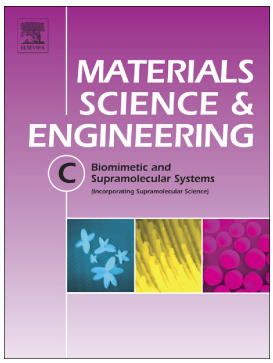

Multiple drug delivery from the drug-implants-laden silicone contact lens: Addressing the issue of burst drug release

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PII: S0928-4931(19)32177-0

DOI: https://doi.org/10.1016/j.msec.2020.110885

Reference: MSC 110885

To appear in: *Materials Science & Engineering C*

Received date: 13 June 2019

Accepted date: 20 March 2020

Please cite this article as: A.R. Desai, F.A. Maulvi, D.M. Desai, et al., Multiple drug delivery from the drug-implants-laden silicone contact lens: Addressing the issue of burst drug release, *Materials Science & Engineering C* (2018), https://doi.org/10.1016/j.msec.2020.110885

Multiple drug delivery from the drug-implants-laden silicone contact lens: Addressing the issue of burst drug release

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Abstract

A fixed combination of bimatoprost/timolol eye drop solution is used to manage the elevated intra-ocular pressure in glaucoma patients, including individuals whose condition is poorly controlled by monotherapy. Eye drop solutions are generally given in high dose, due to poor ocular bioavailability. The high ocular dose of bimatoprost and timolol lead to hyperaemia and systemic cardiac side effects respectively. Here, we introduce multiple implant-laden contact lenses (IM) to passively deliver timolol, bimatoprost and hyaluronic acid at therapeutically relevant doses without high burst release. The drug-loaded implants were individually implanted in the outer periphery of the silicone contact lenses. Atomic force microscopy showed the smooth surface of the implant contact lens, as the implants were inside the contact lens matrix. The implant lens (IM) showed major loss of drugs [timolol = 60.60 %, bimatoprost = 61.75 % and HA = 46.03 %] during the monomer extraction and wet sterilization, while the option of dry radiation sterilization (IM-R lens) and hydration for 24 h prior to use showed relatively lower loss of drugs [timolol = 16.87 %, bimatoprost = 47.95 % and HA = 24.41 %]. The *in-vitro* drugs release data of IM-R lens, showed sustained release for 72 h, with low burst release in comparison to the soaked (SM) and direct drug-laden contact lenses (DL). The *in vivo* drug release data in the rabbit tear fluid showed sustained release using IM-R lens in comparison to the SM lens and eye drop therapy. The burst release with the IM-R lens was many folds reduced, which could bypass the side effects associated with multiple eye drop therapy. The *in vivo* pharmacodynamic study in the rabbit model showed peak and valley profile with multiple eye drop therapy, while IM-R lens showed prolong reduction in intra ocular pressure (IOP) for 120 h. The study demonstrates the application of implantation technology to deliver multiple drug through contact lenses to treat glaucoma.

Key words: Timolol, bimatoprost, hyaluronic acid, silicone contact lenses, implantation technology, animal studies.

1. Introduction

Glaucoma is a chronic disease, which is currently treated using the fixed combination of bimatoprost/timolol eye drops (once daily) to lower the elevated intra-ocular pressure (IOP), including for individuals who are uncontrolled by monotherapy [1]. A three month clinical study using a once-daily dose of bimatoprost/timolol eye drops showed significant reduction in IOP in comparison to the twice-daily timolol eye drop solution [2, 3]. However, the eye drop solutions are administered at high dose (strength), as they show poor bioavailability (1-5%) due to the short precorneal residence time and the physiological & anatomical barriers in the eye [4-6]. The exposure of the ocular surface to such high doses of bimatoprost (1 drop ≈ 15 µg) and timolol (1 drop ≈ 250 µg) lead to hyperaemia [7] and systemic side effects [8, 9] respectively. In addition, the eye drops showed a short duration of action, which requires frequent dosing affecting the routine life style of patients [10, 11]. Thus, a novel approach is needed which can deliver multiple drug at therapeutically relevant doses without initial high burst release to the ocular tissues with improved ocular bioavailability.

The applications of the contact lenses have received a great deal of attention among scientists in the last few decades for the ocular drug delivery, due to its biocompatibility, versatility and low cost [12-15]. In comparison to the eye drop solutions, the ocular bioavailability could be improved many folds using therapeutic contact lenses [16-18]. Substantial research has been devoted to the development of strategies to control the release of drugs from the contact lenses, like conventional soaking methods, molecular imprinting techniques, drug loaded polymeric nanoparticles, drug-PLGA-film, ion-ligand gels and supercritical fluid technology [19-26]. The approaches demonstrated the application of the contact lenses to sustain the release of drugs. However, they may not be optimal for clinical translation due to high burst release and the changes in the desired optical and physical properties of the contact lens material (e.g. transparency, swelling, contact angle) after drug loading [27].

Chi-Chung et al., 2005 prepared timolol soaked contact lenses, which showed improvement in the drug residence time; however the lenses showed high burst release [28]. Jinku Xu et al., 2010 included β-cyclodextrin in the pHEMA hydrogel using puerarin

as a model drug to treat glaucoma. The modified hydrogel showed increase in the swelling behavior and the tensile strength. However, due to high initial drug release, the release rate dropped within few hours [29]. Kuan Hui et al., 2015 developed contact lenses for simultaneous release of timolol and dorzolamide using Vitamin E (physical barrier). The contact lens reduced IOP for 48 h in the rabbit eyes with low dose in comparison to the eye drop therapy [30]. Hyun Jung et al., 2013 dispersed timolol-nanoparticles of propoxylated glyceryl triacylate in the silicone contact lenses to deliver timolol for 30 days. However, the changes in the critical lens properties and drug leaching in the packaging solution remain an issue [31]. Hiratani Haruyuki et al., 2013 applied molecular imprinting technology to develop timolol loaded contact lenses to deliver drug for 24 h. The imprinted lenses showed improvement in the drug residence time in the tear fluid with lower doses than for the eye drop solution [32]. Joseph B. Ciolino et al., 2014 developed latanoprost-eluting contact lenses by implanting the latanoprost-loaded film in the contact lens, which showed prolonged delivery for > 30 days. However, the critical lens properties like mechanical property, water content, ion permeability and oxygen permeability need to be addressed before clinical studies [33, 34]. Herminio et al., 2010 used discontinuous supercritical solvent impregnation technology to load the acetazolamide and timolol maleate in a commercial silicone-based hydrogel contact lenses. The system showed initial burst release and low drug impregnation in the core of the contact lens [35]. Zeeshan Ahmad et al., 2017 used an electrospinning method to engineer timolol-nanofibers which were further coated on the exterior of the contact lenses along with four different permeation enhancers. However, in this method > 85% of drug was released within first 24 h [36]. James D. Brandt et al., 2017 developed a peri-ocular bimatoprost ring which rested on the surface of the inner conjunctiva. The ring extended the release of drug for months. However, a few incidences of eye discharge were observed in the clinical studies. The position of the ring indicates the high chance of nonproductive absorption of drug in the systemic circulation rather than ocular tissues [37, 38]. The above approaches showed that the drug-laden contact lenses could be used to sustain the release of ophthalmic drugs, but the issue of high initial burst release exits and the incorporation of drug or drug loaded formulation changes the transmittance and the physical properties of the contact lenses

[13, 27, 39]. In addition, regular contact lens users have also reported pink eye syndrome at the end of the day; indicating challenges in acceptance as a medical device by the patients [40, 41].

To overcome the issues with therapeutic contact lenses, we have designed a novel preservative free multiple drug loaded implant-laden-contact lens that could uphold the release rate of drugs within therapeutic levels without high initial burst release. The bimatoprost, timolol and hyaluronic acid (HA, comfort agent) loaded implants were implanted in the outer periphery of the silicone contact lens (6 mm inner diameter and 8 mm outer diameter) leaving the centre portion for clear vision [42]. The burst release of drug was prevented to some extent, by entrapping the drugs in the matrix structure of implant. The HA (physical lubricant) release from the implant contact lens will provide comfort to the eyes, as the lens was proposed for extended use. Thus the preservative free implant contact lens could prevent side effects associated with high initial burst release and could improve patient adherence (comfort) to the multiple drug therapy for the treatment of chronic diseases like glaucoma.

2. Materials and Methods

2.1. Materials

Hydroxyl ethylmethacrylate (HEMA), Irgacure® 184 (1-Hydroxycyclohexyl phenyl ketone), ethyleneglycol dimethacrylate (EGDMA), N-vinyl pyrrolidone (NVP), N,N-Dimethyl acrylamide (DMA), and 3-[Tris(trimethylsiloxy)silyl] propyl methacrylate (TRIS, siloxane) were purchased from Sigma-Aldrich Chemicals (MO, USA). Timolol maleate was gifted by Zydus Cadila Pharma Ltd. (Gujarat, India). Bimatoprost was purchased from MSN Laboratories (Telangana, India). Hyaluronic acid was obtained from Shandong Runxin Biotechnology Co., Ltd (Shandong, China). All other reagents were purchased from Sigma-Aldrich Chemical (MO, USA).

2.2. Formulation development

The composition of the monomers used to fabricate the blank contact lens and drug-loaded implants are shown in Table 1.

Table 1

Details of the composition of the base contact lens, direct-drug-laden contact lenses and drug-loaded implants.

Ingredients	Blank contact lens	Direct-drug-loaded contact lens	Drug-loaded Implants		
			Timolol	Bimatoprost	HA
Drug	-	TB+BMT+HA[#]	100 mg	75 mg	60 mg
Irgacure 184	20 mg	20 mg	20 mg	20 mg	20 mg
EGDMA	10 µl	10 µl	10 µl	10 µl	10 µl
DMA	310 µl	310 µl	310 µl	310 µl	310 µl
NVP	10 µl	10 µl	10 µl	10 µl	10 µl
Siloxane	100 µl	100 µl	100 µl	100 µl	100 µl
HEMA	up to 1 ml	up to 1 ml	up to 1 ml	up to 1 ml	up to 1 ml

EGDMA = ethyleneglycol dimethacrylate, DMA = N,N-Dimethyl acrylamide, NVP = N-vinyl pyrrolidone, Siloxane = 3-[Tris(trimethylsiloxy)silyl] propyl methacrylate, HEMA = Hydroxyl ethylmethacrylate. TB+BMT+HA[#] = 8 mg timolol + 4 mg Bimatoprost + 4 mg Hyaluronic acid in HEMA (up to 1 ml).

2.2.1. Fabrication of the drug-loaded implants

The 40 µm thick implants (total thickness of lens was 100 µm) were fabricated using monomer mixtures solution (free radical polymerization technique). Briefly, to fabricate timolol implant; the required quantity of timolol was added in the monomer mixture [Irgacure 184, EGDMA, DMA, NVP, Siloxane and HEMA (up to 1 ml)] and sonicated (15 minutes) to remove any entrapped air bubbles. The timolol-monomer mixture was pipetted between the glass moulds separated by the Teflon spacer (40 µm). The assembly of the moulds was then shifted in the Ultraviolet transilluminiator and the timolol-silicone sheet was cured (polymerized) for 25 minutes at 360-370 nm. The timolol-loaded sheet was punched into a circular ring (8 mm outer diameter and 6 mm inner diameter) using a metallic borer, followed by cutting into the small implants (Fig. 1, A) of 2 mg weight using the cutter. Similarly, the bimatoprost and HA-loaded implants (Fig. 1, B and C) were fabricated. The dry implants were preserved in the desiccators (45 % relative humidity) at room temperature till further use. The quantity of the drugs added in the monomer solution to fabricate the different implants were according to the target dose requirement [timolol (80-90 µg), bimatoprost (40-50 µg), and HA (15-20 µg)] for one week release based on the literature review [22, 42-45].

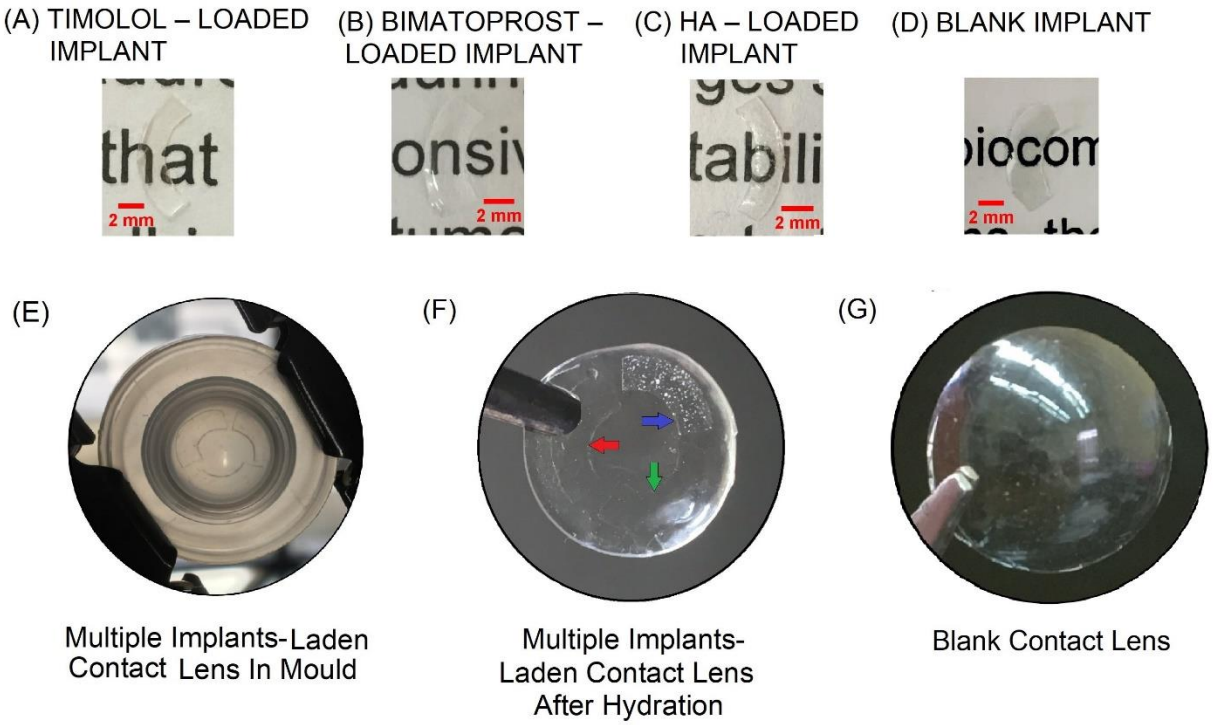

Fig. 1. (A) Timolol-loaded implant, (B) Bimatoprost-loaded implant, (C) HA-loaded implant, (D) Blank implant, (E) Final fabricated (100 µm thickness) implant-contact lens before hydration in the mould, showing 6 mm centre aperture, (F) Hydrated implant contact lens with position of the inner implants margins shown by color arrows (blue arrow shows HA implant (precipitation of HA can be observed). Red and green arrow shows timolol implant and bimatoprost implant respectively) and (G) Blank contact lens.

2.2.2. Implantation of the drug-loaded implants in the contact lenses

The multiple implant-laden contact lenses (IM) were casted using polypropylene mould (14.2 mm outer diameter and 6.5 mm base curve). The timolol-implant, bimatoprost-implant and HA-implant were placed in the cavity of the female mould, keeping a distance of 3 mm (radial) from the center (Fig 1, E). The excess volume of the monomer mixture (base contact lens, Table 1) was added in the female mould and carefully joined with the male mould. The mould was then transferred in the Ultraviolet transilluminator and cured for 30 minutes at 360-370 nm using UV-B light. The multiple implant-laden contact lenses (coded as IM) were removed and stored at 25^{o}C (45% relative humidity) till further use. The transmittance of the implants laden contact lens

was not altered as the implants were placed in the periphery of the lens leaving a region of 6 mm diameter from the center for clear vision.

2.2.3. Direct drug-laden contact lenses

The direct drug-laden contact lenses were fabricated by adding the required quantity of drugs (timolol 8 mg/ml, bimatoprost 4 mg/ml and HA 4 mg/ml) directly in the monomer mixtures (up to 1 ml, using the base contact lens monomer mixture). The lenses were casted using the polypropylene lens moulds. The drug-monomer mixture solution was added in the female mould and the male mould was joined, followed by curing in the ultraviolet transilluminator (360-370 nm) for 30 minutes. The direct drug-laden contact lenses (coded as DL) were removed and stored at 25^{o}C (45% relative humidity) till further use. The strength of the drug concentrations in the monomer mixture solution was selected to achieve sufficient target drug loading for one week release based on preliminary studies (see supplementary files 1 to 3).

2.2.4. Drug-laden contact lenses prepared by soaking method

The soaking studies were performed to compare with the direct drug-laden contact lenses (DL) and implants-laden contact lenses (IM). The blank (without drugs) fabricated contact lenses were extracted to remove the unreacted monomer according to the section 2.3. The drugs (timolol, bimatoprost and HA) were loaded into the contact lenses by soaking in the simulated tear fluid (STF, 2 ml, osmolarity = 295.7 mosmoles/liter, pH 7.4, prepared by dissolving 0.015 %w/v sodium bicarbonate and 0.9 %w/v sodium chloride in deionized water) containing a fixed optimized strength of 2 mg/ml timolol, 100 µg/ml bimatoprost and 1 mg/ml HA. The strength of the drug concentrations in the soaking solution was selected to achieve the sufficient drugs loading for one week based on preliminary studies (see supplementary files 4 to 7). The blank contact lenses were autoclaved (121^{o}C, 15 psi for 30 minutes) in the soaking solution containing drugs and thereafter soaked for a period of 48 h with occasional shaking [46]. At the end of the soaking period, the contact lenses (coded as SM) were blotted to remove the excess of soaking solution from the surface of the contact lenses and were subjected to evaluation studies.

2.3 Removal of the unreacted monomers from the contact lenses

The un-reacted monomers from the blank contact lenses (SM), direct drug-laden (DL) and multiple implant-laden contact lenses (IM) were removed by individually extracting the contact lenses in 5 ml of the boiling water for 15 minutes [47, 48]. The drug loss in the water after extraction was quantified by the developed and validated HPLC methods for timolol [49] and bimatoprost [50, 51]. The HA was quantified by a colorimetric method [52, 53] using Stains All dye at 640 nm. All readings were noted in triplicate.

2.4 Terminal sterilization

The therapeutic contact lenses are typically sterilized using autoclave (wet method) in its packaging solution, which pose the issue of drug leaching, especially with the drugs like timolol, bimatoprost and hyaluronic acid. The SM lenses, DL lenses and IM lenses were terminally sterilized in their packaging/soaking solution (2 ml) by wet process (autoclave at 121°C, 15 psi for 30 min). The drug loss in the packaging solution after sterilization of the DL and IM lenses were determined using developed and validated HPLC methods for timolol [49] and bimatoprost [50, 51]. The HA was determined by colorimetric method [52, 53] using Stains All dye at 640 nm. All readings were noted in triplicate.

To overcome the issue of drug loss during wet sterilization process, the DL and IM lenses were also sterilized by UV-B radiation sterilization using the UV-B lamp (G6T5E, Sankyo Denki Co. Ltd., Japan) at 290-310 nm for 3 h [54]. The radiation sterilized lenses were coded as DL-R and IM-R for direct drug-laden (DL) and implants-laden contact lenses (IM) respectively.

2.5 Characterization of the silicone contact lenses

2.5.1. Swelling study

The contact lenses after fabrication were carefully removed from the moulds and the weights were recorded as dry weight (W_D). The contact lenses were soaked in their respective packaging/soaking solutions for 24 hours at room temperature, thereafter

blotted using filter paper and weight (W_S) again. The percentage swelling was calculated using following formula [55]:

$$\% \text{ Swelling} = \frac{W_S - W_D}{W_D} \times 100$$

2.5.2. Transmittance

The transmittance of the silicone contact lenses should not decrease after drug loading. The transmittance of the centre part (6 mm diameter from the middle/center) of the control contact lenses (without drugs), soaked contact lenses (SM), direct drug-laden (DL) and multiple implant-laden contact lenses (IM) were scanned from 200 nm to 800 nm wavelength using UV-vis spectrophotometer [56, 57]. The transmittance of the implant areas were also determined. The experiment was repeated three times.

2.5.3. Atomic force microscopy (AFM)

AFM was used to observe the surface topography of the SM, DL and IM contact lenses [58]. The implant area was analyzed in case of the IM lens. The marketed contact lens (Freshlook One Day Color Contact Lens by Alcon) was also analysed for comparison. Surface topography of the contact lenses were assessed by AFM NT-MDT (Model No: NT MDT NTEGRA Prima, Russia) in semi-contact mode at 25^{o}C. Contact lenses were adhered to the silicone wafer of root mean square (RMS) roughness 0.062 nm. Images (10 × 20 μm^2) were obtained with silicone probes (resonance frequency: 204-497 kHz) at a scan rate of 1.01Hz. The average roughness (Ra) of the surfaces was obtained using NOVA AFM [59, 60].

2.6. Quantification of the drug loaded in the contact lenses

The amount of drugs (timolol, bimatoprost and HA) present in the SM, DL and IM contact lenses were quantified using HPLC (for timolol and bimatoprost) and colorimetric method (for HA). The contact lenses were individually transferred in the screw capped glass vial holding 25 ml of methanol. These vials were agitated in the incubator shaker at 100 rpm for 10 days at 25^{o}C to extract drugs from the contact lens. After 10 days, the methanol was analyzed for timolol [49] and bimatoprost [50, 51] using the developed and validated HPLC methods. Similarly, to quantify HA, the contact

lenses were extracted using 25 ml of water. Methanol cannot be used for the extraction, as it causes precipitation of the HA inside the contact lens. The HA was quantified by colorimetric method [52, 53]. All readings were recorded in triplicate.

2.7. In vitro drug release study

The *in vitro* release studies of the SM, DL (wet sterilized), DL-R (radiation sterilized), IM (wet sterilized) and IM-R (radiation sterilized) were performed by immersing the contact lenses individually in 2 ml of STF-release media at 34ºC under constant stirring at 100 RPM in the incubator shaker [61-63]. The 2 ml aliquots of the media were withdrawn for drug quantification and replaced with the same volume of the fresh STF periodically. The drugs in the aliquots were quantified by the developed and validated HPLC methods for timolol [49] and bimatoprost [50, 51]. The HA was quantified by colorimetric method [52, 53]. The sampling was carried out until there was no increase in the drug concentration in 2 successive measurements. All readings were noted in triplicate. The release profile of the drugs was evaluated by plotting different graphs like: Percentage cumulative drug release versus time, release rate (ng/h) versus time.

2.8. Animal studies

The animal studies were performed according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) and the study protocol was approved by Institutional Animal Ethical Committee (IAEC) of the Maliba Pharmacy College, Bardoli, India (protocol no.: MPC/IAEC/22/2017). The studies were carried out using New Zealand white rabbits weighing 3 to 3.5 kg (either sex) housed in stainless steel cages at 25 ± 1^{o}C with no restriction of food and water intake.

2.8.1. *In-vivo drug release study*

The *in vivo* drug release studies were conducted to investigate the improvement in drug retention time in the tear fluid using soaked contact lenses (SM) and multiple implant-laden contact lenses (IM-R) in comparison to Ganfort® eye drop therapy (1 drop = 15 µg bimatoprost and 250 µg timolol) [42, 64]. After acclimatization the sterilized contact lenses were placed on the rabbit right eye without anesthesia and the left eye

was kept as a control (n = 6 rabbits for each group). In the eye drop group (n =6), the right eye of the rabbit received the single instillation (50 µL equivalent to 250 µg of timolol and 15 µg of bimatoprost) of Ganfort ophthalmic solution (Allergan) and the left eye was kept as a control. At pre-determined time intervals (1 h, 5 h, 24 h, 48 h, 72 h, 96 h and 120 h), 5 µl of the rabbit tear fluid was collected using the disposable glass capillaries from the *cul de sac* of the rabbit right eye and preserved at -20°C until analysis. The proteins of the tear fluid was precipitated by admixing the tear fluid with a sufficient quantity of the methanol followed by the freeze centrifugation for 20 minutes (Remi freeze-centrifuge, 12,000 RPM). The supernatant was collected and the drug content was quantified by LCMS (TSQ Quantum Access, Thermo Scientific, USA) for timolol and bimatoprost. The HA was used as a comfort agent in the implant contact lens, and the *in vitro* release profile suggested sufficient release of HA from the lens to show comfort [45]. Thus, we have not conducted HA analysis from the tear fluid samples.

At the end of the study, the rabbits were sacrificed for histopathological studies. Both the eyes (control and the contact lens wear, for all the groups) were enucleated and fixed in 10% neutral buffered formalin solution. After embedding in the paraffin, the sections of the cornea were cut using the microtome and stained using the hematoxylin. The cornea was evaluated by the light microscopy at '×400 magnification' for structure of the basement membrane, epithelium and stroma [65-67].

2.8.2. In vivo pharmacodynamic study

The *in vivo* pharmacodynamic study was performed to compare the efficacy of the SM and IM-R lenses with the conventional Ganfort® eye drop therapy (1 drop = 15 µg bimatoprost and 250 µg timolol). The IOP of the rabbit eyes were measured every day (morning, afternoon and evening) and only those rabbits which showed the normotensive pressure without much fluctuation were selected for the study. After acclimatization the sterilized contact lenses were placed on the rabbit right eye without anesthesia and the left eye was kept as a control (n = 6 rabbits for each group). In the eye drop group (n =6), the rabbit right eye received the single instillation (n = 6 rabbits) of Ganfort ophthalmic solution (Allergan) and the left eye was kept as a control. The IOP

was measured at regular time intervals using a Tonometer (Icare® TONOVET rebound tonometer, Finland) [33]. During the entire study ocular discomfort, irritation or ocular toxicity was monitored.

2.9. Statistical analysis

Statistical analysis was carried out using SPSS 21.0 for Windows. T-test (2 tailed) and One-way analysis of variance (ANOVA) was used to compare the different groups, after confirming the normality and the homogeneity of variance.

3. Results and discussion

3.1. Characterization of the contact lenses

3.1.1. Swelling study

The percentage swelling has a direct relationship with the ion permeability, oxygen permeability, and the dimensions of the contact lens [68-71]. Table 2, shows the data of % swelling. The SM contact lenses soaked in the drug solution showed no significant ($p = 0.16$) reduction in the percentage swelling (95.46 ± 1.37 %) in comparison to the control contact lenses without drug (98.85 ± 2.56 %). Thus, the presence of drugs (timolol, bimatoprost, and HA) did not reduce the % swelling (water content) of the soaked contact lenses. Similar results were observed with the DL lenses (96.12 ± 0.99 %, $p = 0.18$). The timolol-implant showed a decrease in swelling behavior (91.82 ± 2.63%, $p = 0.08$), which could be due to the hydrophobic nature of the timolol base and the tight packing of the timolol in the small implant. However, statistically it was not different ($p = 0.08$) in comparison to the contact lens. The HA-implant showed a slight increase in the swelling (102.10 ± 7.01 %, $p = 0.387$), which was due to the hydrophilic nature of the polymer. The multiple implant-laden contact lenses (98.56 ± 2.36%) did not show significant change ($p = 0.88$) in the % swelling in comparison to the control contact lenses, as the implants occupy a limited area of the lens.

Table 2
Data of swelling and % transmittance study. Values are shown as mean ± standard deviations (n = 3).

Codes	% Swelling [Mean ± SD]	p value	% Transmittance [Mean ± SD] at 600 nm	p value
Control contact lens	98.85 ± 2.56	-	98.49 ± 0.58	-
Soaked contact lens [SM]	95.46 ± 1.37	0.16	94.40 ± 1.73*	0.03
Direct drug-laden contact lens [DL]	96.12 ± 0.99	0.18	74.50 ± 1.96**	< 0.01
Timolol implant	91.82 ± 2.63	0.08	95.37 ± 0.66*	0.04
Bimatoprost implant	93.48 ± 6.33	0.24	98.93 ± 0.70	0.41
HA implant	102.10 ± 7.01	0.39	60.37 ± 0.60**	< 0.01
Multiple implant-laden contact lens [IM]	98.56 ± 2.36	0.89	98.47 ± 0.25	0.94

*indicate significant difference ($p<0.05$) with control contact lens.
** indicate very significant difference ($p<0.01$) with control contact lens.

3.1.2. Optical transmittance

The transmittance of the silicone contact lenses were characterized by measuring the transmittance spectra in the range from 200 to 800 nm (Supplementary file 7). Both the control and the multiple-implant lens exhibit near 100% optical transmittance in the visible range. However, the optical transmittance was reduced with the SM and the DL contact lenses. For comparison, the % transmittance values were measured at 600 nm [72, 73] (Table 2). Timolol implant (%T = 95.37 ± 0.66, P = 0.04) and HA implant (%T = 60.37 ± 0.60, P = < 0.01) showed significant reduction in the optical transmittance in comparison to the control contact lenses (% T = 98.49 ± 0.58). The SM (%T = 94.40 ± 1.73) and the DL contact lenses (%T = 74.50 ± 1.96) showed a significant ($p < 0.05$) reduction in optical transmittance in comparison to the control contact lenses. Thus, the presence of drugs inside the lens material makes it unsuitable for therapeutic use, as it reduces the transmittance of the contact lenses. However, the drug-laden implants in the multiple-implant lenses (IM) did not alter the optical transmittance, which was expected as the drug-implants were placed in the periphery of the silicone contact lens for clear vision (Fig. 1, E).

3.1.3. Atomic force microscopy (AFM)

The AFM was used to numerically measure the changes in the surface smoothness of the contact lenses, due to the drugs loading by soaking, direct loading and implantation technique. The AFM report of Freshlook contact lens (marketed by Alcon), SM, DL and IM (all three implant areas) are shown in Fig. 2. The average roughness

(R_a) of the Freshlook contact lens was found to be 116.07 nm, while the SM and the DL contact lens shows lower values of 39.14 nm and 30.61 nm respectively. The average roughness (R_a) of the IM contact lens, i.e. the implant area over the timolol, bimatoprost and the HA implant showed further lower values of 14.59 nm, 11.42 nm and 12.52 nm respectively, which suggest the comfort wear for the patient. The lower values could be due to the absence of the drugs on the surface of the IM contact lens, in comparison to the SM and the DL contact lens. In the previous literature, Vilem et al., 2007 [58] observed the average surface roughness of the various marketed contact lenses in the range of 0.7 nm to 18.8 nm. Similar studies were performed by Maria et al., 2010 [60], and found the average surface roughness in the range of 2.34 nm to 12.99 nm of marketed contact lenses. Thus, the surface smoothness was not affected by the presence of the timolol, bimatoprost and HA implants, instead it was much smoother than the SM and the DL contact lenses. Thus, we expect improved comfort on wearing the multiple implant contact lens (IM) fabricated by the implantation technology.

3.2. Quantification of the drug loaded in the contact lenses

The amount of drugs in the contact lenses/implants was determined to evaluate the loading and uniform dispersion of drug in the lens/implant and the reproducibility of the fabrication process. The drug-loaded values for the SM, DL and IM lenses are shown in Table 3. The uptake of timolol, bimatoprost, and HA from the drug-soaking solution was found to be 83.13 ± 1.01 µg, 44.92 ± 0.89 µg, and 16.81 ± 1.07 µg respectively. Thus, in spite of high drug concentration in the soaking solution, the drug uptake was very low, which indicate the limitation of the soaking method to load drug in the contact lenses. The amount of timolol, bimatoprost, and HA loaded in the DL contact lenses was found to be 90.93 ± 1.20 µg, 66.34 ± 1.61 µg and 18.38 ± 1.28 µg respectively. The IM lenses shows the presence of timolol, bimatoprost, and HA as 85.38 ± 1.64 µg, 50.92 ± 1.26 µg and 26.36 ± 1.33 µg respectively. Thus, the amount of drugs loaded in all the three batches was close to the target values [timolol (80-90 µg), bimatoprost (40-50 µg), and HA (15-20 µg)] and sufficient to produce prolonged reduction in the IOP, however, the systems (batches) should show the sustained release of drugs within a therapeutic

level. The low standard deviation values suggest the reproducibility of the process/procedure to load the drugs.

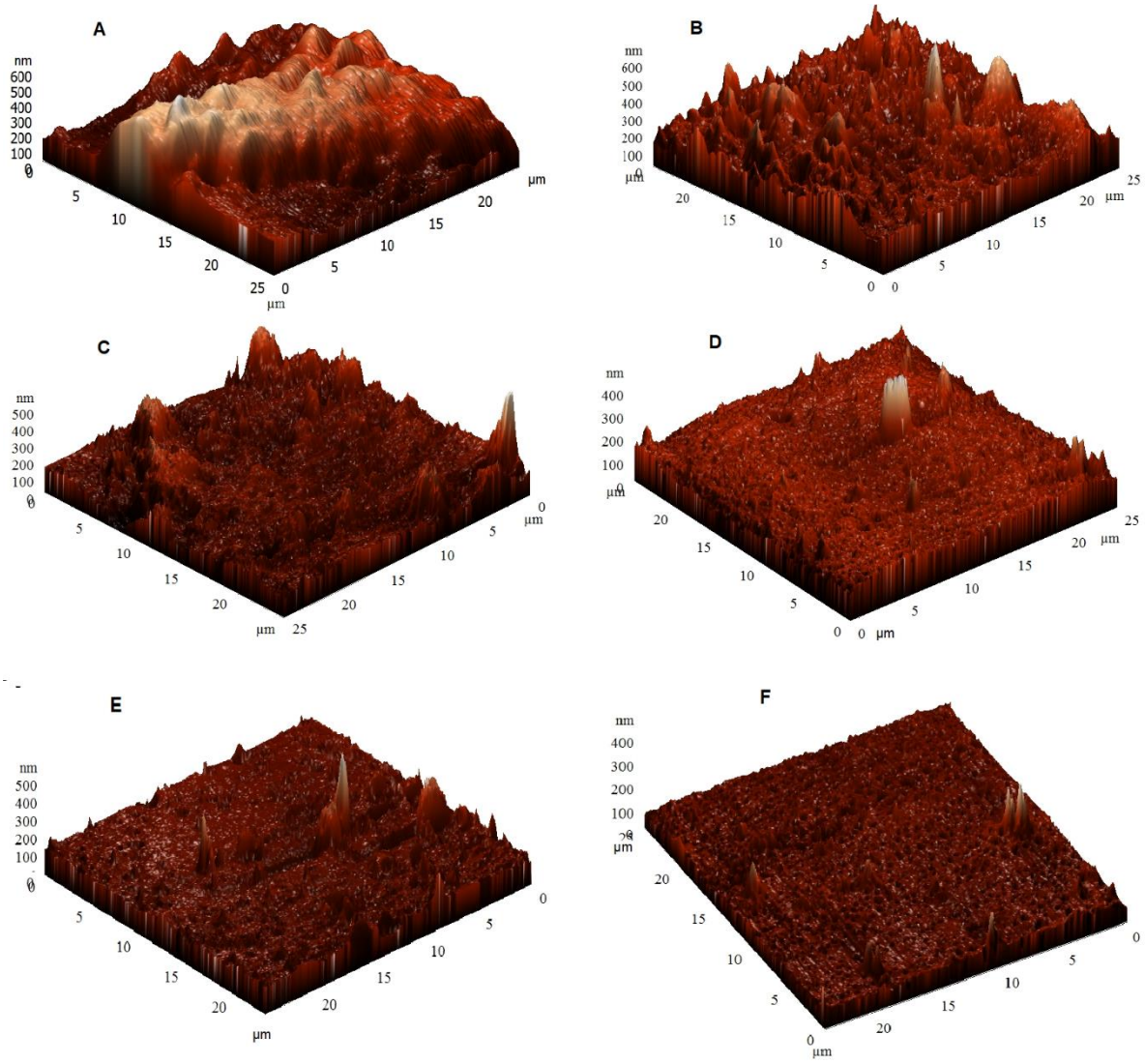

Fig. 2. Three-dimensional image of the surface topography generated by the AFM analysis for (A) Freshlook One Day Color Contact Lenses by Alcon, (B) Soaked contact lens,(C) Direct drug-laden contact lens, (D) Timolol implant, (E) Bimatoprost implant, (F) Hyaluronic acid implant. D, E and F are the images of the implant contact lens areas below which the drug-implant was placed or implanted.

Table 3
Data of drug loading from the SM, DL and IM lenses. Values are shown as mean ± standard deviations (n = 3).

Code	Drug loaded in the contact lenses and implants (µg)		
	Timolol	Bimatoprost	Hyaluronic acid
Soaked contact lens (SM)	83.13 ± 1.01	44.92 ± 0.89	16.81 ± 1.07
Direct drug-laden contact lens (DL)	90.93 ± 1.20	66.34 ± 1.61	18.38 ± 1.28
Multiple implant-laden contact lens [IM]	85.38 ± 1.64	50.92 ± 1.26	26.36 ± 1.33

3.3. Removal of unreacted monomers from the contact lenses

The direct drug-laden contact lenses and the multiple implant-laden contact lenses were treated to remove the un-reacted monomers from the silicone matrix. The percentage loss of drug during the monomer extraction step are shown in Table 4. The results of DL lenses revealed that 61.70 µg (67.85 %) of timolol, 22.11 µg (33.32 %) of bimatoprost and 7.50 µg (40.84 %) of HA was leached out during the monomer extraction step in the boiling water. While, the IM contact lenses show relatively low drug leaching [timolol = 45.88 %, bimatoprost = 33.05 % and HA = 32.85 %] due to the extra barrier of the silicone lens (below and above the implant), which resist the release of drug from the implants. The timolol shows high leaching in comparison to the bimatoprost, due to its higher solubility in the water (timolol = 2.3 mg/ml and bimatoprost = 350 µg/ml). Due to high molecular weight of HA and random coil like structure [74], HA showed the lowest leaching from the lens matrix.

Table 4

Data of timolol, bimatoprost and HA leached during the monomer extraction, steam (wet) sterilization step and radiation sterilization step (Mean ± SD, n=3).

Drug Leaching during monomer extraction step						
Code	Timolol		Bimatoprost		HA	
	µg	(%)	µg	(%)	µg	(%)
Direct drug-laden contact lens	61.70 ± 1.09	67.85 ± 1.20	22.11 ± 0.78	33.32 ± 1.18	7.50 ± 0.41	40.84 ± 2.27
Multiple implant-laden contact lens	39.17 ± 0.08	45.88 ± 0.09	16.77 ± 0.12	33.05 ± 0.24	8.57 ± 0.25	32.85 ± 1.10
Drug Leaching during sterilization (wet) step						
Code	Timolol		Bimatoprost		HA	
	µg	(%)	µg	(%)	µg	(%)
Direct drug-laden contact lens (DL)	7.08 ± 0.15	7.78 ± 0.17	25.02 ± 0.20	37.72 ± 0.30	2.45 ± 0.31	13.35 ± 1.73
Multiple implant-laden contact lens (IM)	12.57 ± 0.81	14.72 ± 0.94	14.61 ± 0.97	28.70 ± 1.48	3.47 ± 0.33	13.18 ± 1.28
Drug loss from radiation sterilized lenses during 24 h wetting (hydration) step						
Code	Timolol		Bimatoprost		HA	
	µg	(%)	µg	(%)	µg	(%)
Direct drug-laden contact lens (DL-R)	36.72 ± 0.89	40.39 ± 0.97	39.56 ± 0.39	59.64 ± 0.59	7.17 ± 0.26	39.00 ± 1.43
Multiple implant-laden contact lens (IM-R)	14.40 ± 0.45	16.87 ± 0.53	24.42 ± 0.55	47.95 ± 1.08	6.43 ± 0.11	24.41 ± 0.17

3.4. The effect of wet sterilization

The leaching of drugs (timolol, bimatoprost and HA) during the wet sterilization step from the DL and IM contact lenses are shown in Table 4. The leaching of timolol from the DL and IM lens was 7.78 % and 14.72 % respectively. The timolol loss from the DL lens was less comparatively, as the major part of timolol (67.85 %) was already lost during the monomer extraction step. The leaching of bimatoprost from the DL and IM lens was 37.72 % and 28.70 % respectively. The IM lens showed less leaching due to the presence of extra barrier as discussed in the monomer extraction (section 3.3). The leaching of HA from the DL and IM lens was 13.35 % and 13.18 % respectively. The issue of drug loss during the wet sterilization method is very challenging for the scientists. In our previous article [75] the issue was addressed by pH-sensitive Eudragit S100 dug-nanoparticles, which we are planning in our future studies.

The data from drug leaching during the monomer extraction and wet sterilization revealed that a large amount of drug was lost during these wet steps, which will restrict the

use of these contact lenses for therapeutic purposes. The total amount of timolol, bimatoprost and HA leached from the DL lenses was 75.63 %, 71.04 %, and 54.19 % respectively, while the IM lenses also showed 60.6 %, 61.75% and 46.03 % leaching respectively. Moreover, the possibility of continuous drug leaching during the storage of lens (shelf life) under the wet conditions in the packaging solution exists, further posing the issue. Thus, it was decided to sterilize the contact lenses (DL and IM) by the radiation sterilization method, which can be used by the patients with prior hydration for 24 h. The 24 h time period is required for the complete hydration and swelling of the contact lens. The drug loss during this hydration period (24 h) is noted in table 4. The IM lens showed relatively less drug leaching in comparison to the DL lens.

3.5. In vitro release study

3.5.1. In vitro release of bimatoprost from the contact lenses

The % cumulative release and release rate (ng/h) profile of bimatoprost from the soaked (SM), direct drug laden (DL, post monomer extraction and sterilization) and implant contact lenses (IM, post monomer extraction and sterilization), and 24 h hydrated radiation sterilized contact lenses (DL and IM lens) are shown in Fig. 3 and 4 respectively.

The cumulative release of bimatoprost from the SM, DL, DL-R (radiation sterilized), IM and IM-R (radiation sterilized) batches are 31.22 µg, 16.03 µg, 21.08 µg, 5.61 µg and 12.95 µg respectively. One should note that the single dose (50 µL volume ≈ 15 µg) of bimatoprost (prostaglandin analogue) eye drop (0.03 % w/v strength Lumigen) solution cause conjunctival hyperaemia [76]. Considering 50 µL volume of single eye drop solution, the single exposure dose which cause hyperaemia is 15 µg. Thus, any burst release amount above 15 µg will show high probability of hyperaemia in human eye.

The SM batch showed very high burst release of 70.59 % (22.04 µg), which can cause conjunctival hyperaemia and other local side effects [77, 78]. The high burst release; indicate the presence of bimatoprost on the surface and the aqueous channels of the contact lens matrix, suggesting the limitation of soaking method for hydrophilic drugs. The drug was detected till 48 h.

The DL contact lenses showed burst release of 72.66 % (11.65 µg), as the major part of drug was lost in the monomer extraction and sterilization. However, the batch still showed sustained release up to 48 h. The DL radiation sterilized batch showed the burst release of 74.04 % (15.61 µg), with improvement of 72 h in the drug release profile. The DL batch showed improved drug release profile in comparison to the SM batch, as the drugs were entrapped inside the matrix of the silicone contact lens, however, the percentage transmittance (74.5 %) value indicate that DL lens cannot be used for therapeutic purpose.

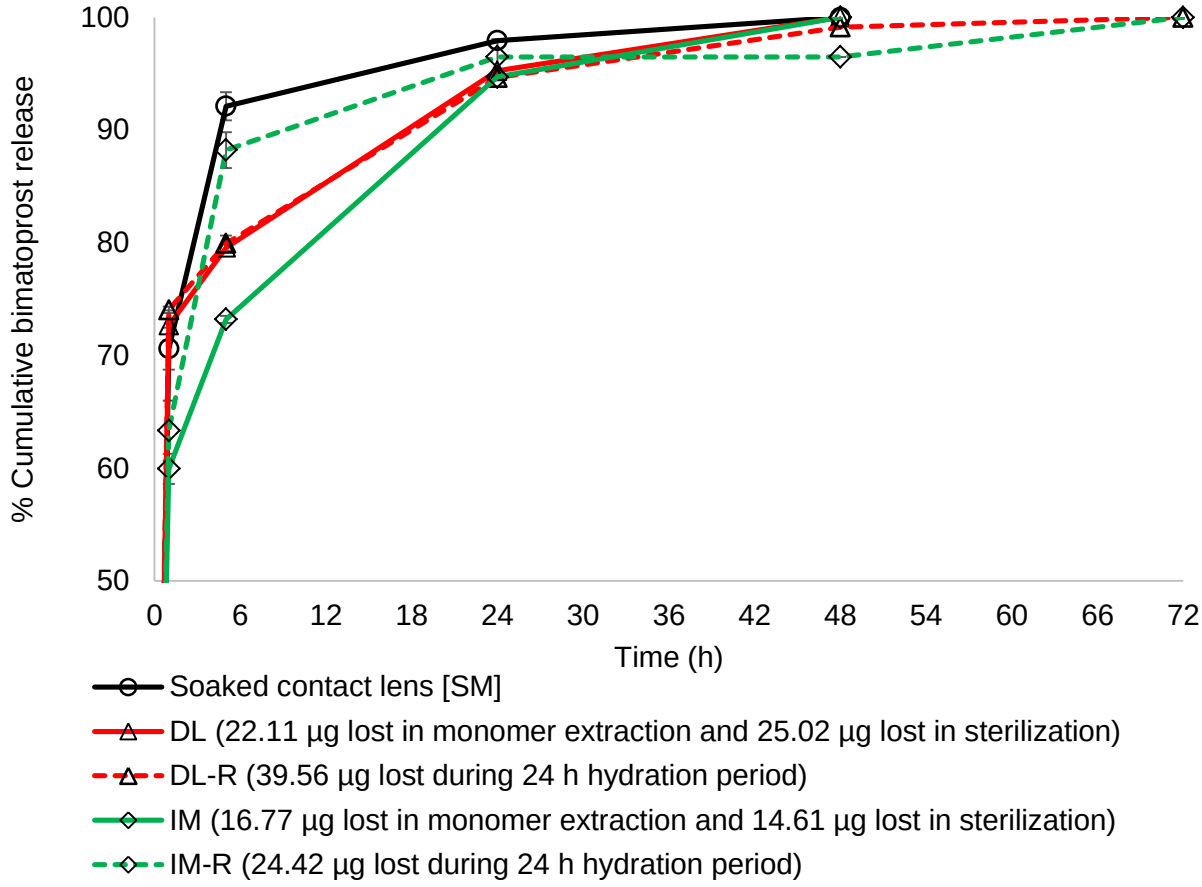

Fig. 3. Percentage cumulative release of bimatoprost from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

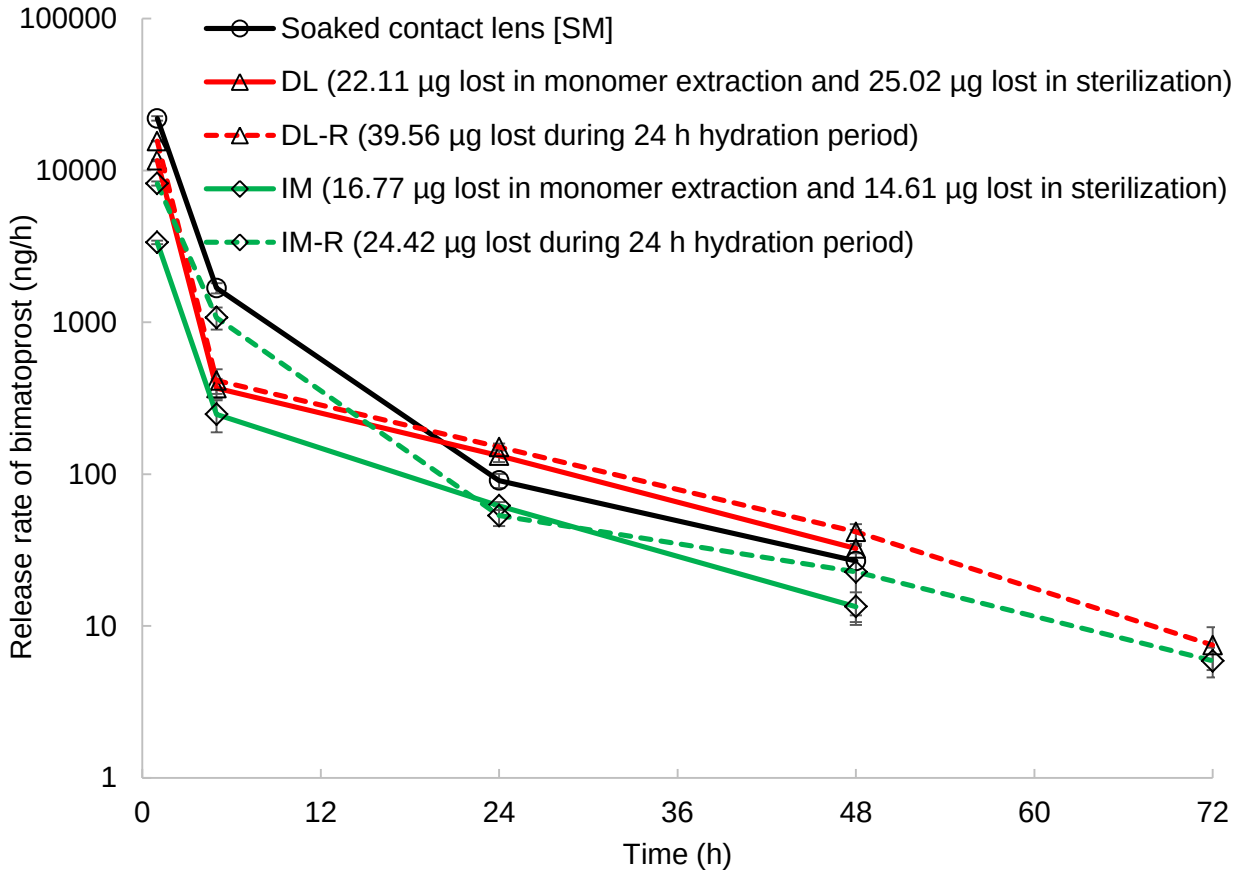

Fig. 4. Release rate (ng/h) of bimatoprost from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

In comparison to the SM and DL lenses, the IM contact lens (post monomer extraction and sterilization) showed lower burst release (59.94 %, 3.36 µg) respectively. Although, the cumulative release was just 5.61 µg, the drug was detected up to 48 h in the flux studies. The IM-R (radiation sterilized) lens showed burst release of 63.34 % (8.20 µg), with a sustained drug release profile for up to 72 h (cumulative release = 12.95 µg). The IM lens showed improved drug release profile in comparison to the SM and DL lenses. The results suggest the controlled release of drug in a two phase pattern. In the first phase, there was a burst release of drug from the aqueous channels, followed by a controlled release from the implant, where the drug was tightly bound, due to the drug-polymer interactions.

3.5.2. In vitro release of timolol from the contact lenses

The % cumulative release and release rate (ng/h) profile of timolol from the lenses are shown in Fig. 5 and 6 respectively. The cumulative release (µg) of timolol from the SM, DL, DL-R (radiation sterilized), IM and IM-R (radiation sterilized) batches was 71.95 µg, 7.81 µg, 16.2 µg, 2.38 µg and 25.64 µg respectively.

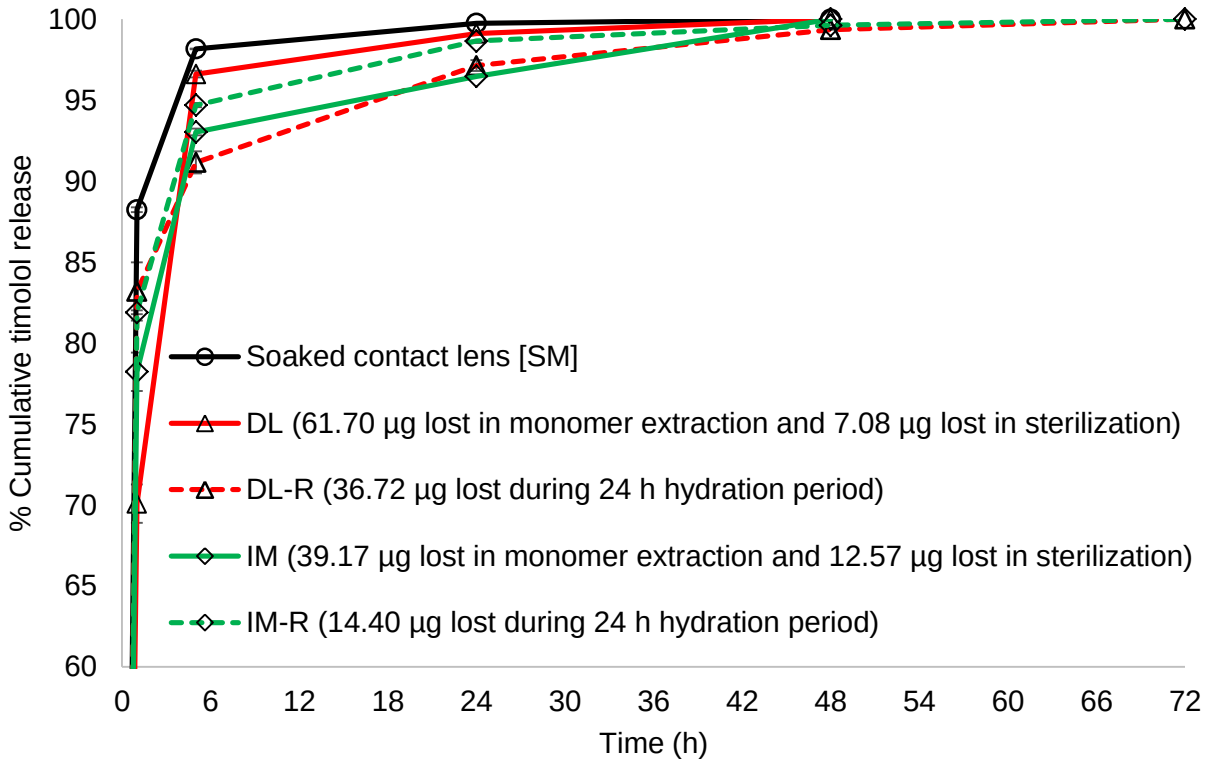

Fig. 5. Percentage cumulative release of timolol from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

The soaked contact lens (SM), showed many fold high burst release (88.23 %, 63.48 µg) of timolol in comparison to DL and IM lenses. The high local concentration of timolol in the tear fluid can enter in the nasolacrimal duct, where it can be absorbed through the nasal mucosa to the systemic blood circulation [79, 80] and can reach a serum concentration > 0.4 ng/ml. If so, the concentration shall be similar to the *ki* for βARs in the heart and lung [81-84], which may lead to cardiac and respiratory side effects. The

high burst release from the SM lens, indicated poor timolol holding capacity by the lens matrix, due to high solubility of timolol in STF. The drug was detected for up to 48 h in the flux studies.

The direct drug-laden contact lenses (DL) showed burst release of 70.08 % (5.47 µg), as the major part of timolol was lost in the monomer extraction and sterilization. However, the batch still showed sustained release for up to 48 h, with cumulative release of 7.81 µg. The data clearly indicate the limitations of direct drug loading, as the major part of timolol was lost during the monomer extraction and wet sterilization steps. The DL-R (radiation sterilized) lens showed burst release of 83.19 % (13.48 µg), with improvement over 72 h in the timolol release profile (cumulative release = 16.20 µg). The release rate data indicate that the DL-R batch was better in comparison to the SM lens, which was expected, as timolol was entrapped inside the matrix of the silicone contact lens.

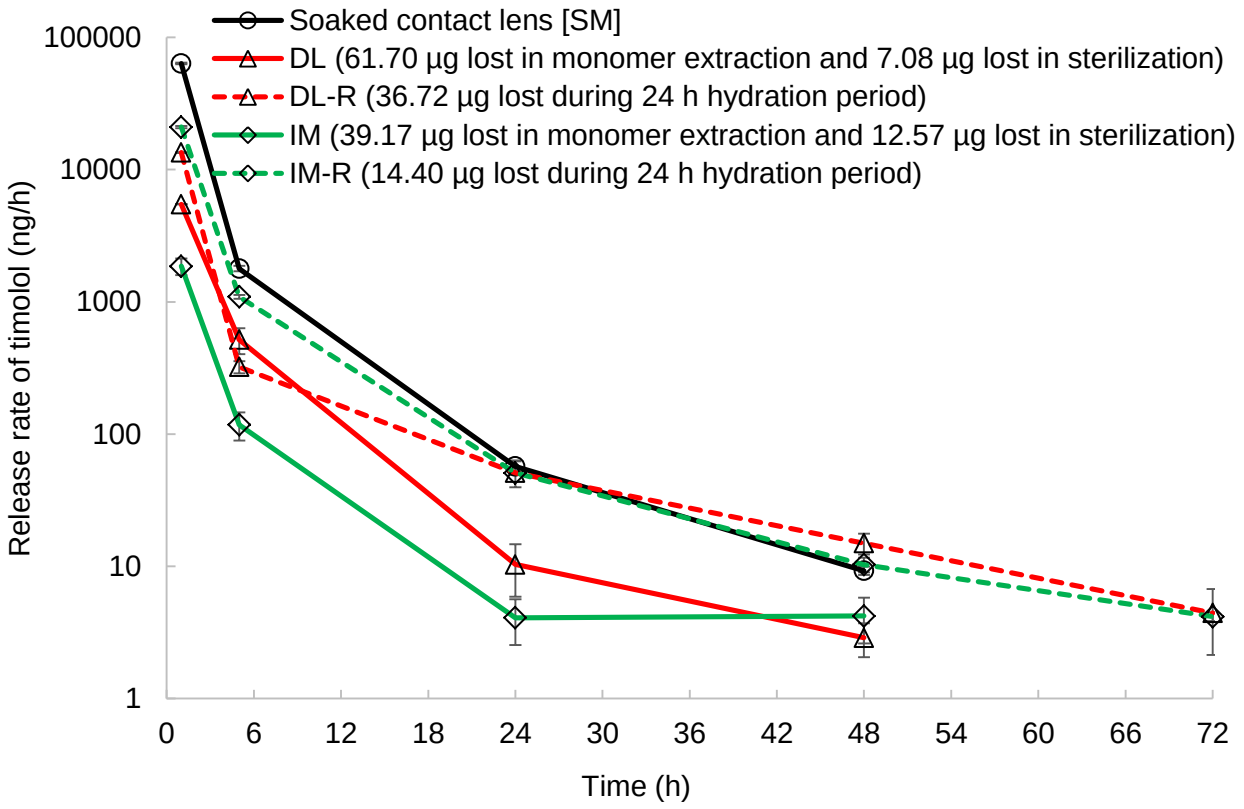

Fig. 6. Release rate (ng/h) of timolol from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

On the other hand, the implant contact lens (IM) showed burst release of 78.23 % (1.86 µg) with only 2.38 µg cumulative release, which cannot be used for the treatment of glaucoma due to low dose. However, the IM-R batch showed 81.89 % (21.00 µg) burst release, which was relatively lower in comparison to the SM batch. The timolol was detected for up to 72 h, with cumulative release of 25.64 µg. Similar to the bimatoprost release, the timolol also showed a first order, two phase release pattern. In comparison to the DL-R lens the IM-R lens showed improvement in its release rate profile, which was expected, due to the extra barrier as discussed previously and the tight packing of timolol in the small implant in comparison to the direct loading of timolol in the entire contact lens matrix (DL batch).

3.5.3. In vitro release of Hyaluronic acid from the contact lenses

The % cumulative release and release rate (ng/h) profile of HA from the lenses are shown in Fig. 7 and 8 respectively. The cumulative release of hyaluronic acid (HA) from the SM, DL, DL-R (radiation sterilized), IM and IM-R (radiation sterilized) lenses was 13.78 µg, 8.51 µg, 10.66 µg, 9.26 µg and 13.63 µg respectively. The soaked contact lens (SM), showed 46.59 % (6.42 µg) of HA release at initial hour, which is preferred for the comfort wear by the patients. The release rate profile showed rapid fall of HA with time, due to high molecular weight of HA which restricts its entry into the matrix of the contact lens during the soaking period. The DL and DL-R contact lenses did not show a significant difference in the HA release profile. The initial burst release was 33.79 % (2.79 µg) and 29.48 % (3.14 µg) with cumulative release of 8.51 µg and 10.66 µg for DL and DL-R batch respectively. The IM batch showed low initial burst of 22.56 % (5.07 µg) with and cumulative release of 9.26 µg respectively, as 46.03 µg of HA was lost during monomer extraction and wet sterilization. The IM-R batch showed sufficient burst release (37.20 %) and improved HA release profile in comparison to DL, DL-R and IM lenses, as only 6.43 µg of HA was lost during hydration of IM-R lens. The controlled release of HA from the IM-R lenses was obvious, due to the high molecular weight and random coil chain like structure of HA, which resisted HA itself crossing the mesh structure of the implant and lens matrix.

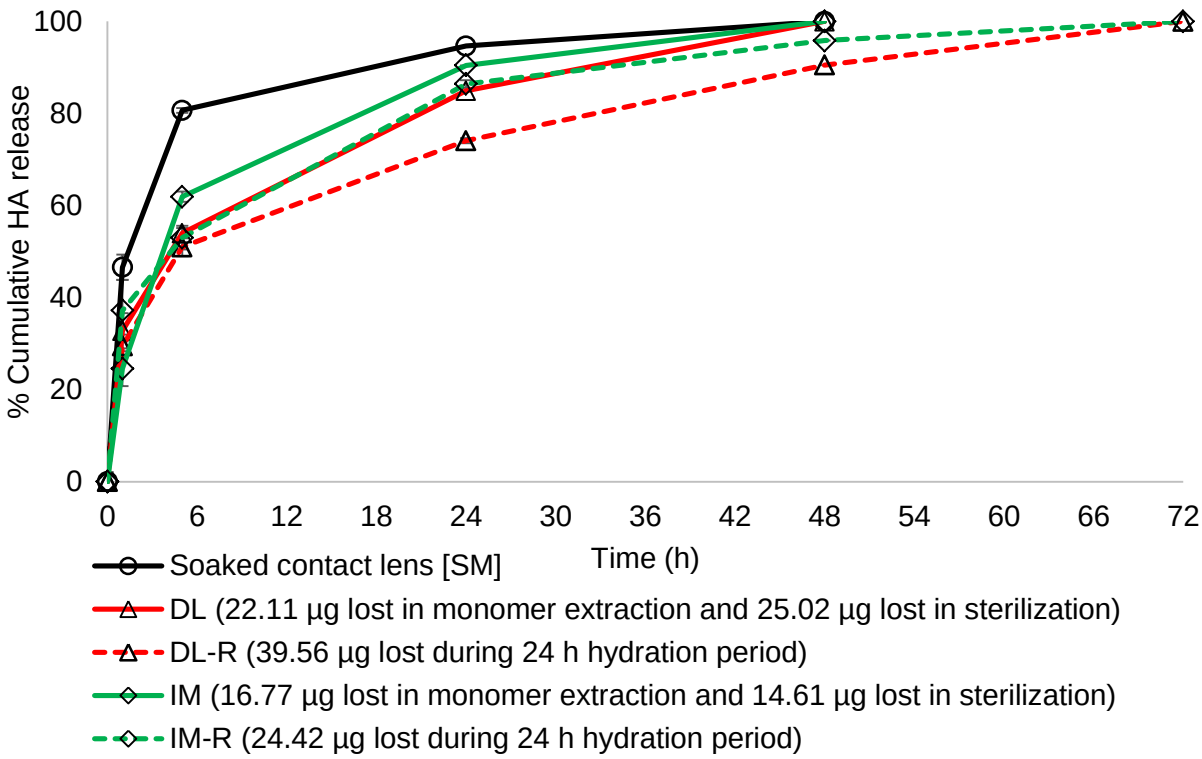

Fig. 7. Percentage cumulative release of hyaluronic acid from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

In summary, the *in vitro* flux results suggest that, the soaking method cannot be used to deliver timolol and bimatoprost due to high burst release which can cause local and systemic side effects. The direct drug-laden contact lenses (DL) and multiple implant-laden contact lenses (IM) showed major loss of drugs during the monomer extraction and wet sterilization. The radiation sterilized direct drug-laden contact lens (DL-R) showed 72 h drug release; however, it cannot be used due to low % transmittance value (74.5%). The radiation sterilized multiple implant-laden contact lenses (IM-R) showed 72 h drug release with satisfactory optical and physical properties. The results clearly suggest the advantage of packaging and dispensing the multiple implant-laden contact lenses in the dry state using radiation sterilization instead of wet sterilization method.

The total amount of drugs (timolol, bimatoprost and HA) released from the contact lenses during the monomer extraction, wet sterilization and flux (54.12 µg timolol, 36.99 µg bimatoprost, 21.3 µg HA) was lower in comparison to the total drug loading (85.38 µg timolol, 50.92 µg bimatoprost, 26.36 µg HA), which may be due to the permanent entrapment of the drug in the matrix structure of the contact lens (less likely), or due to drug-polymer interactions or because the drug release amount was below the limit of detection in the HPLC system.

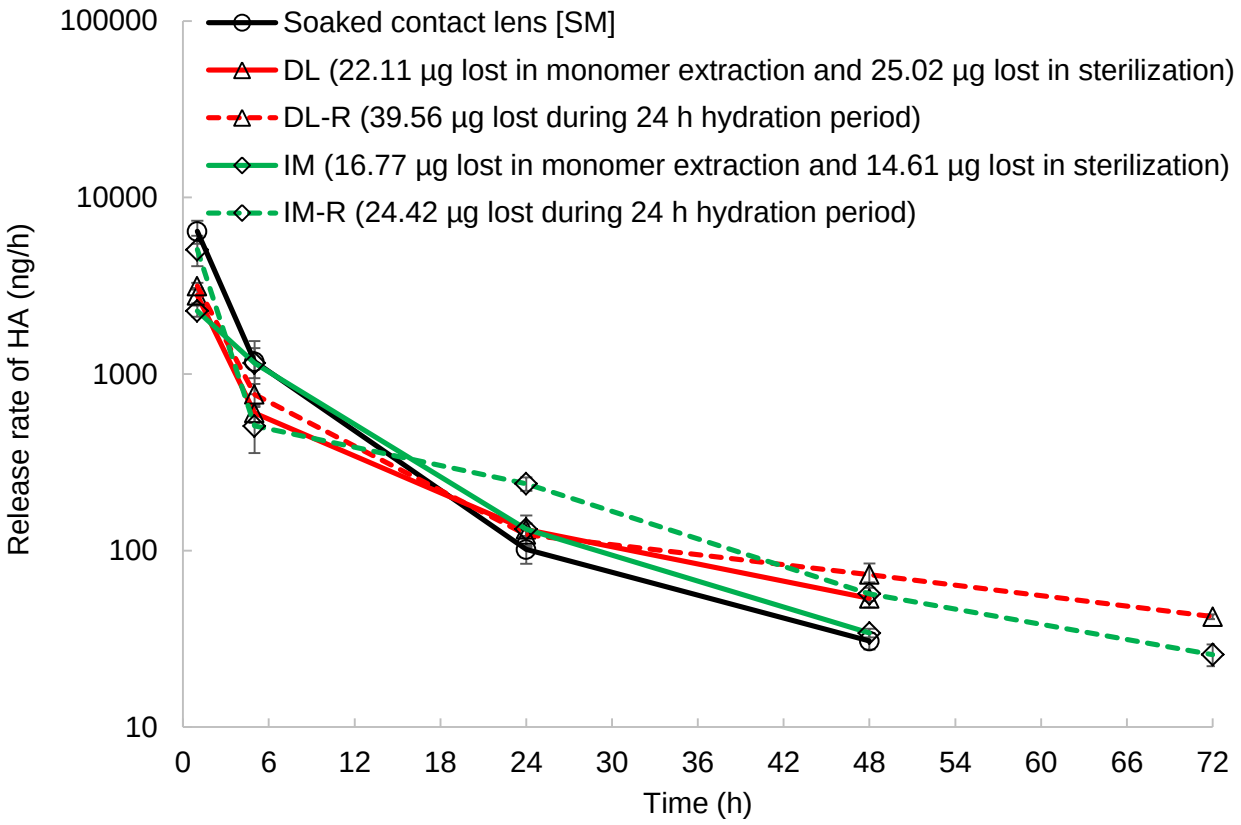

Fig. 8. Release rate (ng/h) of hyaluronic acid from soaked contact lenses (SM), direct drug-laden contact lenses (DL), radiation sterilized direct drug-laden contact lens (DL-R), multiple implant-laden contact lens (IM), and radiation sterilized multiple implant-laden contact lens (IM-R). Values are shown as mean ± standard deviations (n=3).

3.6. Animal studies

3.6.1. In-vivo drug release study

The *in-vivo* drug release studies were carried out to gain an insight into the possible pattern of the drug (timolol and bimatoprost) release from the contact lenses in the rabbit tear fluid [85]. The tear concentration of the drugs (timolol and bimatoprost) achieved after continuous wear of the soaked contact lenses (SM, 83.13 ± 1.01 µg timolol and 44.92 ± 0.89 µg bimatoprost) and the multiple-implant-laden contact lenses (IM-R, 85.38 ± 1.64 µg timolol and 50.92 ± 1.26 µg bimatoprost) were compared with the conventional therapy of combination eye drops (0.5 % w/v timolol + 0.03 % w/v bimatoprost, one drop = 50 µl = 250 µg of timolol and 15 µg bimatoprost, GANFORT®). The timolol and bimatoprost released in the rabbit tear fluid was quantified by LC/MS. The IM-R contact lenses were sterilized by the dry radiation sterilization technique followed by the hydration step in 2 ml of the STF for 24 h (required for complete swelling) that leads to loss/leaching of 16.87 %, 47.95 %, and 24.41 % of timolol, bimatoprost, and HA respectively. The values were lower in comparison to the monomer extraction and the steam sterilization step, where 60.60 %, 61.75 % and 46.03 % of timolol, bimatoprost, and HA were lost respectively.

The initial release of bimatoprost (5 minutes, C_{max}) from the eye drop, SM, and IM-R contact lens was 143.11 ± 14.12 µg/ml, 85.54 ± 16.78 µg/ml and 37.45 ± 12.50 µg/ml respectively (Fig. 9, A). The SM and IM-R lens showed 1.68 and 3.86 folds reduction in the initial burst release in comparison to the eye drop solution. The hyperaemia reported with the use of eye drop solution (0.03% w/v) due to the high local bimatoprost concentration in the conjunctival tissue [86, 87] can be avoided with the use of the implant contact lens. The eye drop solution showed rapid decline in the bimatoprost concentration which was expected, while both SM and IM-R contact lens showed improvement in bimatoprost retention in the rabbit tear fluid. In comparison to the SM lens, IM-R lens showed higher bimatoprost levels in the rabbit tear fluid for up to 24 h. The mean residential time (MRT) of bimatoprost using eye drop, SM and IM-R was 14.45 minutes, 3.95 h and 9.08 h respectively.

The initial release of timolol (5 minutes, C_{max}) from the eye drop, SM, and IM-R contact lenses was 2456 ± 325.45 µg/ml, 93.78 ± 25.32 µg/ml and 31.85 ± 18.98 µg/ml

respectively (Fig. 9, B). The SM and IM-R lens showed 26.18 and 77.11 fold reduction in the initial burst release in comparison to the eye drop solution. The systemic side effects reported with the use of high strength timolol eye drop solution (0.5 % w/v) can be overcome with the use of the contact lens. The eye drop solution showed rapid decline in timolol concentration, while the contact lens showed significant improvement in the timolol retention time in the rabbit tear fluid. Similar to the bimatoprost release profile, the IM-R lens showed higher timolol concentration in the rabbit tear fluid in comparison to the SM batch for up to 48 h. The mean residence time (MRT) of timolol using eye drop, SM and IM-R was 11.47 minutes, 6.61 h and 17.26 h respectively.

The drugs released from the IM-R lens and the standard timolol and bimatoprost showed similar high resolution mass spectra (Supplementary file 8), which confirms the integrity of the drugs released from the lens. The *in vitro-in vivo correlation* (IVIVC) was plotted using the data of percentage cumulative *in vitro* release and percentage cumulative drug retained in the rabbit tear fluid. The IVIVC levy plot (Level A) is shown in Fig. 10. For timolol, the R^2 (correlation coefficient) value for the SM contact lens and the IM-R contact lens was $R^2 = 0.997$ and $R^2 = 0.975$ respectively (Fig. 10, A), which suggest a linear relationship between the *in vitro* cumulative timolol release and the cumulative timolol retained in the tear fluid. Similarly, the high correlation coefficient values were observed for the bimatoprost release (Fig. 10, B) from the SM contact lens ($R^2 = 0.972$) and IM-R contact lens ($R^2 = 0.994$). The high values of R^2 proposed that the *in vitro* drug release study was projecting the *in vivo* presence of drug in the rabbit tear fluid. The histopathological reports of the cornea (Fig. 11) for the control, SM and IM-R groups showed normal non-keratinizing squamous epithelium with normal distribution of the collagen fibers in the corneal stroma. The reports suggest no obvious histopathological alterations in the cornea due to the contact lenses. However, a long term histopathological studies (3 weeks) are required to perform with the implant contact lenses.

Thus, in comparison with the eye drop therapy, the multiple-implant-contact lens showed significantly lower burst release and an improvement in the drug residence time. Therefore, the chances of hyperaemia and the systemic side effects associated with the use of Ganfort® eye drops can be avoided with the use of the contact lenses.

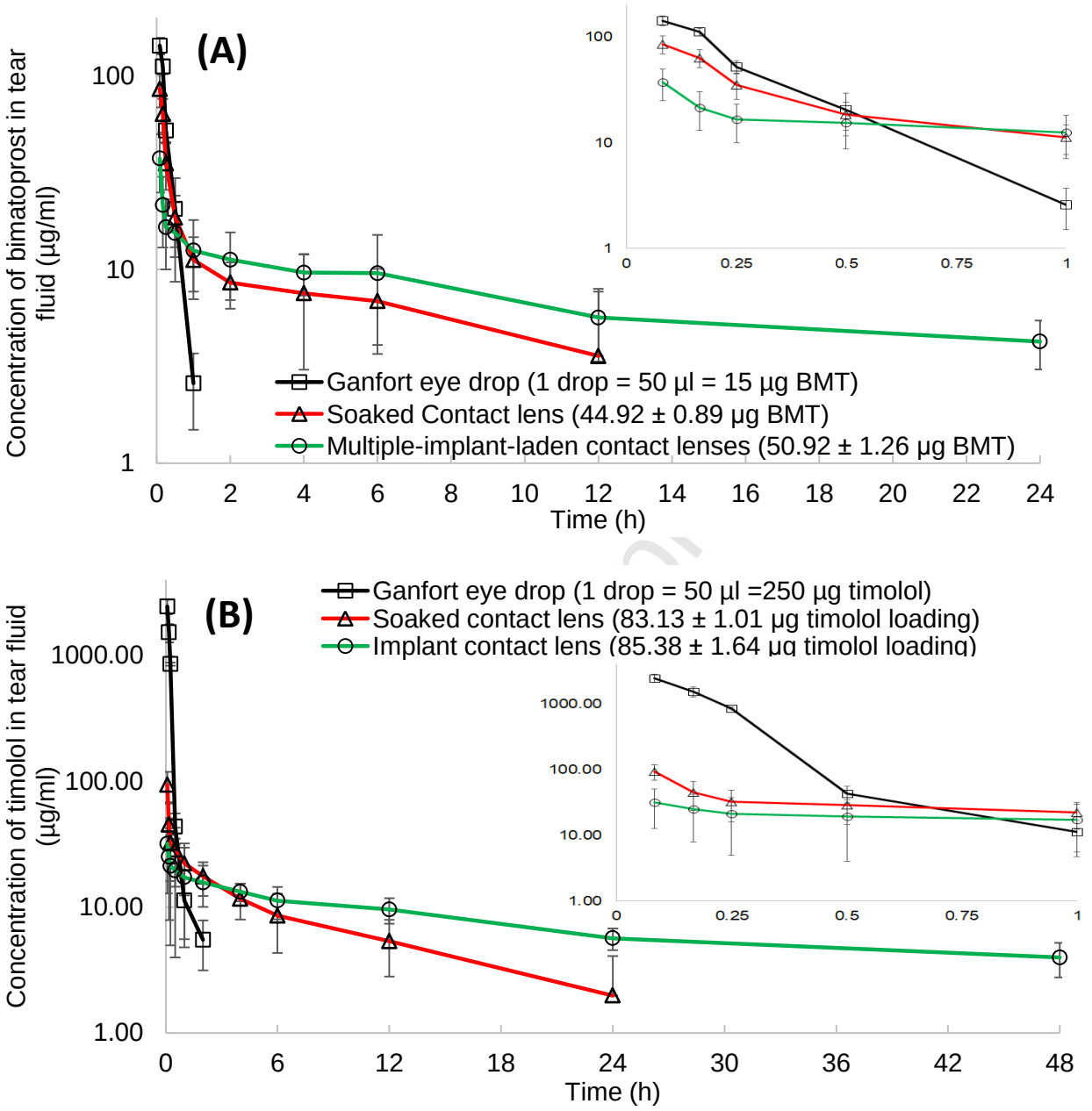

Fig. 9. (A) Bimatoprost tear fluid concentration-time profile after application of radiation sterilized multiple-implant laden contact lenses (50.92 ± 1.26 µg loading, 24.42 µg loss during hydration step), soaked contact lenses (44.92 ± 0.89 µg loading) and single instillation of Ganfort eye drop (50 µl = 15 µg of bimatoprost). (B) Timolol tear fluid concentration-time profile after application of radiation sterilized multiple-implant laden contact lenses (85.38 ± 1.64 µg loading, 14.40 µg loss during hydration step), soaked contact lenses (83.13 ± 1.01 µg loading) and single instillation of Ganfort eye drop (50 µl = 250 µg of timolol). The inset shows the initial 1 h release profile. Each point represents the mean ± standard deviation (n=4).

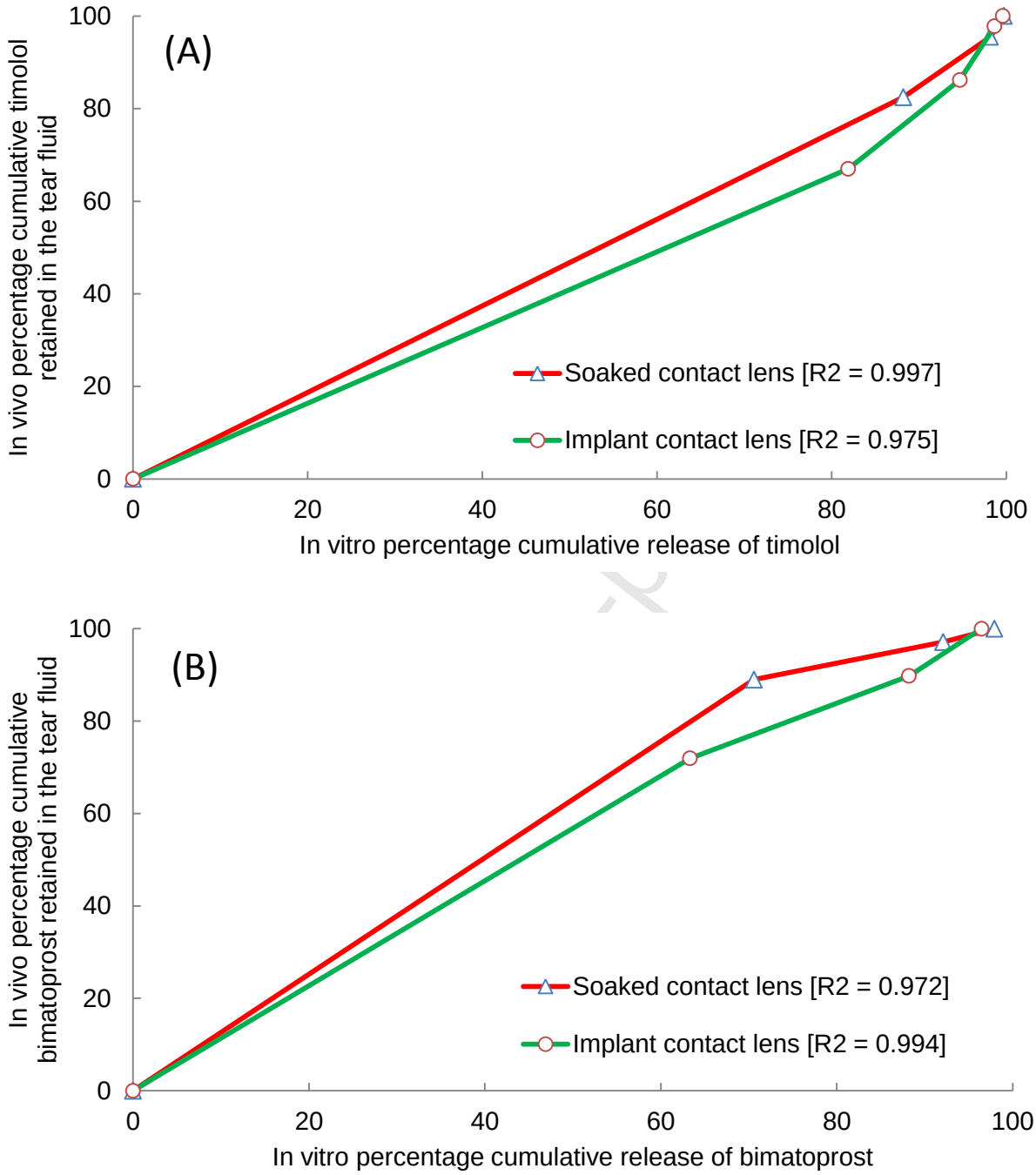

Fig. 10. Levy plot for the relationship between the *in vivo* percentage cumulative of drug retained in the tear fluid and the percentage cumulative of drug released *in vitro*, (A) Timolol (soaked contact lenses, R^2 = 0.997 and implant contact lenses, R^2 = 0.975), (B) Bimatoprost (soaked contact lenses, R^2 = 0.972 and implant contact lenses, R^2 = 0.994), R^2 = correlation coefficients.

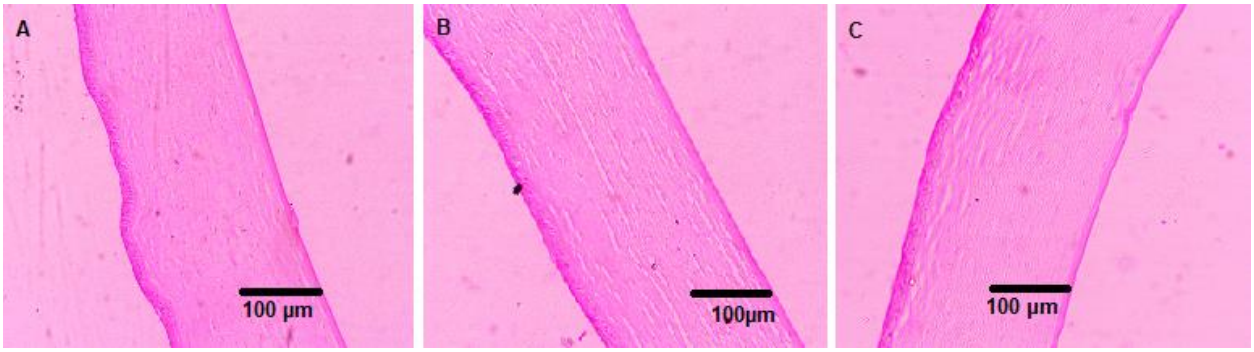

Fig. 11. Histopathological images of cornea, (A) control rabbit eye, (B) soaked contact lens and (C) multiple implant-laden contact lens.

3.6.2. In-vivo pharmacodynamic study

The therapeutic efficacy of the developed multiple-implant-contact lens (IM-R, timolol = 85.38 ± 1.64 µg loading, 14.40 µg loss during hydration step, bimatoprost = 50.92 ± 1.26 µg loading, 24.42 µg loss during hydration step and HA = 26.36 ± 1.33 µg loading) and the soaked contact lens (SM, timolol = 83.13 ± 1.01 µg loading, bimatoprost = 44.92 ± 0.89 loading, and HA = 16.81 ± 1.07 µg loading) to reduce IOP was compared with the multiple eye drop instillation (1 drop of Ganfort® = 250 µg timolol maleate, 15 µg bimatoprost, every 24 h) in the rabbit model (Fig. 12). It should be noted that bimatoprost does not produce any significant effect on the reduction in IOP in the rabbit eyes [88], thus the effect will be mainly due to timolol with lenses and Ganfort® eye drops.

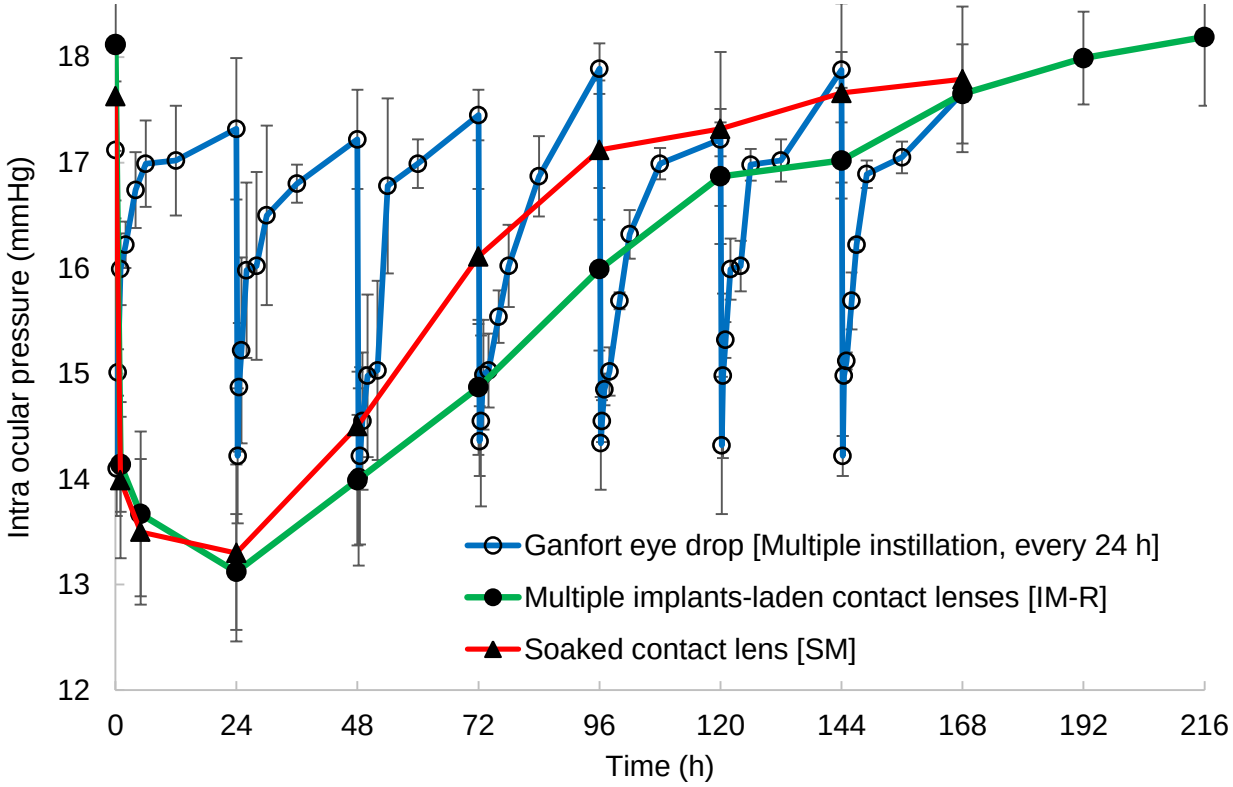

Fig. 12. The change in the intraocular pressure (IOP; mmHg) in the rabbits treated with radiation sterilized multiple-implant laden contact lenses (IM-R, timolol = 85.38 ± 1.64 µg loading, 14.40 µg loss during hydration step, bimatoprost = 50.92 ± 1.26 µg loading, 24.42 µg loss during hydration step and HA = 26.36 ± 1.33 µg loading), soaked contact lenses (SM, timolol = 83.13 ± 1.01 µg loading, bimatoprost = 44.92 ± 0.89 loading, and HA = 16.81 ± 1.07 µg loading) and Ganfort eye drops (1 drop = 250 µg timolol maleate, 15 µg bimatoprost, multiple instillation every 24 h). All the values are reported as the mean ± SD (n = 6).

The normotensive IOP of the rabbits in the eye drop, soaked contact lens and implant contact lens was found to be 17.12 ± 1.64 mmHg, 17.63 ± 2.00 mmHg and 18.12 ± 2.64 mmHg respectively. The Ganfort® eye drop showed maximum reduction of the IOP by 3.10 mmHg (at 15 minutes) from the baseline, however, the effect was not continued and diminished after 6 h reaching back to the normotensive pressure within 12 h. The average reduction in the IOP during the multiple eye drop instillation was 1.28 mmHg (7 days study), with the peak and valley profile. The peak reduction in the IOP (at 24 h) using the SM and IM-R lenses was 4.33 mmHg and 5.00 mmHg respectively. In

comparison to the eye drop therapy (peak and valley), the SM and IM-R lenses showed prolong reduction in IOP for 96 h and 120 h respectively. The average reduction in the IOP with the SM and IM-R lens was 2.87 mmHg (96 h) and 3.45 mmHg (120 h), which was 2 to 3 fold higher than the eye drop treated group (1.28 mmHg, 7 days average). The multiple-implant-contact lens shows better results in comparison to the soaked contact lens, which was expected from the *in vitro* and *in vivo* drug release data. Thus, the multiple-implant-contact lens (without any preservatives) showed much improved drug residence time with prolong reduction in the IOP, which could be due to the drug-depot formation (52) in the thick hydrophilic stroma layer (80% of water) of the ocular tissue. The lens also releases hyaluronic acid along with timolol and bimatoprost, thus the local irritation and the discomfort associated with the contact lens use can be successfully avoided. During the entire study period, no symptoms of any ocular irritation were observed such as conjunctival swelling (redness), discharge or chemosis.

4. Conclusion

The study explored the application of implantation technology to deliver multiple drug without high initial burst release. The multiple implant-laden contact lens (IM-R) did not showed significant alteration in the % swelling and the optical transmittance in comparison to the control contact lens, as the implants occupy a limited area of the lens. The AFM report suggest smooth surface of the implant area of the IM lens, as the implants were inside the contact lens matrix. During the monomer extraction and wet sterilization, the IM lens lost major amount of drugs [timolol = 60.60 %, bimatoprost = 61.75 % and HA = 46.03 %], while the option of dry radiation sterilization (IM-R) and hydration (in STF) for 24 h prior to use showed relatively lower loss of drugs [timolol = 16.87 %, bimatoprost = 47.95 % and HA = 24.41 %]. The *in-vitro* drug release data of the IM-R lens, showed sustained release for 72 h, with lower burst release in comparison to the soaked (SM) and the direct drug laden contact lenses (DL). The *in vivo* drug release data in the rabbit tear fluid showed sustained release with high drug (timolol and bimatoprost) level with the IM-R lens in comparison to the SM lens and the eye drop therapy. The burst release with the IM-R lens was many fold reduced, which could bypass the side effects associated with multiple eye drop therapy. The *in vivo*

pharmacodynamic results showed peak and valley profile with the multiple eye drop therapy, while the SM and IM-R lens showed prolong reduction in IOP for 96 h and 120 h respectively. Thus, the implantation technology demonstrates the application of delivering multiple drug for a prolonged period of time from the contact lenses, with much lower doses and low burst release, which could be a good option in place of multiple eye drop therapy.

Disclosures

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

Acknowledgments

This research project was funded by Uka Tarsadia University, under Research Promotion Scheme (UTU/RPS/2693/2017).

Data availability

Most of the raw data are available in the supplementary files. The other data could also be available on request to corresponding author.

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Graphical Abstract