論文 / 著書情報
Article / Book Information

題目(和文)	単一高分子薬物キャリアの設計と合成および癌標的治療への応用
Title(English)	Design and Synthesis of Self-folding Macromolecular Drug Carriers and their Biological Applications for Tumor-directed Treatment
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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12769号, 授与年月日:2024年3月26日, 学位の種別:課程博士, 審査員:西山 伸宏,三浦 裕,北本 仁孝,田中 克典,神谷 真子,岡田 智
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第12769号, Conferred date:2024/3/26, 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 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	（ 工学 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Current polymeric micelle drug carriers used for systemic administration commonly adopt a shell-core micellar structure, where drugs are encapsulated within the core, and multiple polymer molecules assemble to form the micellar shell. This structure, however, presents two limitations when applied as a DDS: one concerns size minimization, and the other involves the critical micelle concentration (CMC). To overcome the limitations of polymeric micelles as tumor-targeting DDS, a novel self-folding macromolecular drug carrier (SMDC) structure was proposed for cancer imaging and therapy. A macromolecule was designed comprising random hydrophilic segments, hydrophobic segments, and drug-loaded segments. This macromolecule was expected to autonomously form an SMDC through intramolecular self-folding. The size of this SMDC was meticulously controlled within the range of 5 to 10 nm, aiming to exploit the EPR effect and avoid rapid renal excretion, and more importantly, achieve exceptional tumor penetration. Moreover, due to its unique structure, this SMDC does not exhibit a CMC, enabling it to facilitate relatively prolonged and targeted delivery to the tumor site.

Firstly, random copolymers were designed to form the SMDC. A series of random copolymers with hydrophilic segments and hydrophobic segments were synthesized through RAFT polymerization to serve as the backbone of SMDC, where poly (ethylene glycol) methyl ether acrylate (PEGA) was chosen as the monomer for the hydrophilic segments and benzyl acrylate (BZA) was selected as the monomer for the hydrophobic segments. The polymerization process was experimentally indicated to be stable and controllable. BZA_m-$PEGA_n$ samples were processed in aqueous and organic solutions to investigate the relationship between SMDC formation properties (DA and size) and polymer parameters (BZA/PEGA and DP). This clarification aimed to identify the optimal macromolecular structure conducive to the formation of SMDC with a size close to 5-7 nm. To load the imaging/therapeutic agent in the macromolecule, a random terpolymer BZA_m-$PEGA_n$-CEA_k was designed based on the RAFT polymerization process of BZA_m-$PEGA_n$. In these terpolymers, varying amounts of 2-carboxyethyl acrylate (CEA) are employed in place of PEGA in synthetic route of copolymer backbone to incorporate gadolinium chelated tetraxetan (Gd-DOTA) into the macromolecules. And leveraging the identified backbone structure, random terpolymers BZA_m-$PEGA_n$-CEA_k(Gd-DOTA)$_j$ were synthesized and analyzed by SEC-MALS, DLS, SAXS, and TEM. These random terpolymers demonstrated the ability of self-folding to form Gd-DOTA loaded SMDC (SMDC-Gd) in water with the diameter of 5-7 nm.

Subsequently, the *in vitro* and *in vivo* performances of Gd-loaded agents, SMDC-Gds, were investigated and compared. The cell viability assay indicated the non-cytotoxicity of SMDC-Gds and the Gd-leakage assay showed the stability and safety of SMDC-Gds. Then, an *in vivo* biodistribution of SMDC-Gds was investigated to analyze their tumor-targeting proficiency, blood clearance rates, and organ accumulation patterns. According to biodistribution results, SMDC-Gd_4 demonstrated superior performance over SMDC-Gd_{17}, showcasing high tumor targeting accumulation (13.1±4.4%ID/g at 24 h post-injection), relatively low accumulation in organs, and rapid blood clearance. Consequently, SMDC-Gd_4 was selected as the agent for cancer imaging and therapy in this research, the imaging and therapeutic time points were determined to be 24 hours post-administration. An *In vivo* biotoxicity single-dose assay was executed using the selected SMDC-Gd sample and administration dose. The safety of dose at 0.1 mmol/kg on a Gd basis was confirmed by evaluating the blood and plasma indicators of mice intravenously injected by SMDC-Gd_4,

thus it was determined to be the administration dose in MRI and Gd-NCT. The findings from these experiments were pivotal in determining the strategies aligned with the distinctive properties of SMDC-Gd.

Finally, the potential application of the designed SMDC-Gd_4 used as a contrast agent in MRI for tumor-targeted diagnosis and used as drug in Gd-NCT for anti-tumor therapy was explored. The relaxivity (r_1 and r_2) of SMDC-Gd_4 and control groups (PEGA-Gd_4 and Gd-DOTA) were measured and discussed. The higher relaxivity values, both r_1 and r_2, compared to the control groups indicated the suitability of SMDC-Gd_4 for T_1-weighted MRI and suggested that SMDC-Gd could potentially induce high relaxivities with a minimal Gd payload. Subsequently, T_1-weighted MRI scans were conducted according to the schedule established in the preceding results. The strong tumor-targeted contrast enhancement effect of SMDC-Gd_4 in R_1 maps of T_1-weighted MRI was directly observed at 24 hours post-injection, affirming the viability of SMDC-Gd_4 in MRI. Furthermore, the dose-dependence of this enhancement effect was confirmed. This superior performance of SMDC-Gd_4 in T_1-weighted MRI, in comparison to control groups, was attributed to its demonstrated selective accumulation in tumors. Meanwhile, the renal excretion properties of both SMDC-Gd_4 and PEGA-Gd_4 were elucidated by directly observing the R_1 map of kidney and bladder cross-sections during the first hour post-administration. The observed differences in renal excretion properties could be a contributing factor to the notable performance of SMDC-Gd_4 as a contrast agent in MRI. Then, the potential of SMDC-Gd_4 for cancer therapy by Gd-NCT method was explored. When administered at the same dosage used in the MRI experiment (0.1 mmol/kg on a Gd basis), SMDC-Gd_4 did not exhibit a notable therapeutic effect due to the low Gd concentration (41.2 ppm) in the tumor. To address this, the injection schedule was adjusted, involving three consecutive daily injections of equal doses (0.1 mmol/kg on a Gd basis). This modification resulted in an increased intra-tumoral Gd concentration, reaching 91.2 ppm. At such elevated dosage, SMDC-Gd_4 demonstrated a significant tumor growth inhibitory effect. This result suggested that SMDC-Gd_4 could successfully deliver appropriate amount of Gd complexes into tumor tissues and contribute to the anti-tumor effects in Gd-NCT.

These findings demonstrated the feasibility of the developed SMDC-Gd4 in cancer imaging and therapy, furthermore, indicated the outstanding performance and developmental potential of the proposed SMDC structure as a DDS.