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Sustained release of PTX-incorporated nanoparticles synergized by burst release of DOX·HCl from thermosensitive modified PEG/PCL hydrogel to improve anti-tumor efficiency

Shuxin Xu [a,1], Weiwei Wang [b,1], Xijing Li [c], Jianping Liu [a], Anjie Dong [a,d,e], Liandong Deng [a,*]

[a] Department of Polymer Science and Technology, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] Institute of Biomedical Engineering, Chinese Academy of Medical Science, Tianjin 300072, China
[c] Tianjin Institute of Medical and Pharmaceutical Science, Tianjin 300020, China
[d] Key Laboratory of Systems Bioengineering of the Ministry of Education, Tianjin 300072, China
[e] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

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ABSTRACT

As drug therapies become increasingly sophisticated, the synergistic benefits of two or more drugs are often required. In this study, we aimed at improving anti-tumor efficiency of paclitaxel (PTX)-incorporated thermo-sensitive injectable hydrogel by the synergy of burst release of doxorubicin hydrochloride (DOX·HCl). Thermosensitive injectable hydrogel composed of nanoparticles assembled from amphiphilic copolymer poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)-poly(ethylene glycol)-poly(ε-caprolaone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) (PECT) was fabricated. Hydrophobic PTX and hydrophilic DOX·HCl were loaded simultaneously in the thermo-sensitive injectable hydrogel by a two-stage entrapment. Thermosensitive gelling behaviors of drug-loading PECT nanoparticle aqueous dispersions were studied. *In vitro* release profiles of PTX and DOX·HCl and *in vivo* anti-tumor effect by dual drugs from PECT hydrogel were investigated. The results showed that hydrophilic and hydrophobic drugs could be successfully entrapped in PECT hydrogel simultaneously without affecting its thermo-sensitive behavior. *In vitro* release profiles demonstrated the burst release of DOX·HCl and the sustained release of PTX. Anti-tumor effect was improved by a fast and tense attack caused by the burst release of hydrophilic DOX·HCl from hydrogel, which was continued by the sequent sustained release of PTX-incorporated nanoparticles and remnant DOX·HCl. Unintentionally, entrapped in PECT hydrogel, hydrophilic DOX·HCl was observed to have a sustained releasing pattern *in vitro* and *in vivo*.

1. Introduction

Local chemotherapy, an alternative approach to systemic chemotherapy, can improve overall survival and quality of patients' life by increasing the bioavailability of drug to the site of disease, sustaining delivery of drug to the cancerous tissues, and minimizing systemic side effects (Haroun and Brem, 2000; Ko et al., 2013; Wolinsky et al., 2012). It is a promising approach for the treatment of solid tumors. Due to their physico-chemical stability, biocompatibility and controllability, polymer-based drug delivery systems dominate the field of drug carriers for local drug delivery (De Souza et al., 2010; Exner and Saidel, 2008; Jagur-Grodzinski, 2009; Kim et al., 2009; Weinberg et al., 2008). Thermosensitive polymer-based hydrogels that transform from sol to gel state at body temperature, in particular, have been extensively investigated for localized antitumor drug delivery. Injectable *in situ* forming gels have demonstrated numerous advantages, including minimally invasive introduction *in vivo*, drug localization within the tumor site, sustained drug release and improved patient compliance, affording a type of biomaterials potentially used in localized drug delivery (Bajpai et al., 2008; Jeong et al., 1997; Sasaki and Akiyoshi, 2012; Yu and Ding, 2008).

During the past two decades, various thermosensitive *in situ* hydrogels of amphiphilic copolymers based on polyethylene glycol (PEG) and biodegradable polyesters have been synthesized and the relevant investigations *in vitro* and *in vivo* have proved them to be excellent carriers for intratumoral or peritumoral delivery of drugs

* Corresponding author. Address: Tianjin University, Number 92, Weijin Street, Nankai District, Tianjin, China. Tel.: +86 22 27890707.
E-mail address: dengliandong@tju.edu.cn (L. Deng).
[1] These authors contributed equally to this work.

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(Kissel et al., 2002; Li et al., 2012; Moon et al., 2012). Poly(D,L-lactide-co-glycolide)(PLGA) and poly(ethylene glycol)(PEG) triblock copolymer (PLGA–PEG–PLGA) hydrogel (ReGel®) has been proved by the FDA for clinical applications and the formulation (OncoGel) for local delivery of PTX has got through clinical phase I and II studies (DuValla et al., 2009; Vukelja et al., 2007). The thermosensitive PEG and poly ε-caprolactone (PCL) block copolymers have been reported as a local drug carriers and the intratumoral administration of the PTX incorporated PEG/PCL hydrogel exhibited more continuous and efficacious inhibition of tumor growth than Taxol® *in vivo* (Hwang et al., 2005; Hyun et al., 2007; Jiang et al., 2007; Park et al., 2008). In previous study, we developed a thermosensitive hydrogel system based on a novel amphiphilic triblock copolymer, poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)-poly(ethylene glycol)-poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)(PECT) (Wang and Dong, 2012; Wang et al., 2012). The solid powders of PECT at ambient temperature facilitated the weighing and transferring and the PECT nanoparticle aqueous dispersion at certain concentration could perform sol–gel–sol transition behavior with the temperature increase without pre-quenching treatment needed for PEG/PCL hydrogel. In addition, a reconstituted system of thermosensitive injectable hydrogel system assembled from PTX-incorporated PECT nanoparticles (NPs) was fabricated. The efficiency against Ehrlich ascites carcinoma (EAC) tumors of this system had been studied and the result showed that a single peritumoral injection of thermosensitive PTX-incorporated PECT hydrogel was more efficacious than the intravenous injection of Taxol® in inhibiting the growth of EAC tumors in mice and caused fewer off-target side effects over a prolonged time period (Wang et al., 2013). However, the antitumor efficiency of PTX-incorporated PECT hydrogel in the initial stage was not conspicuous compared with Taxol®. It might be due to the linear release of PTX from the hydrogel system and therefore effective drug concentration could not be reached in a short term. Its known that only when the concentration of drug reaches a certain value, does the therapeutic effect emerge.

Burst release refers to the phenomenon that an initial large bolus of drug is released from the formulations, immediately upon placement in the release medium. It is often regarded as a negative consequence of creating long-term controlled release device. In most cases, burst release is prevented or avoided because it can lead to high amount of initial drug delivery and reduce the effective lifetime of the device (Huang and Brazel, 2001). On the other hand, burst release has been utilized to deliver drugs at high release rates as part of the drug administration strategy (Tian et al., 1997). Here, a hypothesis was proposed that a better anti-tumor efficiency could be achieved by taking advantage of the burst release of hydrophilic antineoplastic companied with the sustained release of hydrophobic antineoplastic carried simultaneously in a single formulation. Combining drugs into one single carrier can be accomplished by mixing two distinct drug powders and compressing the mixture into tablet or by creating a carrier structure containing one drug and then coating it with a mixture containing the second drug (Jia et al., 2009; Kumaravelrajan et al., 2010). However, it is difficult to ensure delivery of both drugs to the same location simultaneously at a desired relevant releasing pattern. Combining drugs into one single carrier presents a challenge for encapsulation and release technologies, especially for drugs with different physico-chemical characteristics, such as hydrophilic and hydrophobic actives.

In the present work, PTX and DOX·HCl were chosen as the hydrophobic and hydrophilic drugs respectively to construct a local dual drug delivery system. Thermosensitive injectable hydrogel composed of nanoparticles assembled from amphiphilic copolymer PECT was fabricated. With different loading modes, hydrophobic PTX was incorporated in the hydrophobic regions of nanoparticles and hydrophilic DOX·HCl was entrapped in hydrophilic regions of hydrogel. The local dual drug delivery system was investigated including the thermosensitive gelling, drug release behavior of the PECT formulations *in vitro* and the antitumor activity *in vivo*.

2. Materials and methods

2.1. Materials

PEG (Mn = 1500 g mol^{-1}) was provided by Aladdin company. ε-Caprolactone (CL) from Sigma–Aldrich (Milwaukee, WI, USA) was dried over CaH_2 for 48 h at room temperature and distilled under reduced pressure prior to use. Stannous octoate was used as received from Sigma–Aldrich. 1,4,8-Trioxa[4.6]spiro-9-undecanone (TOSUO) was prepared by our lab. PTX was purchased from Shenyang Tianfeng Biological Pharmaceutical Co. Ltd. (Shenyang, China). DOX·HCl was purchased from Zhejiang Hisun Pharmaceutical Co. Ltd. (Taizhou, China). Dimethyl sulfoxide (DMSO) and acetonitrile were provided by Concord Co. Ltd. (Tianjin, China).

2.2. Animals and tumor cell lines

Balb/C mice (20 ± 2 g, 6–7 weeks old) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and acclimatized for 7 days after arrival. The mice were housed in cages with free access to food and water. EAS cells were incubated by Tianjin Institute of Medical and Pharmaceutical Science (Tianjin, China). All of the animal experiments were performed in accordance with the protocol approved by Tianjin Institute of Medical and Pharmaceutical Science.

2.3. Synthesis and characterization of PECT

PECT was prepared from PEG, CL and TOSUO using a ring-opening copolymerization method as reported previously (Wang and Dong, 2012). The molecular structure and ^{1}H NMR spectrum were depicted in Fig. 1.

2.4. Preparation of PECT hydrogel loading antitumor drugs

The PECT hydrogel loading dual drugs was prepared by two stages as shown in Scheme 1. Firstly, the PTX-incorporated freeze-dried powder was fabricated by nanoprecipitation as reported previously (Wang et al., 2013). Briefly, PTX and PECT were dissolved in tetrahydrofuran, and then the mixed solution was

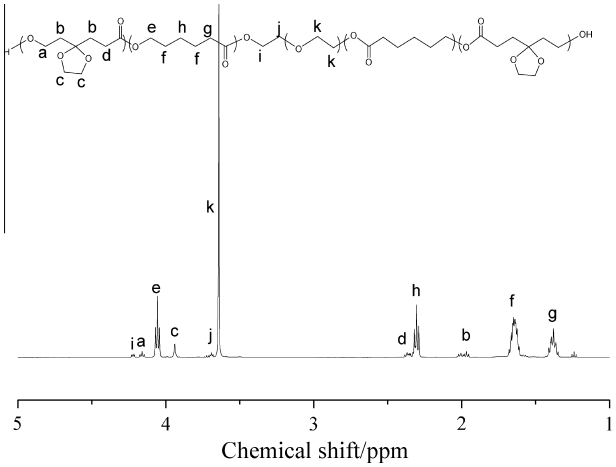

Fig. 1. The molecular structure and ^{1}H NMR spectrum of PECT.