# "Smart", sustained local delivery of siRNA from an injectable scaffold

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**Statement of Purpose:** Small interfering RNA (**siRNA**) are short double stranded RNA that enable **specific** knockdown of a target gene[1]. However, intracellular cytoplasmic delivery is a challenge because uptake occurs via endocytosis, leading to siRNA endo-lysosomal entrapment and lack of bioactivity. Here, we have synthesized polymeric micellar nanoparticles (**NP**s) that possess a positively charged corona that electrostatically condenses siRNA and "smart", pH-responsive behavior tuned to mediate endo-lysosomal escape[2]. Yet, siRNA bioactivity is transient, and there are many applications (i.e. non-healing skin wounds) where sustained siRNA delivery to a local pathological site would be desirable. To this end, we present a novel technological platform for sustained delivery of siRNA-loaded "smart" NPs from an injectable, biodegradable (PUR) scaffold.

**Methods:** siRNA loaded NP synthesis: A diblock-copolymer composed of 2-(dimethylamino) ethyl methacrylate **(DMAEMA)**, 2-propylacrylic acid **(PAA)**, and butyl methacrylate **(BMA)** [DMAEMA-b-PAA-co-BMA-co-DMAEMA], was synthesized by RAFT polymerization targeting 25%DMAEMA, 25% PAA, and 50%BMA in the 2nd block. The polymers were characterized for molecular weight, polydispersity, and composition by size exclusion chromatography **(SEC)** and nuclear magnetic resonance spectroscopy **(NMR)**. NPs were fabricated by dissolving the diblock co-polymer in PBS spontaneously forming micelles and adding siRNA which electrostatically condenses onto the surface. Transmission electron microscopy **(TEM)** and Dynamic Light Scattering **(DLS)** were used for analyzing size and morphology of the NPs.

siRNA gene knockdown was validated *in vitro* by NPs treatment of NIH3T3 fibroblasts and RAW 264.7 macrophages at 50nM GAPDH siRNA for 48h and measuring GAPDH mRNA and protein expression relative to cell number. Cytocompatibility of our NPs was evaluated using an LDH assay after incubating for 24h.

NP-loaded PUR scaffolds were synthesized by reacting polyester triols (900 Da, 60%PCL, 30%PGA, 10%PLA) with lysine triisocyanate **(LTI)**, in the presence of a catalyst, water (blowing agent to create porosity)[3], and lyophilized NPs into the reaction mixture. The free-rise foam was allowed to cure for 20 minutes. Confocal microscopy was used to visualize the distribution of fluorescent siRNA containing NPs in the PUR scaffold.

*In vitro* release kinetics of NPs from PUR were measured for nine days. The PUR scaffolds were immersed in PBS and at set time points, the PBS was collected and replaced. Fluorescence readings were used to measure concentration of siRNA-containing NPs in releasate. TEM was used to confirm the release of intact micelles.

**Results:** siRNA loaded NPs: SEC and NMR confirm an 11.9kDa (PDI=1.04) DMAEMA 1st block with a total diblock size of 31.4kDa diblock (PDI =1.4). The 2nd block was determined to be 51%BMA, 29%PAA, and 20%DMAEMA. DLS and TEM verify the presence of 68.6±13.3nm micelles before and 58.5±10.6nm micelles after electrostatically condensing siRNA at a charge ratio of 4:1 in an aqueous environment. Zeta potential for NPs shows a positive surface charge (16.7 ± 6.9 mV).

Knockdown studies: In studies where GAPDH siRNA was used to validate NP activity, significant knockdown was detected in GAPDH mRNA (Figure 1A) and GAPDH protein. LDH cytotoxicity assay measurements showed no significant cell death caused by NPs in either cell line.

Complex loaded PUR foams: Confocal microscopy indicated a homogenous distribution of fluorescently labeled siRNA-containing NPs throughout all sections of the PUR scaffold (Figure 1B-i1, note dark spots correspond to scaffold pores).

Release kinetics: NP release from PUR scaffolds showed an initial burst release followed by a sustained release over a nine day period (Figure 1B, experiment ongoing for later time points). TEM of releasate confirms the presence of polymer micelles (Figure 1B-i2) indicating the stability of micellar NPs to PUR fabrication encapsulation procedure.

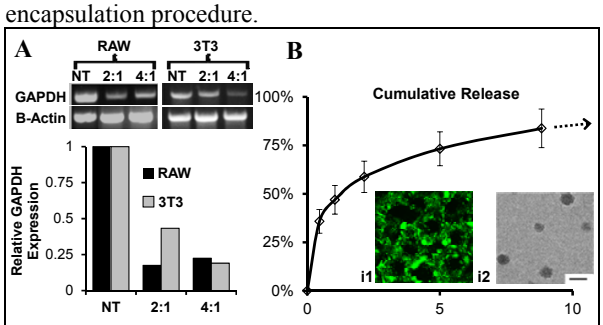


**Figure 1 A:** GAPDH mRNA knockdown by RT-PCR using NPs at charge ratios of 2:1 and 4:1.
**B:** Release kinetics of NPs from PUR.
**i1:** Confocal image of PUR with fluorescent siRNA
**i2**:TEM of micellar NPs in releasate (scale=100nm)

**Conclusions:** Here we present a novel PUR-NP system for sustained, local siRNA delivery. We first validated that the "smart" NP can mediate efficient knockdown in multiple cells lines. PUR-NP scaffolds were then fabricated and found to produce sustained release of intact micellar NPs for 9 days. Experiments to validate releasate siRNA bioactivity are forthcoming. Once verified and optimized, this novel combination of PUR and NPs will provide a porous scaffold template for cell in-growth, ease of delivery (injectability), multiple levels of tunability for release kinetics, and ultimately, the ability to optimize siRNA activity for specific target genes and/or pathological applications. This work is funded by the Vanderbilt Discovery Grant Program.

**References:**

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