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New drug-eluting lenses to be applied as bandages after keratoprosthesis implantation

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ABSTRACT

Corneal tissue is the most commonly transplanted tissue worldwide. This work aimed to develop a new drug-eluting contact lens that may be used as a bandage after keratoprosthesis. During this work, films were produced using poly(vinyl alcohol) (PVA) and chitosan (CS) crosslinked with glyoxal (GL). Vancomycin chlorhydrate (VA) was impregnated in these systems by soaking. Attenuated total reflectance – Fourier transform infrared spectroscopy was used to confirm crosslinking. The cytotoxic and drug release profile, hydrophilicity, thermal and biodegradation as well as swelling capacity of the samples were assessed through in vitro studies. PVA and PVA/CS films were obtained by crosslinking with GL. The films were transparent, flexible with smooth surfaces, hydrophilic and able to load and release vancomycin for more than 8 h. Biodegradation in artificial lachrymal fluid (ALF) with lysozyme at 37 °C showed that mass loss was higher for the samples containing CS. Also, the samples prepared with CS showed the formation of pores which were visualized by SEM. All samples revealed a biocompatible character after 24 h in contact with cornea endothelial cells. As a general conclusion it was possible to determine that the 70PVA/30CS film showed to combine the necessary features to prepare vancomycin-eluting contact lenses to prevent inflammation after corneal substitution.

1. Introduction

Corneal disease is the second most common cause of blindness worldwide. Normally, this illness is caused by degenerative, dystrophic, infectious, and inflammatory disorders. Over the last two centuries, corneal transplantation has arisen as the principal method for visual rehabilitation when corneal damage occurs. In 2010, 42,642 corneal transplants were performed in USA, however, patients have to face long waiting lists, limited tissue source and high rates of grafts rejection (Koplin et al., 2013; Tan et al., 2012; Waldock and Cook, 2000).

Recently, different studies have been done in order to design, produce, and fully characterize artificial corneas (keratoprostheses) that can be used as alternatives to corneal transplantation. Refojo (1969) studied three hydrogels for being used as artificial membranes for corneal surgery. Dohlman et al. (1967) reported the production of glyceryl methacrylate discs to be used as implants for cornea replacement, Chirila et al., (1994) studied the combination of a polymer network for being used for this biomedical application. However, despite the intense research performed in this area, only three materials are commonly used in surgical practice: the Boston keratoprosthesis (Massachusetts Eye and Ear Infirmary, Boston, MA) (Pujari et al., 2011), the AlphaCor (Addition Technology Inc., Des Plaines, IL) (Hicks et al., 2002) and the osteo-odonto-keratoprosthesis also known as the 'OOKP' (originally described by Strampelli, 1963, and then modified by Falcinelli et al., 1993 and Liu, 1998).

Furthermore, patients after being submitted to surgery usually require the continuous use of a soft therapeutic lens (known as a bandage contact lens). These allow the maintenance of an adequate ocular surface hydration, improve patient comfort and protect the eye from necrosis that can be caused by ocular surface exposure (Wong and Yiu, 2012). Endophtalmitis is one of the complications that may arise in the post-surgical period and may result in a considerable loss of vision (Nouri et al., 2001).

The post-surgical therapeutics involves a topical antibiotic prophylaxis (Durand and Dohlman, 2009), commonly using quinolones (e.g., moxifloxacin and gatifloxacin). To further reduce the risk of endophthalmitis, co-administration of vancomycin has

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been also implemented to eliminate gram-positive bacteria resistant to quinolones (Durand and Dohlman, 2009). However, the topical application of vancomycin drops has a limited efficacy due to lacrimation, tear drainage and turnover (Das and Suresh, 2011). Moreover, a certain amount of drug flows with tears into the nasolacrimal ducts and consequently may cause unwanted systemic side effects (Ali and Lehmussaari, 2006). Besides, the instillation of ophthalmic drugs as eye drops on the cornea results in rapid variation in drug concentration limiting their therapeutic efficacy. To overcome such drawbacks, new implantable devices are required to allow a controlled drug release over a long period of time, decreasing drug losses and adverse effects. Additionally, the residence time of the drug in the tear film is also increased (Ludwig, 2005).

The patient subjected to keratoprosthesis will be using soft contact lens in a permanent basis. So the incorporation of the desired drug in the lens composition improves patients' compliance, and allows drug delivery in a sustained noninvasive form. Also, in the presence of contact lens, drug molecules have longer residence time in the post-lens tear film leading to a higher drug flux through cornea and less drug inflow into the nasolacrimal duct (Pattel et al., 2013).

Prototypes of drug-eluting contact lenses, containing fluorescein and ciprofloxacin, have been previously prepared by other authors (Ciolino et al., 2009). These lens were produced with a mixture of poly(lactic-co-glycolic acid) (PLGA) and poly(hydroxyethyl methacrylate) (pHEMA) and were able to perform drug release in therapeutically relevant concentrations for 1 month.

The aim of the present work was to produce drug-eluting contact lenses to be applied as bandages. PVA and CS were selected as raw materials because of their biodegradability and biocompatibility as well as their ability to form films. In order to prepare these systems, GL was used as the crosslinker since previous studies have stated its efficiency for biomedical purposes (Gupta and Jabrail, 2006; Yang et al., 2005).

2. Experimental procedure

2.1. Materials

Acetic acid (1%), amphotericin B, L-glutamine, Eagle's minimum essential medium (MEM), glyoxal (40% wt.%) aqueous solution, hydrochloric acid 0.5 M at 37% (HCl), penicillin G, poly(vinyl alcohol) M_w 13,000–23,000 and 87–89% hydrolyzed, streptomycin, trypsin and lysozyme (from chicken egg white) were purchased from Sigma–Aldrich (Sintra, Portugal). Chitosan (M_w 100,000–300,000) was obtained from Acros Organics. Fetal bovine serum (FBS) was purchased from Biochrom AG (Berlin, Germany). The 3-[4,5-dimethylthiazol-2-yl]-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) and electron coupling reagent phenazine methosulfate (PMS) were purchased from Promega. T-flasks and 96-well plates were purchased from Nunc (Denmark). Vancomycin chlorhydrate was kindly donated by the Ophthalmology Service of the Hospital of the University of Coimbra (Dr. Joaquim Murta).

2.2. PVA and PVA/CS films preparation

The polymeric films (PVA and PVA/CS) were prepared by casting. A PVA solution (5 wt.%) was prepared by dissolving PVA in distilled water. CS solution (2 wt.%) was prepared by dissolving 2 g of CS in 100 mL of an aqueous acetic acid solution (1%). Both solutions were stirred until they became homogeneous.

Two sets of films were prepared, one based on PVA and another combining PVA and CS. The crosslinker agent was GL, as previously mentioned.

Table 1
Ratio of PVA, CS and GL in the films' composition.

Sample	PVA (%wt)	CS (%wt)	GL (%wt/wt$_{polymer}$)
PVA-GL0	100	0	0
PVA-GL20	100	0	20
PVA-GL50	100	0	50
70PVA/30CS-GL10	70	30	10
50PVA/50CS-GL10	50	50	10
CS-GL0	0	100	0

To prepare the PVA based films, the PVA solution was mixed with GL using different ratios, as shown in Table 1. The pH of this mixture was adjusted to 2.5, using an HCl solution. After the homogenization process, the solution was heated in a water bath at 45 °C, for 2 h. Afterwards, this solution was poured into a Petri dish and placed in an oven at 45 °C (≈16 h) to dry. At the end of this period, the obtained films were removed from the Petri dishes and immersed in water for 2 days. A film without crosslinker (PVA-GL0) was also prepared in order to be used as a control sample in the following characterization steps.

PVA/CS films were prepared by using a procedure similar to the one described above. The solutions were homogenously mixed, placed in Petri dishes and then placed in the oven under the same conditions. The ratios between PVA/CS and GL are also described in Table 1. Like in the case of PVA, a CS film without crosslinker (CS-GL0) was prepared in order to be used as a control sample during characterization.

2.3. Attenuated total reflectance – Fourier transform infrared spectroscopy

PVA and PVA/CS films spectra were acquired in the range of 4000–500 cm^{-1}, using a Magma-IR^{TM} Spectrometer 750, operating in ATR mode (Golden Gate Single Reflection Diamond ATR). The samples were previously dried in a vacuum oven at 40 °C and the data collection was performed with a 4 cm^{-1} spectral resolution and after 64 scans.

2.4. Study of water uptake ability (swelling)

The swelling of different films was evaluated in ALF (pH 7.4) at 37 °C and in ALF and water at room temperature. All the samples were previously dried in a vacuum oven at 40 °C and the weight of the dried sample was determined (W_d). The dried samples were then immersed in a constant volume of ALF. At pre-determined intervals (t), samples were removed from the media and weighted at different times until a maximum weight was achieved (W_s). The swelling ratio was evaluated through Eq. (1).

$$\text{Swelling}(\%) = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \quad (1)$$

All the assays were carried out in triplicate and the results were expressed as mean ± standard deviation.

2.5. Water contact angle determination

The water contact angle (θ) measurements were performed at room temperature in an OCA 20 contact angle measurement unit from Dataphysics. All measurements were performed on the air-facing surfaces of the samples using the sessile drop method. Ten measurements on different points were performed on each sample, from which the mean static contact angle and its standard deviation were determined.