審査の結果の要旨

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Active targeting mediated by ligand molecules has been attracting great attention in the field of drug delivery system (DDS) for the purpose of improving the selectivity to diseased tissues and cells. Small compounds directed to cell surface receptors and antibodies binding to proteins overexpressed on targeted cells are principally considered as ligand molecules for promoting targeting. Recently, emerging ligand strategies targeting membrane transporters by using the small molecules that pass through them have attracted much attention. Small molecules which have low binding affinity to the targeted transporters can be utilized as a ligand by taking advantage of multivalent recognition on the surface of nanoparticulated DDS carrier. Therefore, it is crucial to have a versatile platform that allows effective ligand presentation on the surface for achieving multivalent binding of ligands. Polymeric micelles are one of the most comprehensive platforms that can allow precisely controlling ligand installation, as well as being used for DDS to several diseases, such as cancer and central nervous system (CNS) disorders. Previously, glucose-installed polymeric micelles achieved the targeting of cancer cells and brain tissues crossing the blood-brain barrier (BBB) by recognizing glucose transporter-1 (GLUT-1). However, there still remains uncovered structural parameters of ligand installed polymeric micelle which affect to the function of polymeric micelle as a biomaterial and the target recognition ability of ligand molecules. Here, I focused on a versatile micelle platform, *i.e.*, polyion complex micelles (PIC/m), which permits engineering various parameters affecting ligand installation and recognition. To apply PIC/m to this research, the formulation was first optimized to present prolonged blood circulation *in vivo*. This was achieved by fine-tuning the charge mixing ratio of oppositely charged segments that compose the block copolymers forming the micelle. Then, the effect of chain length of the hydrophilic segment in the block copolymers and the influence of the spacer between the ligand and the shell of the micelles on the target recognition were investigated to develop formulations with enhanced delivery to brain tissues.

In **Chapter 1**, the background knowledge of this study was described. A general introduction of nanoparticulated DDS carriers, in particular, ligand-installed polymeric micelle for active targeting, as well as PIC/m, as a platform of DDS carrier for the investigation of general structural parameters regardless of the type of encapsulating drugs was given.

In **Chapter 2**, the structural characteristics of PIC/m prepared with varying charge mixing ratio in oppositely charged segment in the core was described. Although it is widely known that numerous parameters, such as the ionic strength of solvents and environmental pH, as well as the degree of polymerization of block copolymers, affect the physical properties of PIC/m as a versatile biomaterial, the effect of charge mixing ratio of core forming oppositely charged segment has not been investigated yet. Hydrophilic surface properties, such as size distribution, morphology and surface potential, and the structural properties attributed by the core-forming segment, were investigated. Moreover, the performance of PIC/m with different structural features in the core in the biological system were evaluated, and then the essential parameters of PIC/m as a DDS carrier were

proposed. The optimal charge mixing ratio of PIC/m that inhibits the adsorption to the liver sinusoidal walls, and prolongs the circulation in the bloodstream was identified.

In **Chapter 3**, based on the PIC/m preparation condition optimized in **Chapter 2,** the target efficiency of glucose-installed PIC/m (G-PM) as a DDS carrier was evaluated, particularly focusing on their ability to actively penetrate BBB by targeting GLUT-1 *via* glucose ligand molecules. For the purpose of developing the versatile platform of DDS effectively penetrating BBB, it is necessary to obtain quantitative structural parameters that affect the multivalent target recognition of G-PM. The crucial parameters that determine the binding affinity of G-PM to GLUT-1 were defined, as follows: 1) chain length of hydrophilic segment, which is poly(ethylene glycol) (PEG) in this research, and 2) distance between two ligand molecules on the surface of micelles. Long PEG chains with a molecular weight of 12 kDa decreases the target recognition of ligand molecules due to high steric repulsion compared to short PEG chains with a molecular weight of 5- and 2-kDa. G-PM with 2 kDa PEG achieved dramatically increased target recognition when a distance between two ligand molecules was less than 10 nm, so that G-PM with 2 kDa PEG obtains the multivalent effect with higher ligand density.

In **Chapter 4**, G-PM were further engineered with the technique called cocktail PEGylation, which allows increasing the target recognition of ligand molecules conjugated to longer PEG chain. G-PM with 12 kDa PEG could not achieve ligand recognition in **Chapter 3**, but longer PEG chain is preferable for stable micelle formation. Cocktail PEGylation dramatically improved the binding affinity to targeted molecules, as well as brain delivery of G-PM bearing the ligand molecules attached to 12 kDa PEG blocks. My findings indicate that the mixing ratio of short PEG chain critically determines the binding affinity of cocktail PEGylated G-PM to GLUT-1. Thus, precise control of the short PEG chain ratio is required to increase BBB penetration of G-PM with suppressed stacking to brain endothelial cells because of restrained dissociation from GLUT-1.

In **Chapter 5**, the results and significance of each chapter were summarized, and the future perspectives of this study for developing effective therapeutic method of CNS disorders were described.

In this dissertation, by analyzing the effect of structural parameters of PIC/m on the self-assembly process, ligand presentation and targeting capability in biological systems, I was able to improve current knowledge on the preparation of PIC-based nanoparticulated materials, which could allow more efficient and effective carriers with tunable structural and functional characteristics for controlling *in vivo* action. Moreover, the observations of my work provide general information on structural parameters of G-PM to enhance the accumulation in brain, as well as rationalize the structural design of DDS based on G-PM as a carrier of various types of drugs, such as nucleic acids and antibodies, for the treatment of CNS disorders. Furthermore, these findings may contribute to other fields of bioengineering by providing useful structural guidelines for biomaterials employing multivalent ligand recognition. According to the reviewers' comments, this dissertation is eligible for applying a diploma of Ph. D. (engineering).