Journal of Controlled Release 216 (2015) 111–120

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Enzyme sensitive, surface engineered nanoparticles for enhanced delivery of camptothecin

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 3 August 2015
Accepted 10 August 2015
Available online 15 August 2015

Chemical compounds studied in this article:
Caprolactone (PubChem CID: 10401)
Ethylene glycol (PubChem CID: 174)
Camptothecin (PubChem CID: 24360)
Folic acid (PubChem CID: 6037)

Keywords:
Nanoparticles
Matrix metalloproteases
Camptothecin
Targeting
Polycaprolactone

ABSTRACT

To achieve a drug delivery system combining the programmable long circulation and targeting ability, surface engineering nanoparticles (NPs), having a sandwich structure consisting of a long circulating outmost layer, a targeting middle layer and a hydrophobic innermost core were constructed by mixing a matrix metalloproteinase MMP2 and MMP9-sensitive copolymers (mPEG-Pep-PCL) and folate receptor targeted copolymers (FA–PEG–PCL). Their physiochemical traits including morphology, particle size, drug loading content, and *in vitro* release profiles were studied. *In vitro* studies validated that the inhibition efficiency of tumor cells was effectively correlated with NP concentrations. Furthermore, The PEG layer would detach from the NPs due to the up-regulated extracellular MMP2 and MMP9 in tumors, resulting in the exposure of folate to enhance the cellular internalization *via* folate receptor mediated endocytosis, which accelerated the release rate of CPT *in vivo*. The antitumor efficacy, tumor targeting ability and bio-distribution of the NPs were examined in a B16 melanoma cells xenograft mouse model. These NPs showed improved tumor target ability and enhanced aggregation of camptothecin (CPT) in tumor site and prominent suppression of tumor growth. Thus this mPEG-Pep-PCL@FA–PEG–PCL core–shell structure NP could be a better candidate for the tumor specific delivery of hydrophobic drug.

1. Introduction

Drug-loaded nanoparticles (NPs) such as liposomes, micelles, polymeric NPs, and drug conjugates have demonstrated various advantages over free therapeutic molecules [1]. Among them, the amphiphilic polymeric micelles and NPs are particularly attracted much interest in drug delivery system for cancer treatment, because they show significant accumulation at tumor site due to their enhanced permeability and retention effect (EPR) [2,3], and avoid multidrug resistance in cancer cells [4]. However, their clinical application is inadequate, because an ideal drug delivery system can not only avoid the RES capture and enhance circulation time in bloodstream, but also accumulate at the tumor tissue, selectively bind to the tumor cells, escape from the endo/lysosome [5–7].

Therefore, targeting NPs are fabricated by conjugating targeting ligands on their surface, such as folate, to improve the therapeutic index against the tumor [8,9]. Unfortunately, their practical values are questionable because of the compromise between the long circulation time and the targeting ability [10,11]. PEGylation can enhance the circulation of NPs *in vivo*, and strengthen the tumor accumulation of NPs by EPR effect while it shields the targeting property of ligands by weakening the intracellular trafficking of cellular uptake and endosomal escape. So the 'PEG dilemma' is one of the major concerns to design effective drug delivery system [12].

Recently, the precise surface engineering NPs with multifunctional groups, such as responsive ligands, to improve their performance in terms of targeting ability, cellular penetration, circulation longevity, and stimulus sensitivity are highly desirable. By carefully tuning the amount of PEG and targeting ligands on their surface, PLGA NPs having maximally targeted and maximally stealth abilities were obtained, resulting in the most efficient cell uptake *in vitro* and *in vivo* [13]. However, the ratio of PEG to targeting ligand should be carefully calculated, and be re-adjusted when these NPs were used in different person, which made it complicated almost no use in clinic. In our previous report, sandwich-like surface engineered NPs were fabricated through mixing two kinds of polymers to realize the spatiotemporally programmable functions of long circulation and targeting properties by releasing the PEG segment by broking the S–S bond between PEG and polycaprolactone in the intracellular reducing environment generated by glutathione and exposing the targeting ligand, avoiding the short coming of maintaining the balance of PEG segment and targeting ligands, which could significantly enhance the endocytosis and cytotoxicity of these NPs against tumor cells [14]. However, the cleavage of PEG occurs inside the tumor cells and the reduced interaction between the cells and NPs still remains.

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http://dx.doi.org/10.1016/j.jconrel.2015.08.021
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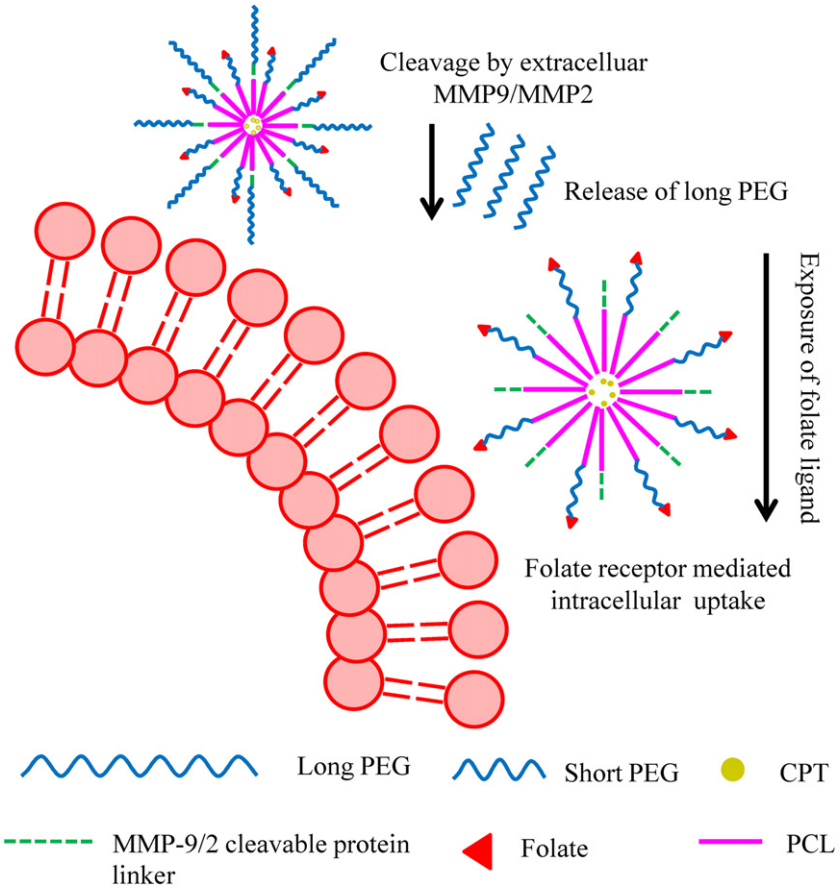
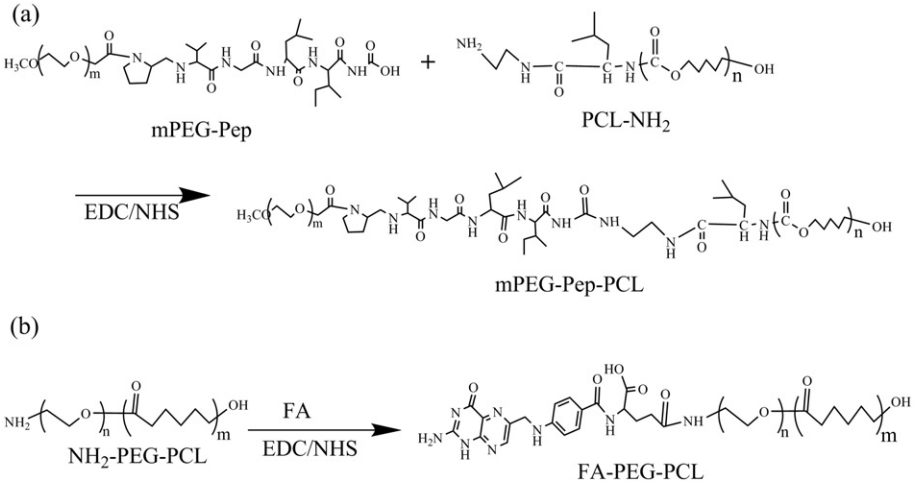


Fig. 1. The structure and delivery process of the nanocarriers.

According to the literature, matrix metalloproteases (MMPs), especially MMP2 and MMP9, are over expressed in almost every type of human tumor microenvironment, and their expression is often associated with tumor progression and metastasis [15–18]. MMP sensitive NPs were successfully applied in the studies of gene delivery and drug delivery system [19,20]. Herein, we reported the fabrication of a kind of sandwich-like surface engineered MMP responsive NPs, combining the programmable long circulation and targeting properties, which were composed of three layers: detachable PEG out layer, middle layer of folate ligands, and PCL core. In this work, two kinds of copolymers were synthesized. One of the copolymer composes of longer PEG and PCL with a MMP2/9 sensitive linker between PEG and PCL (mPEG-Pep-PCL), the other one is PCL–PEG polymer with shorter PEG chain and modified with the tumor cell-specific folate ligand at the end (FA–PEG–PCL). Camptothecin (CPT), a traditional chemotherapeutic drug inducing tumor cells apoptosis after binding to DNA topoisomerase I, was loaded into these NPs [21,22]. CPT is located in the PCL core covered by the folate layer and PEG shell. Upon administration, the NPs are



Scheme 1. The synthetic route of mPEG-Pep-PCL (a) and FA–PEG–PCL (b).