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Cationic block copolymer based nanocarriers for proteins and nucleic acids

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ABSTRACT:

Nanotechnological strategies for therapeutic and diagnostic purposes of life-threating diseases have attracted significant interest of the scientific community over the last decades, due to their beneficial characteristics compared to traditional treatment methods. Diseases such as cancer and diabetes mellitus are affecting more and more the population, so the shift to treatment methods, which are more compatible to human body, less painful and of fewer side effects, is essential. Nanocarriers are able to deliver pharmaceutical substances to human body with greater specificity and selectivity. In addition, they incorporate advanced physical and chemical properties, which enhance the pharmacokinetics and biodistribution, contribute to the high drug-loading efficacy and provide targeting of only damaged cells (Martinelli 2019). Block copolymers are a class of effective nanocarriers for the delivery of biomolecules, since they can be designed and synthesized according to the desirable features that should contain. Size, shape and morphology of the formed nanoparticles can be controlled through the design and synthesis of the block copolymer (Kataoka 2012). Moreover, favorable solubility and colloidal stability in aqueous solutions, as well as high cellular uptake efficiency can be achieved via utilization of block copolymers as nanovectors. Polyelectrolytes are hydrophilic polymers used as nanovectors due to their ability to interact with oppositely charged biomolecules via electrostatic interactions (Sun 2008). Cationic block polyelectrolytes have been used extensively for complexation and delivery of biomacromolecules, such as proteins/peptides and nucleic acids. The result of the interaction is the formation of complexes of nanoscale size. The biomolecules are condensed and protected, while the formed nanoparticles are compact and able to be delivered to targeted cells.

Insulin is administrated to patients suffering from diabetes. When cationic block copolymers are used as nanocarriers for insulin delivery instead of the conventional approaches, the insulin molecules are shielded from degradation and cellular uptake is enhanced (Kamenova 2018). Pippa et al. investigated the formation of complexes between insulin and poly[3,5-bis (dimethylaminomethylene)hydroxystyrene]-b-poly(ethylene oxide) (QNPHOSEO) block polyelectrolyte (Pippa 2015). Light scattering measurements were conducted to determine the properties of the mixed nanoassemblies, such as size, size polydispersity index and surface charge (ζ-potential) in aqueous media and FBS. It was concluded that the insulin/cationic block ratio determines the morphology and the behavior in solutions. Moreover, they noticed that solution parameters, like pH and ionic strength, play a significant role in complex formation. Increase of ionic strength led to charge shielding and decreased electrostatic interactions between components, leading to dissimilar structures of the complexes. Based on circular dichroism and FTIR measurements, structural changes of insulin, after the complexation, did not occur. When complexation experiments were conducted in biological media (FBS), no noticeable difference in the size of the complexes was observed, indicating that the QNPHOSEO copolymer promotes biological stability. Finally, in vitro insulin release studies showed a slow rate of insulin release from the complexes, especially when the higher initial concentration of insulin was utilized, indicating that insulin release can be in fact controlled by the ratio of the two species and the physicochemical conditions occurring during the complexation process.

Cationic block polyelectrolytes have been also utilized for complexation with nucleic acids via electrostatic interactions, between the negatively charged phosphate groups of nucleic acids and positively charged polyelectrolytes. The formed polyplexes between cationic block polyelectrolytes and DNA/RNA act as potential non-viral vectors in gene delivery, aiming to effective therapeutic strategies for the treatment of several intractable diseases. In these terms, Lou et al. developed small nano-sized polyplexes based on poly(vinyl benzyl trimethylammonium chloride) for siRNA delivery (Lou 2017). Small interfering RNAs (siRNAs) are non-coding RNAs, comprised of double stranded oligonucleotides (21-23 base pairs in length) with important functions in gene regulation. siRNAs have been investigated as a novel therapeutic tool, employed for inhibiting the expression of a specific messenger RNA and inducing gene silencing effects (Lam 2015). Cationic polymers based on poly(vinyl benzyl trimethylammonium chloride) (PVTC) homopolymer and two different poly[oligo(ethylene glycol) methacrylate]-b-poly(vinyl benzyl trimethylammonium chloride) (PVTC-b-POEGMA) block copolymers (Haladjova 2016) were evaluated as candidate non-viral vectors for efficient delivery of siRNA in human ovarian adenocarcinoma cells. The cationic polymers were examined for their ability to interact with siRNA and form stable nano-sized polyplexes in aqueous media at different nitrogen/phospate (N/P) ratios. Gel retardation assay indicated high binding affinity of cationic polymers with siRNA. Transmission electron microscopy, dynamic and electrophoretic light scattering were employed to further study polyplexes morphology, size and surface charge, respectively. PVTC-based polymers formed polyplexes with an average diameter smaller than 25nm, positive charge and colloidal stability under physiological ionic strength.

In vitro cytocompatibility and gene silencing activity of the polyplexes were investigated in human ovarian adenocarcinoma cell line (Skov-3-luc). PVTC-based polymers displayed lower cytotoxicity as compared to L-PEI (control). siRNA polyplexes of PVTC and PVTC-b-POEGMA (PP4) induced luciferase gene silencing in the same grade as Lipofectamine 2000 and presented higher gene silencing than L-PEI/siRNA. The cellular uptake of siRNA polyplexes by Skov-3-luc cells was studied by flow cytometry and confocal laser scanning microscopy. The results demonstrate that siRNA polyplexes of the block polymers displayed high cellular uptake and ~50% silencing of luciferase expression in the presence of serum. In summary, PVTC-based polymers have potentials as candidate vectors for siRNA delivery.

Furthermore, amphiphilic block copolymers consisted of a cationic block have been utilized for the encapsulation of magnetic iron

oxide nanoparticles, enhancing multifunctionality of hybrid gene delivery nanocarriers. Haladjova et al. reported the preparation of hybrid polymeric micelles based on the amphiphilic polystyrene-b-poly(quaternized 2-vinylpyridine) block copolymer (PS-b-P2QVP) loaded with magnetic iron oxide nanoparticles (Haladjova 2014).

The polymer-magnetic micelles were allowed to interact and form complexes with linear and plasmid DNA at various P/N ratios. The formed nanosized complexes (so called magnetopolyplexes) exhibit magnetic properties and in the presence of a magnetic field can target on specific tissues. Light scattering techniques indicated the formation of well-defined magnetopolyplexes with particle size below 360nm and zeta-potential values varying from positive to negative depending on the P/N ratios. Moreover, according to TEM results, hybrid micelles and magnetopolyplexes with linear and plasmid DNA have spherical shapes. However, morphological differences were observed depending on the P/N ratios. From the biological aspect, cytotoxicity experiments of the hybrid nanosystems showed low toxicity in human cell lines. The hybrid nanovectors transferred pEGFP-N1 into WISH human cells and presented higher degrees of internalization, compared to commercial transfectants (Lipofectamine, PEI). The application of magnetic field for magnetopolyplexes cellular uptake boosted their transgene expression efficiency and enhanced their ability to escape from the endolysosomal pathway.