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An injectable, thermosensitive and multicompartment hydrogel for simultaneous encapsulation and independent release of a drug cocktail as an effective combination therapy platform

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ABSTRACT

Combination chemotherapy is potent to combat diseases. Simultaneous and segregated delivery of multiple drugs in a single vehicle is essential to achieve this objective. In the present study, an injectable, thermosensitive and multicompartment hydrogel (MCH) was developed by the facile cooperative and incompatible assembly of PEGylated hydrocarbon nanoparticles with PEGylated fluorocarbon nanoparticles. The cooperative assembly behavior was investigated by fluorescence resonance energy transfer (FRET) technology, and the result demonstrated that the incompatible nanoparticle cores possibly accounted for the multicompartment formation in hydrogel. Paclitaxel and doxorubicin could be easily and separately integrated into the different compartments of MCH serving as a sustained drug cocktail formulation. In vitro drug release indicated drugs were liberated in a simultaneous but independent manner without any effect on each other. In vitro and in vivo antitumor activity indicated that peritumoral injection of drug cocktail encapsulated MCH formulation could well achieve the combination effect, which significantly improved the tumor growth inhibition efficiency as well as minimized the drug-associated side effects compared to intravenous injection of free drug cocktail. Furthermore, such a delivery device would allow precise adjustment of drug dosage to the desired effect, achieve spatial–temporal simultaneous and synchronized presence of combination drugs in the target tissue and obviate repeated drug administrations to improve patient compliance. The thermosensitive multicompartment hydrogel cocktail formulation holds great promise for simultaneous and segregated delivery of multiple bioactive agents for sustained combination therapy.

1. Introduction

Cancer is a major public health problem all around the world. One in four deaths in the United States is due to cancer [1]. Cancer therapy has always been tough and challenging. Various treatment strategies including surgical excision of solid tumor, thermal therapy, photo or radiotherapy, gene therapy, immunotherapy and chemotherapy [2–9] have been developed. Adjuvant chemotherapy before or immediately after surgery is often required to promote tumor accessibility and organ preservation during surgery, as well as to inhibit the rapid recurrence and increase of the second primary tumors. Furthermore, chemotherapy is also a preferred selection for many patients who are ineligible for surgical excision. As advanced by nanotechnology, number of drug formulations have been designed and prepared for single drug-induced cancer chemotherapy during the past decades, such as drug-loaded nanoparticles for intravenous injection and drug-eluting implants for locoregional chemotherapy [10–23]. However, a single drug might not be potent enough to kill all cancer cells due to the intrinsic heterogeneity of a tumor and cancer cells exist at different cell divisions or growth stages. Thus, the combination of multiple drugs with different action mechanisms and toxicity profiles is urgently required for cancer chemotherapy. Moreover, the simultaneous administration of multiple drugs was able to minimize the side effects caused by high doses of a single toxic drug and delay the generation of multi-drug resistance, hence, drug combination chemotherapy should exhibit superior therapeutic effect and lead to improved patient survival than any single agent for cancer treatment.

Simultaneous and segregated encapsulation of multiple drugs in a single formulation and the efficient release of incorporated substances are essential for combined therapeutic applications of the synergistic drug cocktail. In this regard, several nanoscale vectors including multicompartment micelles, nanogels, liposomes, and prodrug nanoparticles, have been subjected to prescribed delivery and synchronized release of multiple drugs for cancer combination cancer therapy

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[24–29]. Among these vectors, multicompartment release systems [30–36] have emerged in recent years as an ideal platform for simultaneous encapsulation of multiple drugs in segregated compartments. However, until now, much effort has been paid to develop multicompartment structures [34,37–43] and few such systems could easily succeed in selective encapsulation and controlled release of multiple drugs. One hiding barrier is that the encapsulation demands precise chemical structure adjustment or advanced technologies, especially for separate drug encapsulation. Even though synergistic drugs are encapsulated into the same delivery system, such as polymeric nanoparticles, liposomes, or hydrogels, pharmacokinetic profiles of individual drugs would be dissimilar because of different physicochemical forces affecting the drug release mechanism as well as drug elimination kinetics. And the ideal simultaneous presence of combined drugs at the target site around the same time to maximize the combination therapy is also challenging for combination therapy. Besides, several undesirable side effects associated with combination chemotherapy also hindered the successful translation of these advantages, including the low targeting efficiency in vivo, the strong non-specific drug uptake by healthy organs such as the liver, spleen, kidney, and the potential drug–drug interaction, which would damage normal cells, deteriorate patient health condition, and result in poor patient compliance.

In this study, a facile approach to generate a multicompartment hydrogel was proposed for simultaneous but separate encapsulation of a drug cocktail to act as a combination therapy platform. Different from the previous attempts to deliver hydrophilic or hydrophobic molecules or both by a hydrogel [21,22,44–47], water-insoluble doxorubicin (Dox) and paclitaxel (Ptx) were respectively encapsulated into amphiphilic copolymer nanoparticles and then upon temperature simulation, the hybrid aqueous solution of these nanoparticles with incompatible cores transformed into a semi-solid multicompartment hydrogel achieving the simultaneous and segregated storage. The obtained formulation would ensure the controlled release of each agent at the same location in a desired range of concentration for a long period. In vitro drug release and cell viability were conducted. In vivo antitumor activity of drug cocktail using a multicompartment releasing system was checked and the animal survival and body weight was also recorded. The pathological analysis of tumor and other organs including the liver, spleen and kidney was performed.

2. Materials and method

2.1. Materials

Biodegradable 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) was prepared according to our previous reports [48,49]. Methoxy poly(ethylene glycol) (mPEG, $M_n = 2000$) and 2-(perfluorobutyl) ethyl methacrylate were purchased from Sigma and Apollo Scientific, respectively and used as received. Synthesis and characterization of mPEG-PPFEMA (PEPF) was performed following the previous study [37]. Paclitaxel (Ptx) and doxorubicin (Dox) were received from Shenyang Tianfeng Bioengineering Technology Co., Ltd. and Wuhan Yuancheng Gongchuang Technology Co., Ltd., respectively. Tetrahydrofuran (THF) and dimethylsulfoxide (DMSO) were obtained from Jiangtian chemical company.

2.2. Formation and characterization of Ptx-loaded PECT nanoparticles and Dox-loaded PEPF nanoparticles

Ptx-loaded PECT nanoparticles (NPs) were prepared by nanoprecipitation technology according to our previous procedure [49]. Dox-loaded PEPF nanoparticles were fabricated using the dialysis method. Briefly, Dox (25 mg) and mPEG-PPFEMA (500 mg) were dissolved in DMSO (10 mL) and then the solution was dialyzed in PBS (pH = 7.4, 0.01 M). The aqueous solution of drug-loaded nanoparticles was lyophilized. Ptx and Dox loading efficiency were determined by HPLC and UV–vis spectrum, respectively.

2.3. Fluorescence resonance energy transfer (FRET)-monitored formation of multicompartment hydrogel

The cooperative assembly of PECT and PEPF nanoparticles in water was monitored by FRET technology using 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) as the fluorescence donor and receptor, respectively. DiO and DiI were respectively encapsulated into PECT and PEPF nanoparticles by nanoprecipitation approach with a loading amount around 1% (w/w). Afterwards, these two

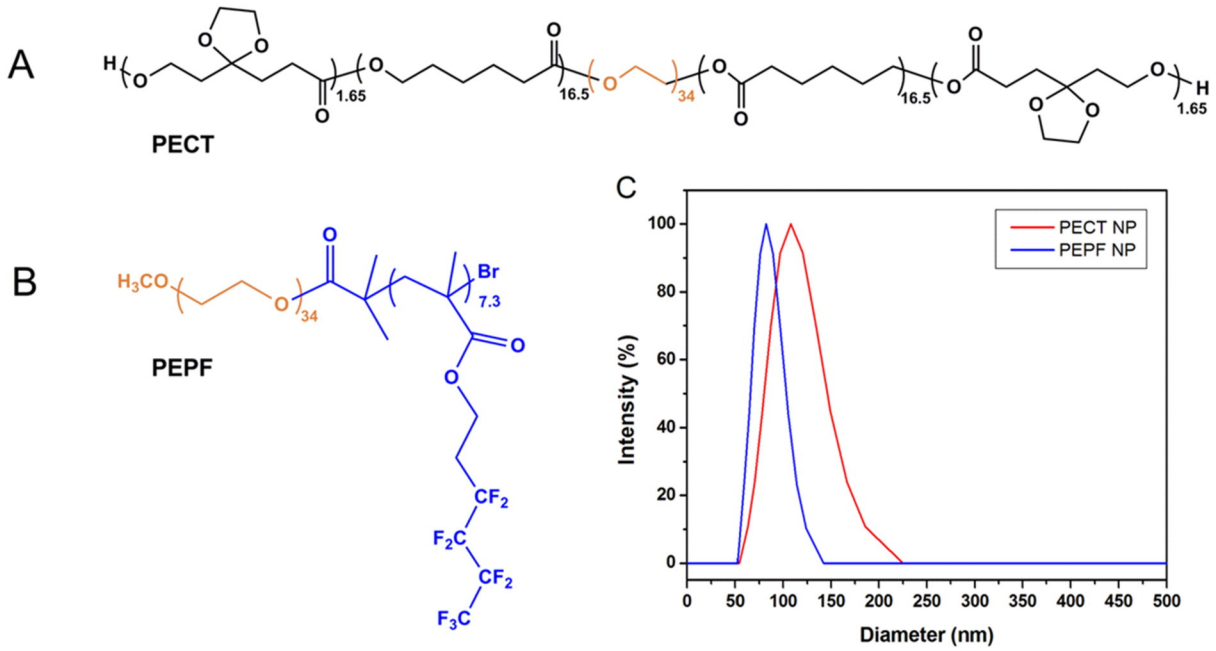

Fig. 1. The chemical structure of PECT (A), PEPF (B) copolymers and the size (C) of drug-loaded PECT or PEPF nanoparticles determined by DLS at a concentration of 1 mg/mL.