Development of Chitosan and Polylactic Acid Based Methotrexate Intravitreal Micro-Implants to Treat Primary Intraocular Lymphoma: An In Vitro Study

Primary intraocular lymphoma (PIOL) is an uncommon but clinically and pathologically distinct form of non-Hodgkin’s lymphoma. It provides a therapeutic challenge because of its diverse clinical presentations and variable clinical course. Currently available treatments for PIOL include intravenous multiple drug chemotherapy, external beam radiation therapy, and intravitreal methotrexate (MTX) injection. Each intravitreal injection of MTX is associated with potentially toxic peaks and subtherapeutic troughs of intraocular MTX concentration. Repetitive injections are required to maintain therapeutic levels of MTX in the eye. A sustained release drug delivery system is desired for optimized therapeutic release of MTX over a period of 1 month. Chitosan (CS) and polylactic acid (PLA) based micro-implants are fabricated for different MTX loadings (10%, 25%, and 40% w/w). First, CS and MTX mixtures are prepared for different drug loadings, and lyophilized in Tygon tubing to obtain CS-MTX fibers. The fibers are then cut into desired micro-implant lengths and dip coated in PLA for a hydrophobic surface coating. The micro-implant is characterized using optical microscopy, scanning electron microscopy (SEM), time of flight-secondary ion mass spectroscopy (ToF-SIMS), and differential scanning calorimetry (DSC) techniques. The release rate studies are carried out using a UV-visible spectrophotometer. The total release durations for 10%, 25%, and 40% w/w uncoated CS-MTX micro-implants are only 19, 29, and 32 h, respectively. However, the therapeutic release durations for 10%, 25%, and 40% w/w PLA coated CS-MTX micro-implants significantly improved to 58, 74, and 66 days, respectively. Thus, the PLA coated CS-MTX micro-implants are able to administer therapeutic release of MTX for more than 50 days. The release kinetics of MTX from the coated micro-implants is explained by (a) the Korsmeyer–Peppas and zero order model fit (R² ~ 0.9) of the first 60% of the drug release, which indicates the swelling of polymer and initial burst release of the drug; and (b) the first order and Higuchi model fit (R² ~ 0.9) from the tenth day to the end of drug release, implying MTX release in the therapeutic window depends on its concentration and follows diffusion kinetics. The PLA coated CS-MTX micro-implants are able to administer therapeutic release of MTX for a period of more than 1 month. The proposed methodology could be used for improved treatment of PIOL. [DOI: 10.1115/1.4026176]

Keywords: primary intraocular lymphoma, methotrexate, chitosan, polylactic acid, sustained release, micro-implant

1 Introduction

In this section we introduce PIOL and its present treatment methods. A background of the present intravitreal sustained release devices is provided and the rationale behind this study is subsequently mentioned.

1.1 Primary Intraocular Lymphoma. PIOL, a non-Hodgkin’s lymphoma of B-cell origin, is a subset of primary central nervous system lymphoma (PCNSL) [1–5]. It has been reported that PIOL occurs in approximately 25% of PCNSL patients [6,7]. PIOL refers to the nonmetastatic malignant lymphoid neoplasia that develops primarily inside the eye [8]. It is distinguished from intravitreal malignant lymphoid tumors and infiltrates that develop metastatically from systemic non-Hodgkin’s lymphoma [8]. Approximately 100 new cases of PIOL were diagnosed in the United States between 2000 and 2005 [1,9,10]. Primary vitreoretinal lymphoma, which accounts for 85% of PIOL cases in the United States, is characterized by accumulation of pale lymphoid cells in the vitreous.
development of tiny dot like extensive geographic pale subretinal infiltrates, and frequent bilateral and common association with independent primary lymphoid infiltrates in the brain (primary central nervous system lymphoma) [8].

1.2 Present PIOL Treatment Methods. Current treatment consists of (a) systemic multidrug chemotherapy [4,5], a treatment in which only a limited quantity of the intravenously administered drugs crosses the blood-retinal barrier and reaches the eye; (b) fractionated external beam radiation therapy, a treatment which has been proven to be fruitful in remission of tumors but can be associated with radiation-induced superficial punctuate keratopathy, keratoconjunctivitis sicca, cataract, and radiation induced cataract (in phakic eyes) [3–5,11]; and (c) multiple intravitreal injections of methotrexate [3–5].

1.3 Intravitreal MTX Injection. At present, one of the accepted treatments of PIOL is repeated intravitreal injections of MTX [2,12–14]. Although MTX injection has proven to be an effective treatment for PIOL, Velez et al. [2] reported that a 400 μg intravitreal dose of MTX provided a therapeutic level of the drug (> 0.5 μM) for only about 48–72 h in nonvitrectomized rabbit eyes. MTX intravitreal injection has a short half-life (t1/2) of 14.3 h [1]. Because of this, repetitive administration of MTX intravitreal is required to maintain cytotoxic concentrations of the drug over a sufficiently long duration to eradicate PIOL.

Frenkel et al. [13] reported that all 26 patients (44 eyes) with PIOL developed conjunctival hyperaemia and some form of keratopathy when treated with intravitreal MTX injection. The nature of keratopathy noticed after the third injection varied from diffuse punctate keratopathy to severe epitheliopathy. These complications subsided when the frequency of injections were reduced to monthly injections from weekly injections.

In another study by Smith et al. [14], the efficacy and safety of intravitreal MTX to treat PCNSL involving the eye was conducted. The complications, which occurred during treatment and follow up, include cataract (73% of 26 eyes), corneal epitheliopathy (58% of 26 eyes), maculopathy (42% of 26 eyes), vitreous hemorrhage (8% of 26 eyes), optic atrophy (4% of 26 eyes), and sterile endophthalmitis (4% of 26 eyes). However, in the review of Rajagopal and Harbour [3], it is mentioned that these complications are reduced in recent times as more experience with the MTX injection is gathered.

Shortcomings of currently advocated protocols for intravitreal MTX injections for PIOL include (a) the need for multiple injections at relatively shorter intervals; (b) likelihood of subtherapeutic intravitreal concentration of MTX between injections; and (c) increased risk of avoidable ocular complications with multiple injections.

1.4 Sustained Release MTX Delivery System. There is a need for a sustained release drug delivery system (micro-implant) for maintaining the therapeutic dosage of MTX over a prolonged time period while avoiding unwanted systemic and local toxicity. In the most recent study of our group by Palakurthi et al. [1], the retinal permeability of MTX in rabbit and human eyes was evaluated. The pharmacokinetics of MTX in the human eye following MTX intravitreal injection and micro-implant were evaluated for better treatment of PIOL. It was suggested that the ideal MTX intravitreal micro-implant would need to administer MTX within the therapeutic window of 0.2–2.0 μg/day for a period of a month or more for improved treatment of PIOL. Sustained release of MTX is also preferred as its time effect (sensitivity of the cells to MTX increase with time) is reported to be greater than its dose effect [15].

The challenges for administering hydrophilic drugs such as MTX in the vitreous environment include preventing rapid release of MTX from the micro-implant matrix and the choice of polymer matrix, which blends well with MTX. The key factors for choosing a polymer matrix for administering hydrophilic drugs are its nature (hydrophilic or hydrophobic nature), biocompatibility, biodegradability, and their mutual compatibility.

1.5 PLA and PLGA Based Intravitreal Micro-Implants. Various intravitreal drug delivery systems have been based on hydrophobic biodegradable polymers like lactic and glycolic acid-based matrices such as poly(lactic acid, polyglycolic acid (PGA), their copolymers, and derivatives poly(lactic-co-glycolic) acid (PLGA) [16–18]. The degraded products of these polymers are metabolized to produce carbon dioxide and water. The issue with existing polymer matrices (PLA, PGA, and PLGA) is that they are hydrophobic in nature and do not blend well with hydrophilic drugs like MTX. Another disadvantage of these hydrophobic (PLA, PGA, and PLGA) matrices is that they degrade very slowly even after the drug has been released, causing local toxicity [19].

1.6 Chitosan as a Polymer Matrix for Intravitreal Micro-Implant. CS, which is known for biocompatible, biodegradable, and nontoxic material, is a potential candidate for drug delivery systems inside the eye [20,21]. CS has been considered as a Generally Recognized as Safe material by the US FDA and is also listed as a food additive in countries like Finland, Italy, and Japan [22]. CS is a copolymer of N-acetylgalactosamine and glucosamine, which is fully or partially N-deacetylated (DS). Derivative of the natural polymer Chitin. CS nanoparticles containing 5-Fluorouracil showed no signs of inflammation or irritation when tested in rabbit eyes [23]. In another study by Yang et al. [24], CS was investigated as a tamponade material to treat proliferative vitreoretinopathy in rabbit eyes. CS showed no significant effect on the histology of the eye and did not affect the intraocular pressure or exhibit any severe inflammatory response, which makes CS a promising choice for intravitreal applications.

The degradation of CS is primarily related to the DA%, and it is also degraded in the presence of hydrolytic enzymes like lysozyme. The presence of lysozyme in the vitreous has been reported by Stainer et al. [25]. The degradation of CS by lysozyme leads to the formation of amino sugars, which are readily metabolized without any toxicity development. CS has also been used as a drug delivery vehicle for MTX in many formulations because of its similar hydrophilic nature [15,26–29].

1.7 CS and PLA Based Intravitreal MTX Micro-Implant. In this study, a CS and PLA based MTX sustained release intravitreal micro-implant is fabricated to treat PIOL using minimally invasive surgical methods. MTX is expected to be released rapidly because of the similar hydrophilic nature of both CS and MTX. The CS-MTX micro-implant is, therefore, coated with a hydrophilic coating of PLA for sustained release of MTX. The hypothesis of this study is that the PLA coated CS-MTX intravitreal micro-implant will administer MTX within the therapeutic window for a period of over one month, thereby enhancing the efficacy of the drug in retarding the progression of PIOL. In this study, we have reported the design, fabrication, and the in vitro characterization of the CS and PLA based MTX micro-implant in a simulated vitreous environment.

2 Materials and Methods

In this section, we first describe the fabrication protocol of the micro-implants. Thereafter, the characterization techniques and the method to determine the in vitro release rate of MTX from the micro-implant are described.

2.1 Fabrication of the Micro-Implant. MTX (MP Biomedical) is mixed with low molecular weight CS (M.W 50,000–190,000 and DA% ≥ 75%) (Sigma Aldrich) in dilute HCl to make different mixtures of 10%, 25%, and 40% w/w drug loadings. These mixtures are then injected into Tygon™ tubing (1/16 in. i.d.). The tubes containing the mixture are lyophilized at a
temperature below ~40°C and pressure below 1200 mTorr for 2 h (Millrock BT48A, Millrock Technology) to obtain CS-MTX fibers. The CS-MTX fibers extracted from the Tygon® tubing are cut into desired micro-implant lengths using a surgical knife under an optical microscope to ensure accurate dimensions of the micro-implant. DL-PLA (M.W. 150,000) (Lactel biodegradable polymers) is mixed in Dichloromethane (Fisher Sci.) to synthesize a 40 mg/ml coating solution. The CS-MTX micro-implants are then dip coated in the PLA coating solution for a hydrophobic surface coating. The dip coating protocol is carried out on both longitudinal directions of the micro-implant to ensure uniform coating on the surface and on two ends of the micro-implant. Each micro-implant is dipped in the PLA solution for 5 s and dried at room temperature for 2 min. This process is carried out three times in each direction, longitudinally. Subsequently, the micro-implants are dried overnight at room temperature in dark conditions. After initial drying, the micro-implants are vacuum dried overnight at 45°C to evaporate the dichloromethane from the micro-implant.

2.2 Micro-Implant Characterization. In this section, the characterization techniques employed to analyze the micro-implant’s material properties are discussed. The characterization techniques that are adopted here are: (a) optical microscopy, (b) scanning electron microscopy, (c) time of flight-secondary ion mass spectroscopy, and (d) differential scanning calorimetry.

Dimensions and Morphology. Optical microscopy (Keyence Digital Microscope, VHX-600) is used to assess the micro-implant’s dimensions and appearance. Scanning electron microscopy (FEI XL 30-FEG, FEI) is used to assess the microstructure and morphology using an accelerating voltage of 15 KV. The micro-implant samples are sputter coated prior to the SEM analysis in argon plasma using an Au-Pd target for 1 min to cause them to be conductive.

Hydrophobic Modification of the Coated Micro-Implant Surface. Time of flight-secondary ion mass spectroscopy is used to assess the hydrophobic modification of the micro-implant’s surface. ToF-SIMS is performed using a ToF-SIMS IV instrument (IONTOF Inc.). Secondary ions are produced from a Ga+ primary ion source at 15 KV accelerating voltage and 1.5 pA current raster over a 200 μm by 200 μm area of the sample. The secondary ions produced are analyzed in high-current bunched mode with analyzer energy of 2 KV. The ion peaks are assigned using Surface-Lab 6 software (IONTOF Inc.). Differential scanning calorimetry is used to measure thermal properties of the micro-implants at physiological temperature ~38°C. DSC is performed at the heating rate of 10°C/min. (DSC6200, Seiko Instruments Inc.).

2.3 Release Rate Studies. The micro-implants are kept in vials containing 5 ml of phosphate buffered saline (PBS; pH 7.4). Each micro-implant weighs ~1 mg. The micro-implants containing 40% w/w MTX contains ~400 μg of MTX, 25% w/w MTX contains ~250 μg of MTX, and 10% w/w MTX contains ~100 μg of MTX. The vials are slowly stirred in a water bath maintained at 38°C. One milliliter of release media sample (PBS) containing MTX is taken out at predetermined time intervals. One milliliter of fresh PBS is added to maintain sink conditions. The concentration of MTX present in 1 ml of release media sample is assayed using an UV-visible spectrophotometer (Cary 50-Bio UV-Vis Spectrophotometer, Varian) at the characteristic MTX wavelengths (258, 302, and 372 nm) [30]. The calibration of MTX absorbance in the UV-visible spectrophotometer is done using MTX standard concentrations in PBS. A calibration curve is derived from the absorbance readings obtained from the MTX standards, and the molar absorbitivity of MTX is determined.

3 Results
The material characterization is first discussed, followed by the release rate study of the micro-implants. Evaluation of drug release is addressed using the characteristic drug release model fitting technique. Results are presented as mean ± standard deviation unless otherwise mentioned.

3.1 Micro-Implant Characterization. Optical microscopy and SEM techniques are utilized to assess the micro-implant appearance, dimensions, and microstructure morphology. Hydrophobicity of PLA coating is evaluated using ToF-SIMS and DSC studies.

Dimensions and Appearance. For micro-implant samples (n = 9; three samples and three readings per sample), the dimensions of the uncoated type and the PLA coated type are measured using an optical microscope. The length and cross-sectional diameter of the uncoated micro-implant are 4 ± 0.04 mm and 0.7 ± 0.03 mm, respectively. The length and cross-sectional diameter of the PLA coated micro-implant are 4.2 ± 0.03 mm and 0.9 ± 0.04 mm, respectively.

The optical microscopy images of surfaces of the PLA coated and the uncoated micro-implants are shown in Figs. 1(a) and 1(b), respectively. Comparing Figs. 1(a) and 1(b), it can be seen that the surface of the PLA coated micro-implant is relatively smoother and more uniform compared to that of the uncoated micro-implant. The optical microscopy images of the cross-sectional view of the PL coated and uncoated micro-implants are shown in Figs. 1(c) and 1(d), respectively. A 100 μm PLA coating can be noticed in the PLA coated micro-implant in Fig. 1(c), which is absent in the uncoated micro-implant in Fig. 1(d). The micro-implants are a yellow color signifying uniform distribution of MTX throughout the CS polymer matrix. Thus, optical microscopy images reveal uniform coating of PLA on the surface of the PLA coated micro-implants.

Morphology and Microstructure. SEM images showing the longitudinal view of the surface of the uncoated and PLA coated micro-implants are shown in Fig. 2. From the SEM images, the porous and irregular CS surface of the uncoated micro-implant can be seen. By coating the micro-implants with PLA, the porous surface gets filled up with PLA and results in a smoother nonporous surface as shown in the SEM images of the coated micro-implant. SEM images of the cross section of the uncoated and the PLA coated micro-implant are shown in Fig. 3. The cross-sectional diameter of the uncoated (0.706 mm) and the PLA coated (0.878) micro-implants are shown in Figs. 3(a) and 3(d), respectively. They are consistent (~2.4% difference) with the results of optical microscopy as shown in Fig. 1. In Fig. 3(b)
and 3(c), the porous internal CS matrix of the uncoated micro-implant is shown. In Figs. 3(d) and 3(e), it is visible that the PLA deposition takes place in the internal voids of the coated micro-implant resulting in a denser internal matrix with reduced porosity. The internal deposition of PLA also plays an important role in the reduction of swelling of the CS matrix and restricting the MTX release.

SEM images showing the longitudinal view of the surface of the uncoated micro-implants with an increasing drug loading are shown in Fig. 4. From the SEM images, it can be concluded that with an increase in drug loading, there is an increase in the quantity of loosely bound drug particles on the surface of the micro-implant. SEM images showing the cross section of the uncoated micro-implants with an increasing drug loading are shown in Fig. 5. It can be seen that the voids of the CS matrix get more filled up with the drug particles with an increase in drug loading.

Time of Flight-Secondary Ion Mass Spectroscopy. ToF-SIMS is used to evaluate the surface chemistry of the coated micro-implants. ToF-SIMS spectra of PLA (MW 150,000), PLA coated 40% CS-MTX micro-implant surface and uncoated 40% CS-MTX micro-implant surface is reported in Fig. 6. Figure 6 shows the characteristic peaks of pure PLA mass fragments (43 [C₂H₃O⁺], 56 [C₃H₄O⁺], 71 [C₃H₅O₂⁺], 73 [C₄H₄O₂⁺], 127 [C₆H₇O₃⁺], 128 [C₆H₈O₃⁺], 129 [C₆H₉O₃⁺], 143 [C₆H₇O₄⁺], and 145 [C₆H₉O₄⁺]) match with that of the PLA coated micro-implant with similar intensities. The characteristic peaks of pure PLA mass fragments and PLA coated micro-implant match with previous study [31].

The spectrum of the uncoated micro-implants does not show the same characteristic peaks (56 [C₃H₄O⁺], 71 [C₃H₅O₂⁺], 73 [C₄H₄O₂⁺], 127 [C₆H₇O₃⁺], 128 [C₆H₈O₃⁺], 129 [C₆H₉O₃⁺], 143 [C₆H₇O₄⁺], and 145 [C₆H₉O₄⁺]) as that of pure PLA mass fragments and PLA coated micro-implant. However, in the spectrum of uncoated micro-implants, there is a match with the spectra of pure PLA mass fragments and PLA coated micro-implant at mass fragment 43 [C₂H₃O⁺], but with a much higher relative intensity than the spectra of the pure PLA mass fragments and PLA coated.
micro-implant. The higher relative intensity from the uncoated micro-implants is probably due to the mass fragment 43 \([\text{C}_2\text{H}_3\text{O}^+]\) being generated from the CS and MTX present on the surface of the uncoated micro-implants. Therefore, we can qualitatively confirm the successful coating of PLA on the surface of the coated micro-implant based on the spectra of Fig. 6.

Glass Transition Temperature \((T_g)\). The purpose of the DSC study is to assess the stability of the constituents of the coated micro-implant in physiological temperature. The DSC study of the PLA coating is done because the \(T_g\) of PLA could be close to physiological temperature depending on the molecular weight. If the coating polymer PLA undergoes glass transition in the physiological conditions, then the PLA coating would soften, affecting the structural properties of the micro-implant, thus, leading to faster drug release [32]. A DSC plot of one of the PLA coated micro-implants is shown in Fig. 7. \(T_g\) is the point where the slope of the endotherm changes. The \(T_g\) values of the PLA coating obtained for the 10%, 25%, and 40% PLA coated micro-implants \((n = 4)\) are 50.2 ± 1.3, 51.3 ± 1.1, and 51.9 ± 2.8°C, respectively. The \(T_g\) values obtained in this study are consistent with previous studies [33]. The DSC study confirms that the PLA coating will not experience glass transition or soften in the physiological temperature \((~38°\text{C})\) inside the intraocular domain. The DSC study of the uncoated CS-MTX micro-implants are not conducted because the decomposition temperature of MTX and \(T_g\) of CS is much higher than the physiological temperature and, thus, would not undergo decomposition and glass transition, respectively. Chadha et al. [34] reported that MTX has no melting transition and decomposes around ~245°C, and the \(T_g\) of CS is reported to be around 140–150°C by Dong et al. [35].

3.2 Release Rate Studies. The details of the drug release rate studies are reported in this section. The calibration of MTX is described followed by the discussion of MTX release rate profiles from the coated and uncoated micro-implants. The drug release
rate data is fitted to pharmacokinetic models to interpret the drug diffusion kinetics.

*Calibration* of MTX. Figure 8 describes the calibration procedure for MTX. Characteristic MTX spectra for different concentrations are shown in Fig. 8(a). The characteristic MTX peaks are at 258 nm, 302 nm, and 372 nm, and the calibration curves for the 258 nm peak, 302 nm peak, and 372 nm peak are shown in Figs. 8(b), 8(c), and 8(d), respectively. The calibration
curve of each peak is obtained by linear regression fitting of the UV-absorbance values for different MTX concentrations. The linear regression is based on terms of correlation coefficient ($R^2$) values. The 258 nm peak of the MTX spectra is used for the release rate experiments, as it provides a sharper deflection compared to the others.

Release Rate Profiles. Release rate profiles of MTX from the uncoated micro-implants are shown in Fig. 9(a). Figure 9(b)
shows release rate profiles of MTX from the uncoated micro-implants in the therapeutic window (0.2–2.0 μg/day). Cumulative release profiles of MTX from the uncoated micro-implants are shown in Fig. 9(c). Release rate profiles of MTX from the PLA coated micro-implants are shown in Fig. 10(a). Figure 10(b) shows release rate profiles of MTX from the PLA-coated micro-implants in the therapeutic window. Cumulative release profiles of MTX from the PLA-coated micro-implants are shown in Fig. 10(c). The mean profile of each type of drug loading is plotted along with the standard error. The summary of release rate characteristics for the uncoated and coated micro-implants for different drug loadings is provided in Tables 1 and 2, respectively.

Release Rate Study of the PLA-Coated Micro-Implants. The mean release rate of the PLA coated CS-MTX micro-implants is $1.8 \pm 0.4 \mu g/day$, $3.2 \pm 0.1 \mu g/day$, and $6.6 \pm 0.3 \mu g/day$ for the 10%, 25%, and 40% w/w drug loadings, respectively, as mentioned in Table 2. The total release duration for 10%, 25%, and 40% w/w PLA coated CS-MTX micro-implants are 58, 74, and 66 days, respectively.

For the 10% coated MTX micro-implant, there is an initial burst release on the fourth day (Fig. 10(a)), then a small secondary burst between the 10th and 20th day and a final burst near the 50th day (Fig. 10(b)). The 10% w/w coated micro-implants exhibit a release rate in the therapeutic window from the 10th day onward up to the 58th day as shown in Fig. 10(b).

For the 25% coated MTX micro-implant, an initial burst release is seen on the third day (Fig. 10(a)). Although there is no prominent secondary burst, there are a couple of bursts between the 20th and 40th day, followed by a major burst between the 40th and 50th day before a final burst around the 70th day (Fig. 10(b)). The 25% w/w coated micro-implants show a release rate in the therapeutic window from the 18th day onward up to the 74th day.

In the case of the 40% coated micro-implant, a significant initial burst release is noticed on the third day (Fig. 10(a)), and then a secondary burst is observed between the 30th and 40th day (Fig. 10(b)). There is no prominent final burst noticed in the release profile of the 40% coated micro-implant. The 40% w/w micro-
implants maintain the release rate in the therapeutic window from the 14th day onward up to the 66th day. Thus, the drug administration can be achieved in the desired therapeutic window for all types of coated micro-implants (10%, 25%, and 40% w/w MTX) for a period of 8–10 weeks.

3.3 Release Kinetics Analysis. Drug release data of all drug loadings of the coated micro-implants were fitted to a zero order equation, first order equation, Higuchi model, and Korsmeyer–Peppas model in order to analyze the mechanism of drug release and diffusion kinetics. The fitting of each model is evaluated based on correlation coefficient (R²) values. The R² values of each model fitting are reported in Table 3.

Korsmeyer–Peppas Model. The Korsmeyer–Peppas model provides an insight into the type of drug release mechanism taking place from swellable polymeric devices [36]. The “n” of the Korsmeyer–Peppas model is estimated from the linear regression fit of the logarithmic release rate data. n > 1 suggests super case II transport relaxational release and also indicates zero order kinetics [37]. The generic equation for the Korsmeyer–Peppas model is as follows:

\[ F = \frac{M_t}{M_0} = K_{tp}t^n \]  

where \( M_0 \) is the initial amount of drug, \( M_t \) is the amount of drug released in time \( t \), \( K_{tp} \) is the Korsmeyer–Peppas release constant, and \( n \) is estimated from linear regression of \( \log F \) versus \( \log t \). \( n \) suggests the type of diffusion. We obtained consistent R² values ~0.99 and “n” values ~1.2 by fitting our first 60% of drug release rate data to the Korsmeyer–Peppas model (Fig. 11(a)), which suggests that the first 60% of the drug release is influenced by swelling and relaxation phenomena of the polymer matrix. The 60% of the drug release takes place in the first 8 days out of the total drug release duration. It may be noted that if we fit the whole range of drug release data to the Korsmeyer Peppas model, then the R² values reduce to 0.82–0.89 and the “n” values vary between 0.62 and 0.73.

Zero Order Equation. The zero order release equation represents a process when the release rate of the drug is independent of the concentration of the drug in the system, and the generic equation for the zero order equation is as follows:

\[ M_t = M_0 + K_0t \]  

where \( M_0 \) is the initial amount of drug, \( M_t \) is the amount of drug released in time \( t \), and \( K_0 \) is the zero order release constant. The range of R² values is between 0.02 and 0.49 when the whole range of drug release data is fitted to the zero order equation. R² values improve to ~0.9 when the initial 60% drug release data is fitted to the zero order equation (Table 3). Therefore, the drug release from the coated micro-implants follows zero order equation for the first 60% of the drug release.

First Order Equation. The first order release equation represents a system where the release rate of the drug is dependent on the concentration of the drug in the system and the generic equation for the first order equation is as follows:

\[ \log M_t = \log M_0 + K_1(t/2.303) \]
where $M_0$ is the initial amount of drug, $M_t$ is the amount of drug released in time $t$, and $K_1$ is the first order release constant. The $R^2$ values are $\sim 0.9$ when the whole range of drug release data is fitted to the first order equation. However, by fitting the drug release data to the first order equation from the tenth day to the end of the drug release ($\sim 60$ days) provides the $R^2$ values of 0.83, 0.94, and 0.98 for 10%, 25%, and 40% coated micro-implants, respectively (Fig. 11(b)). This implies the drug release rate from the coated micro-implants in the therapeutic window, after the tenth day (post-initial burst), is primarily governed by first order kinetics and is dependent on the concentration of the drug in the coated micro-implants. The half-life ($t_{1/2}$) of MTX release from an intravitreal injection is reported to be $\sim 14.3$ h [1] whereas the $t_{1/2}$ of MTX release from the coated micro-implants for the whole range of data is $\sim 240$ h (10 days) (Table 3).

**Higuchi Model.** The Higuchi release equation [38] predicts that the drug release is caused primarily by diffusion mechanism, and the generic equation for the Higuchi model is as follows:

$$M_t = K_{H} t^{1/2}$$

where $M_t$ is the amount of drug released in time $t$, and $K_H$ is the Higuchi constant. The range of $R^2$ values is between 0.7 and 0.91 when the whole range of drug release data is fitted to the Higuchi model. However, fitting the drug release data to the Higuchi model from the tenth day to the end of drug release ($\sim 60$ days) provides the $R^2$ values of 0.99, 0.94, and 0.93 for 10%, 25%, and 40% coated micro-implants, respectively (Table 3). This implies the drug release from the coated micro-implants, after the tenth day (post-initial burst), is primarily governed by diffusion kinetics.

Therefore, it can be concluded that the drug release mechanism primarily follows (i) the Korsmeyer–Peppas model and zero order model for the first $\sim 8$ days where the initial burst takes place and 60% of the drug is released due to swelling of the polymer matrix, and (ii) the first order and Higuchi model from the tenth day until the end of drug release signifying the drug release mechanism being concentration dependent and is primarily caused by diffusion mechanism.

4 Discussion

In this study, the in vitro analysis of a unique CS and PLA based MTX intravitreal micro-implant is reported. It is expected
that such a micro-implant will improve the treatment of PIOL in the future. Each of the PLA coated micro-implants has exhibited sustained release of MTX within the therapeutic window for a period of more than 50 days. This micro-implant can be administered using minimally invasive surgical procedures. The existing treatment method for PIOL, existing intravitreal devices, the influence of PLA coating and drug loading on the sustained MTX release, toxicity from the micro-implants, the assumptions, and the limitations of this study are discussed in detail in the following sections.

Table 1 Summary of release rate characteristics of uncoated CS-MTX micro-implants (n = 3)

<table>
<thead>
<tr>
<th>Implant drug loading (w/w)</th>
<th>Mean release rate ± standard error (µg/day)</th>
<th>Total release duration (hours)</th>
<th>Time of peak release rate (hours)</th>
<th>Peak release rate ± standard error (µg/day)</th>
<th>Start time of release rate within therapeutic limits (hour)</th>
<th>Drug released before therapeutic release rate starts ± standard error (%)</th>
<th>End time of release rate within therapeutic limits (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>88.9 ± 4.8</td>
<td>19</td>
<td>0.5</td>
<td>1414 ± 66</td>
<td>~12</td>
<td>99.2 ± 0.2</td>
<td>~19</td>
</tr>
<tr>
<td>25</td>
<td>188.0 ± 7.9</td>
<td>29</td>
<td>0.5</td>
<td>4314 ± 221</td>
<td>~22</td>
<td>98.8 ± 0.3</td>
<td>~29</td>
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<tr>
<td>40</td>
<td>372.6 ± 7.5</td>
<td>32</td>
<td>0.5</td>
<td>5041 ± 311</td>
<td>~25</td>
<td>98.7 ± 0.4</td>
<td>~32</td>
</tr>
</tbody>
</table>

Fig. 10 (a) Release rate curves from PLA coated Chitosan-MTX micro-implants with different drug loadings. (b) Release rate curves from PLA coated Chitosan-MTX micro-implants with different drug loadings in the therapeutic window (shaded region). (c) Cumulative drug release profile from PLA coated Chitosan-MTX micro-implants.
4.1 Existing Treatment Method for PIOL. PIOL provides a therapeutic challenge. The present protocol of intravitreal injections of MTX to treat PIOL has several drawbacks. The MTX intravitreal injection has a short half-life ($t_{1/2}$) of 14.3 h and, therefore, leads to an uncertainty in the effective duration of therapeutic concentration. Repetitive administrations are required to maintain therapeutic dosage over a period of time, which can also be associated with ocular complications as mentioned before. A sustained release drug delivery system (micro-implant) that maintains the therapeutic dosage of MTX over a prolonged time period may prove to be a more effective and safer treatment method than multiple intravitreal injections.

4.2 Existing Intravitreal Devices. At present, there are no devices for sustained release of MTX or other hydrophilic drugs in the intravitreal domain. However, the majority of the intravitreal micro-implants are based on PLA, PLGA, or similar hydrophobic materials. The existing sustained release intravitreal micro-implants, which are presently FDA approved, are Retisert (Bausch & Lomb) and Ozurdex (Allergan). Retisert is a silicone-based disk shaped nonbiodegradable micro-implant that administers corticosteroid fluocinolone acetonide to treat uveitis and diabetes macular edema (DME) over a period of 30 months. Ozurdex is a pellet shaped PLGA based micro-implant that administers dexamethasone to treat uveitis and DME over a period of 6 months. In all of these devices, the drug administered is hydrophobic in nature, which binds well with a hydrophobic polymer matrix reservoir made of PLGA or silicones. Also, the drug being hydrophobic in nature, it exhibits a sustained release due to its inherent property of limited diffusivity in the vitreous medium of the eye.

In an animal study by our group, toxicity of PLA based MTX microneedle implants were evaluated in rabbit eyes [8]. The micro-implants were well tolerated without any sign of inflammation but degraded rapidly in the in vivo conditions.

4.3 Influence of PLA Coating. PLA coating plays an important role in sustained release administration of MTX and also influences the initial burst release or the peak release rate of MTX. PLA coating imparts hydrophobicity to the surface of the

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Table 2 Summary of release rate characteristics of PLA coated CS MTX micro-implants (n = 3)

<table>
<thead>
<tr>
<th>Implant drug loading (w/w)</th>
<th>Mean release rate ± standard error (µg/day)</th>
<th>Total release duration (days)</th>
<th>Time of peak release rate (days)</th>
<th>Peak release rate ± standard error (µg/day)</th>
<th>Start time of release rate within therapeutic limits (day)</th>
<th>Drug released before therapeutic release rate starts ± standard error (%)</th>
<th>End time of release rate within therapeutic limits (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1.8 ± 0.4</td>
<td>58</td>
<td>4</td>
<td>11.2 ± 6.0</td>
<td>10</td>
<td>62.7 ± 5.3</td>
<td>~58</td>
</tr>
<tr>
<td>25%</td>
<td>3.2 ± 0.1</td>
<td>74</td>
<td>4</td>
<td>21.6 ± 4.3</td>
<td>18</td>
<td>82.3 ± 1.5</td>
<td>~74</td>
</tr>
<tr>
<td>40%</td>
<td>6.6 ± 0.3</td>
<td>66</td>
<td>3</td>
<td>60.4 ± 14.1</td>
<td>14</td>
<td>88.5 ± 1.8</td>
<td>~66</td>
</tr>
</tbody>
</table>

$^a$The half-life ($t_{1/2}$) obtained from the first order kinetics for the whole range of drug release is ~10 days.

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Table 3 In Vitro release kinetic values of MTX from PLA coated CS-MTX micro-implants of different drug loadings

<table>
<thead>
<tr>
<th>MTX loading w/w % (N = 3)</th>
<th>Korsmeyer–Peppas</th>
<th>Zero Order</th>
<th>First Order$^a$</th>
<th>Higuchi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$n$</td>
<td>$R^2$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>10%</td>
<td>0.99</td>
<td>1.22</td>
<td>0.98</td>
<td>0.83</td>
</tr>
<tr>
<td>25%</td>
<td>0.99</td>
<td>1.24</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>40%</td>
<td>0.99</td>
<td>1.24</td>
<td>0.99</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Fig. 11 Fitting of MTX release from the coated micro-implants using (a) Korsmeyer–Peppas equation (for the first 60% of drug release) and (b) first order equation (from the tenth day to the end of therapeutic drug release)
The PLA coating prevents the entry of PBS into the micro-implant. The PLA coating reduces the initial burst of the drug from the micro-implants and influences the sustained release of MTX.

4.4 Influence of Drug Loading. The peak release rate and release duration are governed by the drug loading. The peak release rate of the 40% w/w micro-implants is the highest and that of the 10% w/w micro-implants is the lowest in both coated and uncoated micro-implants (Tables 1 and 2). The high peak release rate indicates an initial burst, which is directly related to the quantity of loosely bound MTX present in the micro-implant matrix, which again depends on the drug loading.

As mentioned in a review article by Fu and Kao [39], a burst is defined as an enhanced dissolution of drug from the polymer matrix, which is due to the rapid release of the surface-associated drug molecules. In this study, a burst is qualitatively characterized by a “bell curve” shaped profile in the release rate plot indicating enhanced rate of drug release (Figs. 10(a) and 10(b)). The initial burst observed in the first ~8 days (Fig. 10(a)) is proportional to the loosely bound drug present on the surface of the coated micro-implant, which is again proportional to the drug loading. Therefore, higher drug loading results in a higher burst release. The increasing presence of loosely bound drug molecules on the surface with increasing drug loading is shown in the SEM images (Figs. 4 and 5).

Bursts are also caused due to polymer swelling, degradation, and erosion of the polymer matrix. Swelling is an important factor, which influences the initial burst, whereas the subsequent bursts are usually due to the degradation and erosion of the polymer matrix as mentioned in other previous studies [18] and our previous study [1]. The initial burst from all three types of coated micro-implants can be explained using the Korsmeyer–Peppas model, which considers the influence of the polymer matrix and the drug loading.

The coated 10%, 25%, and 40% w/w micro-implants produce therapeutic release rate administration until 58, 74, and 66 days, respectively. Since the coated 40% w/w micro-implant contains more quantity of MTX, it is expected that the 40% w/w micro-implant will provide longer drug release duration than the 25% w/w coated micro-implant. However, the 40% coated micro-implant provides shorter release duration of 66 days when compared to 74 days of the 25% coated micro-implant. The optimized polymer to drug (CS:MTX) ratio is expected to influence the maximum duration of the therapeutic release rate. The duration of the release depends on (i) drug loading and (ii) the polymer to drug ratio. Drug loading is responsible for the total amount of the drug, which can be released. Polymer to drug ratio determines how much polymer binding site is available to bind the drug. Higher drug loading results in low polymer to drug ratio, which results in less binding sites and a more loosely bound drug. This leads to a higher initial burst as seen in 40% w/w micro-implant. A higher polymer to drug ratio implies relatively more polymer binding site available to bind the drug as in 10% w/w micro-implant.

Comparing the 25% w/w and 10% w/w micro-implant, the 25% contains higher drug loading and a lower polymer to drug ratio than the 10%. Although the polymer to drug ratio in the 10% (9:1) is higher than the 25% (3:1), the drug loading in the 10% is not sufficient to provide a longer duration of therapeutic release. This is because the 10% micro-implant has relatively more available binding sites and lower availability of the drug. On the contrary, in the 40% micro-implant, the binding sites are all used up, thus, leading to more unbound drug. This results in most loosely bound MTX in the micro-implant’s CS-MTX matrix in the 40% micro-implant as compared to that in 25% micro-implant and 10% micro-implant. As a result, the 40% coated micro-implants show the highest mean release rate and the highest peak release rate, which eventually reduces the overall drug release duration compared to the 25% coated micro-implants. Thus, 25% MTX loading has the optimal polymer to drug ratio or binding site to available drug ratio in order to obtain maximum therapeutic release duration.

4.5 Toxicity From the Micro-Implants. Each uncoated micro-implant weighs ~1 mg. The weight of the MTX (drug) present in the 10%, 25%, and 40% w/w micro-implant is ~100, 250, and 400 μg, respectively. It is reported that an intravitreal MTX injection containing 400 μg MTX provides a therapeutic level of the drug without toxicity for about 48–72 h in both a preclinical setting [2] and in a clinical setting [11,40]. In our study, the micro-implant that has the maximum drug loading (400 μg of MTX) is the 40% w/w MTX, which is comparable to the dosage of the intravitreal MTX injection (400 μg of MTX). This 400 μg of MTX is released over a longer duration (>50 days) from the micro-implant compared to the injection. Therefore, it is expected that the micro-implants containing 40% w/w MTX (400 μg of MTX) in 25% w/w MTX (250 μg of MTX) and 10% w/w MTX (100 μg of MTX) would not cause any toxicity despite the burst release in the initial time points.

In our previous study [1,7] as well as other studies [2], it is mentioned that a release rate of 0.2–2.0 μg/day or a concentration of 0.1–1 μM would be considered as therapeutic. The micro-implants fabricated in this study are able to administer MTX within the therapeutic window (0.2–2.0 μg/day) for a period of more than 50 days and, therefore, should not cause any toxicity.

4.6 Assumptions of the Study. The hydrophilic PLA coating on the surface of the coated micro-implants plays an important role in sustained release of MTX. The PLA coating is carried out using the dip coating method following a manual protocol of predetermined dipping and drying steps. It is assumed that the PLA coating obtained is consistent in every micro-implant. An automated process of dip coating would yield a more consistent coating, thereby minimizing the variability of the pharmacokinetics of MTX.

Further during the coating procedure, Dichloromethane (DCM) is used as a solvent for PLA. DCM is often reported to have toxic effects. Vacuum drying the coated micro-implants ensures evaporation of the DCM. Energy dispersive spectroscopy (EDS) studies of the surface of the micro-implants is performed before and after vacuum drying. EDS studies reveal 0.5% w/w chlorine content in vacuum dried coated micro-implants as compared to 5% w/w chlorine in nonvacuum dried coated micro-implants.

The DSC study of the PLA coating is performed at the heating rate of 10°C/min. The influence of any other heating rate on the Tg has not been studied in this study. The motivation of this study was to confirm that the Tg of the PLA coating is greater than physiological temperature. This, in turn, will avoid softening of PLA coating under physiological conditions. Though the heating rate is expected to have some influence on the resolution of the endotherm peak showing the Tg, the values obtained in our
experiment are consistent with previous work [33]. Thus, it is assumed that the moderate variation in heating rate may not have a significant influence on the Tg of PLA.

Lastly, the fitting of drug release data in the Higuchi model assumes that (i) initial drug concentration in the matrix is much higher than the drug solubility in the polymer matrix, (ii) drug diffusion is taking place from one dimension as one side of the micro-implant is in contact with the vitreal surface, (iii) drug diffusivity is constant, (iv) sink conditions are maintained, and (v) size of the drug particles are much smaller than the thickness of the system.

4.7 Limitations

In the set up of our in vitro release rate analysis, PBS is used to simulate the volume and conditions of the vitreous fluid in the eye. The composition of the vitreous fluid is not exactly replicated by the PBS fluid, which, in turn, could affect the pharmacokinetics. The positioning of the micro-implant in the intravitreal domain of the eye could somewhat influence the drug distribution, which was not assessed in this study. Also, CS is supposed to degrade in the presence of lysozyme. The presence of lysozyme is reported in the vitreous fluid by Stainer et al. [25]. This study does not take into account the effect of lysozymic degradation of CS, which can impact the pharmacokinetics of MTX.

The molecular weight of CS influences the internal structure [27,41,42]. Higher molecular weight yields stronger and tighter structures due to intramolecular and intermolecular linkages. It, therefore, directly impacts the swelling properties and drug release mechanisms. Similar influences in structural properties are also noticed by different molecular weights of PLA [18]. This study can be further improved by analyzing the influence of different molecular weights of PLA and CS on the drug release mechanism.

ToF-SIMS analysis does not provide a quantitative measure of the chemical composition. It is a qualitative measure of the mass fragments generated by the surface when a Ga+ primary ion source at 15 Kev accelerating voltage and 1.5 pA current rasters over a 200 μm by 200 μm surface area of the sample. The spectra of CS and MTX have been ignored, as it is not expected to be on the surface of the coated micro-implant. However, with an increase in drug loading, the relative intensity of the characteristic mass fragments of MTX present on the surface of the uncoated micro-implant is expected to increase.

Lastly, the in vivo feasibility assessment of the micro-implants is at present being tested in rabbit eyes to assess the pharmacokinetics and toxicity evaluation in the in vivo conditions. Based on the results obtained in the in vivo environment, subsequent modifications would be implemented to obtain a more refined prototype design.

5 Conclusion

In this study, a unique CS and PLA based MTX micro-implant is fabricated to improve treatment of PIOL. The uncoated CS-MTX implants are able to administer the drug for approximately 1 day. The PLA coated CS-PTX micro-implants are able to administer the therapeutic release rate of 0.2–2.0 μg/day of MTX for more than 50 days. The PLA coated CS-PTX micro-implant is expected to improve the bioavailability of MTX in the intravitreal domain, as there are no intravitreal sustained MTX delivery devices.

The PLA coating influences the initial burst and the mean release rate of MTX from the micro-implants. The amount of drug loading influences the initial burst and the release duration of MTX from the micro-implants. The release kinetics of MTX from the coated micro-implants is explained by (a) the Korsmeyer–Peppas and zero order model fit (R² ~ 0.9) of the first 60% of the drug release, which indicates the swelling of polymer and initial burst release of the drug from the coated micro-implant, and (b) the first order and Higuchi model fit (R² ~ 0.9) from the tenth day to the end of drug release, implying MTX release in the therapeutic window depends on its concentration and follows diffusion kinetics. The micro-implants fabricated in this study can be used as a possible alternative to MTX intravitreal injection for better tolerance and improved efficacy of MTX. Also, this device would produce minimal toxicity and can be administered using minimally invasive methods.

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References


