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In vitro and *in vivo* evaluation of novel implantation technology in hydrogel contact lenses for controlled drug delivery

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ABSTRACT

Glaucoma is commonly treated using eye drops, which is highly inefficient due to rapid clearance (low residence time) from ocular surface. Contact lenses are ideally suited for controlled drug delivery to cornea, but incorporation of any drug loaded particulate system (formulation) affect the optical and physical property of contact lenses. The objective of the present work was to implant timolol maleate (TM) loaded ethyl cellulose nanoparticle-laden ring in hydrogel contact lenses that could provide controlled drug delivery at therapeutic rates without compromising critical lens properties. TM-implant lenses were developed, by dispersing TM encapsulated ethyl cellulose nanoparticles in acrylate hydrogel (fabricated as ring implant) and implanted the same in hydrogel contact lenses (sandwich system). The TM-ethyl cellulose nanoparticles were prepared by double emulsion method at different ratios of TM to ethyl cellulose. The X-ray diffraction studies revealed the transformation of TM to amorphous state. *In vitro* release kinetic data showed sustained drug release within the therapeutic window for 168 h (NP 1:3 batch) with 150 μg loading. Cytotoxicity and ocular irritation study demonstrated the safety of TM-implant contact lenses. *In vivo* pharmacokinetic studies in rabbit tear fluid showed significant increase in mean residence time (MRT) and area under curve (AUC), with TM-implant contact lenses in comparison to eye drop therapy. *In vivo* pharmacodynamic data in rabbit model showed sustained reduction in intra ocular pressure for 192 h. The study demonstrated the promising potential of implantation technology to treat glaucoma using contact lenses, and could serve as a platform for other ocular diseases.

1. Introduction

Most of the ophthalmic formulations are delivered through eye drops, which need frequent instillation (patient's noncompliance) with high drug loading causing systemic side effects. For example, drug like timolol maleate (non-selective beta blocker) used to treat glaucoma when delivered through eye drop formulation, cause undesirable side effects on cardiovascular system [1,2]. Moreover, eye drop formulations are inefficient due to rapid clearance (low residence time) of drug through the lachrymal duct, which results in low bioavailability (1–5%) [3]. Previous efforts in developing new ophthalmic drug delivery systems are focused on mucoadhesive films, in-situ gels, collagen shields, liposomes, polymeric nanoparticles, inserts, *etc.* [4,5]. Such systems do offer high drug residence time and improvement in bioavailability, yet the issue remains regarding cost, stability, burst release of drug and causes patient non-compliance [6].

Use of contact lenses as a drug delivery device has an increasing interest to improve the efficacy of ocular treatment, due to more than 50% bioavailability in comparison to eye drop therapy studied by many researchers [7–9]. In the last few decades, a number of novel approaches have been advanced for controlled ophthalmic drug delivery using contact lenses. Most of these studies focused on soaking the lenses in concentrated drug solution, molecular imprinting, use of vitamin E, novel polymeric nanoparticles, biodegradable nanoparticles, microemulsion, *etc.* [10,11]. Viviana and co-workers impregnated acetazolamide and timolol maleate in single Balafilcon A contact lens using a discontinuous supercritical solvent impregnation methodology. They failed to incorporate/penetrate the drug in inner region of contact lenses, which resulted in burst release [12]. Jinku Xu developed pHEMA/β-cyclodextrin hydrogels by copolymerization of HEMA with puerarin-β-cyclodextrin complex to treat glaucoma. The incorporation of β-cyclodextrin in the hydrogels resulted in increased swelling and tensile strength. The release data showed high initial burst release, and the release rate fell below therapeutic level within 10 h [13]. Anuj Chauhan and co-workers soaked 200 μg

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of timolol maleate with 25% vitamin E in silicone hydrogel contact lenses. The pharmacodynamic results in glaucomatous beagle dogs showed only 48 h for reduction in IOP [14]. Kuan-Hui Hsu and co-workers developed drug-eluting contact lenses for treatment of glaucoma by releasing two drugs (timolol and dorzolamide) from single contact lens. The lenses showed reduction in IOP for 48 h with the use of vitamin E as physical barrier [15]. Mara and co-workers impregnated acetazolamide into silicone-based soft contact lenses using supercritical solvent impregnation technique. The *in vitro* flux results showed 8 h release without altering physical and optical properties of contact lens material [16]. Andreza was successful in achieving 12 h release of acetazolamide from cyclodextrin pendant-contact lenses [17]. Haruyuki utilized molecular imprinting technique to load timolol in soft contact lenses, and achieved 24 h release from molecular imprinted contact lenses [18]. Chi-Chung developed timolol-contact lenses with o/w type microemulsions using ethyl butyrate and Pluronic F127. The microemulsion showed controlled release of timolol, due to the presence of a tightly packed surfactant at the oil–water interface [19]. To sustain the drug release, Chauhan and his co-workers proposed an approach of in-situ creation of vitamin E as transport barriers for drug molecules. The release of timolol was significantly extended by increasing vitamin E loading from 10 to 40% in contact lenses, while at the same time complicating oxygen and ion permeability [20,21]. Studies have also focused on developing impregnated drug-PLGA (poly[lactic-*co*-glycolic acid]) films in contact lenses to achieve sustained drug delivery for weeks [22]. To treat glaucoma, Ciolino and co-workers [23] designed latanoprost-eluting contact lens by encapsulating film in methafilcon lenses. The *in vivo* results showed sustained release for one month. Key limitations of above methods are alteration in critical lens properties like transparency, mechanical property, water content, ion permeability, and oxygen permeability, which restrict the use of therapeutic contact lenses.

In this work our aim was to formulate and fabricate nanoparticle-laden ring implant in hydrogel contact lenses that can provide extended drug release at therapeutic rates, without affecting optical and physical properties of contact lens. To accomplish this objective we dispersed TM encapsulated ethyl cellulose nanoparticles in acrylate hydrogel (fabricated as ring implant) and implanted the same in hydrogel contact lenses (sandwich system). The hydrogel implant and contact lenses were synthesized using HEMA (hydroxyethylmethylacrylate) monomers by free radical polymerization in the presence of a cross-linker ethylene glycol dimethacrylate (EGDMA) and photo initiator Darocur® polymerized at 365–370 nm (U.V. Irradiation) for 30 min. Using implant contact lenses, drug molecules would have a much longer residence time on cornea (ocular surface), which may improve ocular bioavailability. Furthermore, *in vivo* animal studies were conducted with implant hydrogel contact lenses in rabbits to establish safety and efficacy in glaucoma therapy.

2. Materials and methods

2.1. Materials

Timolol maleate (TM) was kindly supplied by Zydus Cadila Pharmaceuticals Ltd. (Gujarat, India). Hydroxyethylmethacrylate (HEMA), methacrylic acid (MAA), Darocur® (2, 4, 6-trimethyl benzoyl-biphenyl-phosphinoxide), ethylene glycol dimethacrylate (EGDMA), ethyl cellulose (24–27 cP), Pluronic P123, and Polyvinyl alcohol were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Corneal epithelial cell lines ATCC® CCL60™ was purchased from National Centre for Cell Science (Pune, India). Ultrapure water (18.2 MΩ cm) was obtained from synergy U.V. Millipore water purification system. All other reagents were of analytical grade and obtained from Sigma-Aldrich Chemicals (MO, USA).

2.2. Preparation of TM loaded ethyl cellulose nanoparticles

The encapsulated TM nanoparticles were prepared by double emulsion technique. The required amount of ethyl cellulose (100 mg, 200 mg and 300 mg for respective ratios 1:1, 1:2 and 1:3), and Pluronic P123 (100 mg) was dissolved in acetone (30 ml). Separately, 100 mg of TM was dissolved in 2 ml of deionized water. The aqueous drug solution was added drop wise into the above polymeric organic solution placed under probe sonication to obtain a primary emulsion (w/o). The primary emulsion was added drop wise into 30 ml of 1.5% *w*/*v* polyvinyl alcohol solution placed under probe sonication (40% amplitude for a 700 W sonicator) to obtain a secondary emulsion (w/o/w). Finally, organic phase was evaporated under reduced pressure in a rotary evaporator at 40 °C. After evaporation of the solvent, nanoparticles were immediately centrifuged (25,000 rpm, 4 °C for 30 min), and the residue was redispersed in Milli-Q water to remove excess PVA and unentrapped drug. This process was repeated twice, and the final dispersion was subjected to freeze drying at −120 °C for 72 h. Batches were coded as NP-1:1, NP-1:2 and NP-1:3 for 1:1, 1:2 and 1:3 ratios of TM to ethyl cellulose respectively [24].

2.3. Characterization of nanoparticles

2.3.1. Particle size determination

Nanoparticle size distribution was measured using photon correlation spectroscopy (Malvern Nano-ZS, UK). The measurements were performed at a scattering angle of 90° and 25 °C temperature using samples appropriately diluted with ultrapure deionized water to minimize particle–particle interactions. The mean particle size was determined by using disposable polystyrene cuvette.

2.3.2. X-ray diffraction (XRD)

XRD diffraction is a non-destructive analytical technique used for the identification and quantitative analysis of various crystalline forms of molecules. X-ray diffractometer (XPERT-PRO, Analytical, Netherlands) equipped with a compensating slit, Cu K radiation, voltage of 40 kV and a current of 40 mA was used. The angular range (2θ) covered was between 10° and 60°, with a step size of 0.025° and a counting time per step of 0.5 s. The relative degree of crystallinity (RDC) and crystallite size (using Scherrer equation) of drug was calculated as per procedure described in our published research paper [25,26].

2.4. Preparation of TM nanoparticle-laden ring implant hydrogel contact lenses

2.4.1. Procedure for ring implant fabrication

The required amount of nanoparticles was incorporated in pre-monomer mixture and the implants/rings were fabricated by free radical polymerization. Pre-monomer mixture was composed of 46.7% *w*/w hydroxyethylmethylacrylate (HEMA), 0.8% *w*/w methacrylic acid (MAA), 1% *w*/w ethylene glycol dimethylacrylate (EGDMA) and 52% *w*/w water [27]. TM nanoparticles loaded pre-monomer mixture with 0.5% *w*/w photo initiator (Darocur®) was sonicated for 15 min to remove any air bubbles and was poured with the help of micropipette (50 μl volume) between glass plates (mould) separated by 20 μm spacer (Teflon). The mould was then placed in Ultraviolet transilluminator and the implant was polymerized at 365–370 nm for 30 min. The implant was then removed and punched with the help of borer to ring/implant with 6 mm inner diameter and 8 mm outer diameter. The implants were preserved in desiccators (45% humidity) till further use.

2.4.2. Implantation of TM loaded ring in hydrogel contact lens

In step 1; a partially polymerized (curing time = 5 min) contact lens of 50 μm thickness (base curve 6.5 mm and diameter 14.2 mm) was fabricated using cast moulding technique. The contact lens was prepared using pre-monomer mixture and polypropylene (PP) lens mould

(CIBA Vision, Inc.). In step 2; TM nanoparticle loaded ring/implant was placed/sandwiched between partially polymerized contact lenses fabricated in step 1 process. As shown in Fig. 1, excess of pre-monomer mixture was added in female mould holding partially polymerized contact lens (upper part) and the ring was placed. Later the male mould holding partially polymerized contact lens (base part) was placed. In step 3; the mould was placed in ultraviolet transilluminator and polymerized at 365–370 nm using UV-B light for 30 min. The ring implanted contact lenses was removed and well-preserved in desiccators (45% humidity).

2.5. Characterization of TM loaded ring implant hydrogel contact lenses

2.5.1. Terminal sterilization and sterility testing

Terminal sterilization of implant contact lenses were performed using autoclave in glass vial containing 2 ml of simulated tear fluid (STF, 6.78 g/l NaCl, 2.18 g/l $NaHCO_3$, 1.38 g/l KCl, 0.084 g/l $CaCl_2 \cdot 2H_2O$, pH 7.4) at 121 °C, 15 psi for 30 min. After sterilization, STF was analyzed for TM content, to calculate percentage leaching of drug from contact lenses. For negative control, a portion of fluid thioglycollate media and Saboraud's media was incubated for 14 days at 37 °C and 25 °C respectively. For positive control (growth promotion test), fluid thioglycollate media and Saboraud's media were inoculated with micro-organisms (not more than 100 CFU), viz. *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231 at 37 °C and 25 °C respectively. In aseptic condition using laminar flow hood, sterilized contact lens (whole) was directly transferred into 40 ml of each media tube (25 mm diameter × 150 mm height) containing respective media and incubated for 14 days. The medical device under examination complies with the test for sterility, if after 14 days of incubation period, no evidence of microbial growth is observed [28].

2.5.2. Effective ion diffusivity test

Effective ion diffusivity (ionoflux diffusion coefficient, D_{ion}) must be greater than 1.5×10^{-6} mm^2/min for extended wear contact lenses [29], to ensure homeostasis of ion concentration between contact lens and cornea (*i.e.* post lens tear film) and the lens motion would be free from friction and abrasion. Effective ion diffusivity of implant lenses were measured using modified conductivity meter. The measurement of ion diffusivity was done as per Anuj Chauhan et al. [30]. Briefly, implant lens was attached to the bottom of the donor tube (10 mm inner diameter), which was filled with 0.1 M NaCl solution (5 ml) and placed in a receiving chamber (50 ml of ultra-pure de-ionized water). The receiving chamber was placed on the hot plate maintained at 34 °C with continuous stirring (50 rpm). The dynamic conductivity of receptor solution was monitored by conductivity meter and then converted to salt concentration (NaCl) using the calibration plot. The effective ion diffusivity was calculated based on concentration of ions released in receiving chamber with time, and fitting to diffusion model [29,31].

2.5.3. In vitro release kinetics

The release of TM from nanoparticle-laden implant contact lenses was assessed using glass vial containing 2 ml of STF at 34 °C in incubator shaker at 100 rpm for 7 days. At regular intervals (24 h), the STF (2 ml) was replaced with the same volume of fresh STF, to prevent drug saturation. The samples were filtered through 0.22 μm membrane filter, appropriately diluted and analyzed for drug content using HPLC method. *In vitro* release study of nanoparticles (dose equivalent to 150 μg, without loading/entrapping in ring) and nanoparticle loaded ring (without implanting in contact lenses) was also conducted to assess the rate controlling mechanism of TM release from implant contact lenses. The release profile of TM was evaluated by plotting different graphs of cumulative TM release versus time and release rate (ng/h) versus time [32].

2.5.4. Effect of packaging solution

The study was conducted to estimate the effect of time on leaching of TM from implant contact lenses in packaging solution and the effect on *in vitro* drug release (drug release behavior) kinetics. In market, contact lenses are generally packaged in 1 to 2 ml of packaging solution such as 0.9% NaCl or phosphate buffer or STF [33]. The selected implant lenses (NP 1:3 batch) were sterilized in 2 ml of STF and stored at room temperature for 3 months. At the interval of 1 month and 3 months the lenses were removed and analyzed for *in vitro* release kinetics (procedure as described in Section 2.5.3) and the solution was analyzed for TM loss/leached in packaging solution using HPLC method.

2.6. Estimation of therapeutic dose

Ocular dose of TM eye drop (0.125% *w/v* solution) is one drop twice a day. Considering this amount, the daily dose delivered (considering 2 drop ≈ 100 μl) is about 125 μg. The ocular bioavailability through eye drop therapy is about 1% [34], which suggests that the therapeutic requirement is 1.25 μg/day. Therefore, the required dose for contact lens is 2.5 μg/day, considering 50% bioavailability through contact lens.

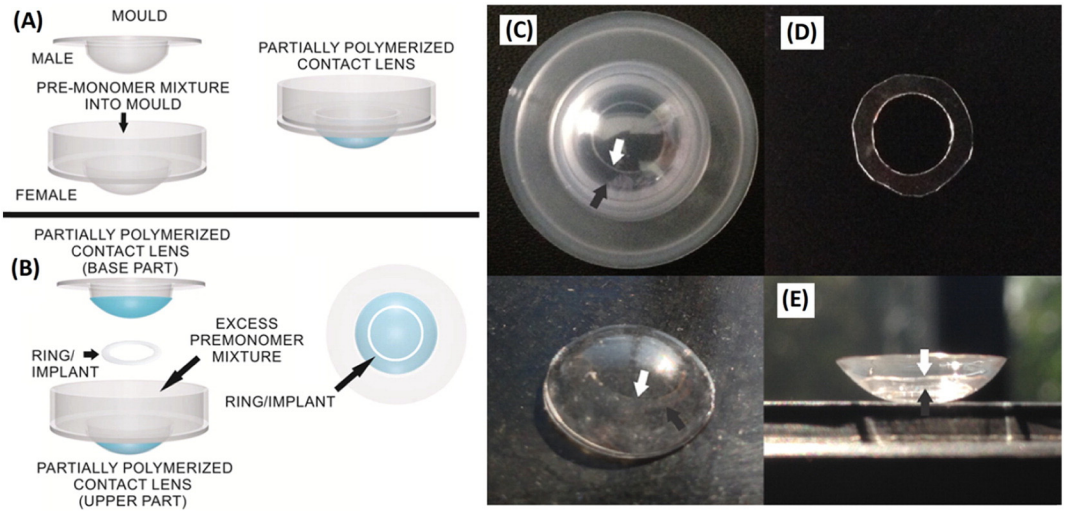

Fig. 1. Illustration of fabricating implant contact lenses using cast mould method. (A) Fabrication of partially polymerized contact lenses (50 μm thickness) using pre-monomer mixture & male and female mould. (B) Implantation of ring (TM-nanoparticle-laden implant) between partially polymerized contact lenses retained on respective mould. (C) Top view image of implant contact lens on male mould shows a clear 6 mm central aperture and a translucent ring. White arrow points to the inner margin of the ring implanted and black arrow points outer margin. (D) Image of TM-nanoparticle-laden ring. (E) Side view of implant contact lens.

Many scientists have demonstrated that, using contact lenses the bioavailability of drug is more than 50% to target tissue [8].

2.7. Ex vivo cytotoxicity study

Cytotoxicity study was performed prior to animal studies, to evaluate whether the leachable extract from the implant hydrogel contact lenses shows any cellular toxicity or not [35,36]. The rabbit corneal epithelial cell lines were used for *ex vivo* cytotoxicity testing. The cells were plated on 24-well flat-bottomed plates, with each well at a density of 1×10^4 cells, and incubated for 24 h at 37 °C in the CO_2 incubator. The cells were cultured in a complete medium consisting minimum essential medium with 10% foetal calf serum and 1% penicillin–streptomycin and incubated at 5% CO_2/37 °C/relative humidity (RH) > 90% for 24 h. After 24 h of incubation (~1 doubling period), the medium was replaced with test extract and again incubated for 24 h (5% CO_2, 37 °C, RH > 90%). Commercial contact lens of Bausch and Lomb (methafilcon) was used in the study for comparison. The cytotoxicity was evaluated using 3-[4, 5-dimethylthiazol-2-yl]-3, 5 diphenyltetrazolium bromide dye (MTT) assay. After 24 h the culture medium was aspirated and cells were washed twice with phosphate buffer solution (PBS). The cells were then incubated with 100 μl of the MTT solution (0.5 mg/ml) in Modified Eagle Medium (MEM) for 2 h at 37 °C. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and the absorbance was measured at 540 nm. The results of the experiments were evaluated to determine cell viability (%).

$$\text{Cell viability } (\%) = \text{T/C} \times 100 \quad (4)$$

where, T/C is the test treatment over control (T/C) values. If viability is reduced to <70% of the control, it has a cytotoxic potential.

2.8. Animal studies

The complete course of experiments were carried out using healthy white New Zealand rabbits of either sex weighing between 1.7–3.2 kg, obtained from the Central Animal House, Maliba Pharmacy College, Bardoli. The animals were housed under standard laboratory conditions of temperature (25 ± 2 °C), maintained on a 12 h light/dark cycle and had free access to food and water. The protocol of study was approved by the Institutional Animal Ethics Committee (IAEC) (Protocol No: MPC/IAEC/14/2014) and experiments were conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals).

2.8.1. Ocular irritation study on rabbit eyes

The TM loaded implant hydrogel contact lenses (NP 1:3) were extracted and the extracts were evaluated for ocular irritation [37]. *In vivo* biological reactivity was evaluated following the single instillation of the material extracts. Based on the ratio of 120 cm^2 (contact lens material): 20 ml of vehicle, the selected lenses were extracted in 0.9% sodium chloride solution (SC) and sesame oil (SO) at 37 °C for 72 h. The vials were agitated occasionally during extraction. The extraction vehicles without contact lenses served as a control blanks. The eyes of each rabbit were examined clinically with an auxiliary light source. A 0.2 ml dose of appropriate test extract was instilled in the lower conjunctival sac of the right eye of each rabbit (six rabbit per extract). The lids were gently held closed for a second following instillation. The corresponding vehicle control was similarly instilled into the left eye. Rabbits were returned to the cages after treatment and examined for ocular reactions with an auxiliary light source at 1, 24, 48, and 72 h after instillation. To detect corneal injury at every regular time intervals, the eyes were treated with florescent stain, flushed with 0.9% NaCl solution, and observed in a dark room under UV light. According to the Draize test [38], ocular irritation (cornea, iris and conjunctiva) was scored: non-irritating (score 0–3); slightly irritating (score 4–8); moderately irritating (score 9–12); and severely irritating (score 13–16). The requirements of the test were met if the test extracts showed no significant irritation compared to reagent control during the observation period.

2.8.2. In vivo pharmacokinetic study

Many times *in vitro* drug release kinetics are poorly correlated with *in vivo* drug release performance, we investigated the ability of the implant contact lenses (NP-1:3) to elute TM safely and effectively in New Zealand white rabbit eyes, in comparison to conventional TM eye drop therapy. This species is commonly used as the size and structure of the rabbits' eyes are close/similar to that of human eyes for contact lens wear [39]. Sterilized TM implant hydrogel contact lenses were carefully placed on cornea under the nictitating membrane without anaesthetization (n = 4 rabbits, left eye control, right eye implant contact lens wear). As the control, 50 μl of 0.5% *w/v* TM eye drop (250 μg TM), was gently instilled in the lower conjuctival sac of the rabbits using a micropipette (n = 4 rabbits). At regular time intervals for 10 days, 5 μl of tear fluid was collected via capillary action from the conjunctival sac using 5 μl disposable glass capillaries. The collected fluid was then treated with 5 μl perchloric acid (0.5 M) to precipitate protein, and finally 500 μl of methanol was added. After vortex oscillation and centrifugation (freeze centrifuge, 10,000 rpm for 15 min) the supernatant was collected and analyzed for drug content. TM in tear samples was quantified by LC–MS (TSQ Quantum Access, Thermo Scientific, USA). The concentration of TM in the tear fluid was measured as a function of time. During study of contact lens wear, the eyes were assessed for any abnormal reactions of cornea and conjunctiva or any signs of ocular discomfort. The measured drug concentration in tear fluid was plotted against time, and AUC (area under the curve) and MRT (mean residence time) were calculated.

2.8.3. In vivo pharmacodynamic study

New Zealand white rabbits were used for *in vivo* pharmacodynamic study to assess the anti-glaucoma activity of the developed implant hydrogel contact lenses (NP-1:3), in comparison with eye drop treatment. All the eyes were examined by portable slit lamp to exclude abnormalities before any procedure and at each time intra ocular pressure (IOP) was measured. The IOP of each eye was monitored daily, only those animals which shows a constant normotensive pressure (IOP = 16.2 ± 0.27 mm Hg) were selected for study. Twelve rabbits were randomized into two groups (n = 6) using the randomization function in Microsoft Excel. In first group (eye drops), each study animal received single drop (50 μl ≈ 250 μg of TM) of commercially available TM 0.5% *w/v* ophthalmic solution (TIMOLET 0.5% *w/v* eye drops) to the right eye and the left eye was kept control. In another group, TM implant contact lenses which were designed to release 150 μg of TM were placed in the right eye and left eye was kept as control. IOP were measured at regular time intervals using a Tonometer (Icare® *TONOVET* rebound tonometer, Tiolat, Helsinki, Finland). During the entire study, ocular discomfort, irritation or ocular toxicity was monitored [39].

3. Results and discussion

3.1. Characterization of nanoparticles

3.1.1. Particle size determination

The zetasizer report of nanoparticle batches is shown in Fig. 2. The size of particles prepared by double emulsion method was found to be in nano range (NP 1:1 = 261 nm, NP 1:2 = 397 nm, NP 1:3 = 340 nm), suggesting the suitability of preparation method. The results also suggest that the particle size is not related with the amount of ethyl cellulose in nanoparticles, and is instead controlled by the other processing parameters (data not shown). The PDI values describe the particle size distribution of the nanoparticles. All PDI values were lower than 0.15, which assures the narrow particle size distribution. In

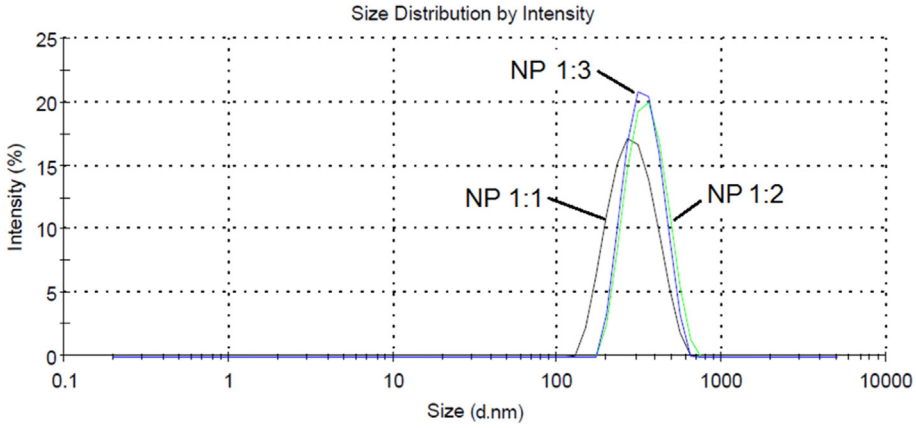

Fig. 2. Zetasizer report of particle size analysis.

the previous work by Ciolino [23], the uniform PLGA films were impregnated in contact lens which might decrease the ion diffusivity and oxygen permeability. While in our case we have loaded nanoparticles in ring which could overcome the issue of ion diffusivity to a certain extent.

3.1.2. X-ray diffraction study (XRD)

The XRD pattern of TM, ethyl cellulose (24–27 cp), and nanoparticle (NP-1:1, 1:2 and 1:3 ratios) batches are presented in Fig. 3. The XRD spectra of drug (TM) showed sharp and strong reflection peaks of crystallinity (highest peak at $2\theta = 19.47$, intensity $= 4054$); whereas the XRD pattern of TM in nanoparticles exhibited reduction in peak intensity compared to pure TM, suggesting decrease in crystallinity and encapsulation of TM in ethyl cellulose nanoparticles. The relative degree of crystallinity (RDC) was determined by comparing the peak height at 19.47 Pos. [2θ] in the diffraction patterns of TM with those of nanoparticles. The RDC for all the samples are shown in Table 1. A decline in the relative degree of crystallinity was observed, as the amount of polymer in nanoparticles increases (*i.e.* from batch 1:1 to 1:3), signifying a better encapsulation of TM in polymer and its conversion to amorphous state. The same is further assured by significant reduction in crystallite size of TM, when encapsulated in ethyl cellulose nanoparticles. The increase in d-spacing values, suggests the incorporation of TM between the planes of ethyl cellulose.

3.2. Sterility testing

Negative control media tubes (fluid thioglycollate media and Saboraud's media) did not show any growth or turbidity after 14 days, which confirmed the sterility of media. The positive control media tubes for both the media showed turbidity, confirming the ability of media to promote the growth of respective microorganisms. No evidence of microbial growth was observed after 14 days in both media tubes containing TM laden implant contact lenses (NP-1:3) under examination, which confirmed the sterility of contact lens.

3.3. Effective ion diffusivity

The data of effective ion diffusivity (ionoflux diffusion coefficients, D_{ion}) are shown in Table 2. The diffusion coefficient of methafilcon contact lenses (control group) was found to be 3.38×10^{-6} mm^2/min, while diffusion coefficient decreases significantly ($p < 0.05$) with implant contact lenses and also with increase in ratios of TM to ethyl cellulose. The decline in effective diffusion coefficient could be due to the presence of hydrophobic ethyl cellulose nanoparticles (comprising 20–25% area of contact lens). The results propose further reduction in thickness and surface area of ring. In the previous work by Ciolino and co-workers [23], the latanoprost-PLGA impregnated contact lenses, could decrease the ionoflux diffusion coefficients. The critical issue is

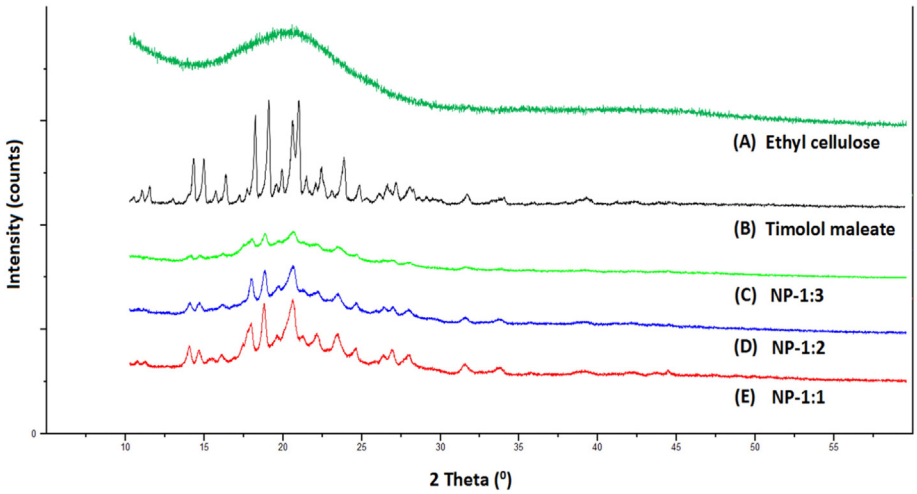

Fig. 3. X-ray diffraction spectra of (a) ethyl cellulose, (b) timolol maleate, (c) NP-1:3, (d) NP-1:2, and (e) NP-1:1.

Table 1
Results of X-ray diffraction spectra.

Type of system	d-Spacing [Å] at 20.17 [2θ] of ethyl cellulose	Intensity [cts] at 19.47 [2θ]	RDC	Crystallite size (Å)
TM	–	4054	–	671.77
Ethyl cellulose	4.398	–	–	–
NP-1:1	4.659	1352	33.35	47.38
NP-1:2	4.675	921	22.71	42.39
NP-1:3	4.758	660	16.28	38.33

addressed in this paper, as even the presence of nanoparticles decreased the effective ion diffusivity. Although there was reduction in ionoflux diffusion coefficients with implant lenses, the values are greater than 1.5×10^{-6} mm^2/min, which confirms that lens motion would be free from friction and abrasion to cornea.

3.4. In vitro release study

The cumulative TM release and release rate (ng/h) profiles in fresh STF from implant contact lenses (NP 1:1, NP 1:2 and NP 1:3) loaded with 150 μg of TM are shown in Figs. 4 and 5 respectively. Percentage leaching of TM after sterilization in 2 ml of STF was found to be 67.67 ± 3.1, 60.47 ± 4.2, and 41.33 ± 2.2 μg from NP 1:1, NP 1:2 and NP 1:3 batches respectively. Thus the major amount of drug was lost/leached during sterilization step, suggesting the limitation of therapeutic contact lenses. The leaching reduces with increase in ratio of drug:ethyl cellulose. As the amount of ethyl cellulose in nanoparticles increases, the barrier thickness (encapsulated wall) also increases, which causes hindrance for drug molecules to cross the barrier (thick wall) to release in simulated tear fluid media. In the previous work by Chauhan [8], the timolol–propoxylated glyceryl triacylate nanoparticle laden contact lenses had exhibited extended drug delivery, but the critical issue of drug leaching during sterilization step was not addressed.

In flux study, all three batches showed initial burst release, which could be due to small molecular weight (size) of drug (TM), solubility of drug in STF (2.4 mg/ml), and relatively high water content of hydrogel contact lens. The burst release was significantly ($p < 0.01$) reduced with increase in ethyl cellulose in nanoparticles. A significant ($p < 0.01$) enhancement in release rate duration was observed from batch NP-1:1 to NP-1:3 (Fig. 5). TM releases up to therapeutic level (105 ng/h) from NP-1:1, NP-1:2 and NP-1:3 batches were found to be 48 h, 84 h and 168 h respectively. The results clearly propose controlled drug delivery (sustained release), using nanoparticle-laden implant contact lenses. It was also observed that, certain part of drug remained permanently trapped (irreversibly bound) inside the nanoparticles.

To determine the rate controlling mechanism (resistance barrier) which controls the release duration of TM from implant contact lenses, we conducted *in vitro* flux study of NP 1:3 batch using nanoparticles (equivalent to 150 μg of TM), ring alone and compared it with implant contact lenses (without sterilization). The cumulative release profiles are shown in Fig. 6. The nanoparticles alone, *i.e.* without loading or entrapping in implant contact lens, showed high initial burst release of 89 μg, and releases entire drug within 48 h. The ring alone, *i.e.* nanoparticle-laden ring without implanting in contact lens, also showed high initial burst release of 65 μg, and gets leached off completely within 72 h. Only the implant contact lens, *i.e.* nanoparticle-laden ring implanted in contact lens, showed relatively low burst release and extended the release duration for 168 h. The sustain release duration was observed in following order; nanoparticles < rings < implant contact lenses. The results suggest the involvement of all three; hydrogel contact lens matrix, implant matrix (ring), and ethyl cellulose nanoparticles in controlling drug release duration. The mechanism of controlled drug delivery, may involve slow diffusion of water (tear fluid) from aqueous channels of contact lens to ring matrix, and subsequent penetration in the tortuous structure (created after leaching of TM) of ethyl cellulose nanoparticles, followed by drug dissolution and diffusion in release media.

Table 2
Data of effective ion diffusivity and cytotoxicity study.

Batch	Ionoflux diffusion coefficient, [$D_{ion} \times 10^{-6}$ (mm^2/min)]	% cell viability
Methafilcon contact lenses	3.38 ± 0.19	94.65 ± 2.18
NP 1:1	2.25 ± 0.15	95.60 ± 2.42
NP 1:2	1.94 ± 0.17	94.60 ± 3.52
NP 1:3	1.61 ± 0.21	91.60 ± 2.42

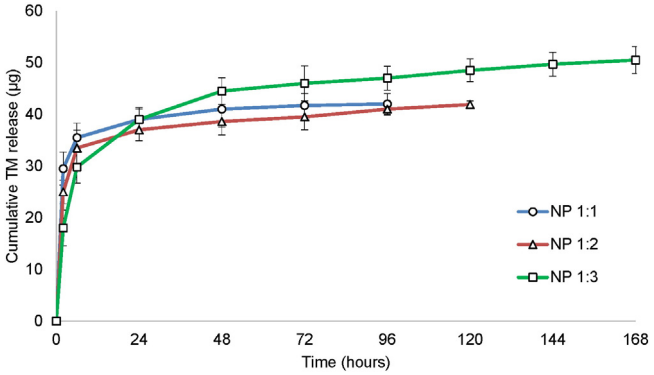

Fig. 4. Cumulative TM release from nanoparticle-laden implant contact lenses. The drug loading in all batches was 150 μg. Values are shown as mean ± standard deviations (n = 6).

3.5. Effect of packaging solution

In packaging study, implant contact lenses were sterilized before storage in packaging solution (2 ml). After 0, 30 and 90 days, the lenses were removed and the TM content in the packaging solution was analyzed using HPLC. On day 0 (*i.e.* on the same day of sterilization), TM leached out due to sterilization was 41.33 ± 3.21%, while analysis data on day 30th and 90th showed loss of 49.33 ± 8.82% and 53.00 ± 7.23% respectively. The results indicate that, drug leached/loss (excluding loss due to sterilization) during storage period, *i.e.* after 30th and 90th days, was 12 μg (8%) and 17.5 μg (11.66%) respectively, which was not significant ($p > 0.05$) considering 150 μg loading.

Fig. 7 shows the *in vitro* release data of implant contact lenses (NP 1:3 batch) after 30th and 90th days of storage in packaging solutions. Though the drug leaching data was not significant, the *in vitro* release profiles showed reduction in release duration of TM with time. The release of TM within therapeutic level (105 ng/h) after 1 and 3 months

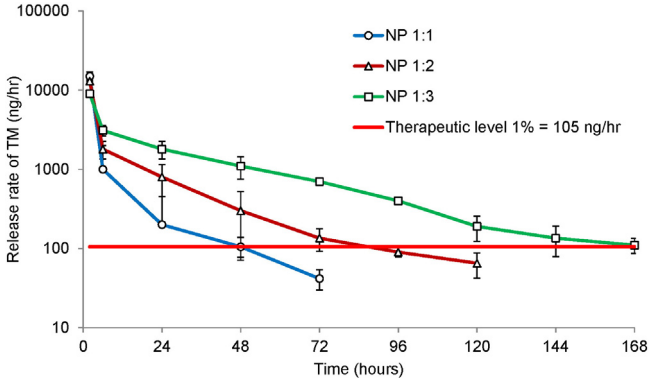

Fig. 5. Release rate (ng/h) of TM from nanoparticle-laden implant contact lenses. Values are shown as mean ± standard deviations (n = 6). Therapeutic level 1% indicates minimum therapeutic drug release rate (105 ng/h) required, considering 1% absorption from 0.125% *w/v* TM eye drops and 50% bioavailability from contact lenses.

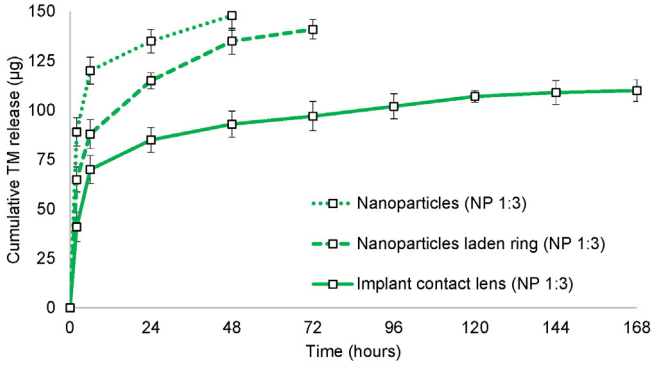

Fig. 6. Cumulative TM release from nanoparticles, ring and implant contact lenses (NP 1:3 batch, without sterilization). The drug loading was 150 μg. Values are shown as mean ± standard deviations (n = 6).

was found to be 120 h and 96 h respectively. The similarity factor f_2 value was found to be 66.17 and 55.12 after 1 and 3 months respectively with reference to zero time (initial period), suggesting similarity in release profiles with time. While, the f_1 value was found to be 13.45 and 26.24 after 1 and 3 months respectively, suggesting the dissimilarity in release profiles after 3 months period [40]. Therefore it is not preferred to supply/market the implant contact lenses in hydrated condition. The option of sterilizing lenses in dry state using radiation sterilization technique and packing under sterile condition and dispensing, will bypass the problem of drug loss during sterilization and storage in packaging solution.

3.6. Ex vivo cytotoxicity studies

Extract of implant contact lenses were evaluated for cytotoxicity by incubating cells in media that contained extract of sample lenses under test, or methafilcon lens (marketed preparation), or vehicle (media only, control group). Cell viability of NP 1:1, NP 1:2, NP 1:3 and methafilcon contact lenses were found to be 95.60 ± 2.42%, 94.60 ± 3.52%, 91.60 ± 2.42% and 94.65 ± 2.18% respectively (Table 2), when compared with cells exposed to media only (control group). Thus, all three batches of TM loaded nanoparticle-laden implant contact lenses were safe for contact lens wear, as the viability was >70%.

3.7. Ocular irritation study on rabbit eyes

The ocular irritation test has been used historically for evaluating *in vivo* biological reactivity, following a single instillation of the material extract in conjunctival sac of the rabbit's eye [41]. The batch NP 1:3 showed the most promising results (minimum drug loss during sterilization and maximum sustained release), thus was chosen for further *in vivo* evaluation. Rabbit's right eye was treated with test extract and left eye received physiological saline or sesame oil as control. Evaluation was performed by macroscopic examination using auxiliary light source. No symptoms of ocular irritation such as opacity of cornea, inflammation or swelling of iris, conjunctivae redness, chemosis, and discharge were observed after instillation of test extract of saline in accordance with control at various time intervals. Redness and chemosis of conjunctivae of grade 1 was observed with sesame oil control and test extract, which was reduced with time (Fig. 8). This may be due to the unctuous/non-polar nature of sesame oil. The eye irritation scores for all groups were less than 1, indicating excellent ocular tolerance of TM laden ring implant hydrogel contact lenses.

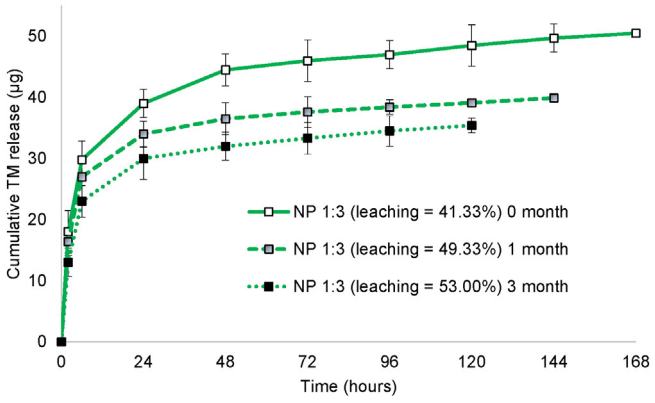

Fig. 7. The profiles of TM release from implant contact lenses (NP 1:3 batch) stored in packaging solution for 1 and 3 months. Values are shown as mean ± standard deviations (n = 6).

3.8. In vivo pharmacokinetic study

The *in vivo* kinetic study was performed to compare the tear fluid concentrations of TM achieved by continuous wear of implant contact lenses (150 μg loading, NP-1:3) with conventional therapy of 0.5% *w/v* TM eye drop (one drop ≈ 50 μl ≈ 250 μg TM) solution (Fig. 9). TM released in tear fluid from implant contact lenses and eye drop treated group was quantified using high-performance liquid chromatography–mass spectrometry (LC–MS). Drug released from implant contact lenses (24 h reading) and pure commercial TM standard (97% *w*/w purity) demonstrated substantially similar high resolution mass spectra (Fig. 10). The concentration of TM released in tear fluid was quantified using standard curve derived from standard TM. The lower detection limit of TM was 10 ng/ml. The LC–MS results confirmed the integrity of drug eluted from the implant contact lenses as TM.

A maximum concentration (C_{max}) of 132.6 ± 15 μg/ml was measured at 5 min after eye drop administration. The concentration vs. time curve was found to be consistent with previous studies that demonstrated rapid decline in concentrations over the first 4 h and little, if any, drug present after 5 h [8,42]. The TM implant contact lenses showed an initial burst release (C_{max} = 6.79 ± 0.52 μg/ml, significantly lower in comparison to eye drop group, $p < 0.01$), followed by a steady TM release in tear fluid, clearly indicating sustained release of TM for 8 days. After 8 days, the TM in tear fluid was not detected on LC–MS. Prolonged retention in the precorneal area using contact lenses, could decrease the dosing frequency of TM, and, in consequence, much

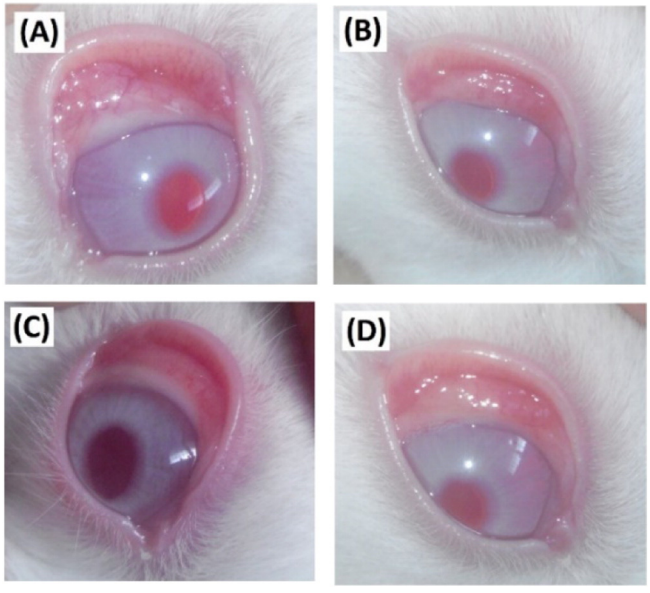

Fig. 8. Images of ocular irritation study after 4 h. (A) Control sesame oil, (B) control saline, (C) test sesame oil, and (D) test saline.

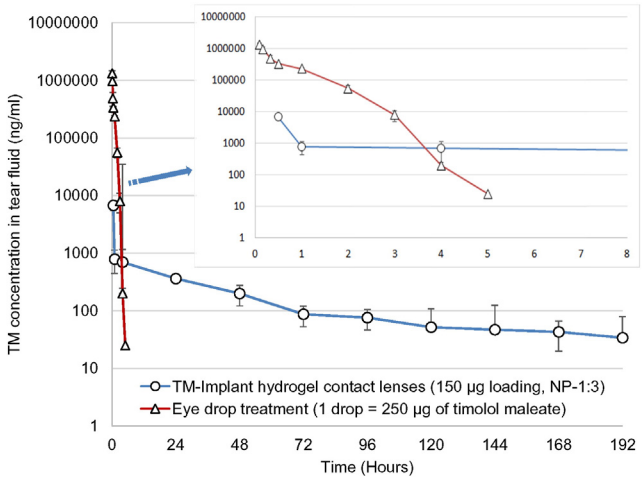

Fig. 9. TM tear fluid concentration-time profile after application of sterilized TM implant contact lenses (150 μg of TM loading, NP 1:3 batch) and single instillation of TM eye drop (50 μl = 250 μg of TM). Each point represents the mean ± S.D. (n = 4). The inset shows the initial 8 h release profile.

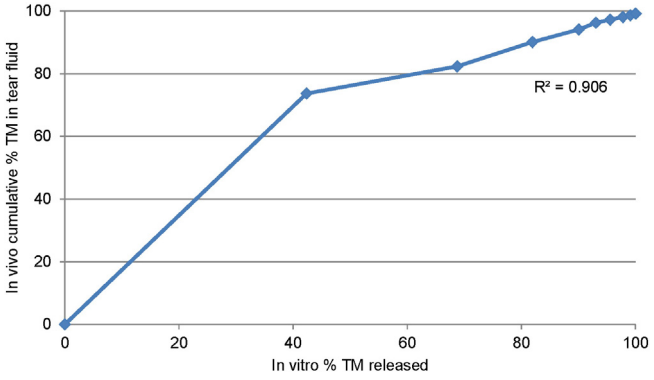

Fig. 11. Levy plot for the relationship between the *in vivo* cumulative percentage of TM in tear fluid over 168 h and the percentage of TM released in vitro over 168 h ($R^2 = 0.906$). R^2 = correlation coefficients.

lower dose of TM is required to achieve the desired therapeutic levels in comparison with 0.5% *w/v* TM eye drop therapy.

The evaluation of IVIVC is essential to demonstrate the ability of in vitro TM released characteristics to forecast the *in vivo* performance of implant contact lenses [43,44]. Fig. 11 represents the level A point to point IVIVC plot (Levy plot) for each time point (168 h), the in vitro % TM released (NP 1:3 batch) against the *in vivo* cumulative % TM in tear fluid. The implant contact lens do show correlation coefficient ($R^2 = 0.906$), between drug release in vitro and drug retained in tear fluid. The linear relationship suggests that the *in vitro* drug release test was foretelling *in vivo* presence of TM in tear fluid from implant contact lenses.

3.9. In vivo pharmacodynamic study in rabbit model

The ability of TM laden ring implant contact lenses (batch NP 1:3) to show extended therapeutic effect (*i.e.* reduction in IOP), *in vivo* was tested in an experimental model using rabbits. The normotensive IOP was 16.03 ± 1.2 mm Hg. No significant day-to-day variations/differences ($p = 0.434$) was observed in the normotensive IOP measurement for each animal. The IOP in the TM eye drop treated group (single instillation) decreased from baseline by 4.4 ± 0.50 mm Hg after 2 h, which was recovered after 12 h to normotensive pressure (Fig. 12). There was no significant variation ($p = 0.298$) in IOP measured in treated eye between animals. No significant difference ($p = 0.342$) was detected among IOP reading at each interval from baseline in the untreated eye (left eye). When TM was delivered by implant contact lenses (150 μg, NP-1:3 batch), the IOP in the treated eye was significantly decreased from baseline by 6.3 ± 1.92 mm Hg after 3 h, which was

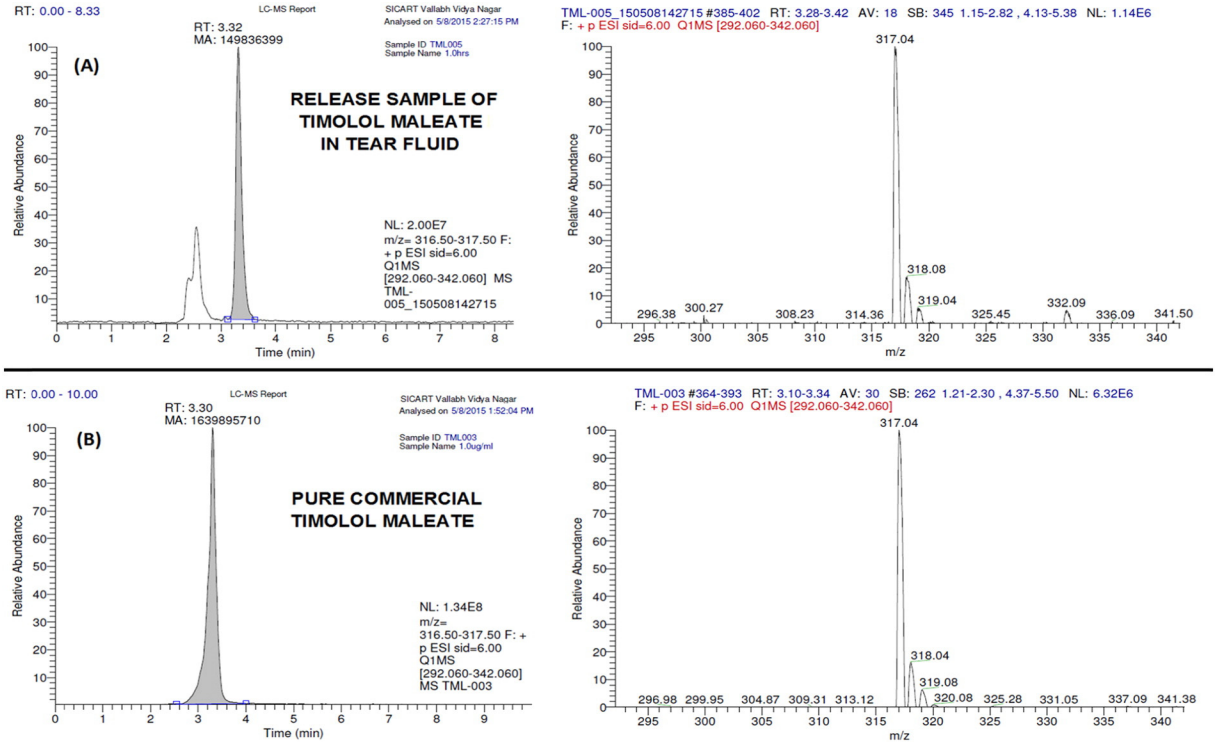

Fig. 10. High-performance liquid chromatography–mass spectrometry (LC–MS) of TM samples. Media containing TM released over 24 h from a TM implant contact lenses (NP-1:3) demonstrated ion chromatograms and annotated high-resolution mass spectra (A) that were substantially similar to those of pure commercial TM (B). The mass spectra of both samples generated the same fragmentation pattern of the protonated ion [TM þ H] þ at m/z 317.04 for TM. m/z = mass to charge ratio.

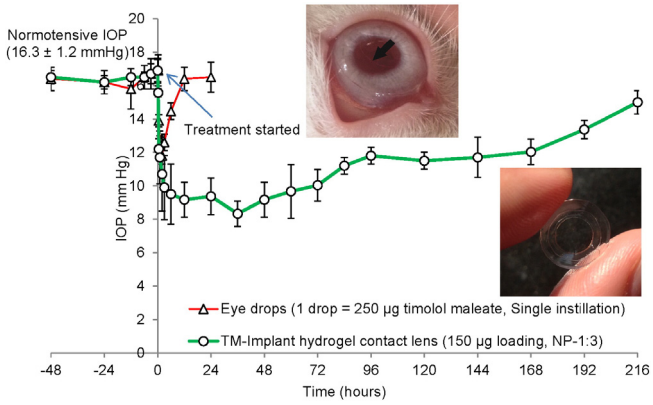

Fig. 12. Change in Intraocular pressure (IOP) in mm Hg in rabbits treated with eye drop (1 drop = 250 µg dose) and TM laden ring implant hydrogel contact lens (NP 1:3, 150 µg TM loading). Each point represents the mean ± S.D. (n = 6).

maintained below normotensive pressure for 192 h. For individual animal, there was no significant day-to-day difference in IOP ($p = 0.130$). For untreated eye (left eye), there was no significant difference among the IOP on any day and the baseline ($p = 0.122$). Thus in comparison to single instillation of eye drop (dose = 250 µg), TM implant contact lens (150 µg) showed 1.3 fold higher reduction in IOP with lower dose. The results suggest the improvement in drug residence time and higher bioavailability of TM using implant contact lenses. *In vitro* release profile showed 168 h therapeutic release (104 ng/h), while *in vivo* study showed 192 h sustained therapeutic effect (reduction in IOP), which could be due to slow release of drug, due to low tear volume in the eye (10 µl) in comparison to 2 ml of *in vitro* release media. Thus, it was successfully proven that the TM laden ring implant contact lenses showed therapeutic effect necessary for treatment of glaucoma for 192 h.

During pharmacodynamic study period (10 days), the animals showed protein adsorption, which produced grade 1 redness in conjunctiva of rabbit eye. The redness was due to protein adsorption, as in ocular irritation test no such redness (irritation) was observed. Thus it was well established that, the presence of ethyl cellulose caused protein adsorption due to its hydrophobic nature. This problem could be circumvented using a biodegradable polymer like PLGA or chitosan. Joseph et al. used PLGA film with HEMA to release latanoprost for 4 weeks [23]. But the issues like degradation of PLGA in the presence of packaging solution with time, the alteration in ion diffusivity due to presence of film, leaching of drug during sterilization conditions, and the stability of latanoprost at 35 °C (during contact lens wear) were not addressed. Dean Ho et al. synthesized TM-loaded nanodiamond nanogels by cross-linking polyethyleneimine coated nanodiamonds and partially N-acetylated chitosan [45]. The system releases TM in the presence of lacrimal fluid lysozyme (content of lysozyme varies from person to person and also during diseased condition), leaving the lens intact. We used this particular polymer system (ethyl cellulose) primarily because of its non-biodegradable nature to sustain the drug delivery. We can also optimize the amount of ethyl cellulose and surface area of ring to prevent protein adsorption in future studies.

4. Conclusion

The study demonstrated the successful application of implantation technology to overcome the alterations in critical contact lens properties and justifies its potential scope in the field of ocular drug delivery. Nanoparticle-laden implant contact lens releases the drug for an extended period of time, likely due to slow diffusion of drug through the polymeric wall of ethyl cellulose nanoparticles and matrix structure of ring and implant. Although, the nanoparticle-laden implant contact lenses loses majority of the loaded drug during sterilization step, NP 1:3 batch showed sustained release for more than 168 h. Packaging study indicates that, implant lenses cannot be stored in hydrated condition (in packaging solution), as the system loses its integrity to show sustained release kinetics. Therefore the implant contact lenses need to be stored in dry state (without hydration) after radiation sterilization. The patients or ophthalmologist can hydrate the dry lenses at the time of use. This issue can also be resolved by adding sufficient (optimized) amount of drug in packaging solution. The presence of drug in packaging solution may prevent leaching of drug, *i.e.* will maintain the equilibrium of drug between packaging solution and contact lens. The implant contact lenses appeared safe in *ex vivo* cytotoxicity and animal study. *In vivo* pharmacokinetic study, showed sustained drug release for more than 192 h in tear fluid, suggesting improvement in drug residence time and higher bioavailability of drug using implant contact lenses. The *in vivo* pharmacodynamic results showed significant reduction in IOP from baseline for 192 h with low drug loading in comparison to eye drop treatment. Within this work, the concept of implantation technology has been shown as a valuable method for sustained ophthalmic drug delivery using contact lenses without compromising optical and physical properties of contact lens.

Disclosures

There are no potential conflicts of interest to disclose for this work.

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References

[1] J.C. Lang, Ocular drug delivery conventional ocular formulations, Adv. Drug Deliv. Rev. 16 (1995) 39–43.
[2] I. Carvalho, C. Marques, R. Oliveira, P. Coelho, P. Costa, D. Ferreira, Sustained drug release by contact lenses for glaucoma treatment—a review, J. Control. Release 202 (2015) 76–82.
[3] G. Novack, Ophthalmic drug delivery: development and regulatory considerations, Clin. Pharmacol. Ther. 85 (2009) 539–543.
[4] R.M. Mainardes, M.C. Urban, P.O. Cinto, N.M. Khalil, M.V. Chaud, R.C. Evangelista, et al., Colloidal carriers for ophthalmic drug delivery, Curr. Drug Targets 6 (2005) 363–371.
[5] H. Gupta, M. Aqil, Contact lenses in ocular therapeutics, Drug Discov. Today 17 (2012) 522–527.
[6] S.K. Sahoo, F. Dilnawaz, S. Krishnakumar, Nanotechnology in ocular drug delivery, Drug Discov. Today 13 (2008) 144–151.
[7] C.-C. Li, A. Chauhan, Modeling ophthalmic drug delivery by soaked contact lenses, Ind. Eng. Chem. Res. 45 (2006) 3718–3734.
[8] H.J. Jung, M. Abou-Jaoude, B.E. Carbia, C. Plummer, A. Chauhan, Glaucoma therapy by extended release of timolol from nanoparticle loaded silicone-hydrogel contact lenses, J. Control. Release 165 (2013) 82–89.
[9] H. Chandasana, Y.D. Prasad, Y.S. Chhonker, T.K. Chaitanya, N.N. Mishra, K. Mitra, et al., Corneal targeted nanoparticles for sustained natamycin delivery and their PK/PD indices: an approach to reduce dose and dosing frequency, Int. J. Pharm. 477 (2014) 317–325.
[10] A. Guzman-Aranguez, B. Colligris, J. Pintor, Contact lenses: promising devices for ocular drug delivery, J. Ocul. Pharmacol. Ther. 29 (2013) 189–199.
[11] K.-H. Hsu, S. Gause, A. Chauhan, Review of ophthalmic drug delivery by contact lenses, J. Drug Deliv. Sci. Technol. 24 (2014) 123–135.
[12] V.P. Costa, M.E. Braga, C.M. Duarte, C. Alvarez-Lorenzo, A. Concheiro, M.H. Gil, et al., Anti-glaucoma drug-loaded contact lenses prepared using supercritical solvent impregnation, J. Supercrit. Fluids 53 (2010) 165–173.
[13] J. Xu, X. Li, F. Sun, Cyclodextrin-containing hydrogels for contact lenses as a platform for drug incorporation and release, Acta Biomater. 6 (2010) 486–493.
[14] C.-C. Peng, A. Ben-Shlomo, E.O. Mackay, C.E. Plummer, A. Chauhan, Drug delivery by contact lens in spontaneously glaucomatous dogs, Curr. Eye Res. 37 (2012) 204–211.
[15] K.-H. Hsu, B.E. Carbia, C. Plummer, A. Chauhan, Dual drug delivery from vitamin E loaded contact lenses for glaucoma therapy, Eur. J. Pharm. Biopharm. 94 (2015) 312–321.
[16] M.E. Braga, V.P. Costa, M.J. Pereira, P.T. Fiadeiro, A.P.A. Gomes, C.M. Duarte, et al., Effects of operational conditions on the supercritical solvent impregnation of acetazolamide in Balafilcon A commercial contact lenses, Int. J. Pharm. 420 (2011) 231–243.

[17] A. Ribeiro, F. Veiga, D. Santos, J.J. Torres-Labandeira, A. Concheiro, C. Alvarez-Lorenzo, Hydrophilic acrylic hydrogels with built-in or pendant cyclodextrins for delivery of anti-glaucoma drugs, Carbohydr. Polym. 88 (2012) 977–985.
[18] H. Hiratani, C. Alvarez-Lorenzo, Timolol uptake and release by imprinted soft contact lenses made of N, N-diethylacrylamide and methacrylic acid, J. Control. Release 83 (2002) 223–230.
[19] C.-C. Li, M. Abrahamson, Y. Kapoor, A. Chauhan, Timolol transport from microemulsions trapped in HEMA gels, J. Colloid Interface Sci. 315 (2007) 297–306.
[20] C.-C. Peng, J. Kim, A. Chauhan, Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing vitamin E diffusion barriers, Biomaterials 31 (2010) 4032–4047.
[21] C.-C. Peng, M.T. Burke, B.E. Carbia, C. Plummer, A. Chauhan, Extended drug delivery by contact lenses for glaucoma therapy, J. Control. Release 162 (2012) 152–158.
[22] J.B. Ciolino, T.R. Hoare, N.G. Iwata, I. Behlau, C.H. Dohlman, R. Langer, et al., A drug-eluting contact lens, Invest. Ophthalmol. Vis. Sci. 50 (2009) 3346–3352.
[23] J.B. Ciolino, C.F. Stefanescu, A.E. Ross, B. Salvador-Culla, P. Cortez, E.M. Ford, et al., In vivo performance of a drug-eluting contact lens to treat glaucoma for a month, Biomaterials 35 (2014) 432–439.
[24] M. Cetin, A. Atila, Y. Kadioglu, Formulation and in vitro characterization of Eudragit® L100 and Eudragit® L100-PLGA nanoparticles containing diclofenac sodium, AAPS PharmSciTech 11 (2010) 1250–1256.
[25] F.A. Maulvi, V.T. Thakkar, T.G. Soni, T.R. Gandhi, Optimization of aceclofenac solid dispersion using Box–Behnken design: in-vitro and in-vivo evaluation, Curr. Drug Deliv. 11 (2014) 380–391.
[26] F.A. Maulvi, S.J. Dalwadi, V.T. Thakkar, T.G. Soni, M.C. Gohel, T.R. Gandhi, Improvement of dissolution rate of aceclofenac by solid dispersion technique, Powder Technol. 207 (2011) 47–54.
[27] F.A. Maulvi, T.G. Soni, D.O. Shah, Extended release of timolol from ethyl cellulose microparticles laden hydrogel contact lenses, Open Pharm. Sci. J. 2 (2015) 1–12.
[28] Glasbey T, Newman SD. Methods and systems for contact lens sterilization. US Patent No. 8109064; 2012.
[29] J. Pozuelo, V. Compañ, J.M. González-Méijome, M. González, S. Mollá, Oxygen and ionic transport in hydrogel and silicone-hydrogel contact lens materials: an experimental and theoretical study, J. Membr. Sci. 452 (2014) 62–72.
[30] C.-C. Peng, A. Chauhan, Ion transport in silicone hydrogel contact lenses, J. Membr. Sci. 399 (2012) 95–105.
[31] F.A. Maulvi, T.G. Soni, D.O. Shah, Extended release of hyaluronic acid from hydrogel contact lenses for dry eye syndrome, J. Biomater. Sci. Polym. Ed. 26 (2015) 1035–1050.
[32] F.A. Maulvi, T.G. Soni, D.O. Shah, Effect of timolol maleate concentration on uptake and release from hydrogel contact lenses using soaking method, J. Pharm. Appl. Sci. 1 (2014) 17–23.
[33] H.J. Jung, A. Chauhan, Temperature sensitive contact lenses for triggered ophthalmic drug delivery, Biomaterials 33 (2012) 2289–2300.
[34] V.R. Kearns, R.L. Williams, Drug delivery systems for the eye, Expert Rev. Med. Devices 6 (2009) 277–290.
[35] R. Mencucci, D.E. Pellegrini-Giampietro, I. Paladini, E. Favuzza, U. Menchini, T. Scartabelli, Azithromycin: assessment of intrinsic cytotoxic effects on corneal epithelial cell cultures, Clin. Ophthalmol. (Auckland, NZ) 7 (2013) 965–973.
[36] M. Dutot, J. Vincent, N. Martin-Brisac, I. Fabre, C. Grasmick, P. Rat, Ocular cytotoxicity evaluation of medical devices such as contact lens solutions and benefits of a rinse step in cleaning procedure, ALTEX 30 (2013) 41–49.
[37] R. Alany, T. Rades, J. Nicoll, I. Tucker, N. Davies, W/O microemulsions for ocular delivery: evaluation of ocular irritation and precorneal retention, J. Control. Release 111 (2006) 145–152.
[38] J.H. Draize, G. Woodard, H.O. Calvery, Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, J. Pharmacol. Exp. Ther. 82 (1944) 377–390.
[39] S. Yu, Q.-M. Wang, X. Wang, D. Liu, W. Zhang, T. Ye, et al., Liposome incorporated ion sensitive in situ gels for opthalmic delivery of timolol maleate, Int. J. Pharm. 480 (2015) 128–136.
[40] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci. 13 (2001) 123–133.
[41] L. Gan, Y. Gan, C. Zhu, X. Zhang, J. Zhu, Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: in vitro and in vivo results, Int. J. Pharm. 365 (2009) 143–149.
[42] H. Sasaki, K. Yamamura, T. Mukai, K. Nishida, J. Nakamura, M. Nakashima, et al., In vivo ocular pharmacokinetic model for designing dosage schedules and formulations of ophthalmic drugs in human, Acta Med. Nagasaki. 42 (1997) 45–50.
[43] J.M. Sankalia, M.G. Sankalia, R.C. Mashru, Drug release and swelling kinetics of directly compressed glipizide sustained-release matrices: establishment of level A IVIVC, J. Control. Release 129 (2008) 49–58.
[44] G. Levy, Effect of dosage form on drug absorption a frequent variable in clinical pharmacology, Arch. Int. Pharmacodyn. Ther. 152 (1964) 59–68.
[45] H.-J. Kim, K. Zhang, L. Moore, D. Ho, Diamond nanogel-embedded contact lenses mediate lysozyme-dependent therapeutic release, ACS Nano 8 (2014) 2998–3005.