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Improved tumor tissue penetration and tumor cell uptake achieved by delayed charge reversal nanoparticles

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ABSTRACT

The high affinity of positively charged nanoparticles to biological interfaces makes them easily taken up by tumor cells but limits their tumor permeation due to non-specific electrostatic interactions. In this study, polyion complex coated nanoparticles with different charge reversal profiles were developed to study the influence of charge reversal profile on tumor penetration. The system was constructed by polyion complex coating using micelles composed of poly (lysine)-*b*-polycaprolactone (PLys-*b*-PCL) as the cationic core and poly (glutamic acid)-*g*- methoxyl poly (ethylene glycol) (PGlu-*g*-mPEG) as the anionic coating material. Manipulation of charge reversal profile was achieved by controlling the polymer chain entanglement and electrostatic interaction in the polyion complex layer through glutaraldehyde-induced shell-crosslinking. The delayed charge reversal nanoparticles (CTCL30) could maintain negatively charged in pH 6.5 PBS for at least 2 h and exhibit pH-responsive cytotoxicity and cellular uptake in an extended time scale. Compared with a faster charge reversal counterpart (CTCL70) with similar pharmacokinetic profile, CTCL30 showed deeper penetration, higher *in vivo* tumor cell uptake and stronger antitumor activity *in vivo* (tumor inhibition rate: 72.3% vs 60.2%, compared with CTCL70). These results indicate that the delayed charge reversal strategy could improve therapeutic effect via facilitating tumor penetration.

Statement of Significance

Here, the high tumor penetration capability of PEG-coated nanoparticles and the high cellular uptake of cationic nanoparticles were combined by a delayed charge reversal drug delivery system. This drug delivery system was composed of a drug-loading cationic inner core and a polyion complex coating. Manipulation of charge reversal profile was realized by varying the crosslinking degree of the shell of the cationic inner core, through which changed the strength of the polyion complex layer. Nanoparticles with delayed charge reversal profile exhibited improved tumor penetration, *in vivo* tumor cell uptake and *in vivo* tumor growth inhibition effect although they have similar pharmacokinetic and biodistribution behaviors with their instant charge reversal counterpart.

1. Introduction

Nanoscale drug delivery systems (NDDSs) are currently efficient tools in enhancing the therapeutic effect of chemotherapeutic agents due to improved pharmacokinetic profile as well as cellular uptake [1–5], and most importantly, preferred accumulation in solid tumors through the enhanced permeation and retention (EPR) effect [6–8]. However, due to the high interstitial pressure and dense extracellular matrix of the tumor tissue [9–11], most of the nanoparticles were restricted in the vicinity of tumor vasculatures after extravasation, resulting in limited therapeutic effect of NDDSs due to inadequate drug exposure over tumor cells. So, efficient tumor penetration has been recognized as another key factor influencing the antitumor performance of NDDSs.

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Surface charge is a key parameter determining the performance of nanoparticles: positively charged nanoparticles could be preferentially taken up by cells via "electrostatic attraction mediated targeting" [12–14], however, this strong adhesion between cationic nanoparticles and bio-interfaces is a double-edged sword which could indeed improve cellular uptake but, in the meantime, severely limit the amount of drug reaching tumor site due to shortened blood circulation [15–17]. Moreover, positively charged nanoparticles would primarily be "arrested" by the cells resided in the periphery of tumor vessels, thus limit their intratumoral distribution [18]. In contrast, neutral or negatively charged nanoparticles could travel longer distances in tumor tissue than the positively charged ones [19,20].

So, to utilize the advantaged cell internalization and avoid the limited tumor penetration of positively charged nanoparticles both induced by non-specific electrostatic interaction, it is hypothesized that NDDSs with delayed charge reversal profile might be capable of promoting tumor penetration without affecting cell internalization. Herein, nanoparticles with different charge reversal profiles were developed based on the platform of nanoparticles with polyion complex (PIC) coatings which showed negligible charge reversal behavior [21]. Manipulation of charge reversal profiles was realized by varying the shell crosslinking degree of cationic micelles self-assembled from poly (lysine)-*b*-polycaprolactone (PLys-*b*-PCL) using glutaraldehyde as the crosslinking agent, prior to electrostatic coating with poly (L-glutamic acid)-*g*-methoxyl poly (ethylene glycol) (PGlu-*g*-mPEG) (Scheme 1). Charge reversal caused by decoating of PGlu-*g*-mPEG under lowered pH was studied by monitoring the changes in ξ-potential and determination of the amount of remained PGlu-*g*-mPEG by GPC. The *in vivo* pharmacokinetics, biodistribution, intratumoral penetration and *in vivo* anti-tumor efficacy of nanoparticles with different charge reversal profiles were compared, and it was found that delayed charge reversal nanoparticles performed better in tumor penetration and tumor-growth inhibition.

2. Experimental section

2.1. Materials

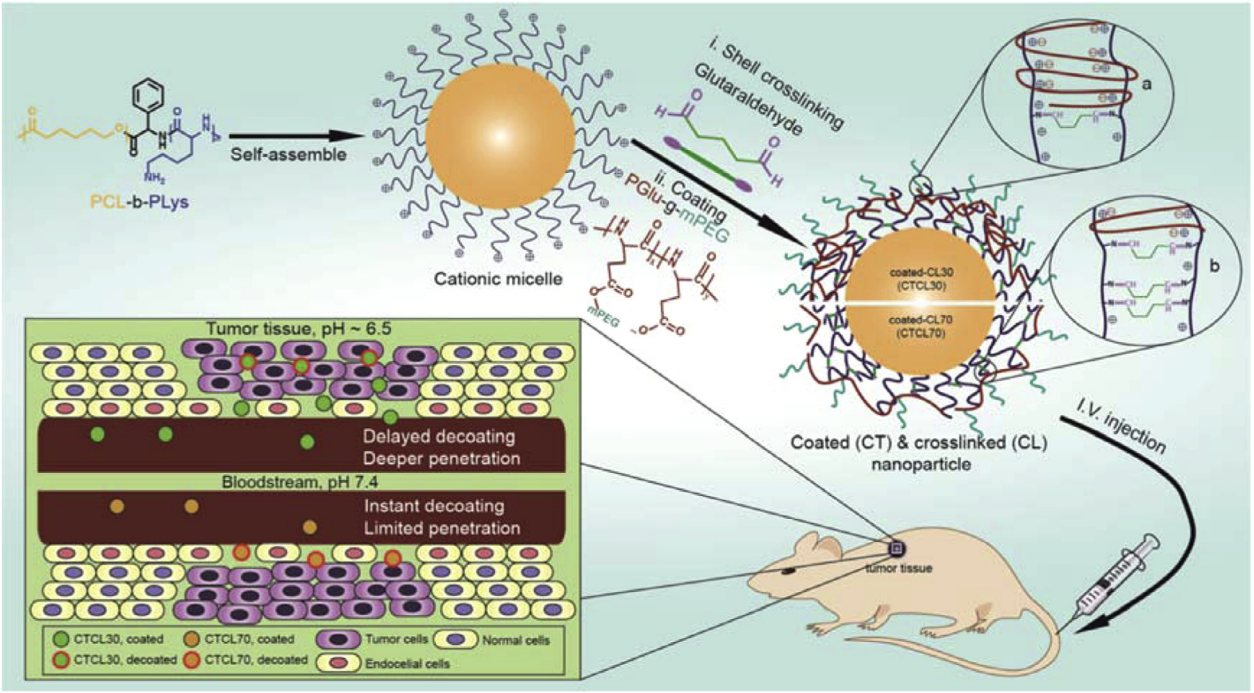
Polycaprolactone and poly (L-glutamic acid)-*g*-methoxyl poly (ethylene glycol) (PGlu-*g*-mPEG, dp_{Glu}: 140, grafting $ratio_{mPEG}$: 2%) were synthesized according to our previous work [22,23]. N-ε-carbobenzyloxy-L-lysine was purchased from Enlai biological technology Co., Ltd. (Chengdu, China). Triphosgene was purchased from GL Biochem (Shanghai, China). Medium chain triglyceride (MCT) was provided by Lipoid KG (Ludwigshafen Germany). Cabazitaxel (CTX) was provided by the medicinal chemistry lab of Yantai University (Yantai, China). Coumarin 6 and rhodamine B isothiocyanate was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). 4′, 6-diamidino-2-phenylindole (DAPI) was purchased from Gen-view Scientific Inc. (USA). Fluorescein isothiocyanate (FITC)-labeled *Lycopersicon esculentum* lectin were supplied by Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.2. Cell lines and animals

The human non-small cell lung cancer A549 cell line and mouse liver cancer H22 cell line were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Sprague-Dawley rats (male, body weight: 200 g ± 20 g) and kunming mice (male, body weight: 20 g ± 2 g) were provided by Liaoning Changsheng Biotech Co., Ltd. (Benxi, China). All animal experiments were approved by the Animal Ethics Committee of Shenyang Pharmaceutical University.

2.3. Preparation and characterization of nanoparticles

Nanoparticles with different charge reversal profiles were prepared using cationic micelles as inner cores that were crosslinked



Scheme 1. Schematic illustration of preparation and tumor infiltration of nanoparticles with different charge reversal profiles. Charge reversal was manipulated by controlling the shell crosslinking degree: a low crosslinking degree indicated slower decoating due to higher chain entanglement in PIC layer and more $-NH_3^+$ available for electrostatic interaction (a); while reduced chain entanglement as well as available amount of $-NH_3^+$ resulted from increased crosslinking degree indicated faster decoating (b). After vessel extravasation, CTCL30 remained coated and gradually reversed surface charge during tumor penetration, this guarantees deeper penetration and higher cellular uptake; while the premature exposure of positive charge limited intratumoral distribution of CTCL70.

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