Materials Science & Engineering C 83 (2018) 121–129

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

High encapsulation and localized delivery of curcumin from an injectable hydrogel

Piao Ning, Shaoyu Lü*, Xiao Bai, Xue Wu, Chunmei Gao, Na Wen, Mingzhu Liu*

State Key Laboratory of Applied Organic Chemistry, Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province and Department of Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

ARTICLE INFO

Keywords:
Injectable hydrogel
High encapsulating capacity
Curcumin
Tumor
Localized drug delivery

ABSTRACT

Most chemotherapy currently available for cancer treatment has limited potential to successful clinical cancer therapy, mainly due to low encapsulating capacity of drugs and unavailable pharmacologically beneficial concentrations at the tumor site. Herein, a novel yet simple strategy is developed to enhance drug encapsulating capacity and localized drug concentration using an injectable hydrogel based on thiolated chitosan (TCS) and poly(ethylene glycol) diacrylate (PEGDA). Almost 100% of encapsulating capacity is achieved when anti-cancer drug curcumin is encapsulated in the system. The interaction of curcumin with PEGDA is determined by fluorescence spectroscopy and the binding constant is calculated, followed by a simulation by a docking study using AutoDock. To improve the anti-tumor activity and achieve effective local concentrations, lysozyme is introduced into the system. Sustained curcumin release in a controlled lysozyme-responsive behaviour is observed, which enables the drug concentration to reach the therapeutic threshold promptly. The system displays efficient intracellular curcumin release to promote cancer cells apoptosis *in vitro*. In addition, the system effectively delays the tumor growth and reduces adverse effects in tumor-bearing nude mice. The strategy of localized, high encapsulation of drug by using an injectable hydrogel would be particularly beneficial with many insoluble anti-cancer drugs.

1. Introduction

Despite our knowledge of cancer biology has advanced significantly, the incidence of some cancers and the cancer death rate are increasing in past decades [1]. Conventional chemotherapeutics associated with intravenous or oral administration always lead to undesired side effects and systemic toxicity. Therefore, localized chemotherapy technology is developed as a key approach to target tumors with enhanced drug bioavailability and reduced systemic toxicity [2–5]. It is a promising approach for delivering anti-cancer drugs directly to the targeted site, and routinely used for the treatment of solid tumors [6–9].

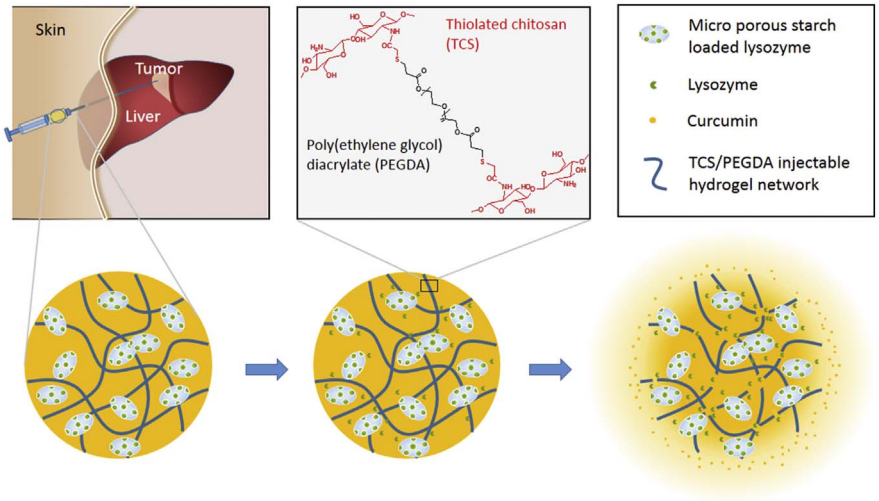
Injectable hydrogels are excellent candidates for anti-cancer drug delivery in local-regional administration owing to their facile drug encapsulation, easy to handle and apply, and minimal discomfort to the patient [10,11]. They provide the potential to deliver anti-cancer drugs locally to the tumor site, and thus leading to low dose requirements and minimal non-target systemic exposure and organ toxicity [12,13]. In addition, the efficacy of the drug can be increased because the need of taking a long journey to reach the target site can be avoided.

Previous studies have reported that various injectable hydrogels were advanced delivery vehicles for therapeutic agents controlled release [14–17]. Unfortunately, the sustained release of drugs for long time always leads to the problem that the concentration of a drug released from hydrogels may not be able to reach the available pharmacologically beneficial concentrations promptly. This may contribute to the development of multidrug resistance (MDR) in tumor cells [18]. In addition, although great effort has been put forth to develop injectable hydrogels for cancer therapy, their real use in clinical applications is still in its incipient stage. The limitation lies in the complicated synthesis process, the possible toxicity of the agents, and the high cost of systems.

Addressing these limitations, we developed an injectable hydrogel based on polysaccharide chitosan and poly(ethylene glycol) diacrylate (PEGDA) in this study. Its preparation process is facile, meanwhile, hydrogel precursor and degradation products are nontoxic. Therefore, the hydrogel has a high potential for clinical application. The injectable hydrogel is expected to release anti-cancer drugs rapidly by destructing the hydrogel network using encapsulated lysozyme. Lysozyme is a secretory product of various leucocytes and epithelial cells [19]. It is well-known to degrade chitosan. Therefore, once the mixture (the hydrogel

* Corresponding authors.
E-mail addresses: lshy@lzu.edu.cn (S. Lü), mzliu@lzu.edu.cn (M. Liu).

https://doi.org/10.1016/j.msec.2017.11.022
Received 9 August 2017; Received in revised form 1 October 2017; Accepted 21 November 2017
Available online 23 November 2017



Scheme 1. Scheme of thiolated chitosan/poly(ethylene glycol) diacrylate (TCS/PEGDA) injectable hydrogel for localized intratumoral delivery of anti-cancer drugs. Micro porous starch was used to adsorb lysozyme, which was expected to improve the anti-tumor activity and induce available pharmacologically beneficial concentrations. Curcumin as an anti-cancer drug was encapsulated in the system.

precursor and drug) is injected in tumor site and a hydrogel has been formed, the lysozyme released from micro porous starch would react with TCS, which cause the hydrogel to destruct and subsequently the drug release. In addition, a number of *in vivo* studies with animal tumors indicated that lysozyme is capable of reducing the mass of the tumor and of increasing the survival time of the treated animals in several situations [20]. Meanwhile, lysozyme is always very well tolerated by the treated animals, even at dosages of 2.500 mg/kg/day. It is expected that the introduction of lysozyme in the hydrogel will improve the anti-tumor activity of the system and overcome the disadvantage of slow drug release from hydrogel on reliance of diffusion. The slow drug release always leads to the problem that the concentration of the drug may not be able to reach the therapeutic threshold promptly [21]. Scheme 1 shows a schematic illustration of the injectable hydrogel and how it works.

A suitable anti-cancer drug delivery system is not sufficient. Most drugs currently available for cancer treatment still remain a large obstacle to successful clinical application, due to their dose-limiting toxicity, inefficiency in treating cancer, intracellular drug-resistance, and expensiveness and thus beyond the reach of the majority [22]. Anti-cancer drugs without these disadvantages are needed. One such agent is curcumin, which is derived from turmeric (*Curcumin longa*). It has a broad range of favorable biological functions, including anti-oxidant, anti-inflammatory activities, and anti-cancer potential against various cancers [23,24]. Curcumin is also found to be pharmacologically safe even at very high doses in a variety of animal models and many human clinical trials [25,26]. In addition, curcumin shows the potential to overcome multidrug resistance in cancer cells due to down-regulating multidrug resistance proteins (MDR) gene expression and P-glycoprotein (P-gp) function [27]. However, curcumin has not been administrated in any clinical trials systemically due to its low water solubility and poor bioavailability [28,29]. Therefore, conventional administration of curcumin is incapable of providing pharmacologically beneficial concentrations in the body for cancer treatment.

Some attempts have been made to encapsulate curcumin in nanoparticles, micelles and hydrogels to improve its solubility, stability and bioavailability [15,30–32]. However, as we known, the solubility of hydrophobic drugs in hydrophilic systems is low. In addition, drugs are commonly encapsulated into polymeric systems through a passive absorption method whereby polymeric systems are added to a drug solution. For hydrophobic drugs, the encapsulating efficiency is limited and a high encapsulating capacity is unattainable. Synthesis of covalent polymer-drug conjugates is another method to improve the drug hydrophobicity. However, only several percent of hydrophobic drugs can be conjugated to make the system water-soluble. In addition, it may not be achievable for all types of drugs, and potential inactivation by covalent attachment may occur [33,34]. Therefore, solubilization of hydrophobic anti-cancer drugs and developing suitable drug delivery systems are still a challenging task in cancer therapy. Here we encapsulated (solubilized without chemical modification) curcumin in TCS/PEGDA injectable hydrogel by simple mixing, which can improve the drug encapsulation capacity and avoid the original activity of curcumin losing after chemical modification. Once curcumin is released from the hydrogel and diffuses into the cytosol, it could immediately exert its biological actions.

2. Experimental section

2.1. Materials

Chitosan (deacetylation degree of 90.8%, molecular weight of 270,000) was supplied by Golden-Shell Biochemical Co., Ltd. (Zhejiang, China). Thioglycolic acid and poly(ethylene glycol) diacrylate (PEGDA, molecular weight of 700) were purchased from Sigma-Aldrich. Corn starch (food grade, molecular weight of 1.28×10 [6]) was supplied by Xuejing Starch Ltd. (Gansu, China). Lysozyme and curcumin were provided from Bio Basic Inc. (Toronto, Canada). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), lactic acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and other chemicals were used as received.

2.2. Synthesis of modified chitosan containing thiol groups (TCS)

Thiolation of chitosan was carried out using a one-pot synthesis procedure. Typically, chitosan (50 mg) was dissolved in 5 mL 5% (v/v) lactic acid solution. A five-molar excess (relative to the carboxylic acid groups in thioglycolic acid) of EDC (2.5 mmol) and NHS (2.5 mmol) were added to the solution and the pH was adjusted to 5.0. After 30 min, thioglycolic acid (0.5 mmol) was added. The reaction mixture was incubated in dark for 3 h at room temperature under stirring. The solution was first dialyzed (molecular weight cut-off 14,000) against 5 mmol L^{-1} HCl for 12 h followed by dialysis against 5 mmol L^{-1} HCl containing 1% NaCl for 24 h, and then dialyzed against 1 mmol L^{-1} HCl for 24 h. After dialysis, the solution was quickly immersed in liquid nitrogen and lyophilized. The conjugated chitosan containing free thiol groups (TCS) was obtained and its structure was characterized by FTIR.

The free thiol content of TCS was determined using Ellman's method. Briefly, TCS was dissolved at 1 mg mL^{-1} in water and mixed with a 10 mM DTNB solution. The absorbance at 412 nm was taken using an UV/vis spectrophotometer (Lambda 35, Perkin-Elmer, America). The thiol content was determined *via* a cysteine calibration curve of thiol content *vs* absorbance.