Biomaterials 30 (2009) 2606–2613

Contents lists available at ScienceDirect

Biomaterials

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

# Dual-drug delivery system based on hydrogel/micelle composites

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ARTICLE INFO

*Article history:*
Received 30 November 2008
Accepted 3 January 2009
Available online 21 January 2009

*Keywords:*
Drug release
Dual-drug delivery
Hydrogel
Micelle
Polypeptide

ABSTRACT

We present a dual-drug delivery system (DDDS) of hydrogel/polypeptide micelle composites in this work. The DDDS was constructed from aspirin (Asp) dispersed poly(vinyl alcohol) (PVA) or Chitosan (CS)/PVA hydrogel and doxorubicin (DOX) loaded poly(L-glutamic acid)-*b*-poly(propylene oxide)-*b*-poly(L-glutamic acid) (GPG) micelles. Independent release behaviors of the two drugs are observed. Asp has a short-term release while DOX has a long-term and sustained release behavior in all the DDDSs. The release of DOX from all the DDDSs is environmentally controlled due to the pH and temperature sensitivity of the GPG micelle. Asp shows the pH controlled release behavior in CS/PVA/micelle DDDS due to the pH sensitivity of CS hydrogel. The releasing profiles were analyzed using a power law equation proposed by Peppas. It reveals that the release of Asp is anomalous transport in all the hydrogel/micelle DDDSs. The release of DOX is Fickian type in PVA/micelle system, and changes to anomalous transport in CS/PVA/micelle system according to the release exponent *n*.



## 1. Introduction

Over the past few decades, drug delivery systems (DDSs) have been developed and studied in great depth to improve the curative effect of drugs [1–4]. DDS can ameliorate the problems of conventional administration by enhancing drug solubility, prolonging duration time, reducing side effect, retaining drug bioactivity and so on [5,6]. A variety of systems such as particulate carriers [7,8], polymer gels [9,10], lipids [11,12], etc. have been used as DDSs. At present, stimuli sensitive DDSs have been an attractive theme for controlled release. The release behaviors of drugs can be easily controlled by surrounding properties, such as pH [13,14], temperature [15,16], ionic strength [17] and electric field [18].

Hydrogels are hydrophilic three-dimensional polymer networks, which contain a large amount of water [19–21]. They are highly permeable to various drugs and the entrapped drugs can be released through their web-like structures [22]. As compared with conventional administration, drugs can prolong their duration time by hydrogel DDS. For example, Park et al. investigated biodegradable elastic hydrogel scaffolds which are based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(ε-caprolactone) (PCL) as a delivery vehicle [23]. These hydrogel scaffolds can offer a suitable environment for the retention of the chondrocytic phenotype and cell therapy. When the hydrogels are stimuli sensitive, they may act as "smart" DDS [24–26]. Wu et al. designed a thermo- and pH-sensitive drug delivery hydrogel system which is composed of *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC)/glycerophosphate (GP) [26]. The polymer composition is a free flowing sol at room temperature but becomes a gel at body temperature, which makes it injectable. The hydrogel is stable at neutral and basic conditions but dissolves at acid condition, which leads to a quick releasing of drug at acid condition and a slow releasing at neutral and basic condition.

Another important DDS is polymeric micelle, which is self-assembled from amphiphilic block or graft copolymers [27–30]. These polymeric micelles show distinct stability in solution [31–33]. The core–shell structure of the micelle can improve solubility of hydrophobic drugs, and protect the incorporated drug from premature degradation [34,35]. When environmentally sensitive (pH, temperature, etc.) functional groups are introduced into these amphiphilic copolymers, "smart" micelles are formed, and they can be used as environmentally controlled drug release system [36–38]. For example, Ko et al. used methyl ether poly(ethylene glycol)–poly(β-amino ester) block copolymer micelles to encapsulate doxorubicin [38]. These micelles show noticeable pH-dependent micellization–demicellization behavior, leading to a quick release at pH 6.4 and a slow release at pH 7.4.

Recently, combined therapy with drugs of different therapeutic effects shows an effective way in treatment of diseases and tissue reborn [39,40]. In order to optimize their effects, different drugs should be used at their optimal dose and different periods in the treatment. One of the main challenges of combined therapy is to

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doi:10.1016/j.biomaterials.2009.01.006

control the release behavior of each drug independently. However, simple drug delivery systems cannot fulfill the needs of such therapy. Therefore, developing the dual-drug delivery systems which can control the release behavior of each drug is desired. However, limited researches on the dual-drug delivery system (DDDS) are reported so far. For example, Lee et al. developed a simple dual-drug-loaded hydroxypropylmethylcellulose (HPMC) matrix tablet which simultaneously contains drug in inner tablet core and outer coated layer [41]. The obtained dual-drug-loaded HPMC matrix tablet shows biphasic release profiles, and can deliver drugs with circadian rhythmic behaviors in the body.

In this work, we present a delivery system of hydrogel/micelle composites as dual-drug release vehicle. The hydrogel is prepared from poly(vinyl alcohol) (PVA) or Chitosan (CS)/PVA. We use PVA hydrogel for its good physical–mechanical properties, and CS hydrogel for its pH sensitivity. Both hydrogels present good biocompatibility for drug delivery. The micelle is prepared from poly(L-glutamic acid)-*b*-poly(propylene oxide)-*b*-poly(L-glutamic acid) (PLGA-*b*-PPO-*b*-PLGA, abbreviated as GPG), which is pH- and thermo-sensitive. Two drugs, aspirin (Asp, water-soluble) and doxorubicin (DOX, fat-soluble) are used as model drugs. DOX is encapsulated into the GPG micelle, while Asp is directly dispersed in the hydrogel. The drug release behaviors of GPG micelle, PVA/micelle DDDS and CS/PVA/micelle DDDS were studied as functions of pH and temperature. The releasing profiles were analyzed by a power law equation to reveal the release mechanisms of drugs.

## 2. Experimental

### 2.1. Materials

Tetrahydrofuran (THF), hexane, 1,4-dioxane were refluxed with sodium and distilled immediately before use. Doxorubicin hydrochloride (DOX-HCl) was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd. Aspirin was supplied by Huayin Jinqiancheng Pharmaceutical Co., Ltd. Poly(vinyl alcohol) (PVA, $M_w = 1750$) was purchased from Shanghai Tianlian Industry of Fine Chemicals Co., Ltd. Chitosan (CS, degree of deacetylation $\geq 90\%$) was obtained from Sinopharm Chemical Reagent Co., Ltd. α,ω-Amino poly(propylene oxide) ($NH_2$–PPO–$NH_2$, $M_w = 4000$) was purchased from Sigma–Aldrich Co., Inc. $NH_2$–PPO–$NH_2$ was dissolved in toluene in a flame-dried reaction bottle, followed by removing the toluene in high vacuum to obtain the initiator used for copolymerization. Cellulose membrane dialysis bag (3500 molecular weight cut-off) was provided by Serva Electrophoresis GmbH. All other reagents are of analytical grade and used without further purification.

### 2.2. Synthesis and characterization of triblock copolymer

Poly(γ-benzyl-L-glutamate)-*b*-poly(propylene oxide)-*b*-poly(γ-benzyl-L-glutamate) (PBLG-*b*-PPO-*b*-PBLG) triblock copolymer was synthesized by ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) initiated by terminal amino groups of $NH_2$–PPO–$NH_2$ [42,43]. $NH_2$–PPO–$NH_2$ (0.2 g; 0.05 mmol) and BLG-NCA (0.71 g; 2.7 mmol) were dissolved in dioxane separately in two flame-dried reaction bottles, then, BLG-NCA solution was added to the solution of $NH_2$–PPO–$NH_2$ via a transfer cannula. The reaction was performed at 15 °C under a dry nitrogen atmosphere. After 72 h stirring, the reaction mixture became a viscous liquid and was poured into a large volume of anhydrous ethanol. The precipitation product was collected and dried under vacuum. The product was purified twice by repeated cycle of dissolution (in chloroform) and precipitation (in anhydrous ethanol) of the product. PLGA-*b*-PPO-*b*-PLGA triblock copolymer was prepared by hydrolyzation of PBLG-*b*-PPO-*b*-PBLG with potassium hydroxide (KOH) [44]. As a brief, 1 g PBLG-*b*-PPO-*b*-PBLG was dissolved in 40 mL THF. In a separate step, a concentrated aqueous solution of KOH (3 mol equivalence with respect to benzyl group) was prepared and added to the solution of the polymer. After 4 h stirring, the mixture was acidulated with excessive HCl, and dialyzed against distilled water for 3 days to remove organic solvents and other small impurities. The product was finally freeze-dried to get PLGA-*b*-PPO-*b*-PLGA powder.

The molecular weight of the block copolymer before hydrolysis was estimated using $^1$H NMR measurements (Avance 550, Bruker). Since the degree of polymerization (DP) of the PPO block is known (69), the total molecular weight of the triblock copolymer PBLG-*b*-PPO-*b*-PBLG can be calculated by the peak intensities of the methylene proton signal (5.1 ppm) of PBLG and the methylene proton signal (3.6 ppm) of PPO in the $^1$H NMR spectrum. The calculation shows that the molecular weight of the original copolymer is 15,800 and the DP of PBLG is 27. The disappearance of methylene proton peak (5.1 ppm) of PBLG segments in the $^1$H NMR spectrum provides the evidence of deprotection of benzyl group from PBLG-*b*-PPO-*b*-PBLG copolymer to form PLGA-*b*-PPO-*b*-PLGA copolymer. The molecular weight of PLGA-*b*-PPO-*b*-PLGA copolymer is calculated to be 10,900 after hydrolysis.

### 2.3. Preparation of drug-loaded delivery systems

To prepare drug-loaded micelle, 3 mg DOX-HCl and 6 mg PLGA-*b*-PPO-*b*-PLGA (GPG) were first dissolved in 10 mL DMF/DMSO mixed solvent (v/v = 4/1). One drop of triethylamine (ca. 0.05 mL) was added to the solution to remove hydrochloride. After stirring at room temperature overnight, the mixed solution was dialyzed against distilled water for 72 h at 20 °C to form the DOX-loaded GPG micelle. The distilled water was replaced every 3–4 h. The obtained drug-loaded micelle solution was diluted, and the final concentration of GPG is 0.3 mg/mL.

To produce PVA hydrogel/GPG micelle DDDS, 1 mg Asp was dissolved in 10 mL PVA aqueous solution (PVA: 50 mg/mL), and then mixed with 10 mL pre-made DOX-loaded GPG micelle solution. The PVA hydrogel/GPG micelle DDDS was then prepared by a freezing–thawing cycle (freezing at −20 °C for 24 h and thawing at room temperature for 3 h) in special moulds [45]. The CS/PVA/micelle mixture was prepared as follows: CS (100 mg/mL, dissolved in 2 vol% acetic acid) and PVA (100 mg/mL) aqueous solutions were mixed with various CS/PVA volume ratios (1/1, 2/3, 1/3 and 1/6). Therefore, the total polymer concentration of all the CS/PVA solutions is 100 mg/mL. And then 1 mg Asp and 10 mL pre-made DOX-loaded GPG micelle solution were added to 10 mL CS/PVA solution. The CS/PVA/micelle solution also went through the freezing–thawing cycle (the same as those applied to the PVA/micelle system) to form the hydrogel/micelle DDDS. All the hydrogel/micelle DDDS samples are cylindrical in shape (ca. 28 mm thick, and 30 mm in diameter).

### 2.4. Characterization of drug delivery systems

The amount of DOX encapsulated in the GPG micelles was analyzed by an ultraviolet–visible spectrometer (UV/vis, Unico UV2102). 8 mL DMF was introduced into 2 mL DOX-loaded micelle solution, the micelles were broken up and the DOX was dissolved in the solution. The characteristic absorbance of DOX at 485 nm was recorded and compared with a standard curve generated from a DMF/$H_2O$ (v/v = 4/1) mixture with DOX concentrations varying from 0 to 100 μg/mL.

The morphologies of DOX-loaded GPG micelles both in solution and in PVA/micelle DDDS were examined using transmission electron microscope (TEM, JEM-1200-EXII), operated with an accelerating voltage of 120 kV. Drops of DOX-loaded GPG micelle solution were placed on a carbon film-coated copper grid and stained by phosphotungstic acid aqueous solution (0.5 wt%). DOX-loaded GPG micelle/PVA solution was pre-stained in solution and dropped to carbon film-coated copper grid, followed by a freezing–thawing cycle. All the samples were dried at room temperature.

### 2.5. Swelling properties

Swelling degrees (SDs) of hydrogels were measured at 37 °C. The fresh made samples (wet) were weighted and immersed in buffer solutions with different pH values. These samples were gently wiped with filter paper to remove the surface solution when taken out from the solutions, then weighted and returned to the same container at pre-determined time intervals. The SD was calculated as follows:

$$\mathrm{SD}\ (\%) = \left(\frac{W_t}{W_0}\right) \times 100 \quad (1)$$

where $W_0$ is the weight of the original hydrogel and $W_t$ is the weight of hydrogel at various swelling times.

### 2.6. In vitro drug release study

A fixed volume of DOX-loaded micelle solution was suspended in dialysis bag, and placed into 10 mL buffer solution with various pH values. PVA/micelle and CS/PVA/micelle DDDS were directly immersed in 10 mL buffer solutions. All the samples were then laid in a shaking bath at 90 rpm, with constant temperature of 20, 30, or 37 °C respectively. The buffer solution was replaced periodically. UV/vis absorbance was recorded at 296 nm (Asp) and 485 nm (DOX); the concentrations of Asp and DOX in buffer solutions were determined according to the standard curves of each drug at corresponding buffer solutions. And then the release amount of the drugs can be calculated.

## 3. Results and discussion

This paper consists of three sections: In the first section, dual-drug delivery system was prepared from PVA hydrogel/GPG micelle composites. Asp is dissolved in the hydrogel, while DOX is encapsulated in the micelle. We investigated the drug release profiles of GPG micelle and PVA/micelle DDDS as functions of pH and