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Research paper

Dexamethasone-loaded poly(ε-caprolactone) intravitreal implants: A pilot study

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Abstract

Purpose: Poly(ε-caprolactone) (PCL) is a biodegradable and biocompatible polymer that presents a very low degradation rate, making it suitable for the development of long-term drug delivery systems. The objective of this pilot study is to evaluate the feasibility and characteristics of PCL devices in the prolonged and controlled intravitreous release of dexamethasone. *Methods:* The *in vitro* release of dexamethasone was investigated and the implant degradation was monitored by the percent of mass loss and by changes in the surface morphology. Differential scanning calorimetry was used to evaluate stability and interaction of the implant and the drug. The short-term tolerance of the implants was studied after intravitreous implantation in rabbit eye. *Results:* PCL implant allows for a controlled and prolonged delivery of dexamethasone since it releases 25% of the drug in 21 weeks. Its low degradation rate was confirmed by the mass loss and scanning electron microscopy studies. Preliminary observations show that PCL intravitreous implants are very well tolerated in the rabbit eye. *Conclusion:* This study demonstrates the PCL drug delivery systems allowed to a prolonged release of dexamethasone *in vitro*. The implants demonstrated a strikingly good intraocular short-term tolerance in rabbits eyes. The *in vitro* and preliminary *in vivo* studies tend to show that PCL implants could be of interest when long-term sustained intraocular delivery of corticosteroids is required.

Keywords: Poly(ε-caprolactone); Implant; Prolonged release; *In vitro* study; Short-term tolerance

1. Introduction

At a time when new active compounds are available for retinal diseases treatment, intraocular controlled drug delivery systems are essential to achieve an ideal pharmaceutical intervention. Polymeric drug delivery systems allow for a sustained and controlled release of the drug, thus optimizing its bioavailability and decreasing potential side effects. Particularly, biodegradable polymers have been extensively studied for ocular drug delivery systems as no surgical procedure is required to remove the empty device [1].

Pharmaceutical intervention for the treatment of diseases affecting the posterior segment tissues of the eye requires repeated intravitreous injection because effective levels of drugs in the vitreous and the retina cannot be achieved through conventional routes of administration [2]. Repeated intravitreous injection however has many drawbacks: (i) patient discomfort and compliance, (ii) cumulated risk of rare but severe complications such as endophthalmitis, retinal tears, hemorrhages and detachments, (iii) cataract, (iv) peak and valley drug levels, (v) toxic risk for ocular tissues when efficacy and toxicity threshold are close. Intraocular implants of biodegradable polymers overcome most of these drawbacks since they maintain stable long-term vitreous concentrations of drugs

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in the therapeutic range. Studies using biodegradable implants containing various agents have been reported [3–11] and dexamethasone biodegradable delivery systems have been developed to the prevention of inflammation after cataract surgery [12–14].

Posurdex (Allergan), a biodegradable polymer matrix prepared with poly-lactide-co-glycolide copolymer (PLGA), releases dexamethasone over approximately 5 weeks and clinical trials have been evaluating its usefulness in persistent macular edema associated with diabetes, retinal vein occlusions, uveitis, and post-cataract surgery. The Phase II clinical trial followed 306 patients who received one of the three treatments: a single implant with either a 350 or 700 μg dose of dexamethasone or observation without drug therapy. The results showed that the patients who had been implanted with the 700 μg dose had the greatest improvement in vision and most of these patients exhibited a three-line increase in visual acuity compared to the control group [15].

Within the biodegradable polymers, aliphatic polyesters, such as poly (ε-caprolactone) (PCL), are of particular interest as they allow for a long sustained and possibly modulated drug release rate [16]. PCL is a biodegradable and biocompatible semi-crystalline polymer having glass transition temperature of −60 °C and melting point ranging between 59 and 64 °C, depending upon its crystalline nature [17,18]. This polymer presents a very slow degradation rate, making it suitable for long-term delivery extending over a period of more than one year. Furthermore, it is biocompatible and very much used in the pharmaceutical and biomedical fields, respectively, as biomaterials (suture, ostheosynthesis material, artificial skin, support of cellular regeneration) or as prolonged drug delivery systems targeting specific tissues within the body [18]. PCL micro- or nanoparticles or solid implants have indeed been widely explored these last years for the administration of drugs by different routes and for the treatment of different diseases [18–23]. However, despite the potential of PCL, its utilization in ophthalmology, especially for the intraocular route, has been poorly explored.

The aim of this study was to develop and characterize biodegradable PCL implants, for intraocular prolonged and controlled release of dexamethasone. The *in vitro* release profile of dexamethasone was investigated and the implant degradation was monitored by the percent of mass loss and surface changes visualization using scanning electron microscopy. Differential scanning calorimetry was used to evaluate the stability and interaction of the implant and the drug.

Finally, the feasibility and short-term tolerance of PCL implants were evaluated in rabbit eye after intravitreous implantation.

2. Materials and methods

Dexamethasone alcohol (MW = 392.5; aqueous solubility at 37 °C = 1.0 mg/ml) and polymer poly(ε-caprolactone) (PCL; MW ~ 14,000; density = 1.145 g/ml at 25 °C) were purchased from Sigma–Aldrich Co. (France). Acetonitrile HPLC grade was purchased from EM Science, Merck KGaA (Germany). Ultrafiltrated water was obtained from Milli Q plus, Millipore (USA). All other chemicals were of analytical grade.

2.1. Preparation by compression of the implants containing poly(ε-caprolactone) and dexamethasone

Firstly, a 25% w/w concentration of the drug (dexamethasone) and the polymer [poly(ε-caprolactone)] was dissolved in a mixture of acetonitrile and distilled water (1:1). The formed solution was then placed in a freezer under −80 °C. Afterwards, the frozen solution was lyophilized (Christ Alpha 1-2 LD, Bioblock Scientific, France). Approximately 200 mg of the obtained powder was compressed in an evacuable KBr die (Shimadzu, Japan), using a hydraulic press SSP-10A (Shimadzu, Japan) at 10 ton/cm^2, during 10 min in the form of 13 mm diameter discs. The obtained discs were, next, cut in the size of implants of 4.0 mm of diameter. The implants were white to off-white in color, presented a rigid structure, and 1 mm in thickness (Fig. 1). The mean weight of the developed devices was 4.01 ± 0.20 mg, corresponding to approximately 1 mg of dexamethasone.

2.2. Content uniformity test for the dexamethasone-loaded PCL implants

For the determination of content uniformity of dexamethasone in the PCL implants, the procedure stated in the general chapter <905> uniformity of dosage units of the United States Pharmacopeia 29 [24] was followed.

Ten implants were selected and weighted. Each implant was dissolved in 100 ml of a mixture of acetonitrile and distilled water (1:1). The amount of dexamethasone was determined by high-performance liquid chromatography, according to the procedure described in the item 2.3.

The obtained values of the amount of dexamethasone in each implant (mg) were estimated and the results were expressed as the percent of the pre-indicated value (approximately 1.0 mg/ml). The relative standard deviation was also calculated.

Fig. 1. Macroscopical view of the developed implant.

2.3. High-performance liquid chromatography method for dexamethasone determination

The amount of dexamethasone in the implants and that released in the *in vitro* study were measured by high-performance liquid chromatography (HPLC) using the method described in the United States Pharmacopeia 29 [24] by a Waters® apparatus equipped with an auto-sampler model 717plus (Waters, USA). A pump (model 515, Waters, USA) was used at a constant flow rate of 1.0 ml/min. A C-18 reversed-phase column (4.6 mm × 100 mm, macropore size of 2 μm) filled with high-purity silica substantially covered with *n*-alkyl chains (Chromolith® RP-18E, Merck KGaA Performance & Life Science Chemicals, Germany) was used. The mobile phase was a mixture of acetonitrile and ultrafiltrated water (45:55). An ultraviolet detector (model 2487, Waters, USA) was used at a wavelength of 254 nm. The validation of the method showed the absence of interference of the incubation medium compounds and the polymer with dexamethasone retention time, discarding the risks of overestimation.

2.4. In vitro release of dexamethasone from the implants of poly(ε-caprolactone)

The United States Pharmacopeia 29 [24] states in the general chapter <1092> the dissolution procedure: development and validation that "sink conditions are defined as the volume of medium at least three times that required in order to form a saturated solution of drug substance. When sink conditions are present, it is more likely that dissolution results will reflect the properties of the dosage form".

The *in vitro* release study was carried out under sink conditions during 150 days. Assuming an aqueous solubility of dexamethasone of 1 mg/ml at 37 °C, sink conditions are achieved with 3.0 ml at least for each implant. Three implants were immersed inside three different tubes containing 3.5 ml of BSS (balanced salt solution, pH 7.4, Bausch & Lomb, USA) each.

The tubes were placed inside an incubator set at 37 °C and 30 rpm. At predetermined intervals, 1.0 ml of the medium was sampled and 1.0 ml of fresh medium was immediately added to each tube. The release profile was evaluated as the cumulative percentage of dexamethasone released in the medium.

The amount of dexamethasone released was measured by high-performance liquid chromatography (HPLC) using the method described in the section 2.3.

2.5. Evaluation of the in vitro mass loss

The *in vitro* mass loss study was carried out during 150 days. Three implants were placed inside three different tubes containing 3.5 ml of BSS (balanced salt solution, Bausch & Lomb, USA) each.

The tubes were placed inside an incubator set at 37 °C and 30 rpm. At predetermined intervals, the implants were retrieved from the media, blotted with wipes to dry off excess water and then dried for 72 h in a vacuum desiccator at room temperature. After, the dried implants were weighted.

The percentage of mass loss was calculated by the ratio between the obtained weight of the implant before and after incubation.

2.6. Scanning electron microscopy analysis

Scanning electron microscopy analysis was used to find information about the *in vitro* degradation of the developed implants by the changes in the implants surface from 0 to 21 weeks.

Morphological changes on the surface of the dexamethasone-loaded PCL implants retrieved from the *in vitro* incubation medium were analyzed by scanning electron microscopy (SEM) using a Stereoscan 440 scanning microscope (Leo Electron Microscopy Ltd., United Kingdom) operating at 15 kV. The retrieved implants were selected at random.

Before visualization, the implants were gently washed with distilled water, blotted with wipes to dry off excess water, and then dried for 72 h in a vacuum desiccator at room temperature. After drying, they were mounted on aluminium stubs using double-sided adhesive tape. Prior to microscopical examination all the samples were sputter-coated with a gold layer under argon atmosphere using a sputter apparatus (Balzers Union SCD 040 unit, Balzers, Germany). The implants surfaces were viewed at 20–1000× magnification and the images were transferred to the computer by means of a Digital Image Transference Interface (DITI). The photomicrographs were adjusted using the software Adobe Photoshop 6.0 and Adobe Illustrator 9.01 (Adobe Systems Incorporated, 2000, USA).

2.7. Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) (TA Instruments, model 2910 Modulated DSC, USA) technique was used to find information about dexamethasone and PCL stability and the possibility of interaction between them.

The thermograms of PCL and dexamethasone before and after lyophilization and their physical and lyophilized mixture (25% w/w of drug and 75% w/w of polymer) were recorded. The thermograms of the final implants were also analyzed.

For the analysis of the substances before preparation of the implants, 3–5 mg of the samples was sealed in aluminium pans. They were firstly heated from 30 to 100 °C (first run), then, cooled from 100 to 20 °C, and heated again from 30 to 300 °C (second run), under nitrogen atmosphere at the rate of 10 °C/min. Calibration of the system was performed using indium standard.

The first run was carried out in order to eliminate any residual solvent that could be present in the samples. DSC curves covering a range of 30–300 °C (second run) were recorded for the evaluation of dexamethasone and polymer stability and dexamethasone–polymer interaction.

For the determination of the glass transition temperature of the polymer in different conditions (non-lyophilized, lyophilized, mixed with dexamethasone and the final implant) the samples were sealed in aluminium pans. They were firstly heated from 30 to 100 °C, then, cooled from 100 to −100 °C, and heated again from −100 to 200 °C (second run), under helium atmosphere at the rate of 10 °C/min (the curves are not shown).

Afterwards, for the analysis of the final implants (except for the determination of glass transition of the polymer), they were sealed in aluminium pans and then heated from 30 to 300 °C, under a nitrogen atmosphere at the rate of 10 °C/min.

2.8. In vivo implantation and preliminary tolerance of PCL implants

In order to evaluate the feasibility of PCL implants and their preliminary *in vivo* tolerance, PCL implants without drug and dexamethasone-loaded PCL implants were implanted in rabbit eyes.

Fauve de Bourgogne, pigmented rabbits (weight range 2.5–3 kg, 10–12 months-old) (Elevage des Pins, Epeigné-sur-Dême 37370, France) were housed and cared for in accordance with the guidelines set forth by the Association for Research in Vision and Ophthalmology (ARVO) [25] for the use of animals in ophthalmic and vision research. Only one eye was used. Rabbits received either the PCL implant without drug ($n = 3$) or the dexamethasone-loaded PCL implant ($n = 3$). The study was approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais (Belo Horizonte, Brazil).

For implantation, the rabbits were anesthetized with an intramuscular injection of xylazine (5 mg/kg) and ketamine (50 mg/kg). The conjunctiva was dissected at the limbus in the temporo-superior quadrant and a 4 mm sclerotomy was performed at 3 mm posterior to the limbus. The PCL implant was inserted through the sclerotomy in the vitreous cavity and the sclerotomy was closed with a 7/0 vicryl suture. The conjunctiva was re-inserted using a 8/0 vicryl suture. No vitreous bleeding occurred during the procedure. The device was not fixed in the vitreous but was not observed any movement of the implant during the period of the study.

The rabbit eyes were followed up at 3, 8, 15 and 30 days post-implantation. Slit-lamp examination and photographs, intraocular pressure measurement, and fundus examination were performed. One rabbit in each group was sacrificed, using lethal dose of pentobarbital, at day 30 and the eyes were fixed in Bouin solution and proceeded for histological analysis. Other rabbits were kept for longer tolerance follow up.

For histology, the fixed eyes were opened transversally at 2 mm posterior to the limbus, the implants and the lens were removed and the remaining posterior segment was embedded in paraffin and sectioned in 7–10 μm thick sections. At the sclerotomy level, sections were stained with hematoxylin–eosin, examined under a Leica (Switzerland) inverted microscope, and photographed with a digital Leica camera.

3. Results and discussion

3.1. Content uniformity test for the dexamethasone-loaded PCL implants

The results of content uniformity showed that dexamethasone presents a uniform distribution in the PCL implants. No unit was outside the range of 85.0–115.0% of the pre-indicated amount of dexamethasone (25% w/w, corresponding to approximately 1.0 mg of the drug per implant). The relative standard deviation obtained was 4.3%.

3.2. In vitro release of dexamethasone from the implants of poly(ε-caprolactone)

Fig. 2 shows the *in vitro* cumulative release profile of dexamethasone during the period of the study.

The *in vitro* release profile obtained from the dexamethasone-loaded PCL implants developed showed that the drug was released slowly possibly controlled by diffusion. According to Merkli et al. [26], the poly(ε-caprolactone) is characterized by a very low hydrolysis rate, which can vary from months to years. A controlled drug release profile is obtained with no significant release burst, which will probably occur after the diffusion period.

Effective dexamethasone concentrations for suppressing various inflammatory processes range from 0.15 to 4.00 μg/ml [27–31] and equivalent and even higher concentrations that could not cause any toxic reactions were achieved by our implant during the period of the study.

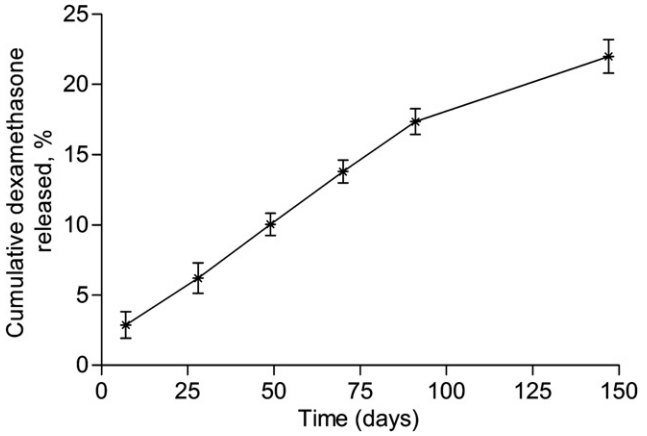

Fig. 2. *In vitro* release of dexamethasone from PCL implants in balanced salt solution at 37 °C: cumulative % of drug released (the values are shown as means ± standard deviation, $n = 3$).

This biodegradable implant released almost 25% of dexamethasone in 21 weeks under sink conditions.

According to the *in vitro* release profile obtained until this moment, it is possible to say that our implant can release the drug for more than one year. But, it is imperative to consider that *in vitro* and *in vivo* different conditions limit our conclusions regarding the release profile and mainly the possibility of occurring toxic reactions.

3.3. Evaluation of the in vitro mass loss

Fig. 3 shows the *in vitro* mass loss of the dexamethasone-loaded PCL implants.

Several factors can affect the rate of degradation of polymers and these include, mainly, the crystallinity, the glass transition temperature and the molecular weight. The water uptake also has been proposed as a tool for regulating degradation rates of polymers, suggesting that absorption of water is accompanied by polymer chain cleavage and mass loss decrease [32].

In almost polyester polymers, in the initial phase of the degradation process, no weight loss is observed. The random cleavage of the polymeric chains leads to an initial decrease of molecular weight without any significant mass loss. In the second phase of polymer degradation it is observed the onset of weight loss that has been attributed to the diffusion of small polymeric fragments from the matrix [18,26]. This initial lag phase, typical for the degradation of polyester implants, has often been explained with the slow penetration of water into hydrophobic matrices. The second phase can be attributed to the hydrolysis of the polymer chain, which occurs by random scission [33].

In PCL matrices, because of the polymer characteristics, the weight loss profile is mainly due to the drug release rather than to the degradation of the polymer. The hydrophobic nature of dexamethasone difficult its release to the medium making its diffusion very slow. After 21 weeks approximately 25% of drug release represents a weight loss of 5%. The overall weight loss of the implant being of 8% in the same period suggests that there was only a 3% weight loss for the polymer. So, according to the obtained results of mass loss, the drug release seems to be due to a diffusion phenomenen influenced by the hydrophobic nature of the drug rather than by the degradation of the PCL. The erosion, in the case of hydrophobic matrices, also plays an important role in the regulation of drug release.

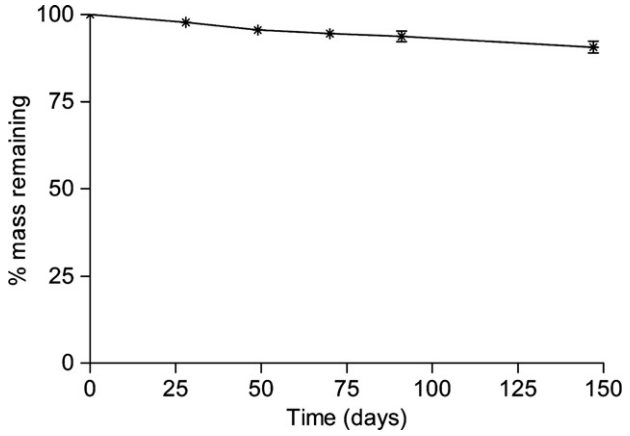

Fig. 3. Changes in mass represented by percent mass remaining of dexamethasone-loaded PCL implants (the values are shown as means ± standard deviation, $n = 3$).

The obtained mass loss profile shows that the developed device presents a very low degradation rate, which can be observed by the high percent of mass remaining in the implants at 21 weeks.

3.4. Scanning electron microscopy analysis

Fig. 4 shows scanning electron photomicrographs of the dexamethasone-loaded PCL implants before and after being placed in the *in vitro* medium. Typical changes in the surface of the developed devices were observed during degradation in the medium.

The surface morphology of polymeric systems as well as the mass loss studies plays an important role in degradation and drug delivery [34]. The pores and channels in the matrices allow drug diffusion possibly not dependent upon polymer degradation. Some studies have revealed that the degradation of polyesters proceeds faster in the center of the device than on the surface [7,35]. Thus, water channels are formed during the degradation process, connecting the surface to the inner part of the implant and allowing drug diffusion throughout the water channels of the polymeric matrix [7]. The pores and channels in the matrices may promote an increased water uptake by the implants, which may consequently accelerate the degradation process. The surface of the dexamethasone-loaded implants was initially smooth, with no evidence of pores or channels. The pores started to appear 7 weeks after implantation and were increased throughout the study. The observed pores can be attributed to voids left behind by the release of the drug or to the absorption of water.

The surface changes observed in this analysis can be compared to the very low degradation rate of the implant found in the study of mass loss where there was a high percent of mass remaining in the implants at 21 weeks.

3.5. Differential scanning calorimetry study

In Fig. 5, the DSC curves of dexamethasone, PCL and dexamethasone mixed with PCL are shown.

In DSC curves the melting peak is clearly observed for PCL and dexamethasone in both conditions: lyophilized and non-lyophilized. Though, in the mixtures, the PCL melting peak is still present and the dexamethasone peak appears much smaller than that of the drug alone.

Table 1 presents the glass transition temperature (T_g) and the melting temperature (T_m) of poly(ε-caprolactone)

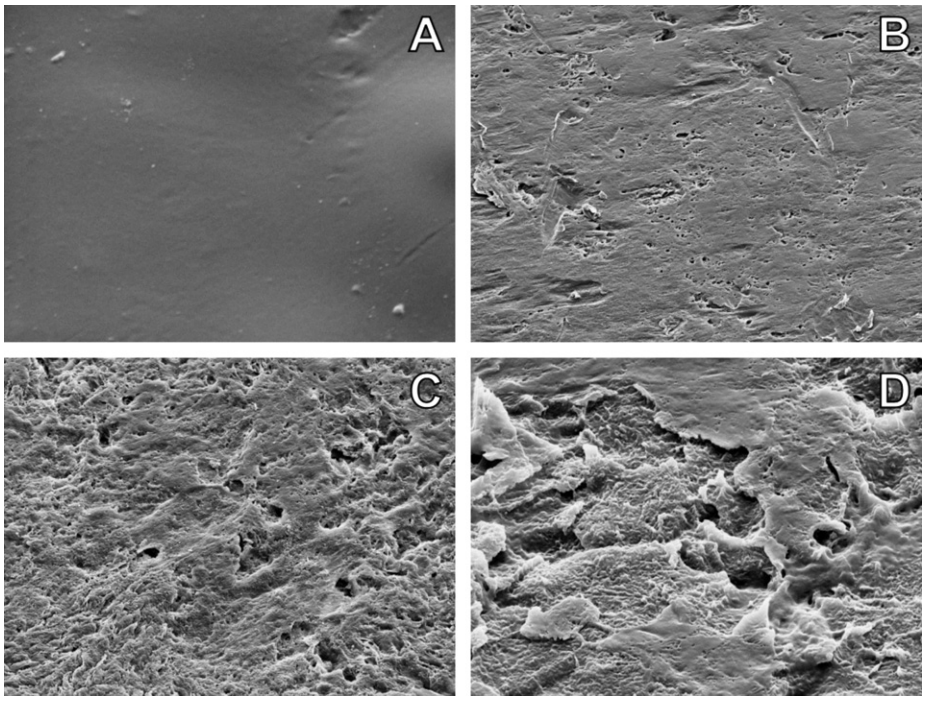

Fig. 4. Scanning electron photomicrographs of the dexamethasone-loaded PCL implants before incubation (A); after 7 weeks of incubation (B); after 14 weeks of incubation (C); and after 21 weeks of incubation (D). All photomicrographs were obtained at 1000× magnification.

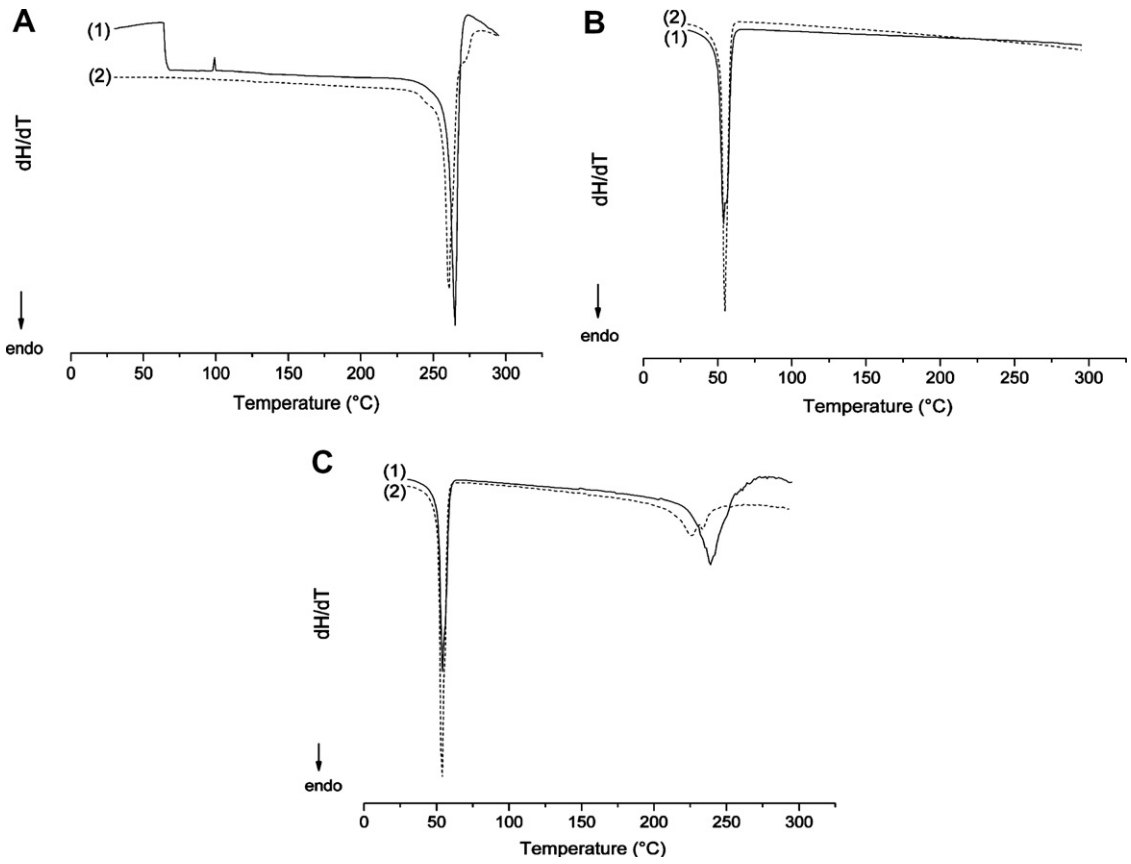

Fig. 5. DSC curves. (A) dexamethasone non-lyophilized (1), dexamethasone lyophilized (2); (B) poly(ε-caprolactone) non-lyophilized (1), poly(ε-caprolactone) lyophilized (2); (C) dexamethasone + poly(ε-caprolactone) physical mixture (1), dexamethasone + poly(ε-caprolactone) lyophilized mixture (2).

at different conditions: non-lyophilized, lyophilized and mixed with 25% w/w of dexamethasone.

The glass transition temperature may be important to define the propensity of amorphous compounds to crystallize at certain temperatures [36]. So, it could be observed that the polymer is completely amorphous before and after the lyophilization process, in which T_g was determined. The obtained values of T_g for PCL (non-lyophilized and lyophilized) were the same, in which no modification in their amorphous state was observed during the lyophilization process, related to state transition. The melting temperature of the polymer also did not change after lyophilization. When the polymer was mixed with dexamethasone it was also not observed significant alteration in these parameters. So, we can suggest that both the lyophilization process and the addition of dexamethasone did not change the PCL characteristics in this study.

Table 2 presents the enthalpy of fusion (ΔH_f), the melting temperature (T_m) and the relative crystallinity of dexamethasone at different conditions: non-lyophilized, lyophilized and mixed with 75% w/w of poly(ε-caprolactone).

It was observed that the melting temperature of dexamethasone alone was not significantly different before and after lyophilization. So, the lyophilization process, in this case, did not change the crystalline characteristics of the drug. The enthalpy of fusion also does not change so much in this situation.

Though, for the mixture of the drug with the polymer a decrease in the melting temperature and a relatively high decrease in the enthalpy of fusion for the lyophilized mixture are observed. The value of the relative crystallinity of dexamethasone in the lyophilized mixture reduces to only 30% from the drug alone in the same condition. In the DSC curves of the lyophilized mixture, it is also observed that the melting peak of dexamethasone is smaller than that of the drug alone. So, we suggest that there is the possibility of interaction between dexamethasone and poly(ε-caprolactone) that affects mainly the drug, as the polymer characteristics did not change significantly.

The parameters determined by DSC for the final dexamethasone-loaded PCL implants showed that the enthalpy of fusion of dexamethasone reduced to 7.1 J/g when compared to the value of the mixture. The glass transition temperature of poly(ε-caprolactone) and the melting temperature of dexamethasone for the final implants did not show any significant modification from the values obtained for the mixtures powder.

As discussed above, the reduction of the enthalpy of fusion of dexamethasone can suggest the possibility of interaction between the drug and the polymer but, as we observed the drug release from the implants in the *in vitro* release study, we cannot affirm that this interaction that may occur would influence the drug release. More studies using different techniques such as thermogravimetry, infrared spectometry and others are necessary to confirm the possibility of interaction. The efficiency of the drug released from the PCL implant, *in vivo*, is being realized to evaluate the influence of the possible drug interaction.

Table 1
Glass transition (T_g) and melting (T_m) temperatures of poly(ε-caprolactone) at different conditions: non-lyophilized, lyophilized and mixed with 25% w/w of dexamethasone

Poly(ε-caprolactone) conditions	T_g[a](°C)	T_m [a](°C)
Non-lyophilized	−66	54
Lyophilized	−66	55
+ Dexamethasone (physical mixture)	−68	54
+ Dexamethasone (lyophilized)	−69	54

[a] T_g and T_m precision is ±1 °C.

Table 2
Parameters determined by DSC for dexamethasone at different conditions: non-lyophilized, lyophilized and mixed with 75% w/w of poly(ε-caprolactone)

Dexamethasone conditions	ΔH_f (J/g)	Relative crystallinity of dexamethasone (%)[a]	T_m (°C)
Non-lyophilized	128.7	ND	261
Lyophilized	100.4	100	265
+ PCL (physical mixture)	38.6	ND	239
+ PCL (lyophilized)	7.4	30	226

ΔH_f, enthalpy of fusion; T_m (°C), melting temperature (precision of ±1 °C); ND, non-determined.

[a] The relative crystallinity of dexamethasone in the lyophilized mixture was calculated by fixing the ΔH_f of dexamethasone lyophilized as 100% of crystallization and considering that the mixture is composed of 25% w/w of dexamethasone.

3.6. In vivo short-term tolerance of the PCL implants into the vitreous cavity

Minimal local inflammation was observed at the surgical site at day 3 and 8 post-surgery in both the PCL implant without drug and the dexamethasone-loaded PCL implant. No significant signs of local inflammation were observed thereafter (Fig. 6A and B). The intraocular tolerance of PCL implants was particularly good with no clinical signs of intraocular inflammation (no cells or proteins in the vitreous or in the anterior chamber) and no cataract formation during the follow-up period (Fig. 6C). Fundus examination using a 3 mirrors Glodman glass allows to visualize the PCL implant, stable at the site of implantation without any migration into the vitreous cavity (Fig. 6D). No significant change in the intraocular pressure was observed before implantation (8 ± 4 mm Hg), and at 30 days post-implantation in the PCL implant without drug group (9 ± 2 mm Hg) or the dexamethasone-loaded group (10 ± 3 mm Hg) ($P < 0.05$, Mann–Whitney U-test).

Histological examination at day 30 after implantation of the PCL implant without drug implant showed healing of the sclerotomy site without fibrotic reaction and minimal cell infiltration (Fig. 7). Similar observations were found in dexamethasone-loaded PCL implants.

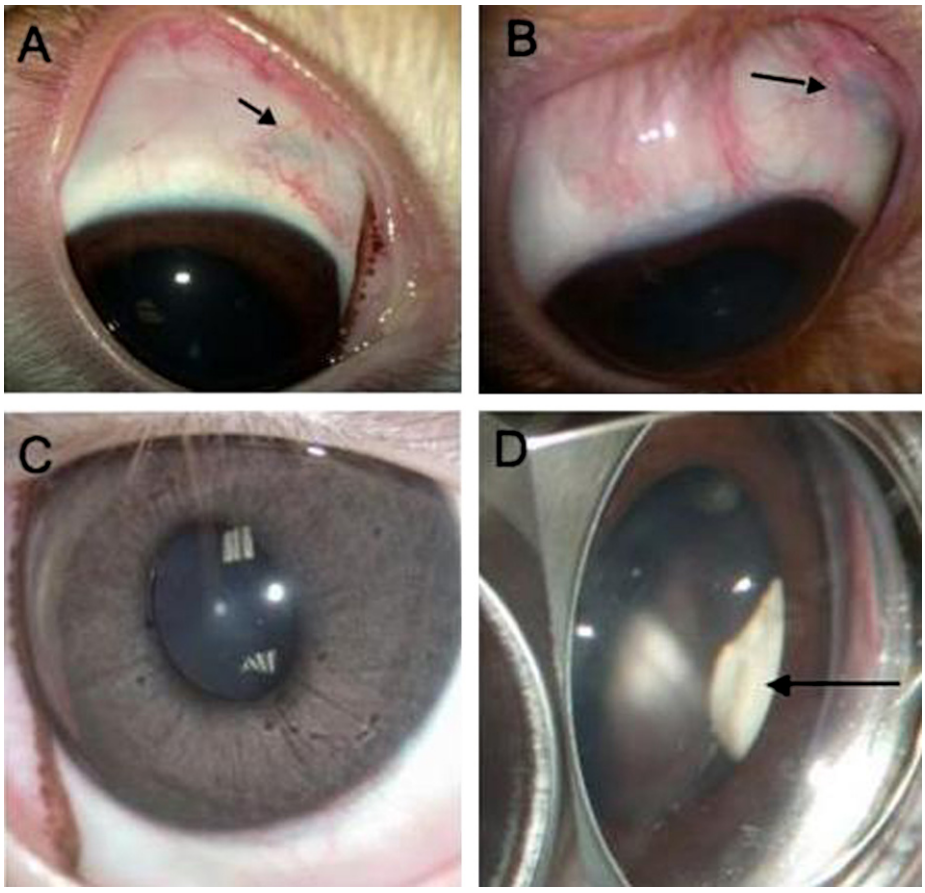

Fig. 6. *In vivo* observation of PCL implant without drug after surgical implantation into the vitreous of pigmented rabbit eyes. Implantation sites at day 8 (A) and 30 (B) after surgery. No clinical inflammation at day 30 after implantation (C) and presence of the implant posterior to the lens at the implantation site using three-mirrors Goldman peripheral retina examination (D).

Drug delivery systems prepared with biostable polymers have to be removed from the eye after complete drug release because they do not substantially degrade *in vivo*, maintaining their structural integrity in the presence of a physiological environment [37]. PCL implants present slow degradation characteristics that can last over years, which make them quasi-biostable. Because of this, the impact of PCL implants in the eye during a long period of time is an important parameter that is being evaluated in long-term tolerance studies.

These observations show that PCL implants can be easily inserted in the vitreous cavity with minimally invasive procedure and that this polymer demonstrates a strikingly good intraocular tolerance in the rabbit eye. Though, these results are preliminary because the PCL matrices showed only a very small sign of degradation after 30 days of implantation and the polymer degradation products can be the main factors that would influence the tolerance of the developed implant. Long-term follow up as well as *in vivo* release kinetics studies are being performed to confirm these preliminary observations.

In summary, our *in vitro* and preliminary *in vivo* studies tend to show that PCL implants could be of interest when long-term sustained intraocular delivery of corticosteroids is required by the clinical condition of the patients. The *in vitro* release profile shows that the dexamethasone-loaded PCL implant allows for a prolonged and controlled release of dexamethasone without any significant burst release. Since 25% of dexamethasone was released in 21 weeks under sink conditions, we can expect for a year of drug release. The very low degradation rate was confirmed by the mass loss and scanning electron microscopy studies. DSC studies showed that the lyophilization process does not cause significant changes in the polymer and the drug characteristics, and that a possible interaction between the two components would not necessarily influence drug release from the polymeric matrix. *In vivo*, the developed PCL implant showed very good short-term tolerance, but long-term studies are required to evaluate their *in vivo* life-time and potential fragmentations at the late stage of degradation. These long-term studies are currently being undertaken.

In conclusion, biodegradable PCL implants may offer a wide range of applications for intraocular drug delivery since controlled and prolonged release over months or even years can be achieved, avoiding the need of repeated intraocular injections. Miniaturization and optimization of implantation procedures are required before any

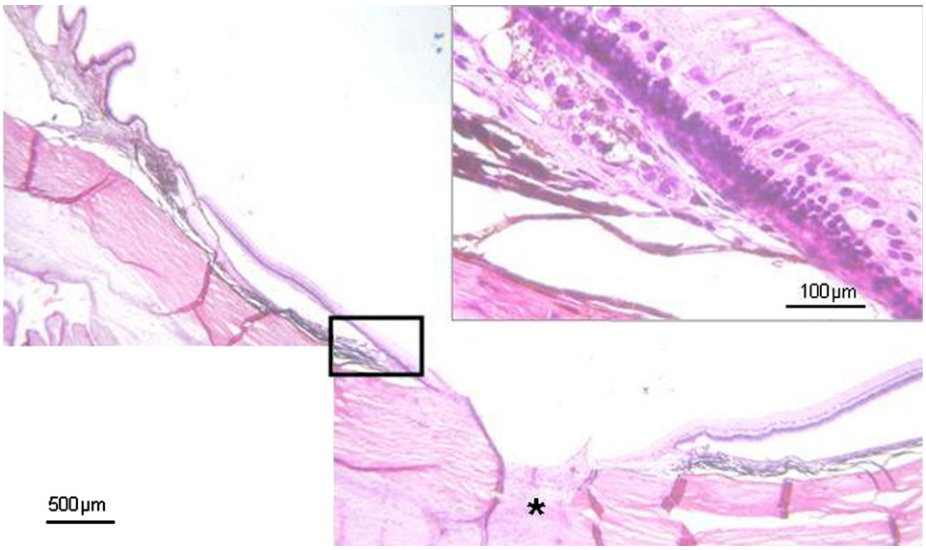

Fig. 7. Histological section (stained with hematoxylin–eosin) of the rabbit eye at 30 days after implantation of the PCL device without drug, showing the implantation site (asterisk) with scleral wound healing fibrosis, but no inflammatory cells in the vitreous cavity or in the retina at the edge of the implantation site (high magnification inset).

clinical applications, but this study demonstrates the feasibility and tolerance of intravitreous PCL drug delivery systems.

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