

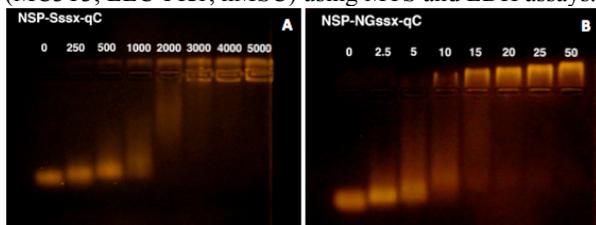
## The Development of Safe and Effective Polymeric Carriers for siRNA Delivery

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**Statement of Purpose:** RNA interference is a powerful tool capable of silencing disease-causing genes and as such, it has a broad scope as a therapy. The biggest barrier preventing it from clinical use, however, is achieving *safe* and *effective* delivery of siRNA to target cells. To this goal, we report the synthesis, characterization and interrogation of cationic nanostructured polymers (NSPs) for the delivery of siRNA. We demonstrate biocompatibility (both *in vitro* and *in vivo*), the ability to bind and release siRNA, internalize into cells and knock down target protein expression.

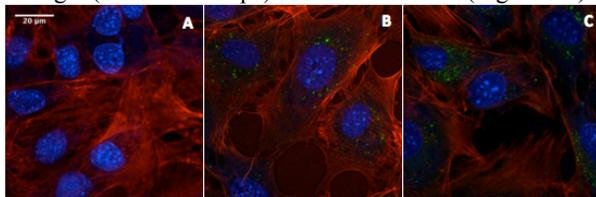
**Methods:** Cationic NSPs with two distinct architectures – stars and nanogels – were synthesized by ATRP(1, 2). *In vitro* cytotoxicity of NSPs was assessed by MTS and LDH assays. Gel electrophoresis was used to determine complexation ratios (polymer to siRNA). Internalization of these polymers into MC3T3 cells was confirmed using confocal microscopy. *In vivo* biocompatibility was determined by NSP loading in Synthes XCM<sup>®</sup> scaffold and implantation in mice for 4 weeks; sections were stained with H&E. Protein knock down was assessed using the Dual-Luciferase Reporter<sup>®</sup> system in S2 cells.

**Results:** NSPs were determined to be biocompatible up to concentrations of 800 µg/mL on multiple cell types (MC3T3, LLC-PK1, hMSC) using MTS and LDH assays.



**Figure 1** – Gel electrophoresis to determine complexation ratio of the cationic star polymer (A) and nanogel (B) with siRNA. Labels represent weight ratio of polymer to siRNA (0:1, 25:1, 50:1, etc.).

The cationic star polymer (NSP-Sssx-qC) complexed with siRNA at a ratio of ~2000:1 (Figure 1A), and the cationic nanogel (NSP-NGssx-qC) at a ratio of ~15:1 (Figure 1B).

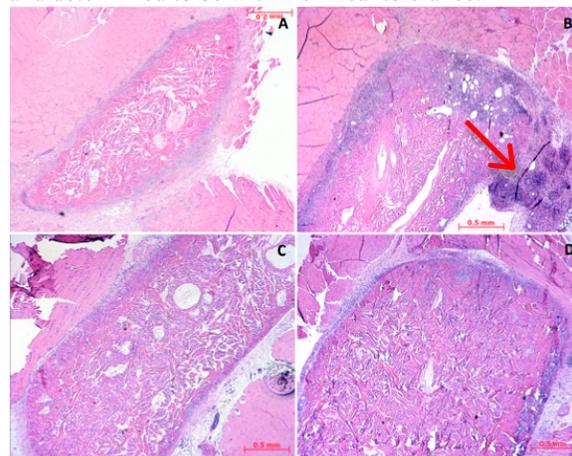


**Figure 2** – Confocal microscopy of MC3T3 cells (A) treated with TRITC-Nanogels at concentrations of 200 µg/mL (B) and 400 µg/mL (C) for 24 hours. Staining: Nuclei | Actin | Nanogel

*In vitro* internalization studies (Figure 2) demonstrated that at concentrations of 200 and 400 µg/mL (Figure 2B-C), a clear nanogel signal (green) is visible inside cells indicating their presence in the cytoplasm.

An *in vivo* analysis of biocompatibility (Figure 3) demonstrated that while there was some inflammation

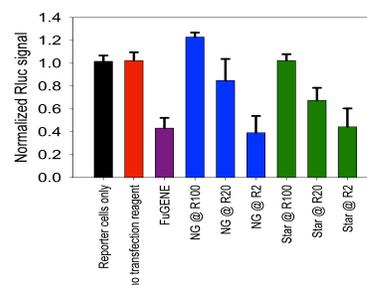
observed in both NSP groups (Figure 3C-D), levels were less than those induced by Freund's adjuvant (Figure 3B), and determined to be within clinical tolerance.



**Figure 3** – H&E staining of samples in the thigh muscle of 8-week old mice. Treatment groups: XCM<sup>®</sup> scaffold (A), XCM<sup>®</sup> + Freund's adjuvant (B), XCM + star NSP (C) and XCM + nanogel NSP (D).

Knock down of target proteins was demonstrated by the Dual-Luciferase Reporter<sup>®</sup> system in S2 cells.

NSP:siRNA ratios of 2:1 (R2) with both the star polymers and nanogels knocked



**Figure 4** – Luciferase knock down studies in S2 cells.

down target luciferase expression roughly 60%. This represented a significant reduction from the negative control (reporter cells only). The knock down achieved was statistically indistinguishable from the commercially available transfection reagent, FuGENE<sup>®</sup> HD.

**Conclusions:** We report the use of novel nanostructured polymers for siRNA delivery, demonstrating high levels of biocompatibility and ultimately, the ability to knock down target protein levels *in vitro*. While the true test of an RNA interference delivery system is the ability to knock down protein expression *in vivo*, our results thus far are promising.

### References:

1. S. E. Averick *et al.*, Preparation of Cationic Nanogels for Nucleic Acid Delivery. *Biomacromolecules*, (Oct 2, 2012).
2. H. Y. Cho *et al.*, Synthesis of biocompatible PEG-Based star polymers with cationic and degradable core for siRNA delivery. *Biomacromolecules* **12**, 3478 (Oct 10, 2011).