PEG-Cloaked, pH-Responsive Nanomicelles for Effective and Biocompatible siRNA Delivery with Intravenous Application

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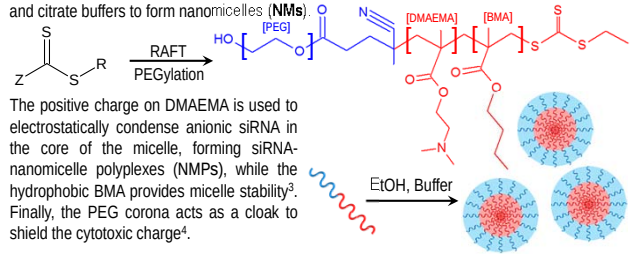
Introduction

Objective: Characterize a biocompatible siRNA delivery vehicle which can be used for stable, intravenous injection for disease treatment applications.

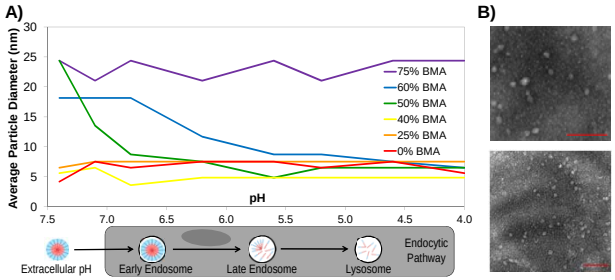
Motivation: Drug development for treatment of cancer and serious diseases was revolutionized by the discovery of small interfering RNA (siRNA) in 1999[1]. siRNA is a potent mediator of cellular expression which can be used to selectively silence genes. However, *in-vivo* siRNA delivery is rendered unfeasible by **kidney filtration**, **enzymatic degradation**, and **membrane impermeability**. Thus, the future of disease treatment hinges on development of biocompatible carriers to deliver siRNA to target cells. Many current efforts are hindered by cytotoxicity problems[2] and ill-suited for therapeutic delivery.

Methods: Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization was utilized to synthesize a pH-responsive block (**red**) consisting of DMAEMA and BMA copolymers. After PEGylation (**blue**), the polymer was dissolved in ethanol, then phosphate and citrate buffers to form nanomicelles (**NMs**).



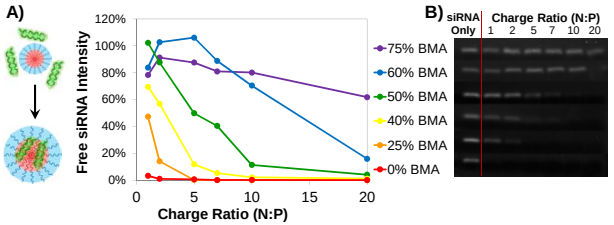
The positive charge on DMAEMA is used to electrostatically condense anionic siRNA in the core of the micelle, forming siRNA-nanomicelle polyplexes (NMPs), while the hydrophobic BMA provides micelle stability[3]. Finally, the PEG corona acts as a cloak to shield the cytotoxic charge[4].

Polymer Characterization



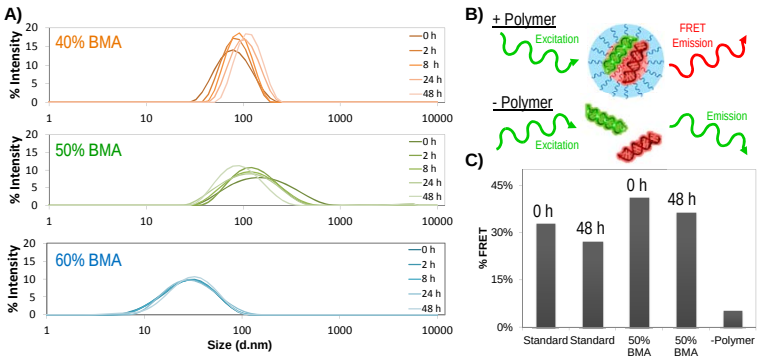
NMs spontaneously disassemble into unimers as pH is lowered. A) Dynamic light scattering (DLS) was used to measure particle size across a pH range designed to model the endocytic pathway. Polymers with 40%, 50%, and 60% BMA are most pH-responsive. B) Transmission Electron Microscopy (scale = 100nm) shows consistency of NM diameter at extracellular pH.

siRNA Binding



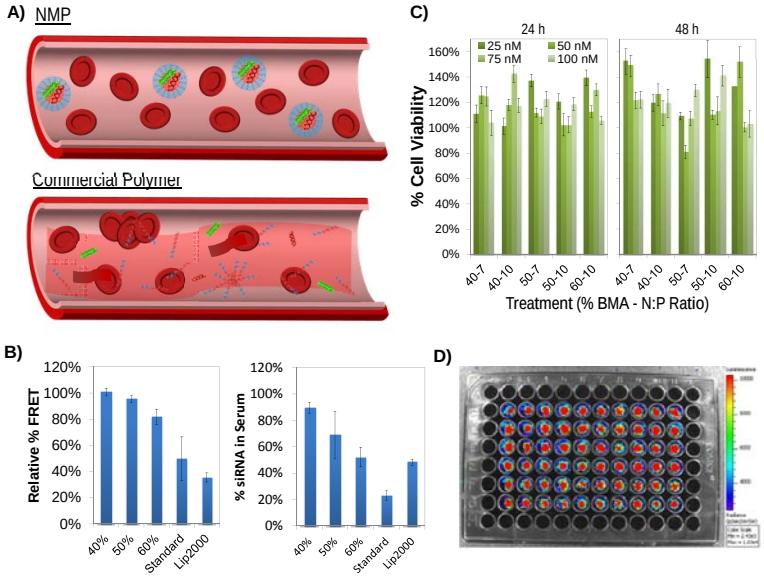
Polymers successfully bind siRNA and form NMPs. A) Electrophoretic mobility shift assays demonstrate that NMP formation depends heavily on N:P ratio – the molar ratio of cationic DMAEMA repeats to anionic phosphate charges on siRNA. B) aligned gel images.

Polyplex Stability



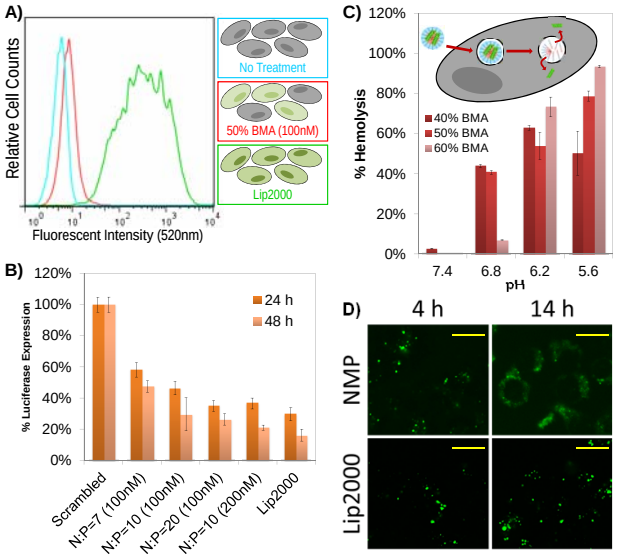
NMPs exhibit long-term stability and effective retention. A) Time lapse DLS measurements on NMPs (N:P=10) illustrate consistent particle size without aggregation or dissociation. B) NMs loaded with Fluorescence Resonance Energy Transfer (FRET) labeled DNA (FAM and Cy5) fluoresce red when excited via green wavelength. In the absence of polymers, the fluorophores are not close enough for energy transfer and green fluorescence is measured. C) Percent FRET was quantified by spectrofluorometry (excitation at 488 nm). Representative data shown indicate that NMPs bind nucleic acid as strongly and effectively as a laboratory standard control polymer[3].

Biocompatibility



NMPs are nontoxic and well suited for IV injection. A) NMPs remain stable and non-destructive in human blood while commercial delivery polymers dissociate and become affixed to red blood cells, leading to pulmonary embolism. B) Micelles loaded with labeled DNA were mixed and incubated with human whole blood. Analysis of blood serum after centrifugation revealed that NMPs remained stable while positive control polymers Lipofectamine®2000 and a laboratory standard[3] dissociated and bound to red blood cells. C) Cell viability remained extremely high after 24 and 48 hours of treatment with various NMP formulations containing scrambled siRNA. D) Representative image depicting uniform populations across different treatments.

Cell Uptake and Delivery

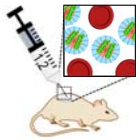


NMPs powerfully and efficiently silence gene expression *in-vitro*. A) Flow cytometry was utilized to quantify siRNA uptake. Characteristic data show significant uptake of positive control Lipofectamine®2000 and slight uptake of NMPs. B) Luciferase-expressing cancer cells were treated with NMPs carrying anti-luciferase siRNA (data for 50% BMA shown). Bioluminescence was measured using an IVIS Imaging System 200 series after 24 and 48 hours and normalized via Bradford protein assay. Treated cells experienced up to 80% reduction in gene expression, matching the knockdown ability of Lipofectamine®2000. C) Representative red blood cell hemolysis data show switch-like behavior as pH is lowered, enabling tightly controlled endosomal escape. D) Confocal microscopy (scale = 10μm) demonstrates that siRNA loaded into NMPs escapes endosomes and diffuses throughout the cytosol. In contrast, siRNA delivered via Lipofectamine®2000 remains distinctly punctate.

Conclusions and Future Work

NMPs provide a powerful yet innocuous tool for siRNA delivery *in-vitro*. Unlike many existing polymers, NMP characterization strongly suggests augmented activity *in-vivo*.

Future work will include blood toxicity studies on mice and *in-vivo* siRNA delivery to tumors via IV injections.



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