Acta Biomaterialia 6 (2010) 1370–1379

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Biodegradable hybrid polymeric membranes for ocular drug delivery

Dharmendra Jain, Edmund Carvalho, R. Banerjee *

Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai 76, India

ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form 20 October 2009
Accepted 2 November 2009
Available online 10 November 2009

Keywords:
Ocular inserts
Ciprofloxacin hydrochloride
Polyvinyl alcohol
Sodium carboxymethylcellulose
Ophthalmic drug delivery

ABSTRACT

Ophthalmic delivery systems such as ocular inserts are useful strategies to improve the ocular bioavailability of topically administered drugs. In the present study polyvinyl alcohol and sodium carboxymethylcellulose based ocular inserts were prepared by solution casting for sustained drug delivery of ciprofloxacin for treatment of topical infections. The polymers were esterified and the formation of ester bonds was confirmed by Fourier transform infrared spectroscopy. The inserts had a smooth structure with a surface roughness of 7.3 nm. Inserts were found to be wettable by simulated tear fluid with contact angle <45°. Mechanical testing results indicated that the tensile strength of polyvinyl alcohol–sodium carboxymethylcellulose (10:2 wt.%) inserts was up to 8.9 ± 1.9 MPa, which is adequate to resist the pressure likely to be exerted during application. In vitro drug release kinetics showed sustained release of ciprofloxacin for up to 48 h from the inserts. Sodium fluorescein-loaded inserts showed higher penetration of the dye in the posterior segment tissues of explanted goat eye balls as compared with an eye drop solution of sodium fluorescein. The inserts were non-toxic to corneal epithelial cells and showed no signs of acute ocular toxicity in in vivo studies in albino rabbits.

1. Introduction

Topical ocular drugs are generally administered in the form of eye drops. The bioavailability of drugs administered as eye drops is severely limited by physiological constraints such as tear turnover and the blinking reflex. Further, drug loss due to naso-lacrymal drainage, conjunctival absorption and protein binding again results in poor bioavailability and systemic side-effects. Consequently, frequent instillation of eye drops is required, resulting in pulsed administration and patient non-compliance [1,2]. Clearly, the main prerequisite for absorption of drugs into the eye is good corneal penetration and prolonged contact time with the corneal epithelium. In this regard, ocular inserts may be considered as promising ocular drug delivery systems. Ocular inserts are solid devices meant to be placed in the conjunctival sac. Because of their prolonged retention and sustained release characteristics, effective therapeutic drug levels can be achieved over an extended period of time using ocular inserts [2–4].

So far different research groups have reported on the loading of various categories of drugs such as antibiotics and β-blockers into ocular inserts composed of single or blended polymers [1,3–9]. However, there has been some concern regarding the toxicity, non-biodegradability and non-biocompatibility of synthetic polymers. Thus we were motivated to develop an insert which is based on a combination of synthetic and biopolymer with well known biocompatibility, biodegradability and sustained release characteristics.

Polyvinyl alcohol (PVA), a water soluble polymer, is frequently used for drug delivery systems and surgical repair because of its excellent mechanical strength, biocompatibility and non-toxicity [10]. It has been used as a component of various kinds of ophthalmic formulations, such as eye drops [11–13], ocular solutions and suspensions [14], as a viscosity enhancing polymer [13,14], as an ointment base [15], as a demulcent in artificial tear substitutes for corneal wetting and the treatment of dry eye syndrome [16,17], as a soft contact lens material [18], as episcleral implants [19] and as vitreous substitutes [20]. Davies et al. developed a 2′-nor-2′-deoxyguanosine-loaded PVA insert for the treatment of experimental herpes keratitis [21]. The insert started dissolving within seconds and was completely solubilized within 1 h. Balasubramaniam et al. determined the antimicrobial performance of low and high molecular weight PVA inserts [22]. Saettone et al. obtained controlled release of pilocarpine for 9 h from Eudragit-coated inserts containing PVA in combination with glyceryl behenate, xanthan gum, jota carrageenan, hydroxypropyl methylcellulose and hyaluronic acid [23].

Sodium carboxymethylcellulose (NaCMC) is a naturally occurring water soluble adhesive polymer and is known for its biocompatible character [24]. It has been used as a component of some ocular preparations as a viscosity-inducing polymer in eye drops [25], and artificial tear substitutes [17]. It has also been used in ocular hydrogels [26,27] and for the preparation of ophthalmic viscosurgical devices [24]. The presence of

* Corresponding author. Tel.: +91 22 25767868; fax: +91 22 25723480.
E-mail address: rinti@iitb.ac.in (R. Banerjee).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.11.001

hydroxyl and carboxyl groups in carboxymethylcellulose makes it attractive for chemical modification. NaCMC alone forms a translucent solution and membrane of low mechanical strength, limiting its application as an ocular biomaterial. Blending of a rigid polymer like PVA with NaCMC for development of inserts may improve the mechanical properties and transmittance (due to PVA) of the inserts while retaining the adhesive and biodegradable character of NaCMC. The molecular structures of polyvinyl alcohol and sodium carboxymethylcellulose are presented in Fig. 1.

Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic having efficacy against Gram positive and Gram negative bacteria and the frequency of spontaneous resistance to ciprofloxacin is very low. Ciprofloxacin is one of the widely used antibiotics in ophthalmology for treatment of blepharitis, conjunctivitis and bacterial keratitis caused by *Staphylococcus aureus*. It is a promising agent for the treatment and prevention of corneal ulcer and endophthalmitis. Flouroquinolones are also effective in the treatment of community acquired pneumonia, complicated skin and skin structure infections like pyodermas and wound infections secondary to trauma [28,29].

The aim of the present work was to develop ciprofloxacin HCl-loaded ocular inserts composed of blends of PVA and NaCMC and evaluate their potential for sustained ophthalmic delivery.

2. Materials and methods

2.1. Materials

PVA (molecular weight 1,250,000 g) was purchased from S.D. Fine-Chem Ltd. (Mumbai, India). Sulforhodamine B (SRB), globulin, lysozyme and porcine mucin were purchased from Sigma–Aldrich (St. Louis, MO). Sodium carboxymethylcellulose (NaCMC) and trichloroacetic acid were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Ciprofloxacin hydrochloride IP was provided as a free gift by Cadila Pharmaceuticals Ltd. (Ahmedabad, India). Glycerol, sodium chloride (extrapure AR), bovine serum albumin (96–98% purity) and ethyelenediamine tetra-acetic acid (EDTA) were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). D-Glucose, calcium chloride, disodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, glacial acetic acid and hydrochloric acid were purchased from Qualigen Fine Chemicals (Mumbai, India). SIRC (rabbit corneal epithelial cells) cell lines were obtained from the National Centre for Cell Science (Pune, India). Minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotic–antimycotic solution, Luria agar and Luria broth were purchased from Himedia Laboratories (Nasik, India). Ninety-six-well tissue culture plates were purchased from Nunc, USA. All other reagents were of analytical grade and were used as received. Milli-Q water was used as the solvent if not otherwise stated.

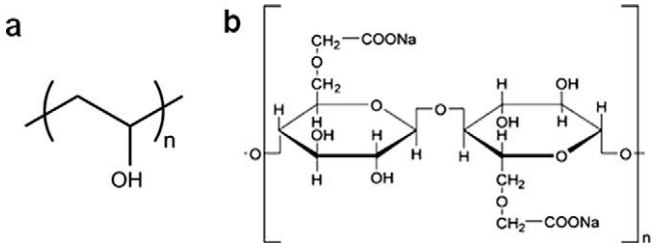

Fig. 1. Molecular structure of (a) PVA and (b) NaCMC.

2.2. Preparation of PVA–NaCMC inserts

PVA–NaCMC ocular inserts were prepared by esterification of the hydroxyl groups of PVA with the carboxyl groups of NaCMC in the presence of hydrochloric acid, similarly to a protocol used by Pal et al. [30] with slight modifications. Three different membranes were prepared by varying the concentration of NaCMC added to the membrane. A clear aqueous solution of polyvinyl alcohol (10% w/v) was prepared by dissolving 1.0 g PVA in 10.0 ml deionized water at 70 °C. Three different NaCMC solutions (2%, 3% and 4% w/v) were prepared by dissolving 100, 150 and 200 mg NaCMC in 5.0 ml deionized water. These were added to previously prepared PVA solution. An aliquot of 500 μl glycerol was added to the above solution as a plasticizer. Esterification between hydroxyl groups of PVA and carboxylic groups of NaCMC was initiated in acidic medium by adding hydrochloric acid. Simultaneously, ciprofloxacin hydrochloride was added to the above solution under magnetic stirring. The resulting blend was stirred at 70 °C for 30 min. The viscous blend so obtained was converted to membranes by casting in glass Petri dishes. The membranes were dried at room temperature. Finally, the dried membranes were carefully removed and wiped with sodium hydroxide solution to neutralize excess hydrochloric acid. The resulting membranes were translucent and soft. Inserts of 6 mm diameter and 0.30–0.40 mm thickness were punched out from the membranes and stored in sealed containers until further use. Unesterified inserts (PVA alone (10% w/v) and NaCMC alone (12% w/v)) were also prepared by same method and served as controls.

2.3. Characterization of the inserts

2.3.1. Inserts characteristics and morphology

The diameters and thicknesses of the inserts were measured with electronic vernier calipers with a sensitivity of 0.01 mm. Five to ten thickness measurements were carried out on each insert and the average was taken. Samples were stored at 27 °C and 50 ± 5% relative humidity prior to analysis. Scanning electron microscopy (SEM) (Hitachi 3400N, USA) and atomic force microscopy (AFM) were used for the morphological evaluation of inserts. For SEM inserts were attached to stubs previously covered with coated tape and sputtered with gold. AFM studies were performed in air, under ambient conditions, using a Nanoscope IV system and E scanner (Digital Instruments, Santa Barbara, CA). Imaging was carried out in tapping mode using oxide sharpened silicon cantilevers and a scanner. Images were acquired at scan rates between 1 and 2 Hz and digitally leveled. A 10 × 10 μm area was scanned from the insert and further zoomed in on for detailed analysis.

2.3.2. Wettability (contact angle)

Contact angle measurement is an indirect empirical method for determining surface energy and wettability. It is extensively used to study surface homogeneity, changes in surface composition and hydrophilicity and/or hydrophobicity. To evaluate wettability, the contact angle of the inserts was measured with simulated tear fluid (STF) in accordance with the procedure of Zhang et al. [31]. STF was prepared by dissolving sodium chloride, lysozyme, D-glucose, globulin and bovine serum albumin in Milli-Q water as per the composition described by Cohen et al. [32]. Contact angles of inserts were measured by the sessile drop technique using a CAM-100 optical contact angle meter (KSV Instruments, Finland), by depositing a 10 μl drop of STF from a microsyringe on the surface of an insert mounted on a glass slide. The image of the drop was analyzed by an automated curve fitting program using in-built software. Contact angle was measured as the average angle between the surface and tangents drawn to the surface of the drop at the right and left extremes. All measurements were made imme-