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| **Histidylated poly(lysine) highly branched polymers for siRNA delivery** |
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| **Background:** siRNA has gained increasing interest in recent years because of its great promise in the field of gene silencing. siRNA works via the RNA interference (RNAi) mechanism, where it can potentially target and silence any gene. In the last few years, several siRNA medicines have been approved. These formulations, however, are mainly lipid-based or bioconjugates and their applications are limited to local or hepatic delivery, with extra-hepatic delivery, transfection efficiency and endosomal escape remaining as great challenges. Highly branched poly(amino acids) are attractive carriers for siRNA because of their versatile characteristics that allow for the synthesis of a wide range of structures based on the desired application. Highly branched lysine polymers have been widely investigated as carriers for siRNA delivery, but they raise concerns of toxicity and low transfection efficiency. The addition of histidine could reduce toxicity, enhance endosomal escape, and improve transfection. In this work, we investigate different approaches for the synthesis of histidylated poly(lysine) highly branched polymers as biocompatible carriers for the safe and efficient delivery of siRNA. |
| **Methods:** We used different approaches to synthesise two types of highly branched histidylated poly(lysine) polymers. Hyperbranched poly-lysine-co-histidine polymers (pKH) were synthesised following a one-pot thermal polycondensation reaction. Poly(lysine) and histidylated poly(lysine) dendrimers were synthesised following solution- and solid-phase synthesis approaches. The structures of these polymers were confirmed by 1H NMR. Polyplex formation was confirmed with gel electrophoresis and particle size and zeta-potential were measured by dynamic light scattering. Cytotoxicity was assessed using PrestoBlue cell viability assay. |
| **Results:** We synthesised several pKH polymers containing different lysine:histidine ratios. pKH polymers showed structural heterogeneity which affected their complexation efficiency with siRNA. As a result, we decided to follow a more controlled approach to synthesise histidylated poly(lysine) dendrimers. We successfully produced poly(lysine) and histidine-capped poly(lysine) dendrimers following solution-phase synthesis. However, NMR analysis showed traces of impurities in the resultant dendrimers. These dendrimers showed efficient complexation of siRNA but polyplexes were slightly polydisperse. Solid-phase synthesis allowed for better purification, higher yield and more flexibility to create various structures of histidylated poly(lysine) dendrimers. We created a small library of histidylated poly(lysine) dendrimers containing different lysine and histidine ratios. All solid-phase dendrimers showed complete complexation efficiency with siRNA and resulted in monodispersed polyplexes <150 nm in size. Polyplexes showed a good cell viability of > 90%. |
| **Conclusions:** Structural variations of hyperbranched pKH polymers have resulted in an irreproducible complexation behaviour with siRNA. Dendrimers made with both solution and solid-phase synthesis showed a better complexation efficiency with siRNA than pKH polymers. However, solid-phase synthesis allowed for higher purity and a better yield than solution-phase. Polyplexes formed with histidylated poly(lysine) dendrimers showed high cell viability. Future work will investigate the transfection and gene silencing efficiency of these polyplexes. |