Development of new delivery vehicles for siRNA using PLGA/PLA and evaluating their efficacy in animal models

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ABSTRACT

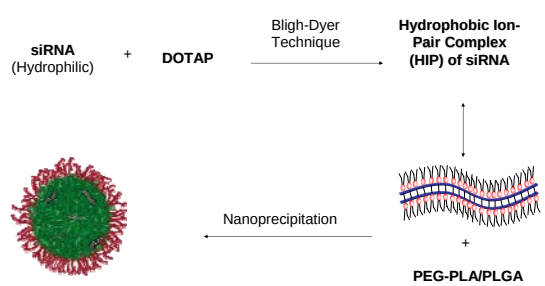
RNAi has had a significant impact on the understanding of gene function and holds tremendous promise for therapeutic applications. However, its utility has been limited by a lack of appropriate *in-vivo* delivery methods and an inability to direct therapies to target tissues. Liposomes and cationic materials are commonly used delivery vehicles for getting nucleic acids into cells, but translation of these approaches to animal models and clinical studies will require further work.

Here we describe embedding siRNA into poly lactic acid (PLA) and poly lactic/glycolic acid (PLGA) polymeric nanoparticles with a PEG coating as a step toward improved *in-vivo* delivery. The particles are designed for prolonged circulation and the PEG coating reduces RES uptake while presenting a platform for attaching ligands/antibodies to enhance target specificity of the nanoparticles.

To demonstrate the feasibility of these nanoparticles for delivering siRNA, we used reporter genes as a target with the intent of targeting viral infections such as Hepatitis-C. Fabrication of siRNA-containing nanoparticles was by nanoprecipitation techniques, and the nanoparticles have been characterized for size, encapsulation efficiency and release kinetics.

To test these particles we have developed a mouse model in which an albumin promoter is used to express *click beetle red luciferase* (CBR) as a liver-specific reporter gene. This mouse exhibits strong bioluminescent signals from the liver, and is an excellent target for validation of delivery of CBR-specific siRNA to this target tissue. The efficacy of the siRNA delivery can be monitored non-invasively using *in-vivo* bioluminescence imaging (BLI) in this mouse model such that longitudinal studies of expression can be performed and delivery methods can be optimized. Development of the appropriate tools for delivery of siRNA, and refining mouse models for rapidly evaluating these tools will be essential if the promise of siRNA is to be realized.

Nanoparticle Fabrication



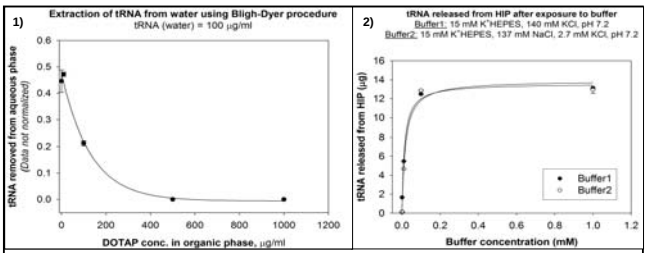


Figure 1: tRNA was used as a model polynucleic acid molecule to test the formation of an HIP complex and its subsequent release in buffer conditions.

1) Formation of HIP Complex: Extraction of tRNA from aqueous phase by DOTAP. 1:1 molar ratios of tRNA and DOTAP is sufficient to extract > 98% tRNA from the aqueous phase.

2) Release of tRNA from HIP complex: When the HIP complex is exposed to a high salt environment, such as HEPES buffer, the ionic interaction between the phosphate groups of the nucleotide and the amine group of DOTAP is disrupted, leading to release of the tRNA.

Sample	Zeta Potential (mV)	Nanofabrication Conditions		Encapsulated siRNA/mg nanoparticle (µg/mg)
		[Polymer + siRNA HIP] (mg/ml)	Non-solvent /Solvent	
NP071 (20k)	-1.58	20 + 0.2	2/1	7.4
NP072 (40k)	-1.1	20 + 0.2	2/1	8
NP073 (40k)	-1.54	20 + 0.2	2/1	8.4
NP074 (40k)	-1.92	10 + 0.1	3/1	8.8
NP075 (40k)	-1.46	20 + 0.4	2/1	16.9

Note: Diameters of the nanoparticles were around 130-180 nm.

Figure 2: Release of siRNA from PLGA-PEG nanoparticles suspended in PBS. PLGA (40k) exhibited a slightly higher encapsulation efficiency as compared to PLGA (20k), whereas the rates of release of siRNA were slower. Most of the siRNA payload appeared to release in 3 days.

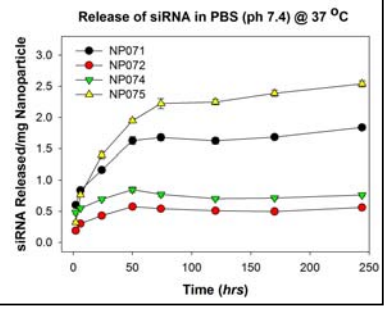


Figure3: PLA/PLGA undergoes degradation due to hydrolysis and yields lactic and glycolic acid subunits. In the process, the nanoparticles form an acidic microenvironment that can be detrimental to the integrity of siRNA that is encapsulated. In order to verify the effect of hydrolytic degradation on siRNA activity, PLA nanoparticles were incubated in PBS for 15 days and then another 24 days, and the efficacy of the siRNA that was released was determined. No apparent decrease was found in the released siRNA. This demonstrated that PLA/PLGA nanoparticles do not significantly impact the functionality of the siRNA payload. **(a)** Gel electrophoresis shows that the siRNA that is released is intact. **(b)** The siRNA retains the ability to knock down gene expression in cells.

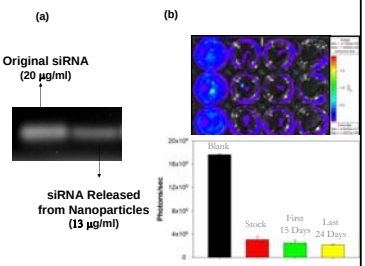
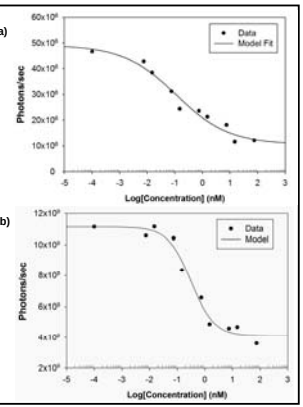


Figure 4: Two liver-specific targets for siRNA are currently under investigation. Successful knockdown of these targets will validate use of the nanoparticles as delivery vehicles of siRNA for a host of liver-specific ailments. **(a)** CV-1 cells were stably transfected to express an Internal Ribosomal Entry Site (IRES) of the HCV coupled to a luciferase reporter. Studies have shown that silencing the IRES region can prevent viral replication. The siRNA exhibited an EC_{50} of 0.11 nM. **(b)** 293 cells were stably transfected to express a CBR luciferase. siRNA directed against the CBR resulted in knockdown of the bioluminescent signal with an EC_{50} of 0.32 nM. Non-specific siRNA did not show any effect on the luciferase expression at any concentration on either cells (data not shown).



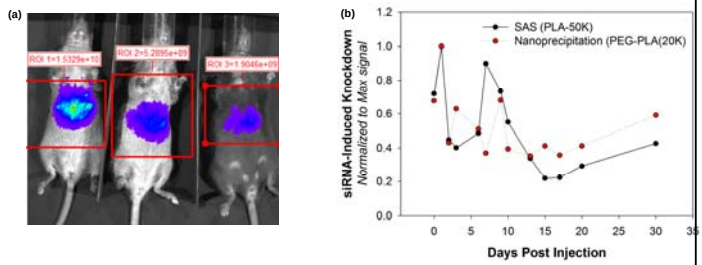


Figure 5: Pilot Studies for siRNA Delivery - Transgenic Alb-CRE mice engineered to express CBR driven by an albumin promoter have been used as a model to validate and quantify delivery of siRNA. Nanoparticles made from PLA and PEG-PLA with encapsulated CBR-siRNA were administered intravenously in 200 µl boluses. **(a)** A representative bioluminescent image of the Alb-CRE mice shows that the predominant signal is emitted from the hepatocytes in the liver where albumin is abundantly expressed. **(b)** Knockdown of CBR expression after administering CBR-siRNA nanoparticles. The effect appears to last for more than a month and measurements are continuing.

Summary:

- We successfully fabricated pegylated-nanoparticles from biodegradable polymers such as PLA and PLGA.
- These nanoparticles encapsulated siRNA which retained their activity upon release.
- The size, encapsulation efficiency and release rates can be controlled by varying different parameters including molecular weights of the polymers, concentrations and additives.
- In our pilot studies, we successfully achieved a knockdown of CBR using the appropriate siRNA encapsulated in our nanoparticles.
- Future work will be focused on labeling the surface of the nanoparticles with ligands such as galactose to enhance uptake into hepatocytes and increasing knockdown of the reporter genes.

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