PEG-PLA/PLGA Nanoparticles for In-Vivo RNAi Delivery

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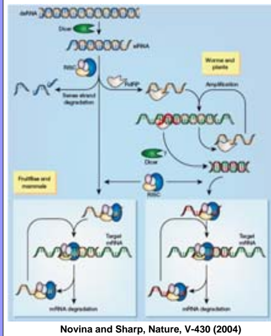
AIM: The potential for nucleic acid-based therapies for a range of diseases is expected to be quite significant. However, their current utility has been limited by inefficient delivery methods and an inability to direct therapies to the target tissue. Presently, liposomes and cationic materials are being investigated as delivery vehicles for nucleic acids. RNAi, including siRNA, miRNA and shRNA, is emerging as a robust new class of therapeutic compounds for controlling gene expression. Numerous examples of gene down-regulation by RNAi have been demonstrated in cell culture studies. Translation of this nucleic acid-based therapy to animal models and clinical studies will require significant advances in delivery. The goal of this project is to develop appropriate in-vivo delivery mechanisms for RNAi that could be used to target specific tissues.

APPROACH: We encapsulate RNAi into polylactic acid (PLA) and polylactic/glycolic acid (PLGA) polymers with a PEG coating. The PEG coating reduces RES uptake and presents a platform for attaching ligands and antibodies to enhance target specificity of the nanoparticles. Encapsulating the RNAi with a biodegradable polymer also helps prevent their degradation by nucleases. In order to demonstrate the feasibility of the RNAi nanoparticles, we target the internal ribosome entry site (IRES) of the Hepatitis-C Virus (HCV) as a means of controlling viral replication in the liver. HCV infection can lead to hepatocellular carcinoma and/or cirrhosis of the liver and an effective antiviral is a critical unmet medical need. We use cell culture and living animal models along with bioluminescence imaging (BLI) to develop the drug delivery systems .

Gene Silencing by siRNA



Novina and Sharp, Nature, V-430 (2004)

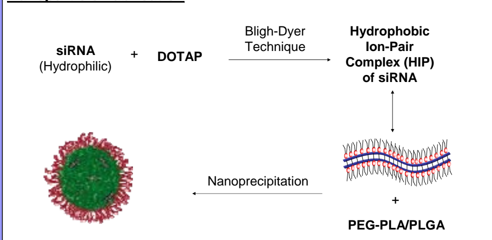
Hurdles in siRNA Delivery:

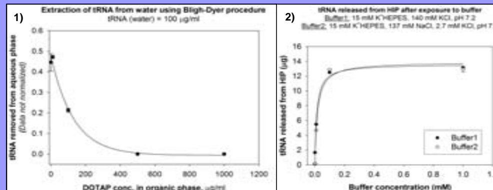
- Excretion
- Serum Degradation
- Non-specific distribution
- Tissue Barriers

Currently Used Approaches:

- Liposomes
- Lipid-based lipoplexes
- Cationic polymers/polyplexes
- Viral delivery

Nanoparticle Fabrication



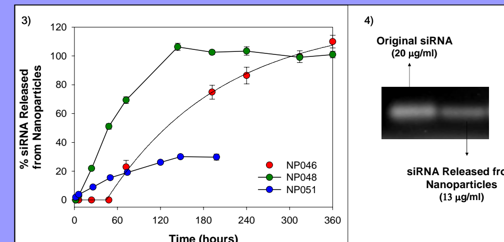


tRNA was used as a model polynucleic acid molecule to test the formation of an HIP complex and its subsequent release in buffer conditions.

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.

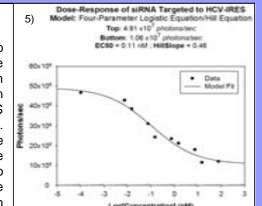
Sample	Diameter (nm)	Nanofabrication Conditions		Encapsulated siRNA/mg nanoparticle (μg/mg)
		[Polymer + siRNA HIP] (mg/ml)	Non-solvent /Solvent	
NP044	107	10 + 0	2/1	0
NP046	92.4	10 + 0.1	2/1	1.75
NP048	177	20 + 0.2	2/1	7.75
NP049	157.6	20 + 0.2	6/5	6.41
NP050	157.4	20 + 0.2	8/5	6.76



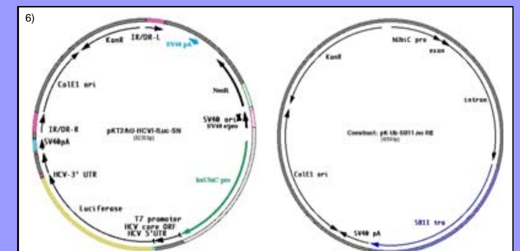
siRNA release from the nanoparticles were analyzed in PBS at 37 °C. As observed in **Figure 3**), release rates can be controlled by varying the fabrications. Polymers such as PLA and PLGA create acidic microenvironments as they undergo hydrolytic degradation. The siRNA recovered from the nanoparticles do not show degradation, **Figure 4**), which may be a result of the DOTAP coating around it.

Cell Culture Results:

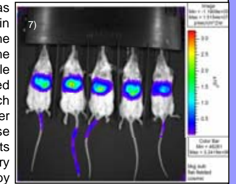
CV-1 cells were stably transfected to express an Internal Ribosomal Entry Site (IRES) of the HCV. Studies have shown that silencing the IRES region can prevent viral replication. The HCV-IRES was coupled to a luciferase reporter. When an siRNA was used to silence the IRES, a decrease in bioluminescence signal from the cells was used to determine the Dose-Response of the system. Figure 5) shows the results from CV-1 cells of an siRNA that was developed to target the HCV-IRES. The siRNA exhibited an EC_{50} of 0.11 nM, whereas a non-specific siRNA did not show any effect on the luciferase expression at any concentration (data not shown).



Bioluminescent Mouse Model for HCV



The HCV mouse model was developed with luciferase as a reporter. The luciferase was coupled to the HCV-IRES, **Figure 6**), wherein successful silencing of this sequence in the mRNA by the siRNA would be reflected in the bioluminescence image. To achieve stable integration in the liver, a transposon-mediated gene delivery system was developed in which the HCV-IRES controls luciferase reporter expression. Co-application of a transposase encoding plasmid with the transposon results in stable intergation into the genome. Delivery of the plasmids to the liver was achieved by hydrodynamic tail-vein injection, **Figure 7**).



SUMMARY:

• RNAi nanoparticles were fabricated by nanoprecipitation, and the nanoparticles have been characterized for size, encapsulation efficiency and release kinetics. These initial results provide useful information for further optimizing the manufacture of PEG-PLA/PLGA based RNAi nanoparticles for in-vivo delivery.

• Cell culture experiments were accomplished to obtain dose-response information of the siRNA targeting HCV-IRES.

• To investigate the delivery and efficacy of these RNAi nanoparticles, we have developed an animal model for control of expression from the HCV-IRES. The animal model would allow for long term studies of a therapeutic RNAi molecule directed against the HCV-IRES and the treatment can be monitored noninvasively using *in-vivo* bioluminescence imaging.