論文 / 著書情報
Article / Book Information

題目(和文)	ポリサルコシン修飾リポソームの調製と細胞内送達および薬物動態挙動
Title(English)	Preparation of Polysarcosine-Coated Liposomes and Their Intracellular Delivery and Pharmacokinetic Behavior
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第12562号, 授与年月日:2023年9月22日, 学位の種別:課程博士, 審査員:小畠 英理,三重 正和,金原 数,丸山 厚,堤 浩
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12562号, Conferred date:2023/9/22, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 コース Life Science and Technology	申請学位（専攻分野）： Academic Degree Requested	博士 （工学） Doctor of
学生氏名： Student's Name	HU MINGXIN	審査員主査： Chief Examiner	Eiry KOBATAKE

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

This thesis deals with the studies on the internalized behavior of liposomes into cells and the pharmacokinetic behavior *in vivo* of polysarcosine (PSar) coated liposomes. PSar is used as an alternative polymer to polyethylene glycol (PEG) for modifying liposomes. The effects of length and density of PSar on liposome surfaces are studied in detail, which is expected to find the optimal PSar chain formulation for liposome surface modification.

Chapter 1 summarizes the development of liposomes in the drug delivery system (DDS) field. The surface modification of liposomes with various hydrophilic polymers is highlighted as a promising strategy to overcome their problems for *in vivo* applications. Especially, the hydrophilic PSar chain has been considered to be a potent alternative polymer of polyethylene glycol (PEG) for decorating nanocarriers. The importance of the optimization of the density and thickness of hydrophilic polymer layer on the polymer-coated liposome surface was introduced.

Chapter 2 demonstrates the cellular uptake efficiency and pathway of PSar-coated liposomes (PSar-lipos) and discusses the effect of composition ratio and chain length of PSar on intracellular delivery. First, PSar-lipid conjugates (PSar-PEs) with three different degrees of polymerization (23mer, 45mer, and 68mer) were synthesized with the open-ring polymerization of sarcosine *N*-carboxyanhydride and the copper-catalyzed azide-alkyne cycloaddition reaction. By co-assembling of PSar-PEs with liposome compositions (phospholipid and cholesterol) through thin film hydration method, PSar-coated liposomes (PSar-lipos) were prepared. The density of PSar was controlled by changing the mixing molar ratios (5 and 10 mol% of PSar-PE in the mixture). Using three PSar-PEs with different lengths, 6 kinds of PSar-lipo samples ($PSar_n$-lipo-m; n and m indicate the degree of polymerization and mixing molar ratio, respectively) were prepared. Then, I confirmed the physicochemical properties including size, morphology, zeta potential, and membrane fluidity of PSar-lipos did not significantly change even in the presence of PSar chains. Next, the cytotoxicity, cellular uptake rate, and cellular internalization pathways of PSar-lipos were investigated. All PSar-lipos with a concentration below 100 μM did not show significant cytotoxicity for HeLa cells within 72 h. All PSar-lipos loaded with doxorubicin could be taken up by HeLa cells *via* the endocytic pathway. $PSar_n$-lipo-10 presented a stronger fluorescence intensity and a higher percentage of cellular uptake at 4 h than $PSar_n$-lipo-5. On the other hand, such a significant difference in cellular uptake was not observed between liposomes with PSar-

PE of the same concentration and different lengths. I concluded the density of PSar chain is a more important factor for the cellular uptake of PSar-lipo than length. Confocal microscope observation proved that PSar-lipos existed in the cytosol. It was confirmed that both $PSar_{23}$-lipos-5 and $PSar_{68}$-lipos-10 were internalized *via* caveolae-mediated endocytosis pathway from the cellular uptake observation with endocytosis inhibitors. These results suggest that PSar-lipo coated by PSar chain with a high density (10 mol%) can exhibit efficient uptake properties in tumor cells.

In Chapter 3, the effects of PSar length and density on the immune response and biodistribution *in vivo* were studied. The physical and chemical properties of liposomes inserted with more PSar-lipid proportions (15-100 mol%) were investigated in this chapter. Transition electron microscopic (TEM) images and Nanoparticle tracking analysis (NTA) indicated that the liposomal sample containing PSar-PE with a higher concentration (>15 mol%) led to the appearance of micelle in addition to PSar-coated liposome. While liposomes modified with different lengths (23, 45, and 68mers) and densities (5, 10, and 15 mol%) formed homogeneous vesicles. Thus, these PSar-lipos were injected into rats for IgM expression, blood circulation, and biological distribution studies. PSar-lipos induced a low IgM expression compared with PEG modified-liposome (PEG-lipo). $PSar_{68}$-lipos-15 showed a slightly higher expression than the other groups of PSar-lipos. Notably, $PSar_{68}$-lipos-15 still showed the longest blood circulation time and attenuated accelerated blood clearance (ABC) phenomenon compared with PEG-lipo. The biodistribution analysis of PSar-lipos indicated that the thicker PSar layer reduced the accumulation into the liver and spleen, resulting in prolonged blood circulation time. These findings provide new insights into the relationship between IgM expression and ABC phenomenon inhibition. It also provides new insights into the pharmacokinetic behaviors of PSar-lipos.

In Chapter 4, I summarized the biological effect of PSar coating on the various properties of liposomes, involving internalization into cells, biodistribution, blood circulation, and immune response which are required in the DDS field. Additionally, the surface conformation and targeted modification of PSar-lipos are discussed as topics of interest for future investigations.

These studies contribute to a more comprehensive research perspective on PSar as an alternative to PEG and give a direction to design the PSar-coated DDS carriers.