polymers after being taken up by the endosome.

Flow cytometry was utilized to help assess the ability of the conjugates to selectively deliver the siRNA to ovarian cancer cells. The uptake between cell lines that were enriched with FR were compared against cell lines that were known to be FR negative or not as abundantly expressed. For qualitative data of siRNA uptake, confocal
microscopy was used. By using a fluorescently labeled siRNA, we were able to see the amount of uptake across all polymers within the cell.

**Results and Discussion**

After performing encapsulation and stability assays, we were able to see that all of our polymers fully condensed siRNA at N/P 5. Therefore, all following experiments with our polymers were performed at N/P 5, 6, and 7. Furthermore, at N/P6, all of our polymers were able to retain and release siRNA better than PEI at pH 7.4 and 4.5, respectively. At the selected N/P ratios, the majority of the sizes of our polymers were under 260 nM (Figure 1); which is the lower limit of macrophage detection in circulation. The zeta potentials were under +20 mV, but no lower than 0 mV, thus avoiding toxicity due to cationic surface charge (Figure 1).

**Figure 1.** Size and zeta potential measurements of all PEI-g-PCL-b-PEG-Fol polymers at N/P ratios 5, 6, and 7. Size and Zeta potentials were measured on a Dynamic Light Scattering instrument.

*In vitro* uptake studies were carried out with flow cytometry and confocal microscopy. Cellular uptake studies with flow cytometry showed comparable uptake to our positive control, PEI, and significantly better uptake than free siRNA. Confocal microscopy showed identical results as seen in the flow cytometry uptake analysis (Figure 2).

**Figure 2.** Cellular siRNA uptake analysis of all tri-block copolymers at N/P ratios 5, 6, and 7.

Preliminary results show that TLR4 knockdown in chemo-resistant cells does lead to sensitivity to PTX and a decrease in cell viability.

**Conclusions**

We have demonstrated that our polymers are effectively able to condense and deliver siRNA and achieve knockdown within the cell. This suggests that our polymers could be a good mechanism to deliver siRNA for oncogene knockdown. Likewise, the knockdown of certain genes such as TLR-4 or MDRG-1 with siRNA, could lead to overcoming chemotherapy resistance within ovarian cancer.

**References**


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