Combinatorial Library of Ternary Polyplexes Enables Identification of Improved siRNA Nano-Formulations

Thomas A Werfel, Martina Miteva, Craig Duvall
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN

Statement of Purpose: Many previous non-viral siRNA vector development efforts have yielded reagents effective for in vitro transfection but that have poor in vivo pharmacokinetics and bioactivity. The current work focuses on development of a siRNA nanocarrier optimized to overcome both cell-level barriers (uptake/endosomal escape) and systemic barriers following intravenous delivery (stability for long circulation time and small size for effective tissue penetration). To this end, we synthesized a combinatorial library with varied polymer compositions that form the polyplex core and PEGylated polymers that form the corona of ternary polyplexes. These ternary polyplexes build from our previous finding that balancing cationic and hydrophobic content in binary polyplexes can enhance both particle stability and endosome escape.[1] Through this ternary complex/combinatorial approach, we were able to systematically study important structure-function characteristics such as polyplex surface PEGylation density, size, stability, and endosomolysis.

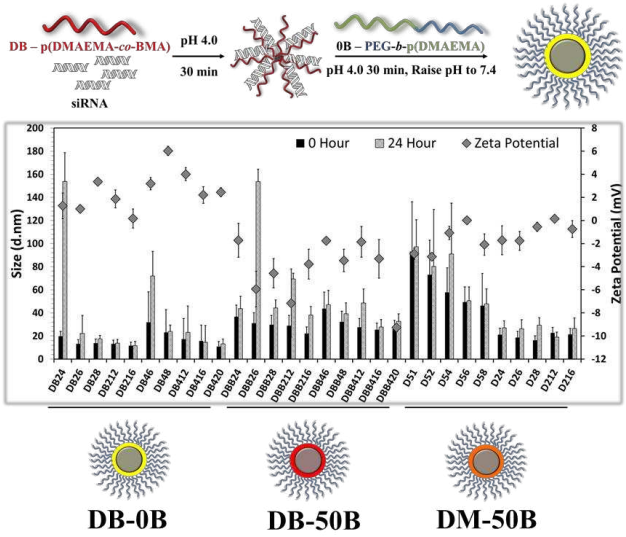

Figure 1. DLS size and zeta-potential measurements show that a subset of ternary polyplex formulations had neutral surface charge with optimal size (~15 – 30 nm) for in vivo applications.

Methods: Poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA, DM), poly[(2-(dimethylamino)ethyl methacrylate)-co-(butyl methacrylate)] (p(DMAEMA-co-BMA), DB), poly[(ethylene glycol)-b-(2-(dimethylamino)ethyl methacrylate)] (PEG-b-p(DMAEMA), 0B), and poly[(ethylene glycol)-b-[(2-(dimethylamino)ethyl methacrylate)-co-(butyl methacrylate)]] (PEG-b-p(DMAEMA-co-BMA), 50B), were RAFT polymerized and characterized by gel permeation chromatography (GPC) and ^{1}H-NMR spectroscopy. Ternary polyplexes were formed at varying N/P ratios and varying ratios of the core (DM/DB) to corona forming polymers (0B/50B) for three classes of formulations: DB core/0B corona [DB-0B], DB core/50B corona [DB-50B], and DM core/50B corona [DM-50B] to produce a library. The siRNA loaded ternary polyplexes were assessed by dynamic light scattering (DLS) for size / zeta potential, the pH-dependent membrane disruption hemolysis assay as a gauge of endosomal escape, flow cytometry for cell uptake, and luciferase assays for siRNA target gene silencing and cytotoxicity.

Results: DB-0B, DB-50B, and DM-50B ternary formulations all formed stable and significantly more compact polyplexes (~15-30 nm) than the 0B or 50B (~100 nm) binary polyplexes. DB-0B polyplexes showed pH-dependent membrane disruption, but DB-50B and DM-50B polyplexes showed better tuned and more switch-like hemolysis triggered between extracellular pH (7.4) and early endosomal pH (6.8). 50B corona ternary polyplexes showed low cytotoxicity, whereas many 0B corona polyplex formulations were cytotoxic, strongly correlating with observation of hemolysis at pH 7.4. In vitro cell uptake was inversely proportional to the degree of PEGylation. The best DB-0B and DB-50B ternary formulations achieved significantly greater siRNA knockdown compared to their 0B and 50B binary parent polyplexes, respectively, as well as previously published ternary complexes.[2,3] Taken together, these results suggest that including the endosomolytic and hydrophobic DB polymer in the polyplex core was the most important variable for in vitro target gene knockdown.

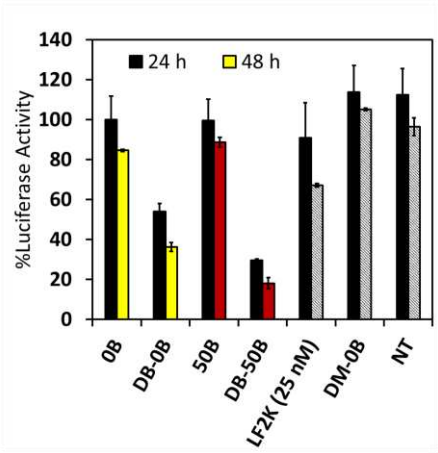

Figure 2. Best performing ternary polyplexes achieved 85% luciferase knockdown, significantly outperforming binary 0B and 50B polyplexes.

Conclusions: A combinatorial screen provided new insights into the relative importance of various design variables for siRNA polyplexes. This approach identified stable, endosomolytic, ternary polyplexes with optimized physicochemical properties (neutral zeta potential and ≤30 nm diameter) that were validated for potent in vitro knockdown and that will be further accessed for delivery, penetration, and bioactivity in tumors in vivo.

References: 1. Nelson, C. E. ACS Nano **7**, 8870-8880, (2013). 2. Kong, W.-H. Journal of Controlled Release **138**, 141-147, (2009). 3. Huang, Y. Biomaterials **33**, 4653-4664, (2012).