Polyion Complex-Coated Polymeric Micelles with Highly Stability as Cell-Specific Drug Delivery Vehicles

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Introduction: Polymeric micelles prepared by amphiphilic block copolymers are of considerable interest in the biomedical field as nanometer-scale drug delivery carriers. Polymer micelles show relatively long-term circulation in the bloodstream after intravenous administration by preventing renal and reticuloendothelial system (RES) clearance. However, there are still problems of instability of such polymeric micelles in body fluids after intravenous injection, due to dissociation under diluted conditions below the critical micelle concentration and interaction with blood components.

We recently reported that polyanion-coated biodegradable polymer micelles prepared via polyion complex (PIC) formation with polyanionic hyaluronic acid (HA) on the positively charged micelles consisting of a biodegradable amphiphilic block copolymer, poly(L-lysine)-*b*-poly(L-lactide) (PLys-*b*-PLLA), HA-coated micelle, exhibited extremely high stability against dilution and colloidal stability (Fig. 1)[1,2]. Using this polyanion-coated micelle system, chemical modification and functionalization of micelle surfaces can be easily achieved by changing the type of outer polyanions, including chemically modified polyanions.

Liver sinusoidal endothelial cells (LSECs) are reported to possess receptors that recognize and internalize HA[3], and liver parenchimal cells have receptors for galactose (Gal). In this study, to evaluate the potential utility of HA-coated micelles as LSEC-specific drug carriers, uptake of HA-coated micelles into LSECs were investigated[5]. Moreover, to achieve specific drug delivery liver parenchimal cells, galactose modified HA-coated micelles (Gal-HA-coated micelle) were prepared, and the specific interaction with HepG2 (liver parenchimal cell derived cancer) cells was investigated.

Methods: FITC-labeled HA-coated micelles were prepared by the method reported previously[2]. Polymeric micelles coated with heparin (Hep) or carboxymethyl-dextran (CMDex) were used as controls. FITC-labeled Hep-coated micelle and CMDex-coated micelle were synthesized by the same methods. Gal-HA-coated micelle was prepared using HA attaching galactose residues. The hydrodynamic diameter (D_h) of the obtained micelles was determined by dynamic light scattering (DLS) measurements at 37 °C using a DLS-7000 photometer. ζ-Potential measurements were carried out on a Zetasizer Nano Z. The uptake of the micelles into LSEC and Kupffer cells were investigated by using a flow cytometer (Beckman Coulter). The interaction of Gal-HA-coated micelle was evaluated by a confocal laser microscope (CLMS) and a flow cytometer.

Results: FITC-labeled HA-coated, Hep-coated, and CMDex-coated micelles could be successfully prepared. All of these micelles showed negative ζ-potential values and were tens to hundreds of nanometers in diameter in water. Fig. 1 shows the results of flow cytometric analysis for the interaction with LSECs and Kupffer cells. Hep-coated micelles and CMDex-coated micelles were efficiently incorporated into both of LSECs and Kupffer cells exhibiting no specificity. Interestingly, almost no HA-coated micelles were incorporated into Kupffer cells (Fig. 1d). Although the uptake efficiency was lower than Hep- and CMDex-coated micelles, HA-coated micelles showed a certain amount of uptake into LSECs. These results suggested HA-coated micelles were taken up into LSECs and could escape from Kupffer cell, which is one kind of RES cells.

Gal-HA-coated micelle was also successfully prepared. The interaction of FITC-labeled Gal-HA-coated micelles with HepG2 cells were investigated by CLMS. Efficient uptake of Gal-HA-coated micelles into HepG2 cells was observed.

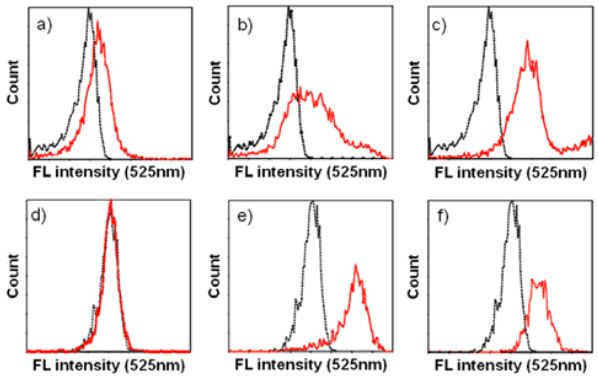

Figure 1. The results of flow cytometric analysis for uptake of HA-coated micelles, Hep-coated micelles, and CMDex-coated micelles into LSECs and Kupffer cells. Black dotted line: cells only; red solid line: cells + micelles. a, b, c: LSECs and d, e, f: Kupffer cells; a, d: HA-coated micelles; b, e: Hep-coated micelles; c, f: CMDex-coated micelles.

Conclusions: It is most important to suppress non-specific interactions for drug delivery vehicles to achieve efficient drug targeting to specific sites (cells or organs). We demonstrated the control of specific and non-specific interactions of the polyanion-micelles by careful selection of the outer polyanions. This system is a good candidate for an effective drug delivery vehicle that not only survives long-term circulation in the bloodstream and has high stability in body fluids, but also has an affinity to specific sites in the body.

References: (1) Ohya Y. et al. Macromol Chem Phys 2010; 211: 1750. (2) Ohya Y. et al. J Contr Rel 2011; 155: 104. (3) Yannariello-Brown J. et al. J Biol Chem 1992; 267: 20451.