Smart Polymeric Micelles as Nanocarriers for Gene and Drug Delivery

Kazunori Kataoka[1,2,3]

[1]Department of Materials Engineering, The University of Tokyo, Tokyo, 113-8656 Japan
[2]Center for Disease Biology and Integrative Medicine, The University of Tokyo, Tokyo, 113-8656 Japan
[3]Center for NanoBio Integration, The University of Tokyo, Tokyo, 113-8656 Japan
kataoka@bmw.t.u-tokyo.ac.jp

Introduction

During the past decade, polymeric micelles have been proven to be useful in drug delivery, and several formulations have been studied in clinical trials. Polymeric micelles are characterized by a size of several tens nm and unique core-shell structure, where the drug-loaded core is surrounded by biocompatible poly(ethylene glycol) (PEG) chains. These characteristics of polymeric micelles allow their longevity in the bloodstream and effective tumor accumulation after their systemic administration. Besides the hydrophobic interaction, an electrostatic interaction between charged block copolymers and oppositely charged macromolecules leads to the formation of the polyion complex (PIC) micelles. This system is potentially useful for the delivery of genes and siRNA. Recent advances in synthetic polymer chemistry and biotechnology allow the development of "smart" gene and siRNA carriers based on PIC micelles equipped with environmentally sensitivity and specific tissue targetability. In this presentation, our recent studies on polymeric micelles as smart nanocarriers for drug delivery will be highlighted.

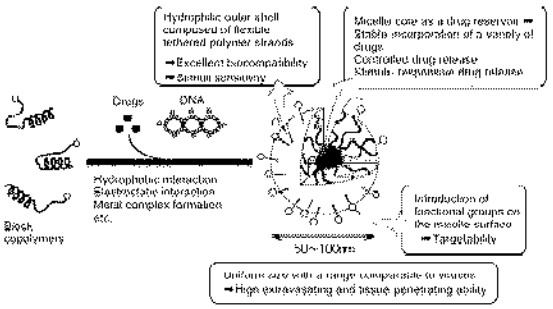

Figure 1 Smart polymeric micelles for drug and gene delivery

Results and discussion

1) Smart micelles for site-specific drug delivery

Recently, we have developed pH-sensitive polymeric micelles, in which doxorubicin (Dox) is attached to the side chain of the core-forming segment of the block copolymers via an acid-labile hydrazone bond (Figure 2)[1]. The pH-sensitive polymeric micelles showed a significant drug release under endosomal / lysosomal low pH conditions (5.0~5.5), while exhibiting no appreciable release under physiological pH conditions[1]. A biodistribution study revealed that the pH-sensitive polymeric micelles showed a prolonged blood circulation due to a minimal leakage of free drug, resulting in the highly selective accumulation in solid tumors [2]. As a result, the pH-sensitive polymeric micelles achieved a significantly higher antitumor activity in C-26-bearing mice over a broader range of injection doses than free Dox [2]. Also, we modified the pH-sensitive polymeric micelles with a folate molecule to construct the polymeric micelles with the tumor-targetability (Figure 3) [3]. It is known that a large

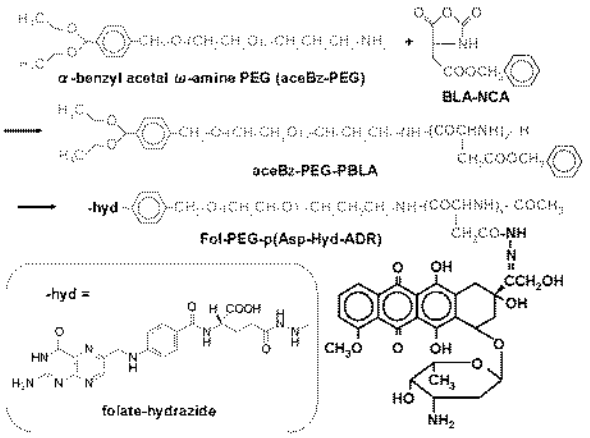

Figure 2 Preparation of the pH-sensitive micelles for the intracellular delivery of ADR

Figure 3 Preparation of the multifunctional polymeric micelles for active drug delivery

number of cancer cells overexpress folate-binding proteins (FBP). The folate-conjugated micelles were more efficiently taken up by the FBP-overexpressing human pharyngeal cancer KB cells than the non-targeted micelles. In the cytotoxic activity assay against KB cells, the folate-conjugated micelles showed a comparable cytotoxicity to free Dox after a 24-h exposure time, whereas the non-targeted micelles had almost a 10-fold lower cytotoxicity than free Dox [3]. It is unprecedented that the folate-conjugated micelles achieved as high cytotoxicity as free Dox despite their different internalization pathways. This result indicates that the use of the folate-conjugated micelles may lower the effective doses over free Dox, improving the safety of the clinical chemotherapy

2) DP-loaded micelles for photodynamic therapy

Photodynamic therapy (PDT), which involves the systemic administration of photosensitizers (PSs) and the following photoirradiation to the diseased sites, is a promising approach for the treatment of malignant tumors and macular degradation. However, it is known that PSs are easily form aggregates, resulting in reduction of the singlet oxygen production efficiency. To prevent the self-quenching of PS, we developed ionic dendritic PS (DPs) (Figure 4), and incorporated them into the PIC micelles [4]. The DP-loaded micelles showed no self-quenching of DP inside the PIC micelles, although each micelle contains an average of 38 DP molecules [4]. No

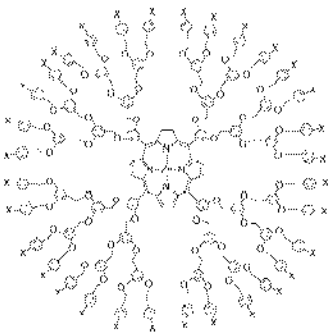

Figure 4 Chemical structure of ionic dendritic porphyrin (DP) (X = COO^-Na^+).

quenching of DP inside the micellar core is attributable to the steric hindrance of the interaction between the dye molecules by the dendritic framework. Interestingly, the DP-loaded micelles showed a 280-fold increase in the photocytotoxicity to LLC cells compared to free DP [4]. In animal experiments, the DP-loaded micelles showed effective accumulation in the choroidal neovascularization (CNV) lesions in the rat eye, and the laser application resulted in a 60-78% CNV occlusion, offering a new nanotechnology-based treatment of age-related macular degeneration (AMD) [5].

3) Smart nanocarriers for gene and siRNA delivery

The success in gene therapy relies on the development of the safe and effective gene carriers. In this regard, PIC micelles (polyplex micelles), which are formed between plasmid DNA (pDNA) and PEG-polycation block copolymers have received much attention due to their small size (~ 100 nm) and excellent biocompatibility. Recently, we synthesized PEG-polycation carrying the ethylenediamine moiety (PEG-PAsp(DET)) at the side chain (Figure 5) [6]. Due to the unique feature of PEG-PAsp(DET) with the

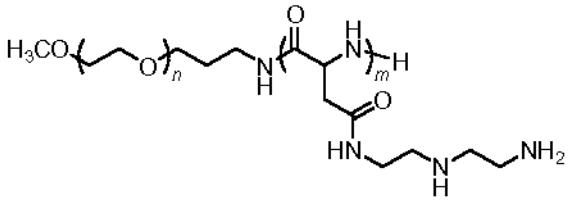

Figure 5 Structure of PEG-PAsp(DET)

regulated location of primary and secondary amino groups, this block copolymer possessed both the sufficient DNA complexation ability and buffering capacity for the efficient endosomal escape of the polyplexes based on the proton sponge effect. The polyplex micelles from PEG-PAsp(DET) showed the excellent gene expressions comparable to ExGen500 in many cell lines; nevertheless, this polymer exhibited remarkably lower cytotoxicity than other transfection reagents. These properties of PEG-PAsp(DET) enabled the transfection to various primary cells [6]. In addition, the PEG-PAsp(DET) polyplex micelles incorporating pDNA encoded with osteogenic factors were implanted to mouse calvaria bone defects in the form of calcium phosphate paste. This implant device transfected adjacent recipient cells and rapidly induced bone regeneration. Thus, the polyplex micelles from PEG-PAsp(DET) might be a useful nanocarrier for local gene delivery.

4) Novel gene carriers for light-induced gene transfer

The site-specific gene transfer in the body is strongly desired; however, the existing vectors might lack the ability to control the gene expression. Recently, a new technology called "photochemical internalization (PCI)" has recently emerged, in which the endosomal escape of the polyplexes is induced by co-incubated PS which photodamage the endosomal membrane, allowing the gene transfection in a light-inducible manner [7,8]. This strategy is quite smart; however, the emergence of significant photocytotoxicity was also found, possibly limiting its further applications for *in vivo* use. To solve this problem, we assumed that the control of the intracellular localization of PSs should be of primary importance, and the photosensitizing property is preferably integrated into the gene delivery system. Recently, we developed novel ternary complexes, in which the pDNA/polycation polyplex is enveloped with the anionic DPc for effective PCI-mediated transfection (Figure 6)[9]. As a result, the ternary complexes achieved more than a 100-fold photochemical enhancement of the transgene expression *in vitro* with reduced photocytotoxicity [9]. The subconjunctival injection of the ternary complexes in rat eyes followed by the laser irradiation resulted in an appreciable gene expression (a variant of yellow fluorescent protein) only at the laser-irradiated site [9]. These results are the first success of the PCI-mediated gene delivery *in vivo*. These light-responsive gene carriers are expected to be useful for the site-directed transfection *in vivo*.

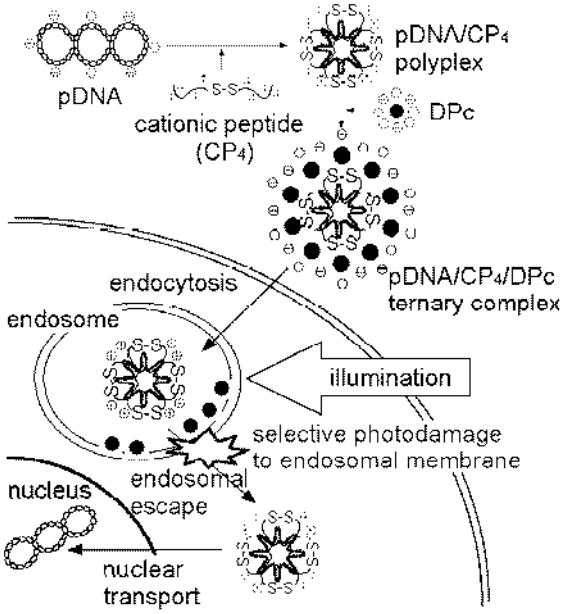

Figure 6 Preparation of the DPc-enveloped ternary complexes and their hypothetical mechanisms in the light-induced gene transfection

References

[1] Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 4640.

[2] Bae, Y.; Nishiyama, N.; Fukushima, S.; Koyama, H.; Matsumura, Y.; Kataoka, K. *Bioconjugate Chem.* **2005**, *16*, 122.

[3] Bae, Y.; Jang, W.-D.; Nishiyama, N.; Fukushima, S.; Kataoka, K. *Mol. Biosyst.* **2005**, *1*, 242.

[4] Jang, W.-D.; Nishiyama. N.; Zhang, G.-D.; Harada, A.; Jiang, D.-L.; Kawauchi, S.; Morimoto, Y.; Kikuchi, M.; Koyama, H.; Aida, T.; Kataoka, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 419.

[5] Ideta, R.; Tasaka, F.; Jang, W.-D.; Nishiyama, N.; Zhang, G.-D.; Harada, A.; Yanagi, Y.; Tamaki, Y.; Aida, T.; Kataoka, K. *Nano Lett.* **2005**, *5*, 2426.

[6] Kanayama, N.; Fukushima, S.; Nishiyama, N,; Itaka, K.; Jang, W. -D.; Miyata, K.; Yamasaki, Y.; Chung, U. -I.; Kataoka, K.; Kanayama, N. *ChemMedChem* **2006** *1*, 439.

[7] Høgset, A.; Prasmickaite, L.; Tjelle, T. E.; Berg, K. *Hum. Gene Ther.* **2000**, *11*, 869.

[8] Høgset, A.; Prasmickaite, L.; Selbo, P. K.; Hellum, M.; Engesæter, B. Ø.; Bondted, A.; Berg, K. *Adv. Drug Deliv. Rev.* **2004**, *56*, 95.

[9] Nishiyama, N.; Iiyama, A.; Jang, W.-D.; Miyata, K.; Itaka, K.; Inoue, Y.; Takahashi, H.; Yanagi, Y.; Tamaki, Y.; Koyama, H.; Kataoka, K. *Nature Mater.* **2005**, *4*, 934.