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Improved design and characterization of PLGA/PLA-coated Chitosan based micro-implants for controlled release of hydrophilic drugs

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ABSTRACT

Repetitive intravitreal injections of Methotrexate (MTX), a hydrophilic chemotherapeutic drug, are currently used to treat selected vitreoretinal (VR) diseases, such as intraocular lymphoma. To avoid complications associated with the rapid release of MTX from the injections, a Polylactic acid (PLA) and Chitosan (CS)-based MTX micro-implant prototype was fabricated in an earlier study, which showed a sustained therapeutic release rate of 0.2–2.0 μg/day of MTX for a period ~1 month *in vitro* and *in vivo*. In the current study, different combinations of Poly(lactic-co-glycolic) acid (PLGA)/PLA coatings were used for lipophilic surface modification of the CS-MTX micro-implant, such as PLGA 5050, PLGA 6535 and PLGA 7525 (PLA: PGA – 50:50, 65:35, 75:25, respectively; M.W: 54,400 – 103,000) and different PLA, such as PLA 100 and PLA 250 (MW: 102,000 and 257,000, respectively). This improved the duration of total MTX release from the coated CS-MTX micro-implants to ~3–5 months. With an increase in PLA content in PLGA and molecular weight of PLA, a) the initial burst of MTX and the mean release rate of MTX can be reduced; and b) the swelling and biodegradation of the micro-implants can be delayed. The controlled drug release mechanism is caused by a combination of diffusion process and hydrolysis of the polymer coating, which can be modulated by a) PLA content in PLGA and b) molecular weight of PLA, as inferred from Korsmeyer Peppas model, Zero order, First order and Higuchi model fits. This improved micro-implant formulation has the potential to serve as a platform for controlled release of hydrophilic drugs to treat selected VR diseases.

1. Introduction

At present, selected cases of several VR diseases, including primary intraocular lymphoma, uveitis and proliferative vitreoretinopathy are being managed by an antimetabolite chemotherapeutic drug, Methotrexate (MTX) (Khalatbari and McCallum, 2003; Velez and Whitcup, 1999; Palakurthi et al., 2010; Sadaka et al., 2016). Currently, in clinical practice, ~400 μg of MTX is being administered as an intravitreal injection (Velez and Whitcup, 1999; Hardwig et al., 2006; Akiyama et al., 2016). MTX, being hydrophilic in nature and with a short half-life of 14.3 h, undergoes rapid clearance in the aqueous vitreous environment (Palakurthi et al., 2010). In order to sustain therapeutic efficacy, several intravitreal MTX injections must be administered, as frequently as two times weekly at the start of therapy. In our prior studies, it has been reported that a sustained release drug delivery device (micro-implant), which maintains a release rate of 0.2–2 μg/day or a concentration of 0.1–1 μM of MTX for a period of a month or more would avert the potential complications associated with a series of intravitreal injections, while maintaining therapeutic efficacy (Palakurthi et al., 2010; Manna et al., 2014). The fabrication of a sustained release device (micro-implant) for hydrophilic drugs, such as MTX, is a challenge because the hydrophilic drugs do not blend well with existing FDA approved lipophilic materials, such as poly-glycolic acid (PGA), poly-lactic acid (PLA) and poly-(lactic-co-glycolic) acid (PLGA), which is a copolymer of PLA and PGA.

Chitosan (CS), considered as Generally Recognized as Safe (GRAS) material by the US FDA (Manna et al., 2014), is a copolymer of N-acetylglucosamine and glucosamine which is fully or partially N-deacetylated (DA) derivative of the natural polymer Chitin. CS, being a biocompatible and biodegradable hydrophilic polymer, is a potential candidate for the matrix of the micro-implant to blend hydrophilic MTX (Kumar et al., 2008; de la Fuente et al., 2010; Li et al., 2011; Nagarwal et al., 2011; Rao et al., 2015) and also known for other biomedical applications (Ramya et al., 2012; Riva et al., 2011; Senel and McClure,

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2004; Wang et al., 2016). Similarly, PLA/PLGA, obtained from lactic acid and glycolic acid monomers, are well known for their application in biodegradable intraocular drug delivery devices and other biomedical applications (Manna et al., 2014; Manna et al., 2015).

Due to the similar hydrophilic nature of CS and MTX, MTX is rapidly released from the CS-MTX micro-implant in the aqueous vitreous environment. A lipophilic surface modification of the hydrophilic CS-MTX micro-implant is imperative to facilitate controlled release of MTX from the CS-MTX micro-implant. In our prior *in vitro* study, PLA was used as polymer for lipophilic surface modification of the CS-MTX micro-implant (Manna et al., 2014). The PLA-coated CS-MTX micro-implant has demonstrated sustained therapeutic release of MTX (0.2–2 μg/day) for a period of 50–70 days *in vitro* (Manna et al., 2014) and also manifested therapeutic concentration of MTX (0.1–1 μM) in rabbit eyes for a period of 33 days without any toxicity (Manna et al., 2016; Manna et al., 2016; Perron and Pagé, 1994). The earlier studies lacked critical evaluation of the influence of different molecular weights (MWs) and composition of the lipophilic coating polymers on the controlled release of MTX and degradation of the micro-implants.

The importance of this study includes: a) evaluation of the influence of different molecular weights (MWs) and composition of the lipophilic coating polymers on the controlled release of MTX from the micro-implant; b) employ additional techniques, such as gel permeation chromatography (GPC), fourier transform infrared spectroscopy (FTIR) and swelling analysis, to characterize the lipophilic coating and its interaction with the structural stability of the micro-implant; and c) improve the therapeutic release duration of MTX for a period of ~3–5 months.

2. Materials and methods

2.1. Preparation of the micro-implant

As described in our prior study, CS-MTX micro-implants are fabricated by lyophilizing CS (M.W. 50,000–190,000 and DA% ≥75%) (Sigma Aldrich, MO) and MTX (Letco Medical, AL), mixed in dilute HCl solution comprising 40% w/w drug loading (Manna et al., 2014).

The polymers used for the lipophilic surface modification in this study are a) different copolymer ratio of PLGA and b) different MWs of DL-PLA. The different types of PLGA investigated are PLGA 5050, PLGA 6535 and PLGA 7525, where the copolymer ratio of PLA:PGA in PLGA are 50:50, 65:35 and 75:25, respectively. The different types of DL-PLA used are that with inherent viscosities 0.67 and 1.16 dL/g in $CHCl_3$ @ 30 °C. The lipophilic polymers are obtained from Lactel Biodegradable Polymers, AL. Each of the lipophilic polymers is mixed in dicholoromethane (DCM) (Fisher Sci., MA) to synthesize a 40 mg/ml coating solution. The CS-MTX micro-implants are then dip coated in the respective coating solutions following the same protocol as reported in the prior study (Manna et al., 2014). The micro-implants are dipped 4 times longitudinally in each direction in the respective lipophilic polymer coating solution.

2.2. Material characterization of the micro-implant

The microstructure of the micro-implant is characterized by optical microscopy (Keyence Digital Microscope, VHX-600) and scanning electron microscopy (SEM) (FEI XL 30-FEG, FEI) using an accelerating voltage of 15 KV. For SEM, the samples are sputter-coated using an Au-Pd target for 1 min. Differential scanning calorimetry (DSC) study is performed at the heating rate of 10 °C/min (DSC6200, Seiko Instruments Inc.) to characterize the glass transition temperature (Tg) of PLGA/PLA at the physiological condition. Additional details of these techniques are provided in our prior study (Manna et al., 2014).

In addition to the above characterization methods, the newer techniques include: a) GPC to characterize the MWs of PLGA/PLA; b) FTIR to evaluate the bonding between the hydrophilic CS-MTX matrix of the micro-implant and the PLGA/PLA coatings; and c) swelling study of the coated micro-implants to simulate the *in vitro* biodegradation of the micro-implant.

2.2.1. Molecular weight of the lipophilic polymers used for coating (GPC)

MWs and polydispersities of the coating polymers are determined by GPC. Samples are weighed on an analytical balance and placed in sample vials where they are diluted to a concentration of 5–15 mg/mL using dimethyl formamide (DMF) with 0.1% LiBr w/v and 0.05% toluene v/v as a flow rate marker. Samples are then placed on a shaker table for 72 h to dissolve and then filtered through a Whatman Anotop 25 syringe filter with a 0.2 μm porosity prior to injection.

The samples are characterized with an Agilent 1200 series HPLC equipped with a PSS Gram (10 μm) guard column and 2 PSS Gram columns (10 μm) (linear range of M.W. = 100–1 × 106 g/mol). A mobile phase of DMF with 0.1% LiBr w/v is used at a flow rate of 0.5 ml/min at 60 °C. Optilab rEX differential refractometer (light source = 658 nm) detector is used and calibrated against poly(methyl methacrylate) standards (850 Da – 2,000,000 Da). ASTRA software v. 5.3.4 is used to determine polymer characteristic values.

2.2.2. Characterization of the lipophilic polymer coating (FTIR)

(%R) FTIR spectra is acquired using the Thermo Fisher Scientific Nicolet 6700 machine with a Smart orbit ATR module for a frequency range of 4000 cm^{-1}–400 cm^{-1}. The samples analyzed are pure CS powder, pure MTX powder, pure PLGA and PLA crystals used for lipophilic coating, uncoated CS-MTX micro-implant and PLGA/PLA–coated micro-implant. The samples are investigated for any possible chemical bonding between the PLGA/PLA coating with the CS-MTX matrix of the micro-implant.

2.2.3. Swelling analysis

Swelling experiments are conducted by placing PLGA/PLA–coated CS-MTX micro-implants (n = 3, for each type of lipophilic coating) in vials containing 5 ml phosphate buffered saline (PBS; pH 7.4). The vials are slowly stirred in a water bath maintained at 37 °C, which is similar to the *in vitro* MTX release analysis (Section 2.3). At pre-determined time points, the micro-implants are weighed after absorbing the PBS on their surface using Kimwipes (Kimtech Science Brand, Kimberly Clark, TX). The swelling index (Swelling index %) for each micro-implant is obtained using the following equation,

$$Swelling\ index\% = ((W_t - W_0)/W_0) \times 100 \quad (1)$$

where, W_0 is the initial weight of the micro-implant at the start of the experiment and W_t is the weight of the micro-implant at time 't'.

2.3. Release rate studies

The set-up of the release rate studies are similar to that reported earlier (Manna et al., 2014); where the micro-implants are kept in vials containing 5 ml of PBS at 37 °C (n = 3 for each type of polymer used for lipophilic coating). At pre-determined time intervals, 1 ml of release media sample (PBS) containing MTX is taken out and replenished with 1 ml of fresh PBS, in order to maintain sink conditions. 1 ml of release media sample is assayed using an UV–Visible Spectrophotometer (Cary 50-Bio UV–Vis Spectrophotometer, Varian) to obtain the concentration of MTX (Ritger and Peppas, 1987). The calibration curve was obtained as reported in our earlier study (Manna et al., 2014), and thereafter the 258 nm peak of the MTX spectra is used for the release rate experiments.

Furthermore, the drug release data of all drug loadings of the coated micro-implants were fitted to the following models in order to analyze the mechanism of drug release and diffusion kinetics. The fitting of each model is evaluated based on correlation coefficient (R^2) values.

Korsmeyer-Peppas model. The generic equation for the Korsmeyer Peppas model is;