論 文 の 内 容 の 要 旨

論文題目

Development of silicated polyion complexes and their characterization for nucleic acids delivery

(シリカ層により被覆されたポリイオンコンプレックスの調製と その核酸デリバリーへの応用に関する研究)

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This dissertation contributes to the enhancement of the functionality of nucleic acids/ polyion complexes delivery systems. Nucleic acids, such as plasmid DNA (pDNA) and short interfering RNA (siRNA), are promising bioactive macromolecules engaged actively in the gene therapy and RNA interference (RNAi) approaches for a potential successful treatment of cancers. Nevertheless, for their effective application in cancer therapy, hurdles of the instability of their naked molecules and/or their delivery systems and their poor cell uptake need to be overcome. Polyion complexes (PICs) prepared from electrostatic interactions between the anionic nucleic acids and cationic polymers are advantageous over other delivery systems in being capable of dynamic associative–dissociative conversions for the loading and release of the nucleic acids. Hitherto, instability of PICs in biological milieu due to immature dissociation or aggregation is still problematic.

This study investigates the concept of shielding the PIC surface by a silica coating layer to enhance its stability and biological activity. The dissertation is commenced by an introductory chapter pertaining to a literature survey of the main concepts upon which this research is based: nucleic acids properties, therapy approaches and clinical applications in cancer treatment, overview of nanoparticle delivery systems with a special emphasis on PIC systems and silica-based systems. The body of the thesis is concerned with two types of PIC systems explored for silica coating: a pDNA/PIC (chapter 2) and a core-shell cross-linked siRNA/PIC micelles system (chapter 3), before elucidating the final conclusions and perspectives in chapter 4.

Chapter 2 results showed a superior effect of silica coating on stability and transfection efficiency for the examined pDNA/polyarginine (PArg) PICs. The pDNA/PArg PICs prepared via the electrostatic interaction between anionic pDNA and cationic PArg were successfully shielded by a silica coating via the condensation of the negatively charged silicic acid and produced negatively charged PICs in the range of 100 nm. The silica coated PICs were stable in the presence of a counter polyanion dextran sulfate. This stabilizing effect was proved to be reversible and to enhance the PIC transfection efficiency on a hepatoma cell (HuH-7).

This successful silica coating of pDNA PICs encouraged testing the same concept on siRNA PICs in Chapter 3. The template PICs were formed by the interaction between anionic siRNA and poly(ethylene glycol)-*block*-poly(L-lysine) with a mercaptopropylamidine side chain (PEG-b-PLL(MPA)) to form disulfide-crosslinked core-stabilized PIC micelles with a small size and a narrow size distribution. The applied silica coating resulted in the formation of silica-coated PIC micelles (SCM) with a size less than 120 nm and a narrow size distribution (polydispersity index < 0.2). Transmission electron microscopy (TEM) and energy dispersive-x-ray scanning TEM confirmed the presence of silica layer on the SCM surface. Surface shielding of core-stabilized PIC micelles increased their stability in presence of counter polyanions. The stabilizing effect did not interfere with the environment sensitivity of this PIC system, *i.e.*, disulfide cleavage in reductive environments. The obtained silica-coated systems allowed good cell viability even at the highest concentration tested (500 nM siRNA) against a cervical carcinoma cell (HeLa), and further, they significantly improved gene silencing efficiency up to 28 % in comparison with non-coated PICs (NSCM) in spite of the significantly lower cellular uptake in 3-hour incubation with the cells. Confocal microscopy observation studies with fluorescently labelled siRNA revealed that the colocalization of SCM with the late endosomes /lysosomes was significantly lower than in case of NSCM, suggesting a facilitated endosomal escape of micelles by silica coating and/or different cellular uptake routes between SCM and NSCM. The cellular uptake studies using selective inhibitors and the pulse and chase intracellular trafficking gave more insights on the internalization process of SCM where it appeared that NSCM and SCM had different endocytosis patterns, through which

more siRNA could be released in the cytoplasm when applied in the form of SCM. In conclusion, this work is presenting a novel approach to stabilize the nucleic acid-incorporating PICs via silica coating.

The surface shielding of PICs by silica coating seems to provide a reversible stabilizing effect for enhanced delivery efficacy of therapeutic nucleic acids and a promising strategy to improve the functionality of PIC systems for *in vivo* applications.