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A PEG-Fmoc conjugate as a nanocarrier for paclitaxel

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ABSTRACT

We report here that a simple, well-defined, and easy-to-scale up nanocarrier, PEG_{5000}-lysyl-(α-Fmoc-ε-t-Boc-lysine)$_2$ conjugate (PEG-Fmoc), provides high loading capacity, excellent formulation stability and low systemic toxicity for paclitaxel (PTX), a first-line chemotherapeutic agent for various types of cancers. 9-Fluorenylmethoxycarbonyl (Fmoc) was incorporated into the nanocarrier as a functional building block to interact with drug molecules. PEG-Fmoc was synthesized via a three-step synthetic route, and it readily interacted with PTX to form mixed nanomicelles of small particle size (25–30 nm). The PTX loading capacity was about 36%, which stands well among the reported micellar systems. PTX entrapment in this micellar system is achieved largely via an Fmoc/PTX π–π stacking interaction, which was demonstrated by fluorescence quenching studies and ^{13}C NMR. PTX formulated in PEG-Fmoc micelles demonstrated sustained release kinetics, and *in vivo* distribution study via near infrared fluorescence imaging demonstrated an effective delivery of Cy5.5-labled PTX to tumor sites. The maximal tolerated dose for PTX/PEG-Fmoc (MTD > 120 mg PTX/kg) is higher than those for most reported PTX formulations, and *in vivo* therapeutic study exhibited a significantly improved antitumor activity than Taxol, a clinically used formulation of PTX. Our system may hold promise as a simple, safe, and effective delivery system for PTX with a potential for rapid translation into clinical study.

1. Introduction

Nanomedicine has been shown to dramatically improve the *in vivo* performance of various anticancer agents through modifying their physiochemical properties, pharmacokinetics, and distribution profiles [1–6]. To date, a variety of polymer- and lipid-based systems have been developed and a few of them are currently being used in the clinic [7–11]. However, it remains a challenge to develop a simple, easy-to-scale up system that provides excellent drug loading capacity and formulation stability.

As a well-developed delivery system, micelles are attractive due to the ease of preparation, and small sizes that can contribute to a reduced rate of elimination from circulation and enhanced accumulation at solid tumors with leaky vasculature based on the enhanced penetration and retention (EPR) effect [12,13]. Most of the current micellar systems are composed of two distinct domains, one being hydrophilic and the other hydrophobic, and drug loading is solely based on the interactions of their hydrophobic domains with the poorly water-soluble drugs [14–16]. While working well for highly hydrophobic/lipophilic agents, these systems exhibit limited effectiveness for drugs with moderate hydrophobicity due to limited drug–carrier compatibility.

Recent studies have highlighted the benefits of introducing other drug-interactive domains into the conventional micellar systems to improve the drug loading capacity and formulation stability through introduction of additional mechanisms of carrier/drug interactions. For example, some studies have demonstrated that inclusion of a hydrotropic domain or entire drug molecule such as doxorubicin can effectively improve the performance of several polymeric systems with respect to drug loading capacity and colloidal stability of drug-loaded micelles [17–21].

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We have recently developed a new concept that introduction of a drug-interactive domain at the interfacial region represents an effective strategy to improve the compatibility between lipid-core micelles and the hydrophobic drugs. Among several drug-interactive domains tested, 9-Fluorenylmethoxycarbonyl (Fmoc) was shown to have an unusual propensity in interacting with many types of agents of diverse structure and water solubility [22]. After interfacially modified with Fmoc groups, polyethylene glycol (PEG)-lipopeptides are active in formulating a panel of dissimilar drugs, ranging from paclitaxel (PTX), steroids, xanthene- and porphyrin-based photodynamic agents, to hydrophobic peptide drugs, with significant improvements in both drug loading capacity and drug retention [23]. These data strongly suggest that Fmoc qualifies as a "formulation chemophor", exhibiting a potent activity in interacting with various pharmaceutical agents and thus a capability of improving carrier–drug compatibility.

In general, it is believed that a large hydrophobic domain such as a lipid chain or hydrophobic polymer is necessary to construct micelle-forming surfactants, and indeed, Fmoc-containing PEG-lipid conjugates were more effective than the counterparts without a lipid motif in formulating a number of hydrophobic agents. Interestingly, a PEG-Fmoc conjugate without a lipid motif, PEG_{5000}-lysyl-(α-Fmoc-ε-t-Boc-lysine)$_2$ (PEG-Fmoc), was found to be highly effective in solubilizing PTX. More surprising is the finding that PEG-Fmoc was significantly more effective than the counterpart with a lipid motif in formulating PTX. This study is focused on characterization of PEG-Fmoc as a simple and effective micellar formulation for PTX. The potential mechanism involved in the drug/carrier interaction between PEG-Fmoc and PTX is also investigated, which may shed insights into the future development of further improved nanocarrier for therapeutic agents.

2. Materials and methods

2.1. Reagents

Paclitaxel (PTX, >99%) was purchased from TSZ Chem (MA, USA). Docetaxel (DTX, >99%) was obtained from LC Laboratories (MA, USA). α-Fmoc-ε-Boc-lysine, di-Boc-lysine, N, N′-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), trifluoroacetic acid (TFA), and triethylamine (TEA) were obtained from Acros Organic (NJ, USA). Monomethoxy PEG_{5000}, monomethoxy PEG_{550}, cholesterol (CHOL), 4-dimethylaminopyridine (DMAP), ninhydrin, and other unspecified chemicals were all purchased from Sigma–Aldrich (MO, USA). Dulbecco's phosphate buffered saline (DPBS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 100× penicillin–streptomycin solution were all purchased from Invitrogen (NY, USA). All solvents used in this study were HPLC grade.

2.2. Synthesis of PEG_{5000}-lysyl-(α-Fmoc-ε-t-Boc-lysine)$_2$ (PEG-Fmoc)

PEG-Fmoc was synthesized largely following our published method [23]. Briefly, 1 equiv. of monomethoxy PEG_{5000} was mixed with excess amount of di-Boc-lysine and DCC in dichloromethane (DCM) with addition of DMAP, and the reaction was allowed at room temperature for 48 h. The mixture was filtered and precipitated in ice-cold ether, followed by washes with cold ethanol and ether to obtain purified PEG_{5000}-di-Boc-lysine. The PEG derivative was then treated with DCM/TFA (1:1, v/v) for 2 h at room temperature, followed by removal of the solvent, precipitation in cold ether, and washes with cold ethanol and ether. Finally, the deprotected PEG_{5000}-lysine-NH_2 was mixed with excess amount of α-Fmoc-ε-Boc-lysine that was pre-

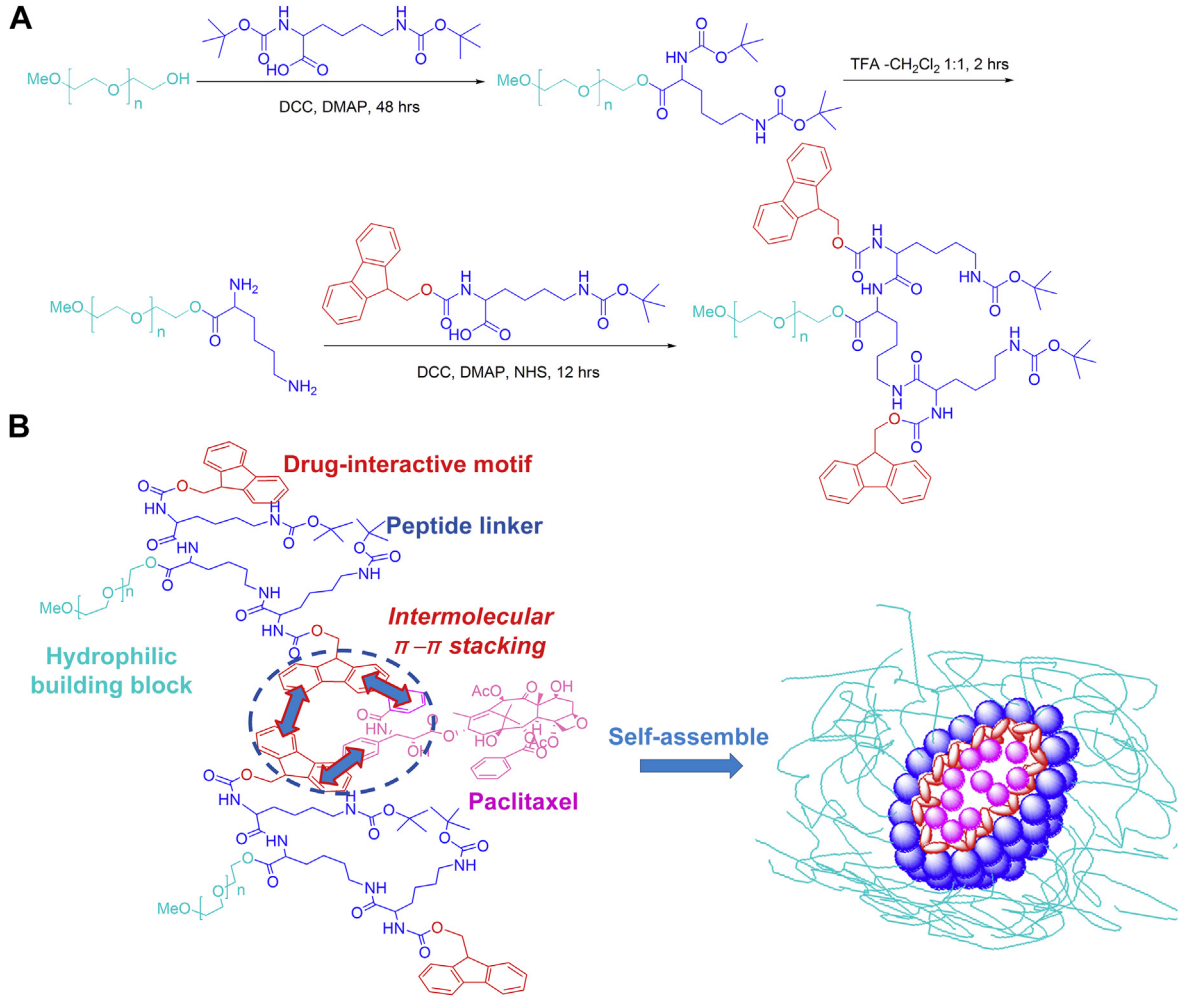

Fig. 1. Synthetic route of PEG-Fmoc (**A**), and schematic representation of self-assembled PEG-Fmoc/PTX mixed micelle based on carrier/drug intermolecular π–π stacking (**B**).