論文 / 著書情報
Article / Book Information

題目(和文)	プラスミドDNAデリバリーを指向したpH変換型ポリ双性イオン導入ナノシステムの開発
Title(English)	Development of Stepwise pH Responsive Polyzwitterion Introduced Nanocarrier for Plasmid DNA Delivery
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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	ライフエンジニアリング 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Philosophy)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

One of the major hurdles of gene therapy is the difficulty in the delivery of a therapeutic gene to the cytosol at the tumor site. The surface modification of nanocarriers using PEG is currently a common strategy for designing gene delivery systems to facilitate stealth function and tumor targeting through the leaky tumor vasculature. However, PEGylation impairs the internalization of gene carriers against cancer cells leading to low cellular uptake and unsound gene transfection efficiency. To overcome such unfavorable influences brought by the PEGylation, this research developed a pH-responsive polyzwitterion-coated micelle using PGlu(DET-Car)-bPEI.

Based on the PBLG backbone, N_3-PGlu(DET-Car) bearing ethylenediamine-based carboxybetaine moiety at the side chain was prepared. Through the investigation of the protonation behavior of N_3-PGlu(DET-Car), this compound displays a regulated ionization of cationic groups, exhibiting pH-responsive characteristics in a reversible manner. In parallel, N_3-PEG, possessing a comparable molecular weight, has been utilized as a non-ionic control within the study.

Moreover, the PMs were formulated by mixing the pDNA and polymer solution at varying N/P ratios. DLS measurements revealed all PMs exhibited uniformed hydrodynamic sizes ranging from 82 - 135 nm with relatively narrow PDI. Importantly, PGDC PM which processed a controlled ionization of cationic moieties exhibited pH-responsive performance in a reversible manner. This stepwise protonation behavior of PGlu(DET-Car) might supply the potential of the charge-switchable property to PMs. The C/A ratio of PGlu(DET-Car) was 1.09 at pH 7.4 which then increased to 1.39 at pH 6.5, suggesting that PGlu(DET-Car) switches its neutral property to cationic at tumorous pH. When exposed to pH 5.5, PGlu(DET-Car) exhibited a C/A value of 1.84, suggesting substantial protonation of the ethylenediamine groups in PGlu(DET-Car). This stepwise increase in the ζ-potential of PGDC PMs with varying N/P rations was attributed to the progressive protonation of the ethylenediamine-based carboxybetaine group with regard to the decrease in pH. PGDC PM prepared at the N/P ratio of 15 represented a desirable characteristic that the ζ-potential values switched from negative at pH 7.4 to positive at pH 6.5. The value of ζ-potential was further amplified at pH 5.5. This charge-conversion behavior of PGDC PM should be applied for recognizing the narrow pH window in the route of gene delivery, i.e., tumor microenvironment (pH 6.5) and endosomes (pH 5.5).

With the above-optimized formulation (N/P = 15), positively charged PGDC PM (+ 6.8 mV) at pH 6.5 promoted electrostatic interactions with the negatively charged cell membrane, resulting in the enhanced uptake and concomitant internalization of PGDC PM in the acidic tumor microenvironment. Additionally, PGDC PM was shown to enhance the endosomal escape, as observed by the consistent results in the colocalization study of Cy5-labeled pDNA with endo-/lysosomes and the calcein assay. This was due to the increment in the cationic charge density of the PGDC shell (value shifts from 1.39 to 1.84), which provides + 15 mV of ζ-potential for PGDC PM at pH 5.5 in the endo-/lysosomal compartment-mimicking conditions. Namely, the electrostatic interactions between PGDC PM and the negatively charged endo-/lysosomal membrane might contribute to the endosomal membrane destabilization in addition to the proton sponge effect. Contrarily, the PEG shell prevented the interactions with the negatively charged membranes, leading to low cellular uptake and endosomal escape of PEG PM. Moreover, the Luc expression is known to be closely interrelated with the intracellular trafficking profile of the PMs. Our PGDC PMs showed enhanced transfection efficiency at pH 6.5 compared to pH 7.4, while other control groups showed no difference, as evidenced by delivering Luc-pDNA into Neuro-2A cells. Overall, we revealed the stepwise charge-switchable property of PGDC PM, thereby facilitating the cellular uptake and endosomal escape, leading to the enhanced gene transfection in vitro.

From the *in vivo* investigation, PGDC exhibited enhanced tumor accumulation and tumor-specific

gene expression, further highlighting the potential of PGDC micelles as effective carriers for targeted gene delivery. The therapeutic capabilities of PGDC PM by using sFlt-1 DNA shed light on the potential of PGDC PM as a vehicle for delivering therapeutic genes and exerting antiangiogenic effects. By trapping vascular endothelial growth factor (VEGF), this gene therapy approach aimed to prevent the formation of tumor vessels. Finally, I conducted comprehensive assessments of the biocompatibility of the PGDC PM to ensure its optimal utilization in future biological contexts. These evaluations are crucial for considering the clinical translation and practical applications of PGDC-based micelles in gene therapy and other biomedical fields.

Overall, PGDC PM has provided valuable insights into the potential applications of PGDC-based micelles in gene delivery and therapy. The findings contribute to the growing body of knowledge in the field of nanomedicine, emphasizing the importance of pH-responsive carriers in improving gene transfection efficiency and targeting specific tissues or diseases. The outcomes of this research open up new avenues for further investigations and potential advancements in the development of safe and effective gene delivery systems.