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Sustained release ophthalmic dexamethasone: *In vitro in vivo* correlations derived from the PK-Eye

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ABSTRACT

Corticosteroids have long been used to treat intraocular inflammation by intravitreal injection. We describe dexamethasone loaded poly-DL-lactide-co-glycolide (PLGA) microparticles that were fabricated by thermally induced phase separation (TIPS). The dexamethasone loaded microparticles were evaluated using a two-compartment, *in vitro* aqueous outflow model of the eye (PK-Eye) that estimates drug clearance time from the back of the eye *via* aqueous outflow by the anterior route. A dexamethasone dose of 0.20 ± 0.02 mg in a 50 μL volume of TIPS microparticles resulted in a clearance $t_{1/2}$ of 9.6 ± 0.3 days using simulated vitreous in the PK-Eye. Since corticosteroids can also clear through the retina, it is necessary to account for clearance through the back of the eye. Retinal permeability data, published human ocular pharmacokinetics (PK) and the PK-Eye clearance times were then used to establish *in vitro in vivo* correlations (IVIVCs) for intraocular clearance times of corticosteroid formulations. A $t_{1/2}$ of 48 h was estimated for the dexamethasone-TIPS microparticles, which is almost 9 times longer than that reported for dexamethasone suspension in humans. The prediction of human clearance times of permeable molecules from the vitreous compartment can be determined by accounting for drug retinal permeation and determining the experimental clearance *via* the anterior aqueous outflow pathway using the PK-Eye.

1. Introduction

Intravitreal (IVT) corticosteroids are routinely used in the treatment of sight threatening conditions in the back of the eye including diabetic macular edema (DME), proliferative vitreoretinopathy, endophthalmitis and uveitis. Administration by IVT injection allows the steroids to bypass the blood-retinal barrier (BRB), leading to higher drug concentrations close to the site of action in the posterior cavity (Cunningham et al., 2008). Common corticosteroids used to treat ophthalmic conditions include dexamethasone, triamcinolone acetonide (TA) and fluocinolone acetonide (FA). Unfortunately, low molecular weight, permeable molecules including corticosteroids that are dissolved in the vitreous rapidly clear from the eye displaying a relatively short $t_{1/2}$ that is of a matter of hours (i.e. 3–7 h) (Haghjou et al., 2013; Thrimawithana et al., 2011; Kwak and Amico, 1992; Laude et al., 2010). Frequent IVT injections to maintain therapeutic drug concentrations can increase the risk of serious adverse reactions including retinal detachment, endophthalmitis and vitreous hemorrhage (Lee et al., 2010).

Steroid suspensions have long been used clinically in an effort to reduce the number of IVT injections. Triamcinolone acetonide (TA) is available as a preservative-free injectable suspension for intraocular use (Triesence®, Alcon). Kenalog® is a TA injectable suspension that is indicated for intramuscular and intra-articular administration; and has been used off-label for many years (Cabrera et al., 2014; Jermak et al., 2007; Behl et al., 1976) for

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treating both anterior and posterior segment ocular diseases. To further increase the duration of action, long-acting corticosteroid implants are now clinically available (Thrimawithana et al., 2011; Choonara et al., 2010; Thakur et al., 2014; Basile et al., 2012; Anon., 2012; Basile et al., 2014; Christoforidis et al., 2012). Corticosteroids are potent, anti-inflammatory agents that are generally stable, poorly soluble and slowly dissolving and so are ideal drug substances for use in longer acting dosage forms (Cima et al., 2014). The volume of the vitreous cavity is approximately 4.2 mL, so non-sink conditions can be exploited to prolong the release kinetics of IVT implants of corticosteroids. Ozurdex® (Allergan, Inc) is a poly(lactic-co-glycolic acid) (PLGA) implant, which releases dexamethasone (0.7 mg) over a 6-month period (Medeiros et al., 2014).

Formulating a corticosteroid in a PLGA matrix allows better control of drug release compared to the dissolution of a free drug suspension. Interestingly, Ozurdex® displays a similar pharmacokinetic (PK) profile between vitrectomised and non-vitrectomised eyes (Chang-Lin et al., 2011) whereas suspensions of TA clear more quickly in vitrectomised eyes (Beer et al., 2003). However, Ozurdex® is a single depot plug, so there is limited flexibility in reducing the duration of action to less than 6-months. Thus, in an effort to exploit the controlled release properties of PLGA for IVT administered dexamethasone with the possibility of varying the dose and duration of action, we describe the preparation of dexamethasone-PLGA microparticles prepared by thermally induced phase separation (TIPS) (Ghanbar et al., 2013).

TIPS has been used to fabricate porous drug vehicles for applications in chronic wound therapy, drug delivery and tissue engineering (Foong et al., 2010). The technique used to produce TIPS microsparticles is rapid and provides high encapsulation efficiency generating spherical particles with rigid outer surfaces and longer shelf-life (Malik et al., 2016). The encapsulation yield for microparticles containing particulate infliximab was $60.4 \pm 5.9\%$ compared with $37.9 \pm 14.4\%$ for microspheres containing an emulsion of infliximab (Foong et al., 2010). Degradable polymeric microparticles can enable a prolonged therapeutic concentration to be available while the porous particle surface aids resorption and reduces the likelihood of autocatalysis associated with solid microparticles. PLGA depots are biodegradable which avoids the need for surgical removal from the eye after drug depletion (Yandrapu and Kompella, 2013). Porous PLGA microparticles are ideal for drug delivery because the amount of polymer per microparticle is reduced compared with solid microparticles of an equal size.

A two-compartment *in vitro* model of the eye, known as the PK-Eye that predicts human clearance times caused by the aqueous outflow pathway (Awwad et al., 2015) was used to determine drug release kinetics of the dexamethasone TIPS microparticles. Aqueous outflow (2.0–2.5 μL/min) is the main cause of mass transfer within the eye (Brubaker, 1982; Toris et al., 1999; Maurice, 2001; Siggers and Ethier, 2012; Ethier et al., 2004). Aqueous humor nourishes the avascular lens and cornea with outflow passing through the front of the eye. The PK-Eye provides a good estimate of human clearance times from the vitreous cavity for protein therapeutics and non-permeable low molecular weight molecules from suspension and depots (Awwad et al., 2015). Permeable low molecular weight molecules are eliminated from the vitreous cavity by both aqueous outflow and permeation through the retina *via* the retinal-choroid-sclera (RCS) pathways. Utilising the PK-Eye to estimate the clearance of low molecular weight, retinal permeable compounds such as steroids requires that we combine *in vitro* outflow clearance data from the PK-Eye, published drug permeability and *in vivo* human data, when available, to develop *in vitro in vivo* correlations (IVIVC). The goal of the work described herein was also to develop IVIVC methodology, which can be used as a surrogate for *in vivo* ocular pharmacokinetic studies during preclinical optimisation to develop sustained release ocular preparations.

2. Experimental

2.1. Materials and instrumentation

Sodium hyaluronate (1.8 MDa) was purchased from Aston Chemicals (Aylesbury, UK) and agar (solubility: 15.0 mg/mL) was obtained from Fluka Analytical (Gillingham, Dorset, UK). Visking dialysis membrane tubing (molecular weight cut off (MWCO) 12–14 kDa) was purchased from Medicell International Ltd. (London, UK). PLGA (75:25, Purasorb PDLG 7507 0.63dL/g) was obtained from Purac Biomatericals (Gorinchem, The Netherlands). Dexamethasone, dimethyl carbonate (DMC >99.9% purity) and dimethylsulphoxide (DMSO) were obtained from Sigma-Aldrich (Dorset, UK).

A 16-channel Ismatec peristaltic pump (Michael Smith Engineers Ltd., Woking, Surrey, UK) was used to generate fluid flow into the PK-Eye. For homogenisation, a T8 Ultra-Turrax homogeniser was used (Ike-Werke, Staufen, Germany). Microparticles were fabricated using a Nisco encapsulator Var D unit, fitted with a stainless steel sapphire tipped nozzle with a 150 μm orifice (Nisco Engineering, Zurich, Switzerland). Scanning electron microscopy (SEM) was achieved with a 7401-high resolution Field Emission Scanning Electron Microscope (Jeol, Tokyo, Japan). Lyophilisation was conducted using an Edwards Micro Modulyo freeze dryer (Thermo Fisher Scientific, Asheville, NC). All analyses were undertaken using an Agilent 1200 series HPLC (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with Chemstation software (Agilent) and a reverse phase Synergi Polar-RP C18, 4 μm, 15 cm column (Phenomenex, Macclesfield, UK).

2.2. Preparation of the PK-Eye for in vitro studies

2.2.1. Design of the model

The design and use of the *in vitro* PK-Eye model has been previously reported (Awwad et al., 2015). Briefly, the PK-Eye is fabricated from plastic (polyacrylate) with anterior (~0.2 mL) and posterior (~4.2 mL) cavities integrated within the model. A washer with a Visking dialysis membrane (MWCO 12–14 kDa) separates the two cavities. The direction of flow and the presence of the dialysis membrane only allows solubilised drug to pass from the posterior compartment into the anterior compartment. The PK-Eye model consists of an inlet port in the posterior cavity for continuous aqueous inflow (phosphate buffered saline, PBS pH 7.4) at a rate of 2.0 μL/min and an outlet port from the anterior cavity for sample collection. An injection port is present in each cavity with a diameter of 2.0-3.0 mm to allow administration of the desired formulation into the model. The model was placed in a pre-heated oil bath at 37 °C to conduct release studies at physiological temperature.

2.2.2. Preparation of simulated vitreous

Agar (0.4 g) and hyaluronic acid (HA) (0.5 g) were each separately mixed in 100 mL of stirred hot water (Kummer et al., 2007). The agar solution was boiled to completely solubilise the agar. After boiling, the hot agar solution was mixed with HA and stirred to give a homogenous mixture to which a few drops of 0.02% sodium azide were added. The solution was left to cool for 24 h at ambient temperature (~25 °C) and formed into a gel-like consistency. The simulated vitreous was then transferred to the PK-Eye model *via* the injection port in the posterior cavity. This combination of agar and HA was found to have a dynamic viscosity

of ~0.6 Pas (Awwad et al., 2015), similar to average human vitreous viscosity, ~0.5 Pas (Soman and Banerjee, 2003).

2.3. Preparation of dexamethasone-PLGA TIPS microparticles

PLGA TIPS microparticles were prepared as previously described (Foong et al., 2010). A 10% weight solution of PLGA (75:25) in DMC (0.5 g PLGA in 5 mL DMC) was prepared with magnetic stirring. Dexamethasone (20.0 mg) was dissolved in DMSO (100 μL) and this solution was added to a solution of PLGA in DMC (5.0 mL, therefore, 0.04 mg dexamethasone per mg of PLGA). The solution was mixed for approximately 10 s using a T8 Ultra-Turrax homogeniser.

Polymer solution was fed into the encapsulator unit *via* a syringe at a flow rate of 1.5 mL/min. The vibration frequency of the nozzle was kept at 1.80 kHz with 70% frequency amplitude. Liquid polymer droplets were collected in a polyethylene beaker containing liquid nitrogen (500 mL). The frozen polymer droplets from the liquid nitrogen were transferred to 50 mL polypropylene containers. Residual DMC solvent in the frozen polymer droplets was removed by lyophilisation for 24 h. Therefore, 165 mg of TIPS-dexamethasone PLGA particles contained 6.6 mg of dexamethasone. The microparticles used for the experiments were sieved to a size range of 250–425 μm and mixed with GranuGEL® (1650 μL) to produce a paste containing 165 mg dexamethasone-loaded particles (therefore, 100 μL of GranuGEL® contained 0.4 mg of dexamethasone).

2.4. Characterisation of TIPS microparticles

2.4.1. Scanning electron microscopy (SEM)

TIPS microparticles were mounted onto aluminium stubs *via* adhesive carbon tabs, sputter coated with 1–2 nm of gold/palladium alloy for 3 min in an argon atmosphere using a high resolution ion beam coater (Gatan Model 681) and viewed by SEM.

2.4.2. Encapsulation and in vitro drug release studies with the PK-Eye

Equivalent amounts of dexamethasone loaded TIPS microparticles were transferred to 1.0 mL syringes and pressure was applied to the syringe to pack the particles. To obtain dexamethasone loading, the dried, packed dexamethasone TIPS microparticles in 0.05 mL volume were then transferred to a glass vial containing DMSO (2.5 mL) and vortexed until completely dissolved. The solution was then diluted 10 times in PBS, pH 7.4. The mixture was sonicated for 15 min and the resulting solution was filtered using a 0.22 μm filter. Dexamethasone concentration was determined with HPLC (240 nm).

The PK-Eye models were assembled using a fresh Visking membrane for each model. The posterior cavity of the model was filled with the appropriate vitreous substitute (PBS, pH 7.4 or simulated vitreous) and the anterior cavity of the model was filled with PBS. Negative controls were conducted with PK-Eye models with the vitreous substitutes containing no particles.

Dexamethasone is poorly water soluble (0.1 mg/mL), so a dexamethasone suspension was prepared in water (0.2 mg/50 μL) to enable direct comparison of the clearance profiles of the suspension and TIPS preparations. Dexamethasone suspension and dry PLGA TIPS microparticles were injected into the posterior cavity of the PK-Eye model. The aqueous outflow was collected from the anterior cavity and was used to determine drug concentrations at different time points. Each sample was filtered using a 0.22 μm filter before HPLC analysis.

2.4.3. Dexamethasone quantification by high performance liquid chromatography (HPLC-UV)

A wavelength of 240 nm was used to detect dexamethasone. The mobile phase comprised 0.1% TFA in water and acetonitrile with a gradient of water:acetonitrile changing from 4:1 to 3:7 over 20 min. An injection volume of 10.0 μL and a flow rate of 1.0 mL/min were used. Dexamethasone showed a retention time of 9.3 min. The calibration curve (R^2 value of 0.998) was prepared with dexamethasone in PBS, pH 7.4 with a concentration range of 0.39–100 μg/mL.

2.5. Establishing in vitro-in vivo correlations (IVIVC)

Use of the PK-Eye model to predict the *in vivo* drug clearance from the posterior compartment was performed with suspensions of two model drugs; triamcinolone acetonide (TA) (solubility 25 μg/mL) and dexamethasone (0.1 mg/mL). TA (4.0 mg) displayed constant concentrations (21–30 μg/mL) in the posterior cavity of the PK-Eye model (Awwad et al., 2015). *In vivo* drug clearance data from the posterior compartment was obtained from published human values (Beer et al., 2003). The PK-Eye overestimates the *in vivo* clearance times of small molecular weight drugs due to the lack of the RCS pathway in the model. Therefore, drug permeation data across the RCS pathway was used with anterior aqueous outflow clearance time from the PK-Eye to predict *in vivo* drug clearance. Details are provided in results and discussion.

2.6. Data analysis

All data reported were conducted in triplicate (n = 3) and results are presented as the mean and standard deviation (±STD). The data obtained were plotted using OriginPro 9.1 (software, Origin lab cooperation, USA). Half-life ($t_{1/2}$) values were calculated according to the best fitting model in OriginPro. First-order kinetic rate constants (k) were derived from the mono-exponential curve and $t_{1/2}$ values were calculated using the equation: $0.693/k$.

3. Results and discussion

3.1. Characterisation of TIPS microparticles

3.1.1. Scanning electron microscopy (SEM)

Spherical dexamethasone-loaded TIPS microparticles were observed within the sieved size range of 250–425 μm (Fig. 1). The surface of the microparticles was smooth, peppered with pores that were generally distributed in chevron-like patterns created by the freezing of the DMC solvent during fabrication process (Blaker et al., 2008). The pore size at the surface of the microparticles was approximately 0.2– 1.0 μm. A large pore was visible on the surface of some of the microparticles, which is a typical feature of TIPS microparticles (Blaker et al., 2008) At higher magnification, amorphous features embedded in the polymer were evenly distributed across the surface of the microparticles, which were likely to consist of dexamethasone since they were not present in unloaded control microparticles.

3.1.2. Encapsulation and in vitro drug release studies with the PK-Eye

The loading efficiency of the dexamethasone loaded TIPs microparticles was determined prior to evaluation in the PK-Eye. Approximately 0.20 ± 0.02 mg of dexamethasone was present in a volume of 50 μL packed dexamethasone TIPS microparticles following the method listed on Section 2.4.2 (Fig. S1). The dexamethasone TIPS microparticles were injected into the

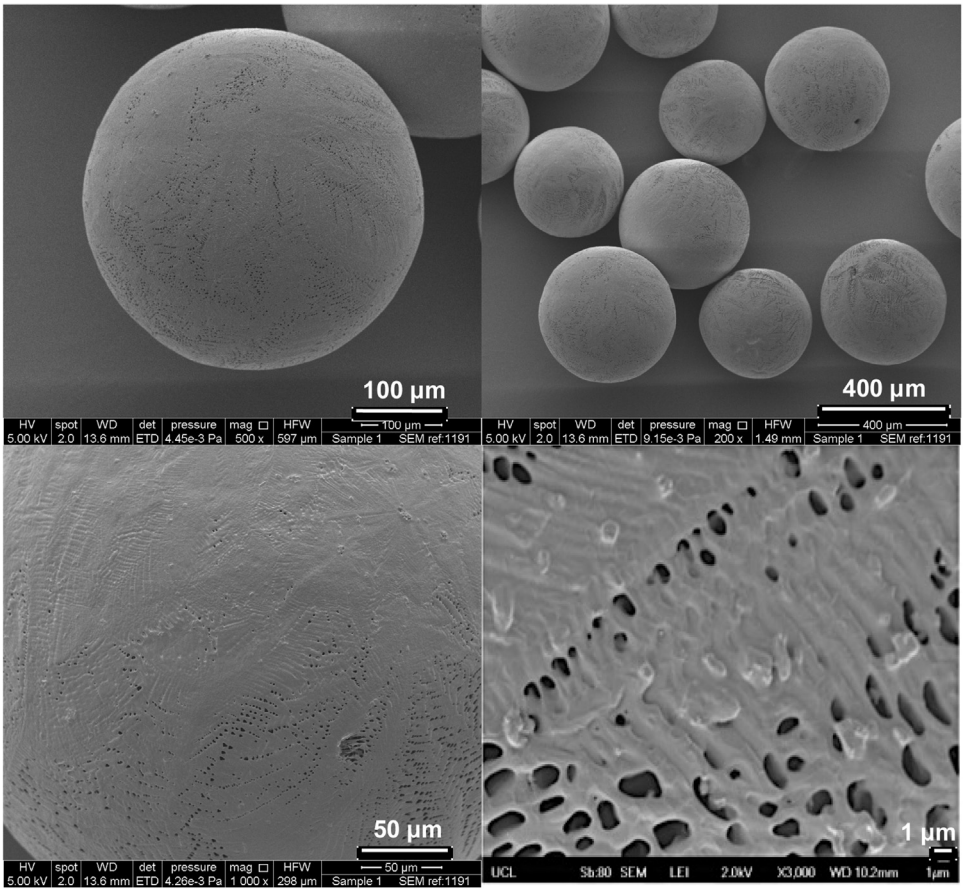

Fig. 1. Scanning electron microscopy (SEM) images of dexamethasone loaded TIPS microparticles. The particles were spherical and the pore size was approximately 1.0 µm in size.

posterior cavity of the PK-Eye (Fig. S2) using a 1.0 mL syringe fitted with a 23G needle.

Dry dexamethasone TIPS microparticle formulations and dexamethasone suspension (control) were injected at an equivalent dose (0.2 mg) into the posterior cavity of the PK-Eye containing simulated vitreous at 37 °C (Table 1 and Fig. 2). The dexamethasone collected from the model outflow was quantified by HPLC (240 nm UV absorbance). Dexamethasone TIPS microparticles (0.2 mg) showed a $t_{1/2}$ of 9.6 ± 0.3 days in simulated vitreous (Fig. 2). The C_{max} and lowest concentration of solubilised dexamethasone detected were 9.3 ± 0.1 µg/mL (21 h) and 1.0 ± 0.1 µg/mL (425 h) respectively. After 160 h, a nearly constant concentration of 1.9 ± 0.7 µg/mL of dexamethasone was present from the outflow of the model. By 500 h (~3 weeks), 78.8 ± 1.2% of the dexamethasone dose had been released. The average amount of solubilised dexamethasone released from the anterior cavity ranged from ~1.0-3.0 µg/mL from weeks 1–3.

In contrast, an equivalent dose of dexamethasone suspension almost completely cleared within 100 h in simulated vitreous (Fig. 2) from the PK-Eye. The dexamethasone C_{max} was 34 µg/mL at 17.6 h and elimination followed first order kinetics with a $t_{1/2}$ of 1.0 ± 0.1 days. Similar drug release kinetics was observed when half the dose of the dexamethasone suspension (0.1 mg, 50 µL) was evaluated in the PK-Eye ($t_{1/2}$: 1.20 ± 0.03 days, Table 1).

Dexamethasone-TIPS microparticles (50 µL) were then evaluated in the PK-Eye using PBS (pH 7.4) in the posterior cavity (Fig. 3)

Table 1
Summary of dexamethasone suspension and TIPS microparticles release kinetics in the PK-Eye at 37 °C.

Type	Injected		Vitreous substitute	Rate constant, k (day^{-1})	Half-life, $t_{1/2}$ (days)
	Mass (mg)	Volume (µL)			
Suspension	0.1	50	PBS, pH 7.4	0.54 ± 0.04	1.3 ± 0.1
	0.1	50	Simulated vitreous	0.60 ± 0.02	1.2 ± 0.03
	0.2	50	Simulated vitreous	0.70 ± 0.07	1.0 ± 0.1
TIPS	0.2	50	PBS, pH 7.4	0.22 ± 0.01	3.1 ± 0.2
	0.2	50	Simulated vitreous	0.07 ± 0.002	9.6 ± 0.3

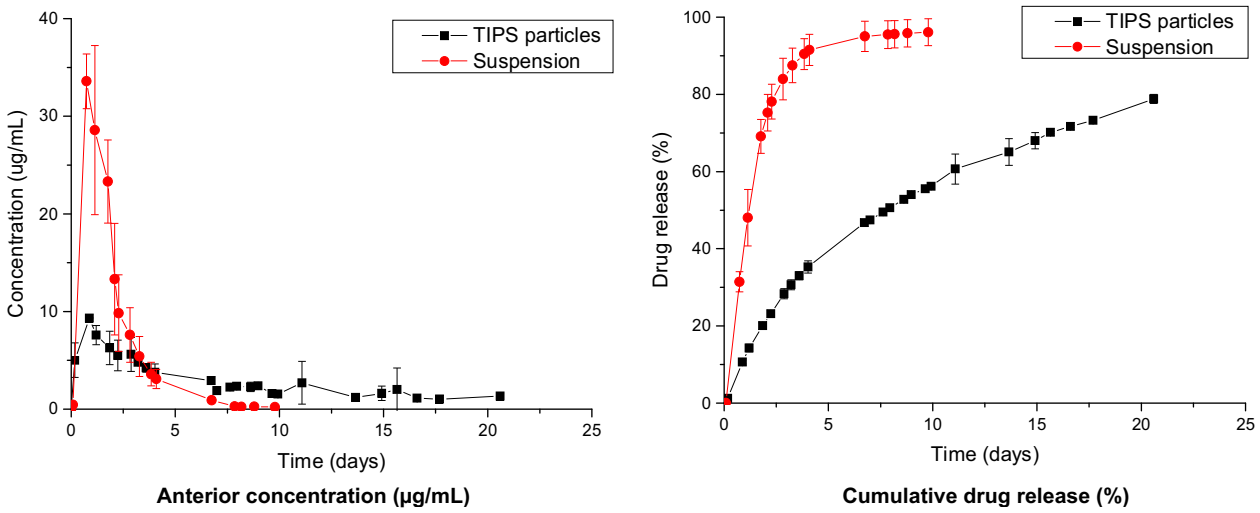

Fig. 2. Release profiles of dexamethasone TIPS microparticles (0.2 mg, 50 μL) and dexamethasone suspension (0.2 mg, 50 μL) in the PK-Eye containing simulated vitreous in the posterior cavity at 37 °C. The dexamethasone dose was not solubility limited and the clearance half-lives ($t_{1/2}$) were 9.6 ± 0.3 and 1.0 ± 0.1 days for the TIPS microparticles and suspension respectively. All results are displayed as the average of the triplicate (n = 3) and its standard deviation (±STD).

and a $t_{1/2}$ of 3.1 ± 0.2 days was observed. The C_{max} and the lowest concentration of solubilised dexamethasone detected were 17.7 ± 8.9 μg/mL (~21 h) and 1.0 ± 0.2 μg/mL (~376 h) respectively. Dexamethasone was still releasing slowly under these conditions with ~99.0 ± 0.4% being released in 3 weeks. A higher burst effect was seen with these particles in PBS compared to simulated vitreous.

The release of dexamethasone in PBS was significantly faster than in simulated vitreous. PLGA undergoes degradation through hydrolysis of its ester linkages to lactic and glycolic acid units, and drug release occurs by both diffusion and erosion. A higher viscosity medium could slow drug diffusion after release from the polymer matrix resulting in longer time periods for the drug to become distributed within the vitreous cavity of the PK-Eye. In contrast, the dexamethasone suspension (0.1 mg, 50 μL) displayed a $t_{1/2}$ of 1.3 ± 0.1 days in PBS, which was similar to what was observed in simulated vitreous ($t_{1/2}$ 1.2 ± 0.03 days) (Table 1). Simulating the composition of physiological fluids in drug release studies improves the capacity to determine IVIVCs for modified release delivery systems, as has been observed in oral drug delivery (Fadda et al., 2009), so the choice of simulated vitreous is important for matrix formulations rather than more simple suspensions that simply dissolve.

3.2. Drug clearance in vitro-in vivo correlations (IVIVCs)

In vitro release studies of ocular formulations have traditionally been conducted in water baths with constant agitation, dialysis membranes on a shaker stand or using USP 4 flow-through cell dissolution apparatus (Barcia et al., 2009; Zhang et al., 2009;

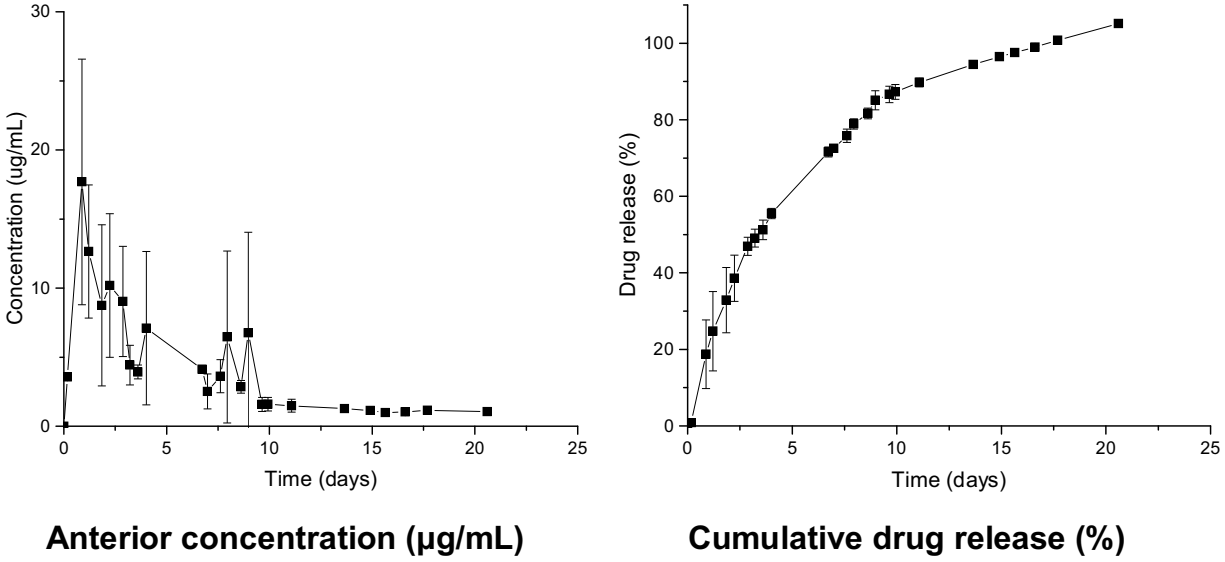

Fig. 3. Release profile of dexamethasone TIPS microparticles (0.2 mg, 50 μL) in the PK-Eye containing PBS, pH 7.4 in the posterior cavity at 37 °C. The $t_{1/2}$ was 3.1 ± 0.2 days. All results are displayed as the average of the triplicate (n = 3) and its standard deviation (±STD).

Chennamaneni et al., 2013). The drawback with these *in vitro* methods is that they do not mimic the human eye in terms of dimensions, compartmentalisation and aqueous outflow. Ocular aqueous outflow plays a major role in drug clearance. The PK-Eye was designed to specifically mimic the aqueous outflow from the eye which is critical to estimate the clearance of non-permeable molecules such as therapeutic proteins (Awwad et al., 2015). The PK-Eye model is also thought to be of value for estimating the dissolution rates of suspensions and the release profiles of implants from the vitreous cavity.

Drug elimination from the vitreous cavity occurs through the RCS pathway and through the anterior hyaloid membrane into the anterior chamber for rapid elimination by aqueous outflow into the conjunctiva. Most low molecular weight drugs are permeable and can clear by both the RCS and anterior routes. Large molecular weight drugs such as proteins, which are also charged molecules, are not RCS permeable, so tend to clear by the anterior outflow route only. Proteins diffuse more slowly in the vitreous liquid compared to low molecular weight drugs and proteins tend to display $t_{1/2}$ over a period of days (i.e. 5-10 days). Low molecular weight non-permeable drugs that are cleared only through the aqueous route will display a longer $t_{1/2}$ (15–30 h) compared to permeable drugs that are cleared by both the anterior and RCS pathways (2–4 h) (Haghjou et al., 2013).

Since the PK-Eye only accounts for the anterior clearance pathway, evaluation of RCS permeable, molecules such as dexamethasone will result in an over estimation of clearance times. Therefore, the clearance times of dexamethasone from the TIPS microparticles as determined from the PK-Eye is an overestimation of *in vivo* clearance. It is necessary to integrate *in vitro* clearance data from the PK-Eye with an estimation of clearance by the RCS pathway by utilising the permeability characteristics of dexamethasone to determine human ocular clearance times Kenalog®, a TA suspension (40 mg/mL) has long been used clinically, so TA was first used to confirm if the PK-Eye could be used to estimate the human clearance time for a molecule that clears by both the RCS and anterior pathways.

3.2.1. Correlating TA clearance from the PK-Eye to human clearance

Human *in vivo* elimination $t_{1/2}$ of IVT administered TA suspension (4.0 mg) is about 15.6 days (~374 h), (Beer et al., 2003) whereas the *in vitro* $t_{1/2}$ clearance from the PK-Eye is 26-28 days (Awwad et al., 2015). The total amount of TA leaving the posterior cavity through aqueous outflow can be estimated from the total inflow volume circulating through the human eye and drug concentration in posterior cavity. With a flow rate of 2.0 μL/min, the total volume (flow rate × time) circulating through the posterior cavity over one drug $t_{1/2}$ (~374 h; ~15.6 days) (Beer et al., 2003) is approximately 44.9 mL. Since TA is injected as a suspension, it is always present in excess in the vitreous compartment and exists at a constant, steady concentration of ~25 μg/mL which is equivalent to the saturation solubility of the drug (Awwad et al., 2015). The amount of drug eliminated from the posterior cavity through only the aqueous outflow over one drug $t_{1/2}$ is therefore 1.122 mg (44.9 mL × 25 μg/mL) (Table 2).

Using the vitreal clearance $t_{1/2}$ of TA in humans (~374 h), we can estimate the amount of TA leaving the posterior compartment through aqueous outflow permeation through the RCS pathway. Since 4.0 mg of TA was IVT injected *in vivo*, then after one $t_{1/2}$ the amount of TA cleared from the posterior segment is 2.0 mg. The difference between *in vivo* and *in vitro* drug elimination after one $t_{1/2}$ is 0.878 mg. Permeability can be determined from Fick's First Law. The concentration gradient across the RCS pathway can be assumed to be constant since sink conditions exist after permeation. The surface area of the RCS was assumed to be equivalent to the surface area of the retinal pigment epithelium (RPE) and taken as 1204 mm^2 (del Amo and Urtti, 2015). Drug permeability across the RCS pathway was calculated to be 2.2×10^{-6} cm/s (Eq. (1)).

$$J = \frac{Q}{SA * t} = P * C \quad (1)$$

where J is drug flux, Q is total drug amount clearing from the vitreous (0.878 mg), SA is surface area of the RCS pathway (1204 mm^2), t is $t_{1/2}$ (374 h), P is drug permeability across the RCS pathway and C is drug concentration in donor compartment (~25 μg/mL).

There is limited data on drug permeability across human RCS. To our knowledge, the only human permeability data available is that for beta-blockers. A study by Kadam et al. (2011) reported the permeability range for eight beta-blockers across the human scleral choroidal retinal pigment epithelium (SCRPE) to be 1.34×10^{-6} to 6.03×10^{-6} cm/s (Kadam et al., 2011). Our value for TA permeability (2.2×10^{-6} cm/s) determined from published human *in vivo* clearance data and the PK-Eye model falls within this range and is closest to the beta-blockers with similar lipophilicity. Thus by combining in *vitro* drug clearance data from the PK-Eye model with drug permeability across the RCS pathway, *in vivo* drug elimination $t_{1/2}$ of small molecules existing in excess amounts in the vitreous chamber can be predicted.

3.2.2. Correlating dexamethasone clearance from the PK-Eye to human clearance

In vivo clearance $t_{1/2}$ of solubilised dexamethasone from the vitreous in humans is reported to be 5.5 h (Gan et al., 2005). At a 0.2 mg dose, the dexamethasone concentration in the vitreous chamber will be decreasing over time, so the time for the total drug amount (Q) to clear *via* aqueous outflow was calculated using Eq. (2) with a time point of 17.6 h from the release studies.

$$Q = Flow \int_0^t C.dt \quad (2)$$

Drug clearance was determined at 17.6 h since this is the time point closest to dexamethasone *in vivo* $t_{1/2}$ (5.5 h), for which we have *in vitro* drug concentrations. Thus at 17.6 h, the amount of drug that had cleared the vitreous *in vitro*, was determined to be 79.5 μg (Fig. 3). *In vivo*, however, the amount of drug cleared from the vitreous compartment after 3 $t_{1/2}$ (17.6/5.5 h) at an equivalent injection dose of 200 μg is 175 μg. Therefore, the amount of drug cleared due to permeation across the RCS pathway is 95.5 μg. Again, since the drug concentration in the vitreous compartment is changing with time, Eq. (3) was used to determine retinal permeability.

$$Q = SA.P. \int_0^t C.dt \quad (3)$$

Table 2
Development of *in vitro in vivo* correlation (IVIVC) with triamcinolone acetonide (TA, Kenalog®, 40 mg/mL).

Parameters	*In vitro*	*In vivo*
Dose of TA (mg)	4.0	4.0
$t_{1/2}$ (days)	28	15.6
Value for 1 $t_{1/2}$ (h)	674	374
Concentration of TA in the PK-Eye (μg/mL)	~ 25	–
Amount of drug eliminated after 1 $t_{1/2}$ (mg)	1.1	2.0

Difference between *in vitro* and *in vivo* clearance: 0.878 mg.
Abbreviations: mg: milligrams; mL: millilitres, $t_{1/2}$: half-life μg: micrograms.

Table 3
Prediction of ocular *in vivo* $t_{1/2}$ of dexamethasone-TIPS microparticles.

Parameters	Values
Dose of dexamethasone (μg)	200
Drug permeability across the RCS pathway (cm/s)	3.3×10^{-6}
Surface area of RCS (mm^2) (del Amo and Urtti, 2015)	1204
Flow rate of aqueous outflow (μL/min)	2.0
Drug eliminated from the PK-Eye (attributable to aqueous outflow) (μg)	68.3
Drug permeation across RCS pathway (μg)	82.0
Total drug cleared from the vitreous chamber (aqueous outflow and permeability) (μg)	150.3

Abbreviations: RCS: retina-choroid sclera. *Note:* Drug elimination and permeation was determined at 96.4 h.

Retinal permeability of dexamethasone was calculated to be 3.3×10^{-6} cm/s. A value for RCS permeability of dexamethasone in humans could not be found, however the reported value in porcine is 2.2×10^{-6} cm/s (Loch et al., 2012). This is in close agreement to the value we calculate using the PK-Eye.

3.2.3. *Prediction of* in vivo *clearance of dexamethasone-TIPS microparticles*

Now that we have shown the PK-Eye can provide a good estimate of IVIVCs, we can use the model to predict the clearance $t_{1/2}$ of sustained release IVT implants. At 96 h, the amount of dexamethasone slowly released from the TIPS microparticles and cleared from the PK-Eye by aqueous outflow was determined using Eq. (2) to be 68.3 μg (Table 3). Drug permeation across the RCS pathway was determined using Eq. (3) to be 82.0 μg.

Assuming first order elimination, the predicted human clearance $t_{1/2}$ of dexamethasone that would be slowly released from the TIPS microparticles was calculated to be 48 h using Eq. (4c) (Equation derived from Eqs. (4a) and (4b)).

$$\ln A_t = \ln A_0 - kt \tag{4a}$$

$$k = 0.693/t_{1/2} \tag{4b}$$

$$t_{1/2} = \frac{0.693t}{\ln A_0 - \ln A_t} \tag{4c}$$

where A_0 is initial amount of drug in vitreous chamber; 200 μg, A_t is drug remaining in vitreous chamber (49.7 μg) and t is time (96 h).

The predicted $t_{1/2}$ of dexamethasone TIPS microparticles (0.2 mg dexamethasone dose) is almost 9 times longer than that of an equivalent dose of dexamethasone suspension. A dexamethasone TIPS microparticle formulation would be expected to require less frequent IVT injection than a dexamethasone suspension. Dexamethasone TIPS microparticles are designed to provide sustained drug release for an intermediate period of time compared to the dexamethasone Ozurdex® implant. Dexamethasone TIPS microparticles thus offer a potential treatment choice for acute ocular conditions of the posterior segment that would require steroid treatment over a period not lasting more than a month.

Integrating drug permeation across the RCS pathway with anterior aqueous outflow from the PK-Eye can be used as a strategy to predict *in vivo* drug clearance of suspensions and implants from the vitreous compartment. Once a permeable, low-molecular weight molecule is solubilised in the vitreous, clearance would be expected to be relatively quick. Our model can be used with other *in vitro* or computational permeability models to develop IVIVCs that include RCS clearance pathways. This is analogous to strategies that are used to develop new dosage forms for oral and pulmonary administration. This can provide useful information when developing novel ocular implants with respect to determination of drug loading, selection of excipients and formulation optimisation. The need for animal studies will be reduced thus accelerating the development of novel ocular formulations for treating diseases of the posterior segment. The PK-Eye model may also be particularly useful for longer term studies, especially if long acting formulations of protein-based drugs are being evaluated where anti-drug antibodies (ADAs) would be expected to develop in animal models. The PK-Eye estimates the human clearance times of protein therapeutics effectively because these charged, large molecular weight drugs are not permeable and clear predominantly *via* aqueous outflow through the anterior chamber (Awwad et al., 2015). The development of effective long-acting formulations of proteins is one of the most important issues in ocular drug delivery today. In addition, the ability to accurately titrate duration of action is important as some drugs such as dexamethasone may have significant side effects in the long term such as an intractable rise in intraocular pressure if delivered to the eye for too long a duration.

With the PK-Eye, quality control efforts can be facilitated and surrogates for bioequivalence studies can be developed for generic drug product approval. Little has been published about drug metabolism in the vitreous cavity, so practical strategies using the PK-Eye to better account for metabolism in the vitreous may need to be developed for some drugs. While estimation of the overall clearance time is important for preclinical development, integration of our results with advanced in silico models to encompass drug distribution, metabolism and permeability across different pathways will add further insight to the fate of drugs from long acting formulations developed for the eye.

4. Conclusions

In this study, biodegradable dexamethasone loaded PLGA TIPS microparticles were fabricated as a sustained release intraocular dexamethasone formulation. Using the PK-Eye which measures drug clearance by the aqueous outflow pathway, the TIPS-dexamethasone formulation displayed prolonged drug release compared to free dexamethasone at an equivalent dose of 2.0 mg. We combined the drug clearance time from the PK-Eye with RCS permeation data to provide a predictive estimate of human *in vivo* clearance for drugs that clear both anteriorly and posteriorly from the eye. The human *in vivo* $t_{1/2}$ of IVT dexamethasone TIPS microparticles is predicted to be 48 h; almost 9 times longer than dexamethasone. TIPS microparticles have the potential to provide sustained dexamethasone release of intermediate duration that may be useful where prolonged, but not over extended release of drugs such as dexamethasone may be required. The prediction of human clearance times of permeable molecules from the vitreous

compartment can be determined by accounting for drug permeation and determining the experimental clearance *via* the anterior aqueous outflow pathway using the PK-Eye.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2017.02.047.

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