Acta Biomaterialia 10 (2014) 3188–3196

Contents lists available at ScienceDirect

Acta Biomaterialia

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

A temperature-induced and shear-reversible assembly of latanoprost-loaded amphiphilic chitosan colloids: Characterization and in vivo glaucoma treatment

Meng-Hsuan Hsiao [a,1], Shih-Hwa Chiou [b,c,d,1], Mikael Larsson [e,1], Kuo-Hsuan Hung [f], Yi-Ling Wang [a], Catherine Jui-Ling Liu [c,d,*], Dean-Mo Liu [a,*]

[a] Department of Materials Science and Engineering, BioICT Consortium, National Chiao Tung University, 1001 Ta-Hseuh Road, Hsinchu, Taiwan 300, ROC
[b] Institute of Pharmacology, National Yang-Ming University and Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan
[c] National Yang-Ming University School of Medicine, No. 155, Sec. 2, Linong Street, Taipei, Taiwan
[d] Department of Ophthalmology, Taipei Veterans General Hospital, No. 201, Shih-Pai Road, Sec. 2, Taipei, Taiwan
[e] Ian Wark Research Institute, University of South Australia, Mawson Lakes Campus, Mawson Lakes SA 5095, Australia
[f] Institute of Clinical Medicine, National Yang-Ming University, No. 155, Sec. 2, Linong Street, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 20 February 2014
Accepted 19 March 2014
Available online 28 March 2014

Keywords:
Amphiphilic chitosan
Shear-reversible
Thermogelling
Glaucoma
Colloidal gel

ABSTRACT

Hydrogels composed of assembled colloids is a material class that is currently receiving much interest and shows great promise for use in biomedical applications. This emerging material class presents unique properties derived from the combination of nanosized domains in the form of colloidal particles with a continuous gel network and an interspersed liquid phase. Here we developed an amphiphilic chitosan-based, thermogelling, shear-reversible colloidal gel system for improved glaucoma treatment and addressed how preparation procedures and loading with the anti-glaucoma drug latanoprost and commonly used preservative benzalkonium chloride influenced the mechanical properties of and drug release from the colloidal gels. The results highlight that incorporated substances and preparation procedures have effects both on mechanical properties and drug release, but that the release of drug loaded in the colloidal carriers is mainly limited by transport out of the carriers, rather than by diffusion within the gel. The developed colloidal chitosan based gels hold outstanding biomedical potential, as confirmed by the ease of preparation and administration, low cytotoxicity in MTT assay, excellent biocompatibility and lowering of intraocular pressure for 40 days in a rabbit glaucoma model. The findings clearly justify further investigations towards clinical use in the treatment of glaucoma. Furthermore, the use of this shear-reversible colloidal gel could easily be extended to localized treatment of a number of critical conditions, from chronic disorders to cancer, potentially resulting in a number of new therapeutics with improved clinical performance.

1. Introduction

Here a highly biocompatible, shear-reversible, injectable drug delivery system based on assembly of amphiphilic chitosan colloids was developed for improved glaucoma treatment. During the development important observations were recorded regarding how drug loading and preparation procedures influenced biomedically relevant properties.

Glaucoma is a major cause of irreversible vision loss and blindness worldwide [1]. It is characterized by permanent damage to the optic nerve, resulting in visual field loss. The damage to the optic nerve is commonly associated with high intraocular pressure (IOP), caused by abnormal drainage of fluid produced in the eye (aqueous humor). Current treatment alternatives are medications and surgeries [2–4], both aimed at lowering the IOP. The surgeries can substantially alleviate the symptoms of glaucoma but involve several latent risks, and many patients still require long-term medical treatment after the surgery [5–8]. Therefore, surgery is not the primary treatment in cases when IOP can be controlled by medications. However, the required medications are lifelong

* Corresponding authors. Address: National Yang-Ming University School of Medicine, No. 155, Sec. 2, Linong Street, Taipei, Taiwan. Tel.: +886 2 2827 5657; fax: +886 2 287 61351 (C.J.-L. Liu), Department of Materials Science and Engineering, BioICT Consortium, National Chiao Tung University, 1001 Ta-Hseuh Road, Hsinchu, Taiwan 300, ROC. Tel.: +886 3 571 2121x55391; fax: +886 3 572 4727 (D.-M. Liu).
E-mail addresses: jlliu@vghtpe.gov.tw (C.J.-L. Liu), deanmo_liu@yahoo.ca (D.-M. Liu).
[1] Contributed equally as first authors.

http://dx.doi.org/10.1016/j.actbio.2014.03.016
1742-7061/

and failure to comply will cause progression of the glaucoma, with worsened vision and possibly blindness as a consequence. Among the medications, the hydrophobic prostaglandin analogue latanoprost is the first-line treatment for glaucoma and ocular hypertension, and was approved by FDA in 2003 [9]. Eye drop formulations of latanoprost usually contain the quaternary ammonium compound benzalkonium chloride (BAK) as an antimicrobial preservative [10–12]. Even though such eye drops are clinically approved and effective, side effects such as ocular discomfort and temporary burning sensation are common [12]. Those side effects and elderly patients failing to follow punctual administration are the most likely reasons for poor patient compliance.

To overcome the side effects and reduce the need for frequent medication, a reliable dosing technology with sustained release of latanoprost over an extended time (weeks to months) would be highly beneficial. From a clinical perspective, an injectable drug depot with a sustained release of latanoprost from the subconjunctival region has been considered an attractive choice. In the very limited literature available, the group of Professor Venkatraman has published two very promising studies where they used an injectable liposome system for sustained latanoprost delivery [13,14]. Not taking anything away from the excellent results in those studies, it is recognized that liposomal drug delivery systems generally have some drawbacks, such as: multiple-step preparation involving hazardous volatile organic solvents (in the large-scale pharmaceutical industry even ethanol can be a concern), changed properties upon storage and risk of fast-burst release from non-encapsulated drugs. In addition, liposomes may enter the circulatory system with systemic and off-target effects as a consequence. To overcome and minimize such issues, a newly developed injectable carboxymethylhexanoyl chitosan (CHC)-based colloidal gel system was evaluated as a latanoprost-carrying depot formulation.

In water, the CHC self-assembles into nanocapsules of about 200 nm in diameter, and the amphiphilic nature of the CHC allows spontaneous and efficient encapsulation of both hydrophilic and hydrophobic drugs, as well as proteins [15–19]. This laboratory has previously demonstrated that, when mixed with β-glycerophosphate (β-GP), the CHC nanocapsules form injectable thermogelling solutions which, upon increased temperature, aggregate into a continuous colloidal network. The gels are composed of a polymer-rich CHC nanocapsule network phase and an aqueous inter-nanocapsule phase, both being continuous throughout the colloidal gels. Furthermore, the gels are highly biocompatible and offer excellent control of drug delivery through the encapsulation of drugs in the nanocapsules [20,21].

Unlike conventional hydrogels, where the continuous network phase is constituted from crosslinked individual polymer chains or phase-separated regions [22–24], colloidal hydrogels, such as the one in this investigation, are formed as a result of colloidal assembly/aggregation of the constituting nanocapsules or nanoparticles [20,25,26]. For such colloidal gels, the packing structure may vary with gelling conditions and kinetics. The packing structure may, in turn, determine or influence rheological, mechanical and drug-release properties of the gels. To the authors' knowledge, there is limited literature available on how gelation conditions and kinetics influence drug release and mechanical properties of drug-loaded colloidal gels. Therefore, in this article, while developing a colloidal CHC-based depot gel carrying latanoprost for glaucoma treatment, rheological properties and drug-release kinetics were investigated for different formulations and preparation procedures. Selected formulations were brought forward for cytotoxicity tests using an MTT assay and in vivo evaluation of biocompatibility and therapeutic efficacy in a rabbit model.

2. Materials and methods

2.1. Materials

Acetonitrile was of HPLC grade and was bought from J.T. Baker. Latanoprost, HPLC-grade dimethyl sulfoxide (DMSO), triamcinolone acetonide, hematoxylin, fetal bovine serum (FBS), trypsin–EDTA, trypan blue, eosin, MTT reagent, glycerol, β-GP and BAK were bought from Sigma–Aldrich. SIRC cells derived from the Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute, Taiwan. Gibco minimum essential medium (MEM) and Gibco antibiotic antimycotic solution were bought from Life Technologies. Phosphate-buffered saline (PBS) solution was bought from UniRegion Bio-Tech (Taiwan). Deionized water was of Milli-Q grade. Carboxymethyl-hexanoyl chitosan (CHC), Mw ≈ 160,000 Da and viscosity ≈ 120 cP (2% solution), was purchased from Advanced Delivery Technology Inc. (http://www.adt-dds.com), Hsinchu, Taiwan, under the name AC-SAC (nanocarrier). Its chemical identity was confirmed to be similar to previously reported CHC using nuclear magnetic resonance imaging (Supplementary material). The molecular structures of CHC, BAK and latanoprost are shown in Fig. 1S.

2.2. Preparation of colloidal CHC hydrogels containing latanoprost/BAK

Colloidal CHC gels containing latanoprost/BAK for glaucoma treatment were prepared as follows: CHC polymer (3 g) was dissolved in deionized water (100 ml) and then cooled in an ice bath. Glycerol (0.5 ml) was added in a 3% CHC solution (8 ml) to prepare 8.5 ml glycerol/CHC solution. Where applicable, latanoprost (500 μg or 5 mg) in DMSO (0.5 ml) was mixed with the glycerol/CHC solution. Subsequently, β-GP solution (33.3% β-GP in 1 ml water), containing 0, 1 or 2 mg of BAK was added to the glycerol/CHC solution under stirring on ice to prepare CHC pre-gel solution containing latanoprost/BAK (CHC gel-(b)). For the investigation into how drug distribution affects the release properties, a different encapsulation method was also used. Briefly, dry CHC (0.24 g) was dissolved in latanoprost-containing solution (9 ml), prepared by mixing latanoprost (500 μg) in DMSO (0.5 ml) with glycerol (0.5 ml) and deionized water (8 ml), and was stirred for 1 day. BAK and β-GP were added to the latanoprost/CHC solution to prepare the CHC pre-gel solution containing 500 μg ml^{-1} latanoprost (CHC gel-(a)). The pre-gelling solutions were generally gelled at 37 °C, to form solid-like CHC colloidal gels. However, to investigate the effect of gelation time on the release properties, CHC gel-(b) was also gelled at 4 °C. To determine the drug encapsulation efficiency (EE), free latanoprost in supernatant and latanoprost encapsulated in the CHC nanocapsules were separated using a centrifuge (Hermle Labortechnik GmbH, Germany) at 12,000 rpm and 20 °C for 15 min. The per cent EE was calculated as:

$$EE = \frac{(A_{total} - A_{remaining})}{A_{total}} \times 100 \quad (1)$$

where A_{total} and $A_{remaining}$ are the absorbance, determined using a HPLC system (Agilent Technologies, U.S.), at 210 nm of the total latanoprost content and the latanoprost remaining in the supernatant after centrifugation, respectively.

2.3. Rheological characterization

The dynamic viscoelastic properties of formed CHC gels with different compositions were determined through rheological analysis using an ARES strain-controlled rheometer (Rheological Scientific, NJ, U.S.) with a parallel-plate fixture (diameter = 41 mm,