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Delivery of cisplatin from thermosensitive co-cross-linked chitosan hydrogels

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ABSTRACT

The aim of the present study was to investigate the *in vitro* cisplatin release from thermosensitive chitosan hydrogels, produced by ionic cross-linking and by ionic/covalent co-cross-linking. For that, two types of cisplatin-loaded hydrogels were prepared: chitosan hydrogels cross-linked with glycerol-phosphate disodium salt (as an ionic cross-linker) and chitosan hydrogels ionic/covalent co-cross-linked (using different amounts of genipin as covalent cross-linker). Both hydrogels were able to be produced *in situ* at physiologic conditions.

Regarding cisplatin release, all hydrogels exhibited a first initial rapid release reaching a maximum at about 3 h. However, the total amount of drug released was only 20% for the ionic hydrogel (without genipin) and about 60–70% for the ionic/covalent co-cross-linked hydrogels. This disparity was explained in terms of the type of cross-linking and hydrogels network structure. Changes in the initial drug loadings (0.6 and 1 mg/mL) as well as in the covalent cross-linker concentrations (0.10% and 0.20%, w/w) did not significantly affect the cisplatin release profiles.

1. Introduction

Cisplatin, a platinum-chelated complex with four ligands (two ammonias and two chlorides), in short CDDP [*cis*-diamminedichloroplatinum (II)], is one of the most effective cytotoxic agents used for the treatment of various malignancies, ranging from solid tumors to melanomas [1–3]. The CDDP, as well as all platinum compounds available for clinical use, is usually administered intravenously. The main problems related with this type of administration are the serious systemic side-effects and the low drug concentrations at cancerous site [3]. Alternative approaches that have been used involve the incorporation of the platinum agent in liposomes [4–6], microspheres/nanoparticles [2,7,8], and polymeric micelles [1]. However, most of these particulate systems have been typically designed for intravenous administration.

To minimize the adverse effects caused by CDDP, it is necessary to deliver the drug directly to the tumor site and to retain it for convenient duration and concentration levels to elicit pharmacological actions as well as to control the release profile.

Recent strategies to deliver the drug to the local environment of a solid tumor involve the use of polymeric hydrogels [9]. Although hydrogels have been extensively explored as drug delivery vehicles [10], much interest has been focused on injectable *in situ* forming hydrogels, since these are capable of forming stable gels at body temperature within a short period after injection [11,12]. Such behavior endows the hydrogel with injectability, most suited for localized and minimally invasive drug delivery, providing, simultaneously, a reservoir for the sustained release of the drug. Chitosan-based systems have been

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extensively employed for this purpose since they present some fundamental properties such as biodegradability, biocompatibility, non-toxicity and the non-inflammatory tendency [13,14].

Although many injectable hydrogel systems have been used for drug delivery, only a few have been applied for anticancer drugs [2]. It should be stressed that in particular the delivery of CDDP poses additional problems due to the relatively small molecular size of this drug that result in a great difficulty in retaining it within the tumor. Many approaches have been proposed to prolong this drug release, namely incorporating in the gel CDDP encapsulated in particulate systems and/or increasing the cross-linking degree of the thermosensitive hydrogel (as for example, using chemical cross-linkers) to improve gel mechanical strength and the network stability [15,16]. However, the chemical cross-linkers mostly used (as for example the glutaraldehyde) originate gels which have difficulty in body clearance and/or could be potentially toxic [2]. Besides, complex and time consuming hydrogel preparation processes have been proposed and/or long gelation times, have been obtained, being the rate of drug release greatly influenced by the gel composition and structure. Indeed, an ideal delivery formulation should offer quick and easy gelation and drug loading preferably in aqueous environment [5].

A novel *in situ* forming chitosan formulation has been recently developed by the present authors [15,17], via coupled ionic and covalent co-cross-linking. The combination of chitosan with two cross-linkers, glycerol-phosphate disodium salt (as an ionic cross-linker) and genipin (as a covalent cross-linker), leads to a thermosensitive hydrogel formed at mild conditions (pH 7 and 37 °C) with improved mechanical and chemical properties compared to those obtained using either cross-linker separately. The hydrogel preparation is quick, easy and water-based: the chitosan solution, pre-neutralized with glycerol-phosphate disodium salt, was subsequently covalently cross-linked with different amounts of genipin (a naturally occurring cross-linking agent, significantly less cytotoxic than glutaraldehyde), at physiologic conditions, to produce *in situ* forming chitosan hydrogels. The rheological characterization of the chitosan-based matrices has been carried out by Moura et al. in an earlier work [15]. *In vivo* studies demonstrated that the gel was rapidly formed and was not delocalized throughout time [17]. Moreover, the genipin load was found to modulate the network structure and performance [15,17].

The aim of the present study was to evaluate the behavior of these thermosensitive hydrogels for local delivery of CDDP, focusing on the effect of the ionic and ionic/covalent co-cross-linking.

2. Experimental section

2.1. Materials

The chitosan used in the experiments (molecular weight $\sim 2 \times 10^5$ Daltons and a degree of de-acetylation of 87%, calculated from the carbon/nitrogen ratio by elemental analysis) was purchased from Sigma–Aldrich, in a powder form. The hydrated glycerol-phosphate disodium salt ($C_3H_7Na_2O_6P{\cdot}xH_2O$; FW = 218.05), used either to adjust the pH of the chitosan solutions and as an ionic cross-linker agent, the *o*-phenylenediamine (OPDA) and the cisplatin ($Pt(NH_3)_2Cl_2$; FW = 300.05) with $\geqslant$99.9% trace metals basis were also obtained from Sigma–Aldrich. Genipin (crystallike powders, reagent grade) was supplied by Challenge Bioproducts Co., Taiwan. Phosphate buffered saline (PBS, pH = 7.4), obtained from Sigma–Aldrich, was prepared by dissolving one tablet in 200 mL of distilled water. All of the other reagents and solvents that were used in this work were of the highest purity commercially available.

2.2. Preparation of chitosan hydrogels loaded with CDDP

As mentioned before two types of chitosan hydrogels were prepared: without and with covalent cross-linker (genipin), respectively known as ionic cross-linked hydrogel and ionic/covalent co-cross-linked hydrogel [15,17].

Briefly, a solution containing 2 g of chitosan in 100 mL of volume was prepared by dissolving the chitosan powder (C) in distilled water that containing 0.5% (by volume) of acetic acid, at room temperature. The pH was adjusted to 7 by the addition of the required amount of glycerol-phosphate disodium salt (GP), previously dissolved in distilled water. The final solution contained 1.5 g of chitosan and 5.5 g of GP in 100 mL of solution. CDDP was then added, under magnetic agitation, to the polymer solution which was finally placed in a polysiloxane mould to produce cylindrical samples (diameter 14 mm). These samples were designated as C/GP. For the hydrogels co-cross-linked with genipin (GE), a similar procedure was followed except in that the genipin powder was added to the neutralized chitosan solution, before adding CDDP. In this case, two different amounts of genipin were used: 0.10% and 0.20% (mass of genipin/mass of total solution), the corresponding samples being designated as C/GP/GE10 and C/GP/GE20, respectively. The effect of the initial concentration of CDDP in the hydrogels (0.6 and 1 mg/mL) was also investigated. These concentrations were selected having in mind the CDDP solubility in water at room temperature (1 mg/mL) [18].

The hydrogels produced for *in vitro* release tests were subsequently cured at 37 °C for 2 h. The rheological characterization of the injectable system carried out in a previous work [15] revealed that this period of time is sufficient to obtain a structured solid matrix required to stand the conditions of the *in vitro* tests.

2.3. Morphological characterization of hydrogels

The microstructure of the chitosan hydrogels was examined using a Scanning Electron Microscope (SEM). For this purpose, the hydrogel samples were frozen at −20 °C and further freeze-dried in a lyophilizer (Snijders Scientific type 2040, Tilburg, Holland) under vacuum (0.50 mbar) at −50 °C for at least 3 days, until all of the solvent had sublimed. The dehydrated samples were cross-sectioned and placed on double-sided tapes, sputtercoated with gold, and observed by SEM (model JSM-5310, JEOL, Tokyo, Japan).