

NEW DRUG DELIVERY SYSTEM FOR OPHTHALMIC USE

Field of the invention

- 5 The present invention relates to the field of sustained release ophthalmic formulations for the delivery of active principles to the ocular surface, in particular for the treatment of pathologies of the cornea.

Background of the invention

- 10 Topical instillation of drugs as eye drops is the preferred and conventional route for the treatment of most diseases of the anterior ocular segment.

- However, this mode of administration is highly inefficient and characterized by a very poor bioavailability. After instillation, the flow of lacrimal fluid moves the drug from the ocular surface to the nasolacrimal ducts in a few minutes and, thus, the majority of it is lost through the naso-lacrimal drainage. Only 5% of the total amount of drug
15 administered is available for a sufficient period of time in contact with the ocular surface to exert a therapeutic effect.

This requires frequent administration of high amount of drug, with very low patient compliance, and renders effective dosage inaccurate.

- Punctal plugs have been used in order to attain effective drug concentration and residence time at the anterior surface of the eye, thus reducing the dose and frequency
20 of administration. These are small medical devices that are inserted into the tear duct of the eye to block the duct and thus prevent the drainage of liquid from the eye. However, they may be not well tolerated by patients and cause complications and such as epiphora, foreign body sensation, infection, pyogenic granuloma, punctal
25 canalicular erosion, dacryocystitis and increased allergy symptoms.

An alternative approach has been the development of sustained release delivery systems that extend corneal residence time of drugs to the anterior segment of the eye, such as viscous solutions, mucoadhesive materials or drug-coated contact lenses.

For example, US20040241207 discloses a contact lens with embedded drug nanoparticles that diffuse through the contact lens into the post-lens tear film when the lens is applied to the eye.

However, contact lenses or other artificial polymers have the inconvenience that they can trigger immune responses in the patients.

In addition to the above, the bioavailability of the drug is further reduced by the physiological barriers of the eye, which greatly limit the drug concentration in the inner layers of the cornea. In particular, the corneal epithelium is lipophilic and presents tight junctions between the cells, which lead to a limited permeation by drug molecules, in particular hydrophilic drug molecules.

It is therefore felt the need of developing a biocompatible, non-immunogenic delivery system that solves the above problems and allows to obtain sufficient concentrations of drug molecules for a sufficient period of time in the anterior portion of the eye.

Summary of the invention

The present inventors have surprisingly found that corneal stroma can be functionalized with polylactic co-glycolic acid (PLGA) microparticles containing pharmaceutically active molecules and used for the controlled release delivery of these molecules to the anterior portion of the eye.

Accordingly, a first object of the invention is a drug delivery system suitable for ophthalmic use comprising a decellularized corneal stroma scaffold having dispersed within and/or bound to the surface microparticles containing at least one pharmaceutically active molecule dispersed in a matrix having a composition consisting for at least 70% of polylactic co-glycolic acid (PLGA).

A second object of the invention is a process for the manufacturing of a drug delivery system according to the first object of the invention.

A third object of the invention is a pharmaceutically active molecule for use in the treatment of pathologies of the front of the eye, wherein said pharmaceutically active molecule is administered by means of a drug delivery system according to the first object of the invention

A fourth object of the invention is a method for the treatment of pathologies of the front of the eye, comprising administering a pharmaceutically active molecule by means of a drug delivery system according to the first object of the invention.

5 Description of the Figures

Figure 1 represents Transmission Electron Microscopy (TEM) images showing the lenticule with no treatment (panel A) and the lenticule after decellularization with 0.1% SDS (panel B), as described in Example 2.

Figure 2 shows images of lenticules obtained by confocal microscopy, as described in
10 Example 5. In details, panel A shows a 3D reconstruction showing the superficial localization of Fluo-MPs (light grey dots) in the lenticule; panel B shows a 2D image showing the Fluo-MPs distribution (light grey dots) between collagen fibers (dark grey). Figure 3 shows the vitro kinetic release of rhNGF from lenticule, as measured in Example 6. The chart shows the cumulative release of rhNGF from loaded PLGA-MPs
15 previously included in the lenticule.

Detailed description of the invention

A first object of the present invention is a drug delivery system comprising a decellularized corneal stroma scaffold having dispersed within and/or bound to the
20 surface microparticles containing at least one pharmaceutically active molecule dispersed in a matrix having a composition consisting for at least 70% w/w of polylactic co-glycolic acid (PLGA).

According to a preferred embodiment of the invention, the corneal stroma scaffold according to the first object of the invention is a graft obtained from the cornea of the
25 patient to be treated or from a donor.

For example, said corneal stroma may be obtained from a donor undergoing a refractive eye surgery, preferably refractive lenticule extraction (ReLEEx) or femtosecond lenticule extraction (FLEEx). Alternatively, the corneal stroma can be of cadaveric donor.

30 According to another preferred embodiment of the invention, said corneal stroma scaffold is an engineered corneal stroma scaffold obtained in vitro.

Preferably, said corneal stroma scaffold is a corneal stroma lenticule.

Preferably, said corneal stroma lenticule has a thickness between 80 and 300 μm , preferably between 100 and 150 μm and/or a diameter between 4 and 9 mm, preferably between 5 and 8 mm, more preferably between 6 and 7 mm.

Preferably, said corneal stroma scaffold is cell-free.

- 5 The absence of cells in the stroma renders it immunogenically compatible and creates pores into the scaffold that incorporate the microparticles.

According to a preferred embodiment, said microparticles are dispersed within the decellularized corneal stroma scaffold.

- 10 According to a preferred embodiment, said microparticles are both dispersed within and bound to the surface of the decellularized corneal stroma scaffold.

Preferably, said matrix of the drug delivery system according to the first object of the invention comprises at least 75% (w/w), preferably 80% (w/w), more preferably 85% (w/w) of polylactic co-glycolic acid (PLGA).

- 15 Preferably, said matrix comprises between 85% (w/w) and 95% (w/w), more preferably 89% (w/w), of polylactic co-glycolic acid (PLGA).

According to a particularly preferred embodiment, said PLGA has a copolymer composition consisting of between 50% (w/w) and 75 % (w/w), preferably 75% (w/w) of lactic acid and between 50% (w/w) and 25% (w/w), preferably 25% (w/w) of glycolic acid.

- 20 According to another preferred embodiment, also in combination with the preceding ones, said PLGA is amorphous.

According to another preferred embodiment, also in combination with the preceding ones, said PLGA has a molecular weight between 4000-15000 g/mol.

- 25 A PLGA particularly suitable for use in the drug delivery system of the invention is for example the product commercialized under the trade name Resomer® RG752H, Poly(D,L-lactide-co-glycolide).

- 30 Preferably, said matrix further contains, in addition to PLGA, one or more additional components selected from: polyethylene glycol, preferably low-molecular-weight grade polyethylene glycol, more preferably PEG400, albumin, preferably human seric albumin (HSA), and/or Trehalose. Preferably, said matrix has a composition consisting

of PLGA, PEG400, HSA and Trehalose, wherein PLGA is present in an amount of at least 70% w/w of the total composition.

According to a preferred embodiment of the invention, said microparticles consist of said matrix with dispersed therein at least one pharmaceutically active molecule.

- 5 Preferably, in order to obtain both an effective incorporation in or binding to the corneal stroma scaffold and a suitable release rate of the pharmaceutically active molecule, the microparticles have a particle size distribution measured by laser diffraction technology between 1 and 15 μm , preferably between 1.5 and 10 μm , more preferably between 1.9 and 7 μm . Preferably, said particle size is measured using the particle
10 size analyzer Mastersizer2000 with the Hydro 2000 μP wet sample dispersion unit (Malvern Panalytical).

The drug delivery system according to the invention is able to release the pharmaceutically active molecule at a controlled rate, preferably for a period of at least one month.

- 15 Preferably, said pharmaceutically active molecule is a protein or a peptide.

Said protein can be of natural origin or synthetically produced, preferably it is a recombinant protein.

- According to a preferred embodiment, said pharmaceutically active molecule is a growth factor or a growth-factor mimetic peptide or protein. More preferably, said
20 pharmaceutically active molecule is a neurotrophin or a neurotrophin-mimetic peptide or protein.

- According to the present invention the term "neurotrophin" refers to proteins, including recombinant proteins, that act as growth promoters for neurons. These include Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-
25 3) and Neurotrophin-4 (NT-4); the term "neurotrophin mimetic-peptide or protein" refers to any peptide or protein, of natural or synthetic origin, capable of acting as an agonist for one or more neurotrophin receptors. For example, a neurotrophin mimetic-protein may be a protein that has mutations or truncations with respect to a natural wild type neurotrophin but is able to activate one or more neurotrophin receptors. The mimetic
30 peptides may be either linear or cyclic peptides.

Particularly preferred pharmaceutically active molecules according to the invention are nerve growth factor and nerve growth factor-mimetic peptides or proteins. Said nerve growth factor is preferably human nerve growth factor, more preferably recombinant human nerve growth factor.

5 The amount of pharmaceutically active molecule dispersed in the microparticle matrix will vary depending on the desired dose to be administered to the anterior part of the eye. Preferably, when said pharmaceutically active molecule is nerve growth factor it is contained in said microparticle matrix in an amount between 30 and 100 ng, preferably between 50 and 90 ng, more preferably between 60 and 80 ng per each mg.

10 According to the present invention, the expression "decellularized corneal stroma scaffold having bound to the surface microparticles" means that the microparticles are held and retained on the surface of the decellularized corneal stroma scaffold by force of attraction that may be of various kind.

A second object of the present invention is a method for preparing a drug delivery system as described above, comprising the following steps:

- 15 a. obtaining a corneal stroma scaffold as above described, preferably a corneal stroma lenticule from a donor cornea as above described;
- b. removing cellular material from said scaffold, if present, thus obtaining a decellularized corneal stroma scaffold;
- 20 c. incubating said decellularized corneal stroma scaffold in a suspension of microparticles containing at least one pharmaceutically active molecule dispersed in a matrix having a composition consisting for at least 70% (w/w) of polylactic co-glycolic acid (PLGA), as described above.

Preferably, in step a. said corneal stroma scaffold is a corneal stroma lenticule with a thickness between 80 and 300 μm , preferably between 100 and 150 μm and/or a diameter between 4 and 9 mm, preferably between 5 and 8 mm, more preferably between 6 and 7 mm.

Preferably, in step b. of the above method according to the invention said cellular material is removed so as to obtain an amount of residual cells in said corneal stroma scaffold lower than 0.5 weight%, more preferably a cell-free lenticule.

Preferably, said cellular material is removed by incubating the lenticule in a solution of a surfactant. According to a preferred embodiment, said surfactant is sodium dodecyl sulfate (SDS), preferably at a concentration between 0.05 and 0.2%, more preferably 0.1%.

- 5 In order to obtain an effective removal of the cellular component from the lenticule, the incubation with the solution of surfactant should be carried out for at least 20 hours, preferably for at least 24 hours.

When the corneal stroma scaffold is a corneal stroma lenticule, the decellularized lenticules obtained in step b. may be incubated with the microparticles immediately
10 after extraction from the donor.

More preferably, the decellularized lenticules obtained in step b are dehydrated and stored at room temperature until use. According to this embodiment, the above method further comprises after step b and before step c., a step b'. wherein the decellularized lenticules are dehydrated, preferably by treatment at a temperature between 50° and
15 70°C, preferably between 55° and 65°C, more preferably at 60°C for between 1 and 3 hours, preferably 2 hours.

The dehydration of the decellularized lenticules is particularly advantageous since it greatly increases the ability of the microparticles to be incorporated in the stroma.

The incubation of the corneal stroma scaffold with the suspension of microparticles in
20 step c. is preferably carried out at room temperature.

Preferably, said incubation is carried out for a period of time between 1 and 5 hours, preferably for 3 hours.

The microparticles of step c. of the method according to the present invention, are obtained by the following steps:

- 25 a. preparing a water/oil emulsion containing the pharmaceutically active principle and polylactic co-glycolic acid.
b. preparing an aqueous solution containing of polyvinyl alcohol doped with the same organic solvent used in the oil phase of step a.
c. mixing the emulsion and solution prepared in a. and in b. and homogenizing, thus
30 obtaining a water/oil/water emulsion.
d. evaporating the organic solvent, thus obtaining precipitation of microparticles;

e. recovering and washing the precipitated microparticles;

f. freeze-drying the microparticles.

Preferably, in step a. the polylactic co-glycolic acid is present in a concentration between 10 and 30% w/v, preferably between 15 and 25% w/v, more preferably
5 between 18 and 22% w/v.

Preferably, said oil phase in step a. is ethyl acetate.

Preferably, in step b. the concentration of polyvinyl alcohol in the aqueous solution is between 0.5 and 2% w/v, preferably 1% w/v.

The drug delivery system obtained with a process according to the second object of
10 the invention has all the features according to the first object of the invention.

As will be demonstrated in Example 6, the drug delivery device according to the invention is able to release at a controlled rate the pharmaceutically active molecule from the microparticles included in the cornea stroma scaffold for a period of time of at least one month.

15 The delivery system according to the invention can be advantageously used to deliver pharmaceutically active molecules to the anterior segment of the eye.

The administration to the patient may be effected in different ways, depending on the specific pathology to be treated.

For example, in case it is necessary that the pharmaceutically active molecule exerts
20 its activity on the inner layers of the cornea, the delivery system may be implanted into a surgically created intrastromal pocket in the eye of the patient, for example formed by means of a femtosecond laser. Alternatively, the delivery system may be applied on the surface of the cornea.

The active molecule in the delivery system will be released at a constant rate, thus
25 maintaining an effective concentration at the site of action throughout the whole treatment.

The delivery system according to the invention is highly biocompatible and non-immunogenic and is therefore well tolerated by the patients.

Accordingly, a third object of the invention is a pharmaceutically active molecule, as
30 described above, for use in the treatment of ocular pathologies, preferably pathologies

of the front of the eye, wherein said pharmaceutically active molecule is administered in a drug delivery system according to the first object of the invention

A fourth object of the invention is a method for the treatment of ocular pathologies, preferably pathologies of the front of the eye, comprising administering a pharmaceutically active molecule in a drug delivery system according to the first object of the invention.

EXPERIMENTAL SECTION

Example 1:

10 Preparation of microparticles loaded with rhNGF

Microparticles formed by a polylactic co-glycolic acid (PLGA) matrix loaded with rhNGF were prepared according to the procedure described below.

A 0.85 mg/mL solution of rhNGF in Formulation Buffer (50 mM Sodium phosphate monobasic monohydrate, 100 mM Sodium Chloride, pH 7.2) was prepared.

- 15 An aqueous solution W1 was prepared containing 150 μ L of the rhNGF solution prepared above, 6.01 mg human serum albumin (HSA) and 150 μ L of polyethylene glycol 400 (PEG400) (ratio v/w/v of 1:40:1).

- 150 μ L of solution W1 were then added to 7.5 mL of a solution O containing 20% w/v of PLGA (Resomer® RG752H) in ethyl acetate and homogenized at 17400 rpm for 3 minutes, in by a homogenizer Ultraturrax t25 basic (IKA), using 8G probe.

- The obtained emulsion W1/O was quickly added to 30 mL aqueous solution W2, containing 1% w/v of polyvinyl alcohol (PVA) (Mowiol® 40-88) doped with 900 μ L of ethyl acetate and homogenized again at 12000 rpm for 30 minutes. The resulting double emulsion (W1/O/W2), water down to wash the tube and recover material, was stirred for 3 hours at 500 rpm, at room temperature using overhead stirrer RW20D (IKA), with a PTFE-coated-2-bladed propeller stirrer, to allow organic solvent evaporation and the precipitation of microparticles. The suspension was collected in two 50 mL polypropylene tubes (Eppendorf Lobind protein) and centrifuged at 7400 rpm, for 15 minutes, at 4 °C. The microparticles were isolated and washed 6 times using 50 mL milliQ water, by centrifugation at 7000 rpm for 15 minutes at 4°C. Afterwards, the microparticles were re-suspended in aqueous solution of trehalose 2%

w/v. The obtained suspension was moved in ISO glass type siliconized Class 1 vials and frozen at -80 °C to be, subsequently, lyophilized. The microparticles produced using this procedure show an encapsulation efficiency of more than 73% (55.5 ng rhNGF/mg microparticles vs theoretical value of 76 ng rhNGF/mg microparticles), with
5 a particle size distribution between 1.9 and 6.9 µm, as measured with the Hydro 2000µP wet sample dispersion method using the analyzer Mastersizer2000 (Malvern). The final composition of the microparticles consists for 89% w/w of PLGA.

Example 2:

Preparation of dehydrated, decellularized lenticles

10 Human corneal lenticles having a thickness between 100 and 150 µm were obtained from SMILE surgery (SMall Incision Lenticule Extraction), according to the procedure described in Sekundo W, Kunert KS, Blum M. Br J Ophthalmol. 2011; 95:335–339, and cryopreserved until further processing.

Later, the lenticles were prepared to be used for functionalization according to the
15 following procedure.

The lenticles were thawed and the cell component of the lenticles was eliminated by incubation in SDS 0.1% solution for 24 hours at R.T. followed by 3 washes in PBS (24 hours each).

As shown in Figure 1, which shows the analysis by Transmission Electronic
20 Microscopy (TEM) of a lenticule before (panel A) and after (panel B) treatment with the SDS solution, a complete removal of the cell component was obtained.

The decellularized corneal lenticles were then dehydrated by treatment at 60°C for 2 hours and stored at room temperature until use.

Example 3:

Functionalization of lenticles

25 The microparticles loaded with rhNGF prepared in Example 1 were used to functionalize the dehydrated, decellularized lenticles obtained in Example 2.

In details, 20 mg of microparticles obtained in Example 1 were suspended in 0.175 mL of 0.9% NaCl and one lenticule was added to the obtained suspension and incubated
30 for 3 hours at R.T. with orbital shaking at 200 rpm.

The lenticule was then removed from the suspension and washed 10 times in 0.4 mL of 0.9% NaCl (up&down).

Example 4

Analysis of functionalized lenticules

- 5 A functionalized lenticule prepared as described in Example 3 was lysed by incubation with 0.2 mL of 5 mg/mL collagenase IA in PBS containing 100 mg/L $\text{Ca}^{2+}/\text{Mg}^{2+}$ at 37 °C for 30 minutes, this step was carried out to specifically lyse the lenticule preserving the already released rhNGF (supernatant) and the whole rhNGF-MPs (pellet). The lysed samples were centrifuged for 10 minutes at 8000 rpm at 4°C.
- 10 The supernatant phase obtained by centrifugation contains the rhNGF already released by the microparticles whereas the pellet phase contains intact MPs. The supernatant phase was collected and immediately stored it at -20° until analysis. The remaining pellet, corresponding to intact microparticles released by the lenticule upon lysis, was treated with 0.05 mL of Ethyl Acetate to lyse the PLGA shell of MPs
- 15 and 0.100 mL of PBS containing 100 mg/L $\text{Ca}^{2+}/\text{Mg}^{2+}$ was added to safely extract the rhNGF. The samples were centrifuged for 5 minutes at 8000 rpm and the aqueous phase collected (Pellet phase). The Supernatant and Pellet phase were each diluted 1:3 in PBS containing 100 mg/L $\text{Ca}^{2+}/\text{Mg}^{2+}$ and analyzed by ELISA assay to detect the concentration of rhNG.
- 20 The supernatant phase contains the rhNGF already released by the MPs whereas the pellet phase contains intact MPs. The results obtained from the analysis of three lenticules are shown in Table 1 below

Table 1:

Sample		Concentration rhNGF in each phase (pg/mL)	Total concentratio n of rhNGF (pg/mL)	rhNGF free&released/ rhNGF in MP
Lenticule 1	Supernatant (free&released rhNGF)	6387	7617	84%
	Pellet (intact MPs)	1230		16%
Lenticule 2	Supernatant (free&released rhNGF)	3455	4384	79%
	Pellet (intact MPs)	929		21%
Lenticule 3	Supernatant (free&released rhNGF)	3904	4639	84%
	Pellet (intact MPs)	735		16%

The results obtained demonstrate that the ratio between the rhNGF released and that
 5 contained in the microparticles is highly reproducible in the different lenticules.

Example 5:

Distribution of rhNGF-MPs in lenticules

Fluorescent PLGA microparticles (Fluo-MPs) (Supplied by S.I.C. #LGFG5000) were
 used to test the interaction of the microparticles with the lenticule.

10 5 mg of Fluo-MPs were suspended in 0.175 mL of 0.9% and one lenticule was added
 to the obtained suspension and incubated for 3 hours at R.T with orbital shaking at 200
 rpm.

The lenticule was then removed from the suspension and washed 10 times in 0.4 mL
 of 0.9% NaCl (up&down).

In order to evaluate the binding to or inclusion of the Fluo-MPs in the lenticule and their location, the collagen in the lenticule was stained by immunofluorescence using the protocol reported below.

The lenticule was fixed in paraformaldehyde 4% for 10 min, washed 3 times in PBS 1X for 5 min and then incubated for 1 hour with Anti-Collagen I primary antibody (Abcam, Cat# ab34710) in BSA 1% prepared in PBS 1X. It was then washed again 3 times in PBS 1X for 5 min and incubated for 1 hour with Anti-rabbit Cy3 secondary antibody (Jackson ImmunoResearch, Laboratories, Cat# 111-165-144) in BSA 1% prepared in PBS 1X and washed 3 times in PBS 1X for 5 min.

The immunostained lenticule was finally mounted on coverslip and analysed by LMS-800 Zeiss confocal microscope.

The data obtained, shown in Figure 2, demonstrate that the microparticles are mainly bound at the surface of lenticule.

Example 6

In vitro kinetic release of rhNGF from MPs included in lenticule

The in vitro release kinetic of rhNGF from the functionalized lenticules was evaluated with the following procedure.

A functionalized lenticule obtained as described in Example 3 was incubated in a tube containing 0.150 mL of PBS at 37°C with 80 rpm orbital shaking (LoBind tubes were used to preserve rhNGF)

Time points were set at t=0, 2, 4 or 24 hours and t=2, 5, 7, 9, 12, 14, 16, 19, 21, 23 or 25 days.

At every time point, the lenticule was removed from the initial tube and moved to the new tube containing 0.150 mL of PBS and incubated again at 37°C with 80 rpm orbital shaking.

Each sample was immediately stored at -20°C;

After 1 month the samples were analyzed, without dilution, by ELISA assay to evaluate the rhNGF title.

As shown in Figure 3, the rhNGF release from MPs included in the lenticule is sustained for about one month. Particularly, the neurotrophin is quickly released in the first 24 hours and the release is constantly maintained up to the end of the experiment.