PM29

Porous and Safe Ocular Implants Developed by $scCO_2$ Foaming/Mixing for Increased Degradation Rate

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ABSTRACT

The use of biodegradable poly(ε-caprolactone) (PCL) based intraocular implants for the controlled delivery of drugs to treat several ocular pathologies has been reported in recent years. However, the PCL complete degradation takes normally more than 2 years. This work aims to develop new PCL-based intraocular implants presenting a faster hydrolytic degradation rate by applying a "green" supercritical carbon dioxide ($scCO_2$) foaming/mixing method (SFM) to increase their porosities, and to test their safety for the retina using *in vivo* techniques. These new implants were also tested as controlled release devices for the drug 2-Cl-IB-MECA, a selective adenosine A_3 receptor agonist with therapeutic potential for the treatment of retinal diseases. Glycofurol (G) was used as a safe compatibilizer and plasticizing agent. Mixtures of PCL:Drug:G (92:0:8 and 66:26:8, wt.%) were processed by SFM (318.15 K and 20 MPa for 2 hours, depressurization rate of 2 MPa/min) to obtain cylindrical implants (2×0.46 mm^2). Additionally, drug-free implants were also prepared for comparison purposes by a two-step hot-melting process (HM, 1 atm) at 323.15 K (0.5 h) followed by 353.15 K (0.5 h). Accelerated hydrolytic degradation tests showed a faster degradation rate for the higher porosity and surface area drug-free SFM-processed implants, attaining 50% of mass loss after 31 h to 59 h for the HM-processed materials, and 100% (full degradation) around 69 h to 81 h. Electroretinography and optical coherence tomography were performed *in vivo* for the analysis of retinal function and structure, respectively, in animals with surgically inserted drug-free SFM implants in the vitreous. The presence of the SFM implants in the vitreous did not change retinal function and structure. Drug-loaded SFM implants presented an extended release of 2-Cl-IB-MECA in water of around 30 days. Thus, we have successfully developed new and safe PCL-based intraocular implants having faster degradation rates and with the potential of extended release of drugs by using the "green" SFM methodology. These results also suggest that 2-Cl-IB-MECA-loaded PCL-based SFM implants might be envisaged as a new strategy to deliver the A_3 receptor agonist to the retina for the treatment of chronic retinal diseases, thus avoiding the typical, more invasive and less patient-compliant topical injections that are currently being used in clinical practice.

INTRODUCTION

The efficient delivery of therapeutic drugs to treat ocular diseases is a challenging task mostly due to the unique anatomy and physiology of the eye [1, 2]. Topical drug delivery is commonly used for being non-invasive and easily applied by the patient [1]. However,

drug bioavailability is low [1], requiring higher frequency of administration that could lead to patient compliance problems [3, 4]. Thus, the more invasive periocular and intraocular injections are also applied, since they increase the local drug concentration and diminish the delivery of the drug to off-target sites [2]. However, frequent injections may be needed to maintain therapeutic drug levels, which may promote complications such as retinal detachment, cataract, endophthalmitis, iritis, uveitis, intraocular haemorrhage and increased intraocular pressure [2, 5].

The development of controlled drug delivery systems based on polymeric implants has the potential to circumvent some of the aforementioned drawbacks [5, 6]. These systems can increase patient compliance by using suitable polymers and by tuning the implants physicochemical and morphological properties (e.g., porosity, surface area, degradation rate) to control and maintain therapeutic drug levels for extended periods of time. Intraocular implants based on biodegradable polymers are particularly interesting by advantageously avoiding the surgical removal after application [6]. These polymeric implants are typically manufactured by hot melt-pressing, extrusion, injection moulding or solution casting [5]. However, these methods normally use harmful solvents and/or operate at processing conditions (e.g., temperature, pH) that could promote the degradation of polymers, drugs and other additives [5, 7]. Most of these disadvantages can be avoided by applying the supercritical carbon dioxide ($scCO_2$) foaming/mixing method (SFM). Poly(ε-caprolactone) (PCL) has been extensively applied as a biocompatible and bioresorbable polymer in pharmaceutical and biomedical applications due to its tailorable mechanical properties and ease of shaping and processing (at relatively low temperatures) to enable appropriate pore sizes, and due to the controlled delivery of drugs contained within the matrix [6, 8].

The present work aims to develop and to characterize novel and highly porous PCL-based intraocular implants, prepared using the "green" SFM methodology, and to be applied for the extended release of drugs and for a faster PCL hydrolytic degradation rate. Glycofurol (G) is a relatively nontoxic and non-irritant material at levels normally used for pharmaceutical excipients, presenting a LD_{50} (lethal dose for 50% of the test sample) of 3.5 mL/kg (mouse, IV). Thus, glycofurol is a safe and FDA-approved excipient, being used as a drug-polymer compatibilizer and polymer plasticizer [9]. The new implants were evaluated for the incorporation yields and for the release of the drug 2-Cl-IB-MECA, a selective adenosine A_3 receptor agonist, since the activation of this receptor has been demonstrated to protect the retina, particularly retinal ganglion cells [10]. An *in vitro* degradation study of drug-free implants, at accelerated alkaline conditions, was performed in order to compare and to correlate the observed degradation rates with the implants final properties and employed processing methods (SFM vs. hot-melting, HM). Finally, optical coherence tomography and electroretinography recordings were performed to evaluate the safety for the retina of surgically inserted drug-free SFM implants in the vitreous of Wistar rats.

MATERIALS AND METHODS

Physically mixed combinations of PCL:Drug:G (92:0:8 and 66:26:8, wt.%) were introduced into polyurethane micro-cylinder moulds and were processed by SFM (20 MPa, 318.15 K, 2 h), using a depressurization rate of 2 MPa/min. The employed experimental SFM set-up and the followed general procedures were previously described [7]. Additionally, a drug-free PCL:G (92:8, wt.%) mixture was also processed for comparison purposes by a two-step hot-melting (HM) method (1 atm) at 323.15 K (0.5 h) followed by 353.15 K (0.5 h). The processed materials were removed from moulds and cut into cylindrical implants with dimensions of around 2×0.46 mm, length × diameter.

The hydrolysis degradation patterns of drug-free PCL:G (92:8, wt.%) implants prepared by SFM and HM were studied *in vitro* at accelerated conditions by adapting the method developed by Lam et al. [11]. Samples (3 replicates for each process) were kept in 5 M sodium hydroxide (NaOH) solution (310.15 K and 100 rpm) and weighted (m_i) at defined time intervals. The percentage of mass loss, Δm(%), was determined by $\Delta m(\%) = 100 \times (m_0 - m_i) / m_0$, where m_0 is the initial mass.

Samples processed by SFM from initial mixtures of PCL:Drug:G 66:26:8 wt.% were tested to determine the drug incorporation yields and the kinetics of drug release in water by a chromatographic method (HPLC-DAD). Samples (2 replicates) were kept in sealed vials in a thermoshaker at 310.15 K and 100 rpm. For the incorporation quantification, methanol was used and replaced till a negligible amount of drug was detected (less than 0.5% of the cumulative drug). In contrast, the results of the drug release experiments in water were represented by the percentage of released 2-Cl-IB-MECA over time, in which $Released\ Drug(\%) = 100 \times M_t/M_0$, and M_0 is the mass of drug loaded and M_t is the amount of drug released at a given time.

Wistar rats housed in certified animal house facilities, with temperature- and humidity-controlled environment and under a 12-h light/12-h dark cycle, were provided with standard rodent diet and water ad libitum. All procedures involving animals were approved by the animal welfare committee of the institution and are in agreement with the Association for Research in Vision and Ophthalmology statement for animal use.

Wistar rats (8 weeks old) were anesthetized with 2.5% isoflurane in 1 l/min O_2, as previously described [12], and SFM drug-free PCL:G (92:8, wt.%) implants were inserted in the vitreous body. The animals were randomly assigned into sham-operated group or implanted group. Electroretinography and optical coherence tomography (OCT) were used to analyse the effects of the implants to the retinas after 4 weeks.

RESULTS

Figure 1 shows the *in vitro* erosion patterns at accelerated alkaline conditions obtained for these drug-free implants.

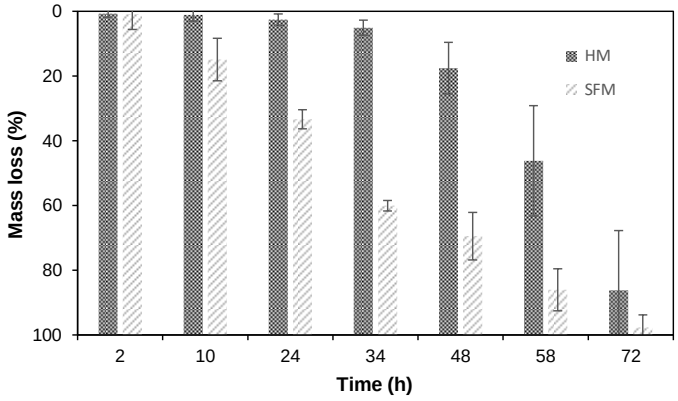

Figure 1. Percentage of mass loss versus time (h) in 5 M NaOH solution for PCL:G 92:8 (wt.%) implants prepared by: (▩) HM and (▨) SFM.

The results for the percentage of mass loss versus time obtained at accelerated conditions are expected to follow similar trends to the *in vivo* erosion of the materials [11]. Thus, this initial study allows to determine the effect of the proposed SFM methodology on the PCL erosion. The mass loss of HM materials was negligible for the first 34 h. Then, an apparent linear pattern was followed for the three replicates, with a relatively high variability. These findings are consistent with a bulk erosion mechanism, defined by a homogeneous reduction of the molecular weight of the PCL polymeric matrix [11, 13].

In contrast, mass loss of SFM implants followed a seemingly near linear pattern right from the beginning of the test, in line with a surface erosion mechanism in which the molecular weight of the PCL polymeric matrix remain constant [11, 13]. Thus, results confirm the SFM process to significantly decrease the degradation time of PCL materials by attaining 50% of mass loss after around 31h to 59 h for the HM methodology, and 100% (full degradation) around 69 h to 81 h. The observed differences for the mass loss rates are probably explained by morphological differences. In particular, the higher surface area of SFM materials [14] allows additional reaction sites for the hydrolysis to occur at the surface.

Incorporation yields of drug loaded SFM implants (PCL:Drug:G, 66:26:8 wt.%) were around 91 ± 12%, showing a relatively high variability promoted by the process. Glycofurol and 2-Cl-IB-MECA are probably soluble to a certain extent in $scCO_2$ and thus may be extracted/removed during processing, partially explaining the observed variability. An extended release of drug in water of around 30 days was obtained. This result corresponds to a drug release of around 1.6 ng/day, a value of the same order of magnitude of the amount delivered by a daily intravitreal injection of 3.3 ng [10].

Electroretinography was used to assess retinal response to light, as a measure of retinal function after 4 weeks post-surgery. The presence of the SFM drug-free intraocular implants did not change the response of the retinas to light, when compared with the contralateral (implant free) eye (Figure 2).

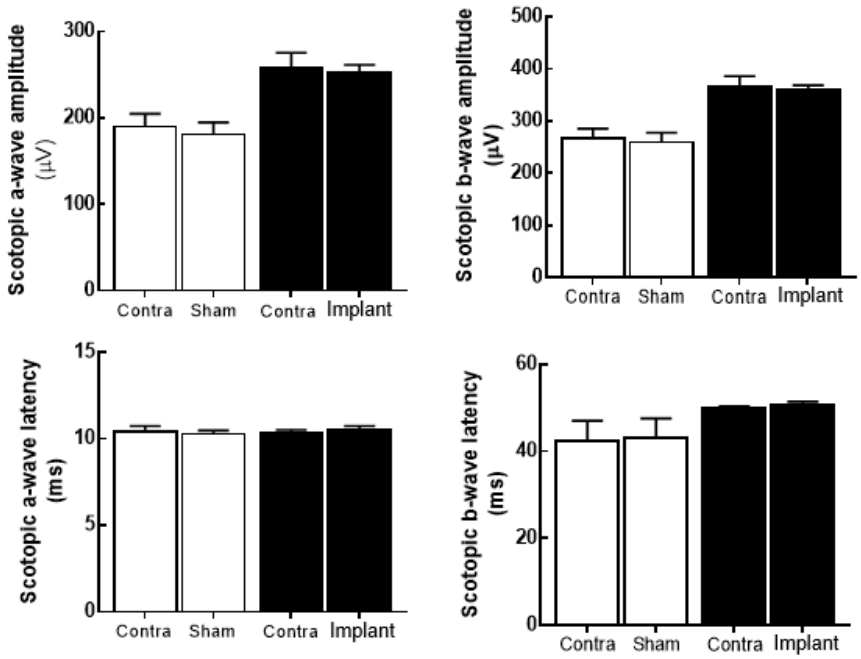

Figure 2. The intensity-response functions relatively to the scotopic a-wave amplitude and latency and to the scotopic b-wave amplitude and latency at 4 weeks after sham procedure or implantation of SFM drug-free PCL:G 92:8 (wt.%) implant. Contra: contralateral eye; Sham: sham-operated eye; Implant: implanted eye.

Moreover, OCT that was used to determine the retinal structure *in vivo*, revealed that the presence of SFM drug-free implants in the vitreous of Wistar rats up to four weeks did not cause major structural changes in the retinas (Figure 3).

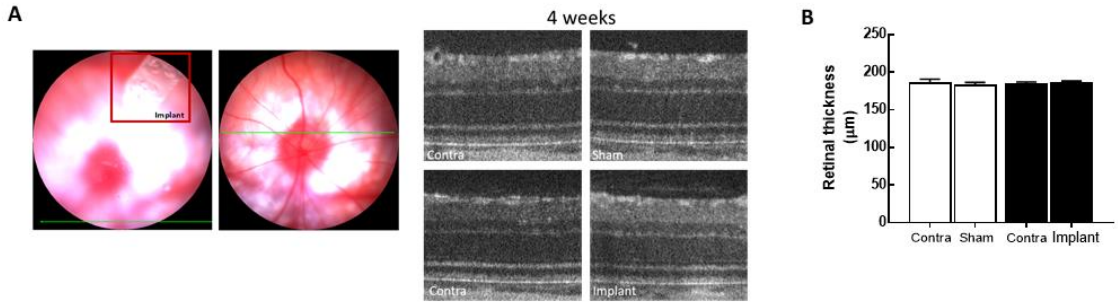

Figure 3. (A) Representative fundus image of a rat eye showing the implant inside the eye and the line scan (green line). Representative retinal OCT images at 4 weeks after sham procedure or implantation of SFM drug-free PCL:G 92:8 (wt.%) implant are depicted. **(B)** Total retinal thickness were measured and the results are expressed as mean ± SEM. Contra: contralateral eye; Sham: sham-operated eye; Implant: implanted eye.

CONCLUSIONS

A new porous PCL-based intraocular implants for the release of drugs was successfully developed by using the "green" $scCO_2$ foaming/mixing (SFM) method and the compatibilizer/plasticizer glycofurol. These processing strategies avoid the use of harmful solvents and of processing temperatures that could promote the degradation of the mixture components (polymers, drugs and other additives), and assure solvent-free materials and equipment after processing. The increased surface area and porosity of SFM processed materials allows additional reaction sites for the hydrolysis to occur at the surface, leading to higher erosion rates at accelerated alkaline conditions when compared to typical hot-melting processing methodologies. The drug-free SFM implants were found to be safe and tolerable by the Wistar rats, since no changes on retinal function and structure were reported. Implants loaded with 2-Cl-IB-MECA presented an extended release in water of around 30 days, corresponding to an average release of around 1.6 ng/day, a value of the same order of magnitude of the amount delivered by a daily intravitreal injection of 3.3 ng. Therefore, the new, safe and faster hydrolytic degrading SFM implants can potentially be used to deliver 2-Cl-IB-MECA to retinal cells, replacing the need of regular intravitreal injections, for relatively short time periods (less than 12 months), in the treatment of chronic retinal diseases. This strategy would increase patient compliance and avoid possible complications such as retinal detachment, uveitis or intraocular haemorrhage.

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