Screening of Novel Hydrophobically Modified Triblock Copolymers for Improved Stabilization of siRNA Polyplexes

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Introduction

Our lab has shown that diblock polymer-based siRNA nano-polyplexes (si-NPs), containing zwitterionic phosphorylcholine (PMPC) as the corona and a copolymer of dimethylamino ethyl methacrylate (DMAEMA), and butyl methacrylate (BMA) as the core, function as efficient vectors for siRNA delivery. These polymers possess several desirable properties including lack of toxicity, pH dependent endosomal escape, long circulation half-lives, and high levels of tumor cell uptake and silencing activity. Here, we hypothesized that a third, hydrophobic block of polypropylene sulfide (PPS), would yield si-NPs with higher stability and biocompatibility due to added hydrophobicity and core stabilization.

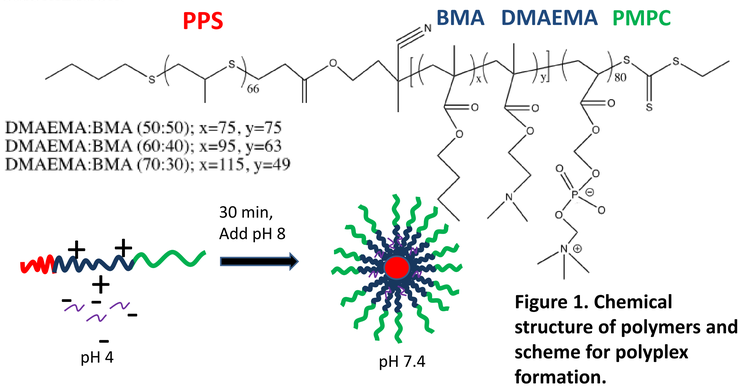
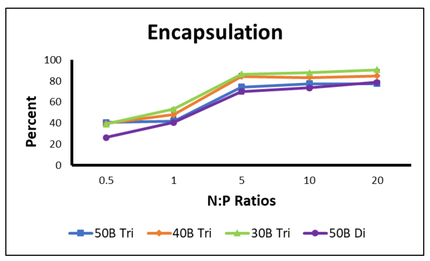


Figure 1. Chemical structure of polymers and scheme for polyplex formation.

RiboGreen Assay



All polyplexes efficiently encapsulate siRNA after N:P 10 and the higher DMAEMA ratio correlate with higher encapsulation.

Figure 2. Ribogreen study of polymers at various N:P Ratios

Dynamic Light Scattering

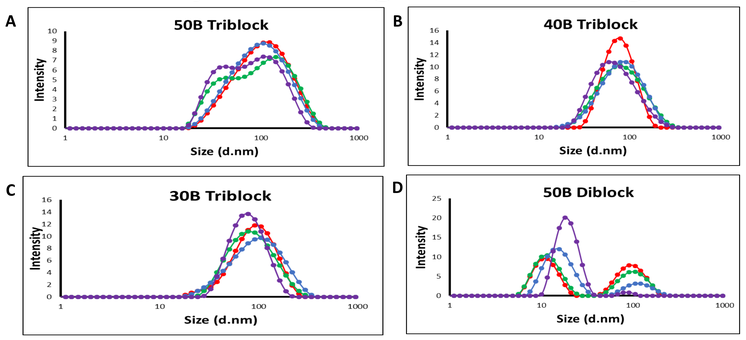
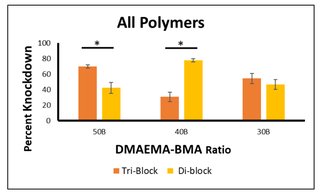


Figure 3. Dynamic Light Scattering (DLS) of polymers at varying pH Concentrations: pH 5.6 (red), pH 6.2 (green), pH 6.8 (blue), pH 7.4 (purple).

Unloaded polyplexes demonstrate pH-responsiveness.

Luciferase Knockdown Assay



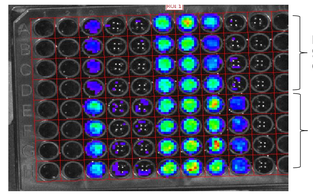
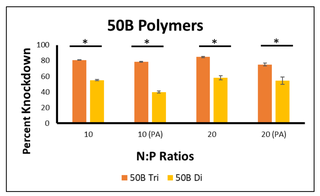


Figure 4. Knockdown of luciferase gene in MDA-MB-231 cells. (24 Hours)



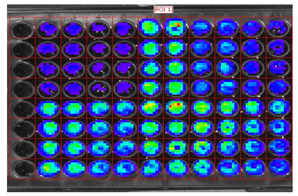


Figure 5. Knockdown of luciferase gene in MDA-MB-231 cells. Includes Palmitic Acid siRNA (PA) (24 Hours)

PPS block helped improve luciferase knockdown for the 50B polymer. Trends were different for 40B and 30B polymers due to toxicity.

Nanocarrier siRNA Loading Stability

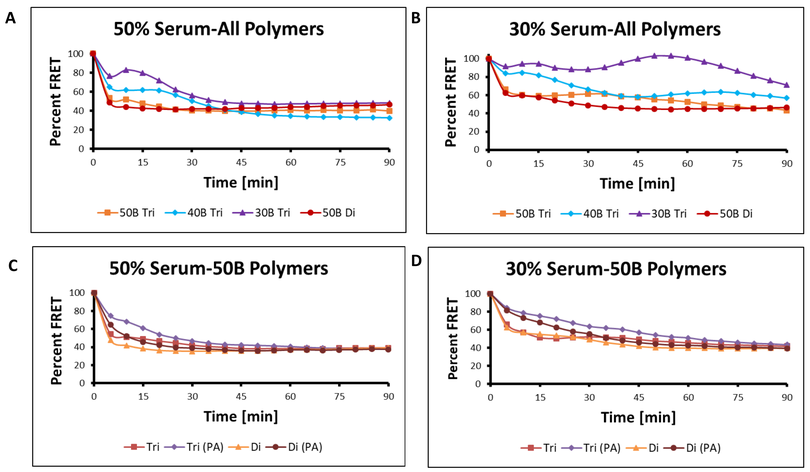


Figure 6. Fluorescence Resonance Energy Transfer Assay (FRET) shows stability of nanoparticles in FBS serum at different concentrations: All polymers study (A,B) and Tri vs. Di study with palmitic acid(C,D). All samples were at an N:P molar ratio of 20.

Triblock copolymers showed better stability as compared to the diblock model. Palmitic Acid improved stability for the 50B triblock.

Toxicity

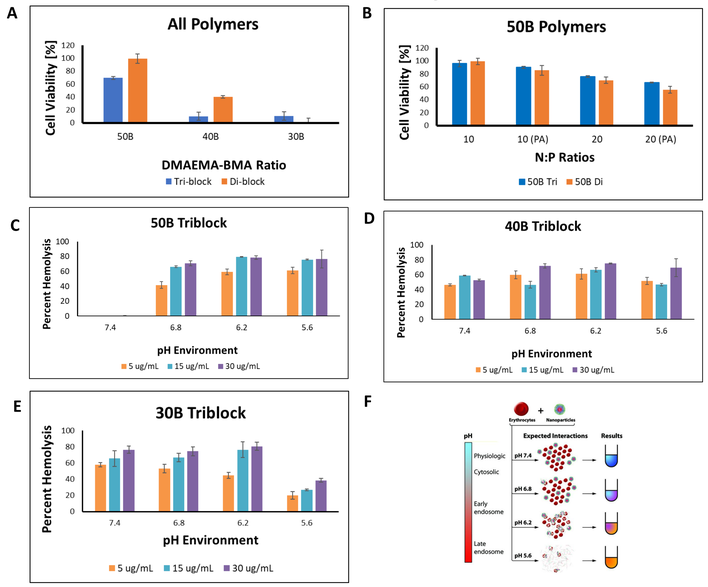


Figure 7.(A,B) Luciferase Viability Assay performed on MDA-MB-231. (C,D,E) Hemolysis Assay performed on Human Red Blood Cells with different concentrations. (F) Hemolysis mechanism.

Conclusions

- Hydrophobic third block shows impact in stability for the nanoparticles allowing for longer circulation life.
- PPS block increases knockdown efficiency for the 50B copolymer.
- PPS block in addition with palmitic acid siRNA-conjugates increase core stability.

Future Work

Further research for this library of copolymers would be an *in vivo* characterization to determine if the triblock has better circulation than the diblock. It would also be important to test different hydrophobic third blocks to improved stability. It would also be necessary to characterize the different lengths for the third block and finding the correct ratio of hydrophilic to hydrophobic units in the polymer.

References

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