Materials Science and Engineering C 69 (2016) 144–153

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Cyclodextrin-polyhydrazine degradable gels for hydrophobic drug delivery

Esmat Jalalvandi, Jaydee Cabral, Lyall R. Hanton, Stephen C. Moratti *

Department of Chemistry, University of Otago, Dunedin 9054, New Zealand

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 24 May 2016
Accepted 16 June 2016
Available online 16 June 2016

Keywords:
Hydrogel
β-cyclodextrin
Inclusion complex
Hydrophobic drug delivery

ABSTRACT

An injectable and biocompatible hydrogel system was designed for hydrophobic drug delivery. This hydrogel consisted of degradable polymers with cyclodextrin (CD) moieties. CD groups were used to increase the solubility of a hydrophobic molecule (nicardipine) in an aqueous solution through the formation of the inclusion complex. Two sets of gels were prepared by mixing oxidized dextran (DA) and CD functionalized polyhydrazine (PH) at physiological conditions and different level of crosslinking via hydrazone bonds. Cytotoxicity studies on the gels and their components confirmed the biocompatibility of these materials. Gel-30 with higher crosslinking density showed a two week degradation period whereas this period was 10 days for gel-10, with lower crosslinking density, to complete degradation. The results from swelling tests and rheological measurements were also found to be dependent on crosslinking density of the hydrogels. Release profile of the hydrogel displayed a sustained release of nicardipin up to 6 days for gel-30 and a 4 day release with initial burst release for gel-10.

1. Introduction

The low water solubility and lipophilic character of many different drugs is a big problem in many pharmaceutical applications, resulting in low bio-absorption and low therapeutic efficiency [1–3]. Drug solubilisation using colloidal carriers, such as liposomes and surfactant micellar structures has been a common solution. Typically, drug-loaded micelles or liposomes are dispersed into aqueous solutions of crosslinked networks during the synthesis processes [4,5]. The limitations of this solubilisation strategy often include poor shelf-life and stability which may change optical and mechanical properties of the hydrogel, difficulty in targeting specific tissues and rapid clearance by phagocytic cells of the reticuloendothelial system [6–8].

As a way of addressing the above problems, hydrogels have been extensively investigated in the drug delivery area [9,10]. These three dimensional networks composed of synthetic and/or natural water soluble polymers often show good biocompatibility and mechanical properties resembling biological tissues [11]. There are many types of hydrogels used for controlled or sustained drug delivery, ranging from chemically, physically and supramolecularly crosslinked systems. Conventional drug delivery methods such as oral delivery are not suitable to control the rate of drug delivery. These modes of drug administration experience difficulty in succeeding steady-state drug concentrations and pharmacokinetic profiles [12]. Of the different types of crosslinking techniques, *in-situ* forming hydrogels are of particular interest in drug delivery area. The precursors of *in-situ* forming hydrogels are injectable fluids which are presented into the body in a minimally invasive manner prior to solidification at the targeted site. These systems allow an effective and homogeneous encapsulation of bioactive agents such as drugs and cells. These injectable systems undergo sol-to-gel transitions through chemical and/or physical crosslinking at physiological conditions. The use of biodegradable polymeric system in controlled release drug delivery is highly desired, since the matrix needs to be degraded and eliminated from the body and there is no need for removing them afterwards [13–15]. Due to the highly hydrophilic character of the hydrogels, the loading and the release of hydrophobic drugs from these networks can be challenging. An insufficient therapeutic amount of loaded drug, drug aggregation or a rapid release profile which leads to side effects are some of the problems encountered [6,16,17].

The ability of cyclodextrins (CD)/or modified CDs to form inclusion complexes with hydrophobic guest molecules is well-known. This ability is generated by the internal hydrophobic cavity of the CDs which can accommodate small lipophilic molecules [18,19]. However, these complexes are very labile, and dilution leads to instant de-complexation. This means no sustained drug release can be observed from a typical CD:drug (host:guest) complex [20,21]. One way to address this issue is incorporation of CDs into chemically crosslinked hydrogels which might help prevent fast de-complexation and give a more sustained release, but retaining good loadings [16,22,23].

Gels can be used preformed or set *in-situ*. The latter is preferred for drug release as the gel can be injected and set inside the body. There

* Corresponding author.
E-mail address: smoratti@chemistry.otago.ac.nz (S.C. Moratti).

http://dx.doi.org/10.1016/j.msec.2016.06.058
0928-4931/

are several prior examples of CD-based hydrogels, however many of them are preformed and non-biodegradable. In many cases the use of toxic crosslinking agents limited their biological application [24–26]. Alvarez et al. developed a preformed hydrogel system based on the copolymerization of poly(hydroxyethylmethacrylate) and glycidyl methacrylate with grafted β-CD for sustained release of diclofenac over a two week period [27]. Kros et al. prepared an *in-situ* gelling hydrogel composed of thiol-functionalized β-CD and maleimide-functionalized dextran. In this system β-CD acts as both the crosslinker and the hydrophobic site to accommodate *trans*-retinoic acid. The release rate of the drug was essentially limited by its solubility and continued for two weeks without showing an initial burst effect [16]. Rodriguez-Tenreiro et al. developed a new hydrogel based on cyclodextrins crosslinked with ethyleneglycol diglycidylether under mild conditions, to be used as carriers of an amphiphilic drug. This preformed hydrogel showed high ability to load and sustain the release of diclofenac for several hours [17]. Another preformed hydrogel was made by Nielsen et al. Vinyl derivatives of CDs were added to a blend of poly vinylpyrrolidone and poly ethyleneglycol dimethacrylate and cured by UV light to obtain a hydrogel system with CD moieties covalently incorporated into the matrix. This matrix was used to retard the release of ibuprofenate [28]. However, the preparation of *in-situ* forming hydrogels which are biodegradable and biocompatible to deliver hydrophobic drugs over an extended period is still needed.

Herein, an injectable system has been designed and developed for delivery of nicardipine (NIC). NIC is an antihypertensive drug with low solubility in alkaline media. It is rapidly absorbed, extensively presystemically metabolized and excreted in the urine and faeces, mainly as inactive metabolites [29]. Since the duration of its action can be extended by prolonging the absorption interval, the design of a controlled release formulation is required [30]. This hydrophobic drug has been administered intravenously, subcutaneously and transdermally [31, 32]. Formation of inclusion complex with β-CD has been suggested to improve the solubility of NIC in aqueous solution [33,34]. Due to the poor solubility of NIC in aqueous phases and other routes of administration, it has been chosen as a model drug in this work. Dextran is a natural polysaccharide widely used in hydrogel preparations in biomedical applications due to its biodegradability and biocompatibility. It can be oxidized to give dextran aldehyde (DA) which allows crosslinking and gel formation [35]. A CD-functionalized polyhydrazine (PH) was the second polymer used in this work. This polymer is a derivative of polysuccinimide, which is a biodegradable and non-toxic material [36]. Two sets of *in-situ* formed hydrogels with different crosslinking densities were prepared by mixing aqueous solutions of DA and PH. Gelation occurred rapidly *in-situ* via hydrazone linkages between the aldehyde and pendant hydrazine functional groups. These injectable networks were found to be non-cytotoxic and successfully encapsulated NIC via their CD moieties. Release profile of NIC from the gels exhibited a long term sustained release over a period of 4–6 days.

2. Experimental section

2.1. Materials

β-CD (99% ELSD) and NIC (98%) were purchased from AK Scientific. Hydrazine hydrate, ethylenediamine (EDA), thiazolyl blue tetrazolium bromide (MTT) and 10% sodium dodecyl sulphate (SDS) were purchased from Sigma-Aldrich. 2-Mercaptoethanol was from Merck Millipore (Molecular Biology grade). L929 mouse fibroblast cells (NTCC clone 929, passage 9), Dulbecco's Modified Eagle's Medium (DMEM), trypsin-EDTA, penicillin-streptomycin were purchased from ATCC (Manassas, VA, USA). Fetal calf serum (FCS) was acquired from Invitrogen Canada. All other reagents and solvents were of analytical grade procured from Acros Organics and used without further purification.

2.2. Synthesis of dextran aldehyde, 6-amino-6-deoxy-β-CD and polysuccinimide and polysuccinimide

Dextran was oxidized using sodium periodate to obtain DA as described previously [37,38]. Briefly, dextran (4 g, 24.67 mmol) was dissolved into 60 mL distilled water. Sodium periodate (1.28 g, 5.98 mmol) was dissolved into 40 mL water and added dropwise into dextran solution over 30 min. The mixture stirred for 4 more h and then dialysed against distilled water for 72 h. DA with 20% oxidation degree was collected after freeze drying and kept at 5 °C for further use. Typical yield of this reaction was between 62 and 70%. Synthesis of 6-amino-6-deoxy-β-CD (EDA-β-CD) was carried out in two steps according to the reported procedure [39,40]. In the first step, a monotosyl derivative of β-CD was prepared by the reaction of *p*-toluene sulfonyl chloride with β-CD in water. The second step involved reaction of monotosyl β-CD with excess of EDA at 80 °C in a sealed pressure tube for 6 h followed by precipitating into large amount of acetone. After filtration, EDA-β-CD was collected in 63% yield. High molecular weight polysuccinimide (PSI) was obtained by the thermal polycondensation of aspartic acid according to reported procedure [44,42]. Briefly, L-aspartic acid (10.0 g, 77.1 mmol) along with phosphoric acid (2.5 g, 25% eq by weight) were dispersed into sulfolane:mesitylene mixture (1:3) and refluxed at 180 °C in a Dean-Stark water trap system for 7 h. The final product was filtered and washed with a mixture of water and methanol. The filtrate was then dried at 90 °C for 20 h to obtain PSI.

2.3. Synthesis of cyclodextrin functionalized polyhydrazine (PH)

Solid EDA-β-CD (1.45 g, 1.23 mmol) was added to a solution of PSI (1.00 g, 10.3 mmol) in DMF (20 mL) and stirred for 36 h. Ethanolamine (ETA, 0.360 g, 5.97 mmol) was added and allowed to react overnight. Next, excess hydrazine hydrate (0.65 g, 10.3 mmol) was added to the reaction mixture and stirred overnight. The final mixture was then dialysed in 3.5 kDa-cut off tubing for 48 h to remove DMF and unreacted initial materials. Lyophilization of the dialysed solution at −40 °C resulted in a yellow powder of PH in typical yields of 65–73%. Two different sets of PH were synthesized with 30 mol% hydrazine (PH30) and 10 mol% hydrazine functionalities (PH10). Fig. 1 illustrates the synthetic routes for PSI, PH and DA.

2.4. Preparation of hydrogels

Individual solutions of PH and DA (10% w/v) in PBS (pH 7.4) buffer were prepared and mixed in equal volumes at room temperature in 5 mL syringe tubes with the tips cut-off. So, the final shape of the gels was cylindrical (height: 2 cm, diameter: 1.2 cm). The Schiff-base reaction between the hydrazine in the PH polymers and the aldehyde groups in DA resulted in hydrazone bond formation (Fig. 1(c)). This crosslinking reaction is reversible, which allows for a route for biodegradation. Gel-30 was made by mixing the same volume of the 10% DA and 10% PH30 and gel-10 was prepared by mixing an equal volume of the 10% DA and 10% PH10.

2.5. Characterization

Molecular weight (M_w) of the polymers was measured by Gel permeation chromatography (GPC) using a Polymer Laboratory PL-GPC 50 (Varian Australia Pty. Ltd., Australia) at 30 °C through a Shodex OH-pak mixed C column (Phenomenex NZ. Ltd., New Zealand). Anhydrous DMF and sodium phosphate buffer (pH 7.4) were used as eluent for measuring the M_w of PSI and PH/DA respectively. 1H NMR spectra were recorded on a Varian Inova Spectrometer at 400 MHz using D_2O (DCl) or DMSO-d_6 solvents. Rheological measurements were performed using a Thermo Haake Rheostress rheometer (RS1, Thermo Electron Corporation, Waltham, MA) at 37 °C. After mixing the precursors, the mixture was immediately loaded onto a lower stationary thermostatted