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Preliminary characterization of dexamethasone-loaded cross-linked hyaluronic acid films for topical ocular therapy

J.A. Calles[a,b,c], A. López-García[a,d], E.M. Vallés[b], S.D. Palma[e], Y. Diebold[a,d,*]

[a] Institute of Applied Ophthalmo-Biology (IOBA), University of Valladolid, 47011 Valladolid, Spain
[b] PLAPIQUI, CONICET, National University at Bahía Blanca (UNS), 8000 Bahía Blanca, Argentina
[c] Department of Biology, Biochemistry and Pharmacy, UNS, 8000 Bahía Blanca, Argentina
[d] Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
[e] UNITEFA, CONICET, National University of Córdoba, X5000HUA Córdoba, Argentina

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ABSTRACT

The aim of this work was to design and characterize cross-linked hyaluronic acid (HA)–itaconic acid (IT) films loaded with dexamethasone sodium phosphate salt (DEX) for topical therapy of inflammatory ocular surface diseases. Films were chemically cross-linked with polyethylene glycol diglycidyl ether (PEGDE), then physical and mechanical characterization by stress–strain, X-ray diffraction, X-ray fluorescence spectrometry and swelling assays was conducted. A sequential *in vitro* therapeutic efficacy model was designed to assess changes in interleukin (IL)-6 production in an inflamed human corneal epithelial (HCE) cell line after film exposure. Changes in cell proliferation after film exposure were assessed using the alamarBlue® proliferation assay. Experimental findings showed desirable mechanical properties and *in vitro* efficacy to reduce cell inflammation. A moderately decreased proliferation rate was induced in HCE cells by DEX-loaded films, compared to commercial DEX eye drops. These results suggest that DEX and HA have opposite effects. The sequential *in vitro* therapeutic efficacy model arises as an efficient tool to study drug release from delivery systems by indirect measurement of a biological response.

1. Introduction

The ocular surface is affected by a number of inflammatory disorders. Some can be classified as acute and mild, such as seasonal allergic conjunctivitis and transient infectious conjunctivitis, others as chronic and/or more severe, such as vernal keratoconjunctivitis, atopic keratoconjunctivitis, dry eye syndrome and cicatrizing autoimmune conjunctivitis, involving corneal damage and leading to visual loss (Holland et al., 2013).

The systemic use of corticosteroids to treat ophthalmic inflammatory diseases was widely introduced in the 1950s (Raizman, 1996). However, several systemic and ocular-specific adverse effects, such as cataracts and increased intraocular pressure, were reported after a few years of this clinical practice (Becker and Mills, 1963; Covell, 1958; Urban and Cotlier, 1986). Dexamethasone (DEX) is one of the well-known resources used to treat inflammatory processes. It is a synthetic glucocorticoid with potent anti-inflammatory and immunosuppressive effects, being commonly used to treat inflammation of the anterior structures of the eye.

The high complexity of eye anatomy represents an important challenge in the development of new drug delivery systems. Topical administration is the preferred administration pathway for structures of the front of the eye, such as the cornea and conjunctiva, where the pre-corneal tear film and corneal epithelium represent an important barrier that any drug delivery system has to overcome. Traditional eye drop formulations have important limitations, leading to a reduction of their therapeutic capacity, which is usually affected by blinking and tear drainage and replacement reducing drug bioavailability in the pre-corneal area (Ding, 1998; Kompella et al., 2010; Washington et al., 2001). Research and development in this area of Pharmaceutical Sciences is a strong field of scientific and technological interest.

In recent years, several efforts were focused on optimizing corticoid delivery to ocular structures while minimizing systemic adverse effects, leading to a wide range of topical drops, ointments,

* Corresponding author at: Institute of Applied Ophthalmo-Biology (IOBA), University of Valladolid, Paseo de Belén 17, 47011 Valladolid, Spain.
E-mail address: yol@ioba.med.uva.es (Y. Diebold).

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delayed-release vehicles and intraocular, periocular and oral corticosteroid preparations (Boddu et al., 2010; Gan et al., 2010; Kassem et al., 2007; Kiernan and Mieler, 2009; Kompella et al., 2003; McGhee, 1992). The chemical form of the drug can be very important for ocular bioavailability. Changing the salt can affect the solubility and lipophilicity of the drug. For example, DEX acetate ester has the preferred solubility and partition coefficient properties for corneal permeation compared to the very water-soluble phosphate salt or very lipophilic freebase. However, the phosphate salt is preferred for eye drop formulations because of its water solubility (Gibson, 2004). Despite the growing number of reported approaches, systemic and local ocular adverse effects remain still high (Bielory et al., 2010; Carnahan and Goldstein, 2000; Chew et al., 2011; Holland et al., 2009; Pavesio et al., 2010).

An additional limitation of traditional eye drops is related to the need to maintain the sterility, and ensuring stability and security of the formulation throughout the treatment period. Benzalkonium chloride (BAK) is the most commonly used ophthalmic preservative; however, it is less and less used because of its reported side effects on patients (Noecker, 2001). Single-dose containers appear as the best alternative; nevertheless, the market cost of these formulations is near fivefold higher than that of multi-dose formulations.

Some of these drawbacks can be overcome by using solid, dry, bioadhesive biopolymer-based systems capable of remaining attached to the conjunctiva while delivering the drug in a preservative-free fashion. Thus, pre-corneal contact time lengthening, an increase in drug biodisponibility into ocular structures, and a reduction in drug elimination rate can be obtained.

The use of biopolymers for drug delivery systems has been the subject of numerous reports in scientific literature (Diebold et al., 2011; Lehr and Haas, 2002). These systems, or the materials used to produce them, should gather some desirable characteristics like zero or minimal biological effects, no toxicity or contamination due to chemical residues, and rapid degradation or excretion. Hyaluronic acid or hyaluronan (HA) is a high molecular mass linear polymer consisting of alternating units of N-acetyl-β-D-glucosamine and β-D-glucuronic acid. It is a naturally occurring biodegradable, non-toxic, non-immunogenic and non-inflammatory biomaterial, widely used in medical practice for many pathological conditions. The well-known biocompatibility of HA makes it a suitable material for different ophthalmic applications, such as enhanced contact lenses wettability (Fonn, 2007), eye drops (Miyauchi et al., 1993), surgery (Polack, 1986), tear film stabilizer (Hamano et al., 1996; Prabhasawat et al., 2007), and in the treatment of dry eye (Johnson and Murphy, 2006; Sand et al., 1989) and other ocular disorders (Aragona, 2004; Stuart and Linn, 1985). We have previously reported the preparation of HA–itaconic acid (IT) cross-linked films with polyethylene glycol diglycidyl ether (PEGDE), which were well tolerated by corneal cells both *in vitro* and *in vivo* (Calles et al., 2013). In this work we characterized HA-based films loaded with DEX to evaluate their potential for ocular delivery, using an *in vitro* model of corneal inflammation.

2. Materials and methods

2.1. Materials

HA sodium salt (Mw: 1,560,000 Da) was purchased from Parafarm® (Buenos Aires, Argentina). Dimethyl sulfoxide (DMSO), PEGDE (average Mn = 500) and DEX were purchased from Sigma–Aldrich Corp. (St. Louis, MO, US). Fetal bovine serum, penicillin, streptomycin, epidermal growth factor, insulin, and Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F-12) were provided by Invitrogen-Gibco (Inchinnan, UK) and alamarBlue® reagent was acquired from AbD Serotec (Oxford, UK). Culture plates were purchased from Nunc (Roskilde, Denmark). The enzyme-linked immunosorbent assay (ELISA) kit to measure interleukin (IL)-6 was purchased from Gen-Probe Incorporated (San Diego, CA, US). Commercially available preservative-free (Dexafree) and BAK-preserved DEX (Colircusí) eye drops were obtained from Laboratoires Théa (Clermont-Ferrand, France) and Alcon-Cusí (Barcelona, Spain), respectively. All other chemicals were of extra pure grade.

2.2. Film synthesis

Films were synthesized from HA/IT/PEGDE solutions by a previously described homogeneous cross-linking method (Calles et al., 2013), where IT and PEGDE were used as chemical cross-linker agents in a twice-distilled water solution. The amount of those reagents was adjusted to produce (1:1:2) molar ratios and the HA solution concentration was 2% (w/w). After a 24 h reaction period under slight stirring at room temperature (RT) (21–23 °C), gels were cast at RT under an extraction hood as circular films of 7.0 cm diameter. DEX was incorporated into the HA films during the cross-linking process to achieve a 0.4% concentration w/w (DEX-loaded film). DEX concentration was chosen according to literature (Calles et al., 2013) to achieve transparent films suitable to be used in cell culture experiments. Obtained films were flexible and clear (Fig. 1). Different size and shape samples were cut for film characterization.

2.3. Physicochemical characterization of films

Films were physically and mechanically characterized in terms of: (a) stress–strain; (b) X-ray diffraction; (c) X-ray fluorescence spectrometry; (d) swelling and (e) oxygen permeability.

Film thickness (mm) was measured using a digimatic caliper MDC-1″SFB (Mitutoyo Corporation, Kanagawa, Japan). Five measurements were made for each different film in central and peripheral areas. The stress–strain properties of the films were studied in 4 × 1 cm rectangular samples using an Instron 3369 tester (Norwood, US) in traction mode at 2 mm/min at RT.

X-ray diffraction and X-ray fluorescence spectrometry analyses were made in unloaded- and DEX-loaded film samples using a Philips PW1710 X-ray diffractometer and a Philips MagiX spectrometer (Amsterdam, The Netherlands), respectively. IT, HA and DEX powders were used as controls. X-ray diffractograms were performed for diffraction angles (°2θ) from 2 to 70 using a copper tube anode. After X-ray fluorescence spectrometry, further semi-quantitative analysis was performed by using IQ + Standardless software from PANalytical (Almelo, The Netherlands).

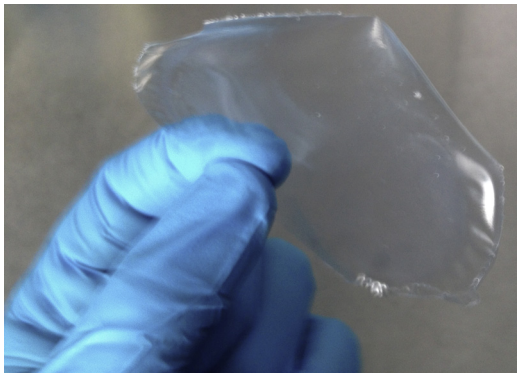

Fig. 1. DEX-loaded films (7.0 cm diameter).