International Journal of Pharmaceutics 501 (2016) 96–101

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Nanoparticle cross-linked collagen shields for sustained delivery of pilocarpine hydrochloride

Yosra Agban[a,c], Jiaxin Lian[b], Sujay Prabakar[b], Ali Seyfoddin[c], Ilva D. Rupenthal[a,*]

[a] Buchanan Ocular Therapeutics Unit, Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand
[b] Leather and Shoe Research Association of New Zealand, P.O. Box 8094, Palmerston North 4446, New Zealand
[c] School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form 11 January 2016
Accepted 27 January 2016
Available online 29 January 2016

Keywords:
Collagen shields
Cross-linking
Metal oxides
Nanoparticles
Ocular drug delivery

ABSTRACT

Glaucoma is a common progressive eye disorder which remains the second leading cause of blindness worldwide. Current therapy involves frequent administration of eye drops which often results in poor patient adherence and therapeutic outcomes. The aim of this study was to overcome these limitations by developing a novel nanoparticle cross-linked collagen shield for sustained delivery of pilocarpine hydrochloride (PHCl). Three metal oxide nanoparticles (NPs); titanium dioxide (TiO_2), zinc oxide (ZnO) and polyvinylpyrrolidone (PVP) capped zinc oxide (ZnO/PVP), were evaluated for their cytotoxicity as well as shield transparency before selecting ZnO/PVP NPs as the ideal candidate. Cross-linked collagen shields were then characterized for their mechanical strength, swelling capacity and bioadhesive properties, with ZnO/PVP NP cross-linked shields showing the most favorable characteristics compared to plain films. The shield with the best properties was then loaded with PHCl and *in vitro* release of zinc ions as well as PHCl was measured without and with further cross-linking by ultraviolet irradiation. The concentration of zinc ions released was well below the IC_{50} rendering them safe for ocular use. Moreover, collagen shields cross-linked with ZnO/PVP NPs released PHCl over a period of 14 days offering a promising sustained release treatment option for glaucoma.

1. Introduction

With recent developments of therapeutic agents and the increasing prevalence of ocular diseases, the eye has become an important target for drug delivery. Topical administration of eye drops remains the most common method for the treatment of anterior segment disorders and although the surface of the eye is readily accessible, drug molecules have to overcome several barriers to maintain an effective drug concentration at the target site. Glaucoma is a common progressive eye syndrome affecting more than 60.5 million people worldwide (Quigley and Broman, 2006). Current management mainly involves the application of intraocular pressure (IOP) lowering eye drops; however, due to their low bioavailability they are only effective when administered frequently. This results in poor patient adherence to therapy. Therefore, to overcome this limitation, advances in drug delivery aim at enhancing the bioavailability by increasing the drug retention time on the corneal surface (Cholkar et al., 2013).

To achieve this, a number of natural polymers have been incorporated into drug delivery vehicles to create viscous eye drops or flexible films (Bhattarai et al., 2010; Calles et al., 2013; Lai, 2010; Pahuja et al., 2012; Ribeiro et al., 2015; Rupenthal et al., 2011a,b; Widjaja et al., 2014). Such polymers offer the advantage of being biodegradable, highly compatible and comprise similar mechanical properties to the body's natural tissues (Pahuja et al., 2012). Collagen is a natural protein polymer which makes up the majority of the cornea and has been used extensively for various ocular applications such as collagen shields (Mitra, 2013). Collagen shields were first introduced to the market in the 90s (Erwin, 1990) and can function as corneal bandage lenses for ocular surface protection following corneal surgery or trauma as well as drug delivery devices (Willoughby et al., 2002). They have been successfully marketed in promoting wound healing after corneal procedures (Marmer, 1988) and in relieving dry eye conditions (Shaker et al., 1989). Moreover, they have been investigated for drug delivery and when administered together with topical drug solutions have shown to increase the contact time between the drug solution and the corneal surface by serving as a reservoir. Shields loaded with steroids (Hwang et al., 1989; Sawusch et al., 1989) and antibiotics (Phinney et al., 1988; Sawusch et al., 1988;

* Corresponding author.
E-mail address: i.rupenthal@auckland.ac.nz (I.D. Rupenthal).

http://dx.doi.org/10.1016/j.ijpharm.2016.01.069
0378-5173/

Taravella et al., 1999) pre and post operatively have shown an equal or enhanced drug bioavailability in the anterior eye segment and a faster healing rate when compared to conventional formulations (Marmer, 1988). However, over the years the idea of using collagen shields in ocular drug delivery has become less popular due to various limitations including a reduction in visual acuity (Willoughby et al., 2002) as they are not fully transparent as well as safety concerns with regards to the cross-linking agents. Moreover, they could only enhance drug bioavailability for brief periods of time (Friess, 1998).

To overcome some of the limitations associated with conventional collagen shields this study aimed to use metal oxide nanoparticles (NPs) of titanium dioxide (TiO_2), zinc oxide (ZnO) and PVP capped ZnO (ZnO/PVP) as cross-linking agents for collagen and evaluate the physicochemical properties and drug release characteristics of the produced films. While replacing possibly toxic conventional cross-linking agents such as glutaraldehyde, these metal oxide NPs also offer broad spectrum antibacterial properties (Emami-Karvani and Chehrazi, 2011; Wei et al., 1994) and the ability to generate radical oxygen species when irradiated with blue light (400–470 nm) resulting in further cross-linking (Lipovsky et al., 2009) thus making them excellent candidates for ocular devices.

2. Materials and methods

2.1. Materials

Pilocarpine hydrochloride (PHCl) was purchased from Sigma-Aldrich (New Zealand). 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT), Minimum Essential Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), streptomycin and non-essential amino acids were all purchased from Life Technologies (New Zealand). Dimethyl sulfoxide (DMSO) was purchased from AppliChem GmbH (Germany). Hydrochloric acid was manufactured by AnalaR (Global Science, New Zealand). Calcium chloride ($CaCl_2$), sodium chloride (NaCl), magnesium chloride ($MgCl_2$) and sodium hydrogen carbonate ($NaHCO_3$) used for the preparation of simulated tear fluid (STF) were all purchased from Scharlau (Spain). All other chemicals and reagents were of analytical grade. All aqueous solutions were prepared with water obtained by reverse osmosis of demineralized water (Milli Q Unit, Millipore, USA). ARPE-19 cells were purchased from American Type Culture Collection (ATCC, USA). Human Corneal Epithelial Cells (HCEC) were extracted from donor tissues supplied by the New Zealand National Eye Bank housed within the Department of Ophthalmology at the University of Auckland, New Zealand. Collagen used in this study was limed split extracted Type I collagen (Singh et al., 2011) and was assessed for purity using SDS polyacrylamide gel electrophoresis. ZnO/PVP NPs were prepared by adding PVP to a solution of zinc acetate dehydrate in ethanol with continuous stirring. The solution was then hydrolyzed by adding potassium hydroxide in ethanol at 60 °C and NPs were precipitated by adding *n*-hexane (Guo et al., 2000). ZnO NPs were synthesized using the same protocol without the addition of PVP, while TiO_2 NPs were prepared by adding titanium tetraisopropoxide solution drop wise into deionized water containing acetic acid under continuous stirring at room temperature for 24 h.

2.2. Particle size, zeta potential, polydispersity index (PDI) and cytotoxicity of NPs

Nanoparticle size, zeta potential and PDI were assessed by light scattering spectroscopy using a Nanosizer (ZEN3600, Malvern, UK). The cytotoxicity of the NPs was evaluated using the MTT assay on two cell lines; human corneal epithelial cells (HCECs) and human retinal pigment epithelium (ARPE-19) cells. The initial cell density was 1×10^4 cells/well. Cells were allowed to adhere for 24 h to a 96-well tissue culture plate at 5% CO_2– 95% atmospheric air at 37 °C. Cells were then exposed to varying concentrations of each NP solution ranging from 0.625 to 60 μL/mL and cultured for another 24 h before incubation with MTT for 4 h. The absorbance was measured at 570 nm and the cell viability was calculated using the following equation: *Cell viability (%) = Absorbance (test)/Absorbance (control).*

2.3. Preparation of collagen shields

Cross-linked collagen shields were prepared using the solvent casting method. An amount of 3.8 g of 1.65% w/w collagen solution was poured into a 39 mm diameter petri dish. NP suspensions were sonicated for 10 min prior to adding them to the collagen at different ratios ((1:0.25), (1:0.5) and (1:1) w/w). Petri dishes were stored at 4 °C to remove any trapped air bubbles and prepared shields were then left to dry overnight at room temperature. For UV cross-linked shields the collagen-NP mixture was irradiated with blue light (400–470 nm) for 20 min via the laser lamp of a confocal laser scanning microscope (Olympus, USA). Drug loaded shields were prepared by adding a 1% w/v PHCl solution to the collagen-NP mixture prior to casting resulting in cut film strips containing 0.87 mg of PHCl.

2.4. Transparency

The transparency of the shields was determined using a UV–vis spectrophotometer (Libra323PC, Biochrom, UK) at 600 nm. Distilled water was used as the reference.

2.5. Swelling ratio

The swelling ratio was investigated to determine the cross-linking density of the shields. Shields were initially weighed and then placed into a petri dish with simulated tear fluid (STF) at pH 7.4 (Weyenberg et al., 2005), maintained at 32 °C to mimic physiological conditions. After 6 h, when the equilibrium point was reached, shields were retrieved and excess surface moisture was carefully removed with a filter paper. Swollen shields were reweighed and their swelling ratio was calculated as follows: *Swelling ratio* $(\%) = [(W_t - W_0)/W_0] \times 100$; where W_0 is the initial weight of the shield and W_t is the weight of the swollen shield at time t.

2.6. Mechanical properties

The tensile strength of the shields was evaluated using a TA-XT plus texture analyzer (Stable Microsystems Ltd., UK). Shields were cut into 30 × 10 mm rectangular strips and held longitudinally between two clamps. They were then pulled at 2 mm/s and the force required to break the shields was recorded. The tensile strength was then calculated as follows: *Tensile strength* (g/mm^2) = *Breaking force of shield (g)/cross-sectional area of shield* (mm^2).

2.7. Ex vivo bioadhesion studies

The bioadhesive properties of the shields were also evaluated on the TA-XT plus texture analyzer using fresh bovine corneas. Shields were cut into 5 mm diameter circles and attached to the probe using double-sided adhesive tape. The cornea was secured on the tissue holder and the probe was lowered until the shield was in contact with the cornea for 50 s. The bioadhesive strength of the shields was determined by measuring the maximum force required to detach the shield from the corneal surface at a speed of 2 mm/s.