**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 target specific tissues.

**APPROACH:** We encapsulate RNAi into polyactic acid (PLA) and polyactic/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**

**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**

- siRNA (Hydrophilic) → DOTAP
- Bligh-Dyer Technique
- Hydrophobic Ion-Pair Complex (HIP) of siRNA
- Nanoprecipitation
- PEG-PLA/PLGA

**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 cell 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 EC50 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, as shown in 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 integration into the genome. Delivery of the plasmid 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.