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Positively-Charged Di-Block Copolymers for Delivery and Controlled Release of Nucleic Acids

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Extended Abstract

Therapeutic gene(s)-encoding nucleic acids (NAs) are promising pharmaceuticals for many human diseases, but their rapid enzymatic degradation, low cell-penetration efficiency, and lack of specific targeting limit their clinical application. Therefore, there is a growing demand for a biocompatible vector capable of providing delivered genes with efficient transfection. One way to efficiently deliver NAs *in vivo* is the use of synthetic polycationic vectors. However, it is challenging to design such a structure that will help the NAs overcome all extra/intra-cellular barriers and ensure sufficient and persistent production of therapeutic protein in the target cell. The development of such vectors was the subject of this work. Specifically, we focused on the preparation of positively-charged vectors based on di-block copolymers, synthesized by reversible addition-fragmentation chain transfer (RAFT). The positively charged blocks consisted of methacrylamide monomer units containing various types of ionizable groups (primary, secondary, tertiary, or quaternary amino group, and phosphine group) attached to the backbone via pH-sensitive hydrazone linkage. This bond was designed to resist at the neutral pH (~7.4) mimicking conditions in the bloodstream, but to disintegrate at the mildly acidic pH (~5.5) mimicking the intracellular environment. In addition, some positively charged blocks contained ~20 mol% of hydrophobic *N*-butyl methacrylamide comonomer units to enhance vector internalization. The second block was a hydrophilic electroneutral polymer, either based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) or 2-methacryloyloxyethylphosphorylcholine (MPC), increasing the stability of the vector during transport. The prepared di-block copolymers were mixed with a model non-coding NA (calf thymus DNA) or a plasmid (encoding mCherry protein) to form the hydrophilic polymer-stabilized polyelectrolyte complexes. The saturation of the charges of NAs and polycations, defining the formation of the complexes, was monitored by electrophoresis and fluorescence spectroscopy. The hydrodynamic sizes of the complexes, measured above the charge saturation ratio of the polyelectrolytes by dynamic light scattering, ranged from 75–585 nm depending on the type of NA and the polycation used. According to the preliminary results, the most stable complexes form polycations with a quaternary amino group, with charge saturation in the ratio of 2/1 positive/negative charge.