Design of Semi-Randomized Zwitterionic Peptides to Prevent Serum Aggregation of Polymeric Nanoparticles

Clyde Overby and Danielle S.W. Benoit
Department of Biomedical Engineering, University of Rochester

Statement of Purpose: Delivery remains the primary barrier to the development siRNA into clinical therapies. Polymeric nanoparticles (NPs) are one of the most promising approaches to delivering siRNA therapeutically. However, recent data indicate these NPs exhibit poor systemic delivery properties due to protein absorption and aggregation, which can lead to NP clearance via opsonization [1,2]. Various surface modifications to NPs have been explored to prevent aggregation including poly(ethylene glycol) (PEG) conjugation, which can lead to immunological reactions [2], and zwitterionic (ZI) polymers (including peptides) [3,4], which are not well-characterized in polymeric NP applications. Incorporating ZI peptides onto NPs is an attractive option with several potential advantages over other ZI polymers, including biodegradability, the potential for secondary structure, and precise control over individual amino acid (AA) residues. However, preliminary data (not shown) suggests that highly ordered ZI peptides may increase polymeric NP aggregation in storage and in serum. We propose that semi-randomized ZI peptides with low self-aggregation potential will reduce NP self-aggregation when conjugated to their surfaces and retain general anti-fouling properties. Herein data will be presented on the design, synthesis, and characterization of a semi-randomized ZI peptide-NP conjugate to prevent protein adsorption.

Methods: A diblock copolymer comprised of a poly(dimethylaminoethyl methacrylate) (DMAEMA) first block and a random tercopolymer second block of DMAEMA, butyl methacrylate (BMA), and propylacrylic acid (PAA) was synthesized via reverse addition fragmentation chain transfer (RAFT) polymerization [5], which self assembles into NP under aqueous conditions (**Fig 1A**). Peptide sequences were generated with a random seeding and scored using an inverted peptide aggregation prediction algorithm [6]. A Monte Carlo algorithm was used to score the peptide sequences with lowest self-aggregation potential by analyzing a sample of discrete AA sequences to identify the sequence with the lowest self-interaction potential, SDDSKDDKDSKDSKK with 50% of S randomly substituted with T and 50% of D randomly substituted with E (rZP). rZP synthesis was performed using solid phase peptide synthesis (CEM Liberty), with each randomized portion of the sequence represented as molar ratio mixtures of AA. rZP synthesis was confirmed through mass spectrometry and ^{1}H-NMR. The NP was conjugated with rZP to form rZP-NP via the chain transfer agent terminal carboxylic acid and the unprotected terminal amine of the peptide via carbodiimide chemistry (**Fig 1B**). Conjugation was confirmed through o-phthalaldehyde analysis. NP size was determined via dynamic light scattering (DLS) at 1 mg/mL in phosphate buffer solution before and after the addition of 10% fetal bovine serum (FBS). In the aggregated state, siRNA release is hindered, and thus was examined via a heparin displacement assay.

Results: Conjugation of rZP to NP reduces aggregation by 75% in 10% FBS (**Fig 2**) compared to unmodified NP, reducing the aggregate diameter from 129.3 ± 26.9 nm to 26.5 ± 0.4 nm. Furthermore, in 10% FBS rZP-NP had <0.1% aggregates above 200 nm by volume while NP had >5%. Preliminary data suggest ~ 60% improvement in siRNA release of rZP-NP post-aggregation over unmodified NP.

Conclusions: Data suggest semi-randomized ZI peptides reduce protein adsorption-mediated aggregation of polymeric NPs. Current efforts are focused on cellular uptake of rZP-NP conjugates for optimization, siRNA delivery and gene knockdown studies, and preparation for in vivo biodistribution and pharmacokinetics analyses.

Funding: T32GM118283, R01 DE018023, R01 AR056696.

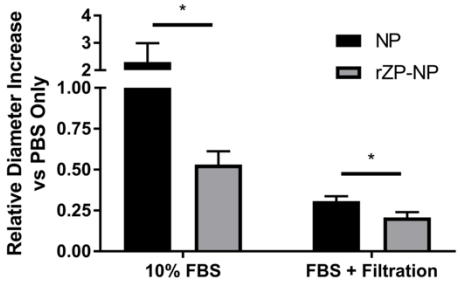

Figure 2: Comparison of NP and rZP-NP Z-avg sizes before and after 10% FBS addition (via DLS). * indicates $p < 0.05$

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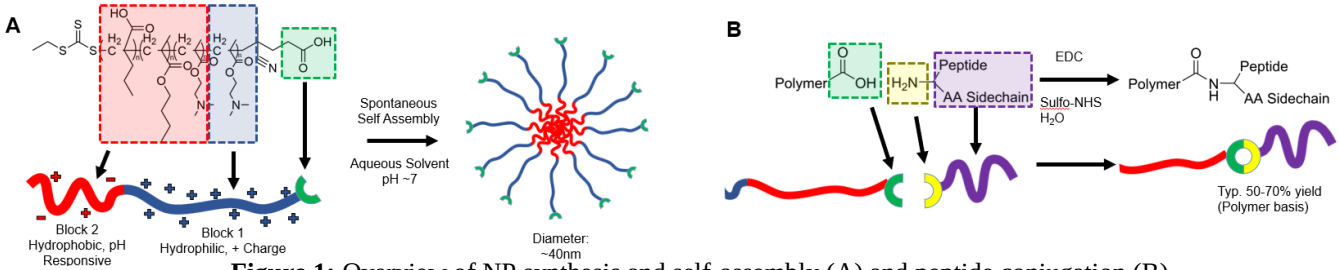

Figure 1: Overview of NP synthesis and self-assembly (A) and peptide conjugation (B).