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Novel biodegradable polyesteramide microspheres for controlled drug delivery in Ophthalmology

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ABSTRACT

Most of the posterior segment diseases are chronic and multifactorial and require long-term intraocular medication. Conventional treatments of these pathologies consist of successive intraocular injections, which are associated with adverse effects. Successful therapy requires the development of new drug delivery systems able to release the active substance for a long term with a single administration. The present work involves the description of a new generation of microspheres based on poly(ester amide)s (PEA), which are novel polymers with improved biodegradability, processability and good thermal and mechanical properties. We report on the preparation of the PEA polymer, PEA microspheres (PEA Ms) and their characterization. PEA Ms (~15 μm) were loaded with a lipophilic drug (dexamethasone) (181.0 ± 2.4 μg DX/mg Ms). The *in vitro* release profile of the drug showed a constant delivery for at least 90 days. Based on the data from a performed *in vitro* release study, a kinetic ocular model to predict *in vivo* drug concentrations in a rabbit vitreous was built. According to the pharmacokinetic simulations, intravitreal injection of dexamethasone loaded PEA microspheres would provide release of the drug in rabbit eyes up to 3 months. Cytotoxicity studies in macrophages and retinal pigment epithelial cells revealed a good *in vitro* tolerance of the microsystems. After sterilization, PEA Ms were administered *in vivo* by subtenon and intravitreal injections in male Sprague–Dawley rats and the location of the microspheres in rat eyes was monitored. We conclude that PEA Ms provide an alternative delivery system for controlling the delivery of drugs to the eye, allowing a novel generation of microsphere design.

1. Introduction

Most of the diseases affecting the posterior segment of the eye are related with visual impairment and blindness. The effective treatment of these pathologies is one of the major challenges in drug delivery as most of them are chronic and multifactorial. Among them, aged-related macular degeneration, diabetic retinopathies and glaucoma produce irreversible visual damage and blindness [1]. These diseases are becoming more and more prevalent in the aging populations, and nowadays tens of millions of patients are affected worldwide. Depending on the disease, the medications should be delivered to the retinal cells, retinal pigment epithelium or choroid. Furthermore, therapeutic concentrations of the active substance in the intraocular target site have to be maintained during a long period of time.

Due to the ocular barriers, it is difficult to deliver effective drug concentrations to the posterior tissues of the eye using non-invasive routes such as topical or systemic administration [2]. It is well known that after topical administration only very low drug concentrations are reached in the retina and choroid [3]. This is due to the obstacles of drug penetration that include the short residence time of formulations on the ocular surface, the presence of tissue barriers (cornea, lens, conjunctiva, sclera), and flow mediated drug loss factors (conjunctival blood flow,

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aqueous humor flow) that limit the drug access to the retina and choroid. Although systemic administration is used to deliver some drugs to the eye (*e.g.*, corticosteroids), this route is restricted by the systemic toxicity of the drugs and reduced access to the target site, mainly due to the blood-aqueous and blood-retinal barriers [2].

The most effective method of drug delivery to the back of the eye is through intraocular administrations, mainly intravitreal injections. However, intravitreal administration is an invasive mode of drug delivery and it is sometimes associated with adverse effects (endophthalmitis, hemorrhages, damage of lens or retinal detachment) and it requires frequent visits of the patients to the clinics. Besides, most low molecular weight drugs have short intravitreal half-lives (2–10 h), so they have to be administered frequently to be clinically feasible. Controlled drug delivery systems, such as nano- and microcarriers, as well as implants, able to release and maintain effective active substance levels over long periods of time, would prolong the dosing interval to months [4,5]. Biodegradable micro- and nanoparticulate systems are emerging therapeutic tools as they can be administered as a conventional injection by periocular (subconjunctival, subtenon, juxtascleral) and intraocular routes and they are cleared from the site of administration over time.

Ophthalmic drug delivery systems can be made with a variety of biodegradable materials such as polyesters (lactide and glycolide copolymers, polycaprolactones, poly(β-hydroxybutyrates)), polyamides (including natural polymers such as collagen, gelatin and albumin), heteropolysaccharides (chitosan) or lactic and glycolic acid polymers and copolymers, among others. Poly-lactic-co-glycolic acid (PLGA) has been widely used for the development of a number of drug delivery systems, such as the intraocular commercialized implant loaded with dexamethasone (Ozurdex®). The advantages of biodegradable implants over the non-erodible devices in the clinical practice have promoted the interest in novel polymers adequate for intraocular drug delivery purposes.

We have studied a new generation of microspheres based on poly(ester amide)s (PEA). The PEAs are amino acid containing biodegradable polymers combining ester and amide groups in the polymer chain. This chemical structure contributes to improved biodegradability, processability and mechanical properties of the materials (*via* intra- or inter-chain hydrogen bonding interactions though its amide groups). Furthermore, an important polymer feature is that the current composition of PEA predominantly degrades through a surface erosion mechanism [6]. These materials have already demonstrated good biocompatibility showing little or no inflammation both *in vitro* and *in vivo* [7] including in an ophthalmic setting [8]. Extruded PEA fibrils have been implanted in both periocular (subconjunctival) and intravitreal routes in a rabbit experimental model. Readouts after 1, 3, 5 days and 2, 4, 8 weeks have shown excellent material tolerance and tissue biocompatibility. Further work [9] investigated blood and cellular *in vitro* responses of PEA. The findings of the study revealed that monocytes adherent to PEA secreted reduced levels of the pro-inflammatory interleukins (IL)-6 and IL-1β into the culture supernatant relative to those on comparative polymers but secreted significantly higher amounts of the anti-inflammatory mediator, IL-1 receptor antagonist. A PEA coating on cardiovascular stent has been reported in a phase III, 2-armed clinical study [10]. The 24-months follow up of this study reports absence of Major Adverse Cardiac Events (MACE) and suggests that the tested PEA-coated stent is safe [11].

The present work is focused on the study of the ability of biodegradable polyesteramide to form microspheres for ophthalmic drug delivery purposes. Microspheres present several advantages among other ophthalmic drug delivery forms for different reasons: (a) drugs encapsulated in microspheres are protected from degradation and physiological clearance, (b) the release kinetics of the drug can be adjusted by varying the technological parameters of these systems and (c) microspheres can be injected as a suspension using conventional needles (27-34G) without surgery [12].

This study shows the synthesis of polyesteramide (PEA) polymers and the preparation, sterilization, "*in vitro* tolerance" and delivery characteristics of microspheres (Ms) made from PEA and loaded with a lipophilic drug model (dexamethasone). After studying the impact of gamma sterilization on the properties of these systems, we have built a kinetic ocular model with *in vitro* release data to predict *in vivo* drug concentrations in a rabbit vitreous model. Then, we studied the impact of the different reagents, solutions and processes required to perform histological procedures, on the properties of Ms, in order to establish the most appropriate inclusion techniques for *in vivo* studies. Finally, we have analyzed the behavior of PEA Ms after injection in the subtenon space and in the vitreous humor of rats with the aim of determining whether or not a satisfactory amount of PEA Ms were placed in the desired locations.

2. Material and methods

2.1. Material

Polyvinyl alcohol 67 kDa (PVA) was provided by Merck (Darmstadt, Germany). Acetonitrile (ACN), dichloromethane (DCM) and methanol (MET) were purchased from Sigma-Aldrich (Schenelldorf, Germany). Tetrahydrofuran (THF) was supplied by Teknokroma (Barcelona, Spain). Super gradient acetonitrile (ACN) was purchased from Lab-Scan (Madrid, Spain). Dexamethasone (DX), dimethyl sulphoxide (DMSO) and 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (Schnelldorf, Germany). Freshly produced MilliQ water (W) was used in all the experiments. Poly-(D,L-lactide-co-glycolide) PLGA ratio 50:50 (35 kDa; Resomer 503) was purchased from Boehringer Ingelheim GmbH (Ingelheim am Rhein, Germany). Polyvinyl alcohol 72 kDa (PVA) and anhydrous DMF were obtained by Merck KGaA (Darmstadt, Germany). Polyethyleneimine (PEI) microspheres were supplied by Micromod (Rostok, Germany). Unless noted otherwise, cell culture reagents were provided by Life Technologies (Carlsbad, CA, USA). Macrophages (RAW 264.7) and human retinal pigment epithelial cell lines (ARPE-19) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2. Synthesis of polyesteramide copolymers

The polymer in this study is a biodegradable poly(ester amide) based on α-amino acids, aliphatic dicarboxylic acids and aliphatic α-ω diols. Among this class of materials the AA–BB hetero-chain polymers offer the greatest versatility in terms of molecular level design to tailor drug release properties. The selected PEA is depicted on Fig. 1 and it comprises three types of building blocks randomly distributed along the polymer chain.

The polymer was synthesized according to a procedure reported previously [13]. Briefly, the polymer was prepared *via* solution polycondensation of di-p-toluenesulfonic acid salts of bis-(α-amino acid) α,ω-diol diesters, lysine benzyl ester and di-N-hydroxysuccinimide sebacate in anhydrous DMF. The use of pre-activated acid in the reaction allows polymerization at low temperature (65 °C) affording side-product free polycondensates and predictable degradation products [14]. The polymer was isolated from the reaction mixture in two precipitation steps. The polymer was characterized by ^{1}H NMR spectroscopy and THF based GPC relative to polystyrene standards.

2.3. Polymer characterization

^{1}H NMR spectra were obtained on a Bruker Avance 500 MHz Ultrashield NMR; samples were recorded in ethanol d_6.

Molecular weight and molecular weight distributions of PEA were determined by GPC equipped with RI detector. Samples were dissolved in THF at a concentration of approximately 5 mg/mL and were run at a flow rate of 1 mL/min at 50 °C. The molecular weights were calibrated to a narrow polystyrene standard calibration curve, using Waters Empower software.