Simulation of the hydrodynamic conditions of the eye to better reproduce the drug release from hydrogel contact lenses: experiments and modelling

A.F.R. Pimenta[1,2], A. Valente[1], J.M.C Pereira[2], J.C.F Pereira[2], H.P. Filipe[3], J.L.G. Mata[1], R. Colaço[2], B. Saramago[1], A.P. Serro[1,4]

[1] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

[2] IDMEC, Mechanical Engineering Department, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

[3] Hospital das Forças Armadas - Polo de Lisboa, Azinhaga dos Ulmeiros, Paço do Lumiar, 1690-020 Lisboa and Hospital dos SAMS, R. Fialho de Almeida, 1070-128 Lisbon, Portugal

[4] Centro de Investigação Interdisciplinar Egas Moniz, Instituto Superior de Ciências da Saúde Egas Moniz, Quinta da Granja, Monte de Caparica, 2829-511 Caparica, Portugal

* Corresponding author

e-mail: anapaula.serro@tecnico.ulisboa.pt

Abstract

Currently, most *in vitro* drug release studies for ophthalmic applications are carried out in static sink conditions. Although this procedure is simple and useful to make comparative studies, it does not describe adequately the drug release kinetics in the eye, considering the small tear volume and flow rates found *in vivo.*

In this work a microfluidic cell was designed and used to mimic the continuous, volumetric flow rate of tear fluid and its low volume. The suitable operation of the cell, in terms of uniformity and symmetry of flux, was proved using a numerical model based in the Navier-Stokes and continuity equations. The release profile of a model system (a hydroxyethyl methacrylate-based hydrogel (HEMA/PVP) for soft contact lenses (SCLs) loaded with diclofenac) obtained with the microfluidic cell was compared with that obtained in static conditions, showing that the kinetics of release in dynamic conditions is slower. The application of the numerical model demonstrated that the designed cell can be used to simulate the drug release in the whole range of the human eye tear film volume and allowed to estimate the drug concentration in the volume of liquid in direct contact with the hydrogel. The knowledge of this concentration, which is significantly different from that measured in the experimental tests during the first hours of release, is critical to predict the toxicity of the drug release system and its *in vivo* efficacy.

In conclusion, the use of the microfluidic cell in conjunction with the numerical model shall be a valuable tool to design and optimize new therapeutic drug loaded SCLs.

Keywords: Controlled drug release; Hydrogels; Contact Lenses; Microfluidics; Numerical simulation

INTRODUCTION

Topical administration of ocular medication through eyedrops constitutes the most used form of treatment of the diseases that affect the anterior segment of the eye. It is estimated that this conventional dosage form accounts for approximately 90% of the commercial ophthalmic formulations [1,2]. The use of eye drops has numerous advantages, namely the ease of administration, patient compliance and low cost. However, these conventional ocular formulations show low bioavailability due to physiological and anatomical constraints of the eye. Typically, only a small fraction of the administered dose (1-7%) is absorbed due to spillage from the eye, lacrimation and tear turnover, nasolacrimal drainage, metabolic degradation, and/or non-specific absorption [3,4]. Moreover, as a result of the tear drainage, part of the administered drugs are absorbed into the systemic circulation and may reach important organs, causing eventual side effects [5,6].

Several strategies have been attempted to overcome these problems, including the use of emulsions, ointments, suspensions, aqueous gels, *in situ* thermosensitive gels, nanomicelles, nanoparticles, liposomes, dendrimers, nanosuspensions, microneedles, implants and soft contact lenses (SCLs) [7–9]. Among these, SCLs have raised special attention, due to their high degree of comfort, biocompatibility and prolonged contact with the eye. More than 125 million people worldwide use contact lenses, which corresponds to a market of about $7.6 billion [10]. These devices are generally used to correct a number of vision impairments and for cosmetic purposes, but may also be used for therapeutic reasons. In certain situations, clinicians prescribe contact lenses to relief the ocular pain, promote the corneal healing, provide mechanical protection and support and maintain the corneal epithelial hydration [11]. They have also potential to be used as drug delivery devices [12,13], although till date there are no contact lenses loaded with drugs available in the market. So far, SCLs are only used in association with eyedrops, and in this case, they must be placed in the eye immediately after the application of the drops to increase the drugs residence time in the eye. This procedure improves the drugs permeation and absorption through the cornea but still requires the patient's compliance and ability to self-administer the medicine.

Efforts have been made in the last decades to develop drug loaded SCLs that ensure *per si* a sustained drug release, allowing to enhance the drug bioavailability in the eye, and thus, to improve the efficacy of treatments. Several approaches are described in the literature, including the modification of the composition of the base materials by

incorporation of monomers/compounds that enable to tailor the hydrogel properties [12,13], molecular imprinting with the drugs to create "cavities" in the polymeric matrix which affect the reloading/swelling of the hydrogels and the respective drug release [14] and grafting of ligands to the hydrogel matrix which form inclusion complexes with the drugs [15]. The possibility of creating two-layer contact lenses with an inner drug-bearing biodegradable polymer film was also investigated [12]. Other researchers incorporated in the hydrogels drug-loaded nanostructures, like nanoparticles and liposomes [1,16,17].

All these studies involve *in vitro* drug release experiments which are typically carried out in static conditions. It is normally assumed that the release occurs in infinite sink conditions[1] and the accumulation of drug in the solution surrounding the hydrogel is negligible, which is not always true. In fact, the low solubility of some drugs, the small volumes of release (e.g. 2-10 mL) and experiments performed without stirring often used in this type of experiments, may compromise the infinite sink conditions [18,19]. It should be stressed that in the eye, sink conditions can be maintained if the drug clearance is high. However, the total clearance mechanism (including lacrimal turnover and absorption by conjunctiva) is complex and difficult to simulate in *in vitro* studies. Static conditions are generally used due to the simplicity of the experimental procedure and they are useful to compare different systems. However, they are far from simulating the real release conditions, since they do not match the ocular *in vivo* flow conditions. In a normal situation, the human eye contains a tear volume that ranges from 6.2 to 30.0 μL [20–22] and the tear flow rate assumes values between 0.9 and 2.1 μL/min [23]. The use of contact lenses increases the tear turnover to values of the order 1.4-4.3 μL/min [23]. In order to predict in a more reliable way the drug release kinetics in the eye, it is crucial to develop microfluidic models that mimic, as close as possible, the hydrodynamic conditions of the eye.

As far as the authors know, only a reduced number of papers have addressed this issue in the development of new therapeutic drug loaded SCLs. The group of M.E. Byrne used two microfluidic devices with different geometries [4,24] to study the drug release behavior of SCLs materials under physiological flow rates. The results were compared with those obtained in static conditions. It was concluded that under flow conditions similar to those of the human eye, the release time of the drugs increased, and more

[1] The term **sink conditions** is usually defined as the volume of medium at least greater than three times that required to form a saturated solution of a drug substance.

sustained release profiles were obtained. More recently, Jones *et al.* [25] achieved similar conclusions. They compared the release of two drugs from commercial contact lenses, in static and dynamic conditions, and concluded that experimental parameters, such the volume of release and the flow rate, have a significant influence on the measured release profiles. Although the work of these groups has contributed to draw the attention to the importance of reproducing more closely the eye conditions in *in vitro* tests, in no case a detailed characterization of the microfluidic devices operation was done. The use of numerical models yields information on the fluid flow inside the devices which is not accessible from experimental measurements. Furthermore, these models would allow predicting the impact of changing the inner chamber volume and/or the flow rate, on the drug release profiles.

In this work, a microfluidic cell was designed and built to mimic the tear flow rate and to approximate the *in vitro* drug release conditions to those found in the eye. Due to operational limitations, the volume of the inner chamber, where the hydrogel is placed, is higher than the tear film volume in the eye (while the minimum volume of the cell chamber that could be achieved was 45 μL, the maximum tear film volume in the eye is 30 μL). A conventional hydroxyethyl methacrylate-based hydrogel (HEMA/PVP) for SCLs loaded with an anti-inflammatory (diclofenac) was used as model system to obtain an experimental release profile using the microfluidic cell. This release profile was compared with the one obtained in static conditions. The fluid dynamics in the microfluidic cell was fully characterized through numerical simulation using a computational model. The fitting of the numeric model to the experimental drug release profile allowed the parametrical characterization of the system and the use of a release volume closer to the lower limit of the tear film volume. The *in vivo* drug release efficacy of the studied model system was predicted, taking into account the estimated drug concentration in the tear film volume.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), 2,2'-azobis(2-methylpropionitrile) (AIBN), phosphate saline buffer (PBS, pH 7.4), phosphoric acid, monopotassium phosphate, diclofenac sodium (DCF), was all purchased from Sigma-Aldrich. Poly(viny thlpyrrolidone) (PVP K30, Kollidon VR 30) was kindly provided by BASF. Acetonitrile and methanol were purchased from Fisher

Scientific. A Millipore Milli-Q water purification system was used to get distilled and deionized (DD) water.

Hydrogels preparation

To prepare HEMA/PVP based hydrogels (98/2, w/w), an appropriate amount of the crosslinker EGDMA was dissolved in HEMA (hydrophilic monomer) to obtain a concentration of 80 mM. Then, the mixture was degassed by ultra-sounds (5 minutes) and bubbled with a gentle stream of nitrogen (15 minutes) before the addition of AIBN (initiator) to a final concentration of 10 mM, and PVP (hydrophilic additive) to a final concentration of 0.02 g/mL. The solution was magnetically stirred for about 2 hours to obtain complete dissolution of PVP and injected into a mold consisting of two silanized glass plates (silanization procedure described in [26]) separated by a spacer of polyurethane. The polymerization reaction was performed at 50 °C for 14 hours, followed by 24 hours at 70 °C. The obtained hydrogel sheets were washed over 5 days, with DD water renewed three times a day, to remove unreacted monomers and to facilitate the cutting of the samples used in the study. The hydrated samples (thickness 0.30 mm) were cut with a leaker of diameter 1.4 cm and dried overnight in an oven at 35 °C.

Drug loading and drug release

The dry hydrogel samples were loaded with diclofenac (DCF) by soaking in the drug solution (prepared with PBS) with a concentration of 1 mg/mL, for 38 hours, at ambient temperature, in the dark.

For the *in vitro* static drug release experiments, drug loaded samples were immersed in 4 mL of PBS solution in closed vessels, at 36 °C, under stirring (180 rpm). At predetermined time intervals, 800 µl aliquots of the supernatant were collected and replaced by the same volume of fresh PBS solution.

In vitro dynamic drug release experiments were done in a microfluidic cell fabricated with in poly(methylmethacrylate) (PMMA). The microfluidic cell is constituted by one central feeding pipe and from eight radial exit pipes converging in a collector ring (Fig. 1a). The cell was designed with a cylindrical inner chamber of 45 µL. This volume is slightly superior to the volume of the tear fluid present in the eye (6.2-30.0 µL) [20–22], but, due to operational constraints it could not be smaller.

[Insert Figure 1]

Fig. 1 a) Schematic representation of the microfluidic cell used to approximate *in vitro* to *in vivo*'s release conditions and b) experimental apparatus used in the dynamic release experiment

In order to approach the physiological conditions, the drug release experiments in the microfluidic cell were performed at 36°C and a continuous flow of PBS of 3 μL/min was used. This flow rate is within the range of values found for contact lenses wearers (1.4-4.3 μL/min [23]). At predetermined time intervals, the out flow solution was collected to be analyzed. All *in vitro* release experiments were carried out in triplicate.
The concentration of diclofenac in the collected samples was determined using a high performance liquid chromatograph (HPLC) with a Jasco UV–vis detector and a C-18 column Nova-Pak Watters, at the wavelength of 276. The mobile phase, consisting of phosphoric acid, acetonitrile and methanol (40/48/12 in volume), was introduced into the column at a flow rate of 1 mL/min and a pressure of 14 MPa.

Numeric flow modulation

The flow inside the microfluidic cell was modeled through the numerical solution of the Navier-Stokes and continuity equations using the StarCCM+ simulation package. The fluid was considered to be incompressible and with constant properties: density of 0.9937 g/cm^3 and dynamic viscosity of 0.0692 mPa.s at 36 ºC. The background fluid (tear fluid) was represented by the buffered saline (PBS). The drug flows in the tear fluid volume as a passive scalar. The passive scalar model is used since the drug properties do not affect the solution properties, due to the very low concentration of the drug. Diclofenac diffusivity in PBS, determined by Nuclear Magnetic Resonance (NMR), is 1.4x10^{-9} m^2/s [27]. Effective diffusivity in the hydrogels materials was determined by fitting the numerical model to the experimental release data obtained with the microfluidic cell (inner chamber 45 μL). The lens is modeled as a porous media with hydrodynamic permeability of 10^{-14} m^{2}s^{-1}Pa^{-1} [28,29] and a polymer volume fraction in the swollen state of 0.62. The used mesh of trimmed topology comprises about one million control volumes, with higher cell density in the zone near the lens and tear film. The time step was 60 seconds. The effect of the mesh size and time stepping on the results was analyzed indicating independence on the used numerical parameters.

RESULTS AND DISCUSSION

Comparison of static and dynamic release conditions

As previously referred, static conditions are used in drug release studies due to the simplicity of the experimental procedure. In Figure 2, the fractional cumulative DCF mass profile obtained in static conditions is compared with the one obtained using the microfluidic cell.

[Insert Figure 2]

Fig. 2 DCF fractional cumulative mass release in static and dynamic conditions (microfluidic cell) from HEMA/PVP hydrogel

The release kinetics is greatly affected by the release conditions: in static conditions more than 90% of DCF is released upon the first 24 hours; in the inner chamber of the microfluidic cell the release progresses at a steady rate, reaching 90% of total release at approximately the 100^{th} hour. Such differences in the drug release profiles can be understood through the analysis of the driving forces for drug release. The decrease of the release medium volume (from 4 mL, used in static experiments, to 45 µL in the microfluidic cell) leads to distinct concentration gradients between the drug loaded hydrogel and the release medium. In static experiments the drug concentration in the release medium is well below the drug solubility limit, which is 6-9 mg/mL for DCF in PBS [30]. In these conditions, the driving force produced by the gradient of concentrations between the hydrogel and the supernatant is maximum. Additionally, the mechanical stirring during the release experiment contributes to obtain a homogeneous medium and causes the disruption of any surface boundary effect. In turn, in microfluidic conditions, the more sustained release could be a consequence of the lower gradient of the drug concentration resultant from the small volume of release and flow conditions. Similar conclusions were achieved by other authors who compared the results in static conditions and under flow [4,25,26]: the release time of the drugs increased under flow, being significantly affected by the volume of release and the flow rate.

Static conditions can be useful for an initial evaluation of a drug releasing system performance, as well as to make comparisons between different systems. Nevertheless, one should take in consideration that in static conditions the release environment is far from replicating the hydrodynamic conditions of the eye, as our results show.

A microfluidic system designed to approximate the *in vivo* conditions (volume and renovation rate) can provide more reliable information about the drug delivery system behavior, particularly in what concerns release rates and release amounts.

Evaluation of the microfluidic cell operation

In order to ensure that the designed cell respects the fluid mechanics principles, e.g. avoiding dead volumes, the cell operation was evaluated through the numerical flow modulation described above.

In terms of fluid dynamics, considering a flow rate of 3μL/min, the flow in the inner chamber of the microfluidic cell is found to stabilize due to the Stokes nature of the considered flow. Also, the unsteady diffusion of the drug into the inner chamber (further studied) appears to have a negligible effect in the fluid dynamic, again due to the very low concentration, both in mass and volume terms.

The fluid flows in the cell uniformly, as shown in the Figure 3a where some streamlines are drawn. The color is related to the local fluid velocity. Symmetric and very regular paths are followed by the fluid from the inlet pipe to the eight exiting pipes. In the leeward side of the returning pipes the velocity field also displays a regular pattern, therefore no dead zones are present in the flow. Vector field details are presented in Figure 3b. From this figure one can verify the flow overall uniformity and alignment in the inner chamber.

[Insert Figure 3]

Fig. 3 a) Representation of the paths of fluid inside the microfluidic cell with a central entry point and eight radial exit points and b) vector field details in a cross section of the cell

Impact of the inner chamber volume on drug release profiles

Figure 4 presents the concentration profile of DCF released from HEMA/PVP hydrogel obtained with the microfluidic cell, whose inner chamber volume is 45 μL. The numerical model was fitted to the experimental data, setting the hydrogel properties (hydrodynamic permeability and porosity) and the external medium properties (density, viscosity and DCF diffusivity in PBS) - see values in Numeric flow modulation section.

Although a large deviation between the data and the fitting is observed in the first 24 hours of release (r^2=0.734), the quality of the fitting greatly improved after that (r^2=0.964).

From the fitting, the effective diffusivity of DCF in the HEMA/PVP hydrogel was estimated to be 1.35 x10^{-13} m^2/s.

Since it was not possible to design a microfluidic cell with an inner chamber volume inferior to 45 µL, the numerical tool was used to predict the drug release profile in a volume of 7 µL, which is close to the minimum value of the tear film, considering the value estimated for DCF diffusivity in HEMA/PVP. Both estimated profiles (for 45 µL and 7 µL) are also presented in Figure 4.

[Insert Figure 4]

Fig. 4 Concentration profile of DCF released from HEMA/PVP at the exit point of the microfluidic cell and adjustment obtained using the numerical model for cells with inner chambers of 45 µL and 7 µL. Zoom for the first hours is included

It can be observed that the profiles estimated by numerical simulation are not significantly affected by the inner chamber volume. The initial lag observed in these curves does not find correspondence with the experimental data, because the experiment with the microfluidic cell started with the device filled with PBS to ensure that no air bubble was trapped inside. The first aliquot collected consists of PBS that was forced to pass through the inner chamber at an higher velocity, and thus, will drag some drug. This initial step could not be considered in the simulations. The obtained results allow us to conclude that our microfluidic cell is able to simulate the range of the human eye tear film volume (6.2 to 30.0 µL).

Efficacy of diclofenac loaded SCLs

It is important to stress that the results of simulation presented in Figure 4 are concentrations of DCF at the exit point of the system, since in the experimental assay this is the collection point of the aliquots for drug quantification. However, to approach the *in vivo* conditions, we should consider the concentration of DCF in the inner chamber (that represents the tear film volume), to estimate the efficacy of drug loaded SCLs. As far as the authors know, all the reported studies using microfluidic devices to

study drug release from SCLs rely on the measurement of drug concentrations at the exit point.

The drug concentration in the inner chamber can be estimated through the numerical simulation. In Figure 5, the predicted concentration profile of DCF for the 7 µL inner chamber of the microfluidic cell is shown. The concentration profile in the eye, resultant from application of commercial DCF eyedrops, according to the recommend posology, is also represented. This profile was estimated considering the application of 1 drop of DCF commercial ophthalmic solution (e.g. Voltaren Ophthalmic®, 1 mg/ mL) each 4 hours during the day (the posology recommended for treatment of a post traumatic inflammation) and assuming that a volume of 7 µL of an eyedrop remains in the eye and the tear renovation rate is 3 µL/ min.

[Insert Figure 5]

Fig. 5 Concentration profiles of DCF released from HEMA/PVP at the exit point and at the 7 µL inner chamber of the microfluidic cell, obtained using the numerical model. The concentration profile in the eye resultant from application of DCF eyedrops is also shown

As expected, the periodic application of the eyedrops leads to a saw shape drug concentration profile. In contrast, in the case of the drug loaded hydrogel, although the concentration profile at the inner chamber presents an initial burst, after the first few hours, a more sustained delivery is achieved. The maximum concentration achieved in the inner chamber is slightly higher than that obtained by application of the eyedrops. One should note that the chosen inner chamber volume is close to the lower limit of the tear film volume in the eye, whereby, higher tear film volumes should lead to lower burst values. The comparison of the concentration profiles correspondent to the inner chamber and the exit point shows a significant difference during the first 2 hours which faints afterwards.

To assess the efficacy of the drug release system herein studied, the therapeutic amount which has to be delivered to the eye was estimated. As mentioned above, when eyedrops are applied, only 1-7% of the administered dose is absorbed. Considering an eyedrop of 34 µl [31] and an effective absorption of 5%, the recommend posology delivers, in one day, 8.5 µg (5 x 1 drop). Taking this value into account, one can predict

for how long the studied hydrogels release a therapeutic amount of drug. In Table 1 the DCF mass released per day from a SCL, obtained by numerical simulation for the inner chamber, is shown. An average dry weight of 30 mg was assumed for the SCL.

[Insert Table 1]

The results analysis suggests that the HEMA/PVP SCLs loaded with DCF shall be effective during ≈ 5 days.

It should be underlined that the studied system (drug + hydrogel) is a model system. Thus, several aspects may require optimization to develop a commercial therapeutic device. For example, instead of using a HEMA-based, which has a low oxygen permeability, we could use the newer silicon hydrogels that, due to their higher oxygen permeability would be more suitable for a prolonged drug release [13]. Also, the loading procedure could be modified to adjust the amount of drug released. However, the focus of this work is not the optimization of a specific drug delivery system, but rather finding a way to better simulate the behavior of the drug loaded lenses *in vivo*.

In conclusion, the use of the microfluidic cell to access the drug release profiles in hydrodynamic conditions which are closer to those found *in vivo* (in particular volume and renovation rate), associated with the application of the numerical model that predicts the drug concentration in the tear film volume, shall be a valuable tool to design and optimize new therapeutic SCLs.

CONCLUSIONS

In the last years, an increasing number of studies has focused on the development of drug delivery systems for ophthalmic applications, based on SCLs. In almost all those studies, the *in vitro* drug release profiles are obtained in static conditions, very different from those found in the eye.

In this work, a microfluidic cell was designed to simulate the hydrodynamic conditions of the eye, namely the low volume of tear fluid and its continuous renovation. The release of a non-steroid anti-inflammatory commonly used in ocular therapy (diclofenac) from a conventional HEMA-based hydrogel for contact lenses was investigated. The drug release profiles in static conditions and obtained with the microfluidic cell were compared. As expected, the release time of the drugs increased under flow.

The flow inside the microfluidic cell was characterized using a computational fluid dynamics numerical model based on the Navier-Stokes and continuity equations. It was demonstrated that the fluid flows in the cell uniformly without dead zones, which testifies the adequate operation of the cell.

The numerical model was fitted to experimental data obtained with the cell, using as adjustment parameter the diffusivity of the drug in the hydrogel. The model was then used to evaluate the effect of changing the volume of liquid in contact with the hydrogel, on the drug release profiles. The reduction of the volume from 45 μL (the volume of the inner chamber of the cell used) to 7 μL (≈ the lower limit of the tear volume in the eye) led to minor differences in the concentration profiles of the solution collected from the cell, demonstrating that the designed microfluidic cell may be used to simulate lower volumes, within the tear film volume range.

The numerical model was applied to estimate the drug concentration in the volume of liquid in direct contact with the hydrogel, which, in the first few hours, is significantly different from that measured experimentally (from solutions collected at the exit point of the microfluidic cell). This estimated concentration simulates the tear film concentration and allows a more reliable prediction of the *in vivo* efficacy of the drug loaded hydrogel than that based on common static drug release experiments.

ACKNOWLEDGEMENTS

Acknowledgements are due to Fundação para a Ciência e a Tecnologia for funding through the projects PEst-OE/QUI/UI0100/2013, M-ERA.NET/0005/2012 and support, through IDMEC, under LAETA, of project UID/EMS/50022/2013. A.F.R. Pimenta acknowledges Fundação para a Ciência e a Tecnologia for the PhD Grant SFRH/BD/52334/2013. The authors also thank Professor J.R. Ascenso (CQE-IST, University of Lisbon) for the NMR measurement.

The authors declare that there are no conflicts of interest.

REFERENCES

1. Gulsen D, Li C-C, Chauhan A, Dispersion of DMPC liposomes in contact lenses for ophthalmic drug delivery. Curr Eye Res. 2005;30(12): 1071–80.
2. Nguyen D, Hui A, Weeks A, Heynen M, Joyce E, Sheardown H, Jones L, Release of Ciprofloxacin-HCl and Dexamethasone Phosphate by Hyaluronic Acid Containing Silicone Polymers. Materials. 2012;5(4): 684–98.
3. Gaudana R, Ananthula HK, Parenky A, Mitra AK, Ocular drug delivery. AAPS J. 2010;12(3): 348–60.
4. Ali M, Horikawa S, Venkatesh S, Saha J, Hong JW, Byrne ME, Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. J Control Release. 2007;124(3): 154–62.
5. ElShaer A, Ghatora B, Mustafa S, Raid A, Contact lenses as drug reservoirs & delivery systems: the successes & challenges. Ther Deliv. 2014;5(10): 1085–100.
6. Salminen L, Review: systemic absorption of topically applied ocular drugs in humans. J Ocul Pharmacol. 1990;6(3): 243–9.
7. Bengani LC, Chauhan A, Extended delivery of an anionic drug by contact lens loaded with a cationic surfactant. Biomaterials. 2013;34(11): 2814–21.
8. Bourlais CL, Acar L, Zia H, Sado P, Needham T, Leverge R, Ophthalmic drug delivery systems--recent advances. Prog Retin Eye Res. 1998;17(1): 33–58.
9. Kuno N, Fujii S, Recent advances in ocular drug delivery systems. Polymers (Basel). 2011;3(1): 193–221.
10. Nichols JJ, Contact Lenses 2014. Vol. 30, Contact Lens Spectrum. 2015. p. 22–7.
11. Rubinstein MP, Applications of contact lens devices in the management of corneal disease. Eye. 2003;17(8): 872–6.
12. Hehl EM, Beck R, Luthard K, Guthoff R DB, Improved penetration of aminoglycosides and fluorozuinolones into the aqueous humour of patients by means of Acuvue contact lenses. Eur J Clin Pharmacol. 1999;55(4): 317–23.
13. Kakisu K, Matsunaga T, Kobayakawa S, Sato T, Tochikubo T, Development and efficacy of a drug-releasing soft contact lens. Invest Ophthalmol Vis Sci. 2013;54(4): 2551–61.
14. Ciolino JB, Hoare TR, Iwata NG, Behlau I, Dohlman CH, Langer R, Kohane DS, A drug-eluting contact lens. Invest Ophthalmol Vis Sci. 2009;50(7): 3346–52.
15. Nicolson PC, Vogt J, Soft contact lens polymers: an evolution. Biomaterials. 2001;22(24): 3273–83.
16. Yañez F, Martikainen L, Braga MEM, Alvarez-Lorenzo C, Concheiro A, Duarte CMM, Gil MH, de Sousa HC, Supercritical fluid-assisted preparation of imprinted contact lenses for drug delivery. Acta Biomater. 2011;7(3): 1019–30.
17. dos Santos J-FR, Alvarez-Lorenzo C, Silva M, Balsa L, Couceiro J, Torres-Labandeira J-J, Concheiro A, Soft contact lenses functionalized with pendant cyclodextrins for controlled drug delivery. Biomaterials. 2009;30(7): 1348–55.

18. Alvarez-Lorenzo C, Yañez F, Barreiro-Iglesias R, Concheiro A, Imprinted soft contact lenses as norfloxacin delivery systems. J Control Release. 2006;113(3): 236–44.

19. Gulsen D, Ophthalmic Drug Delivery through Contact Lenses. Invest Ophthalmol Vis Sci. 2004;45(7): 2342–7.

20. Tieppo A, Boggs AC, Pourjavad P, Byrne ME, Analysis of release kinetics of ocular therapeutics from drug releasing contact lenses: Best methods and practices to advance the field. Cont Lens Anterior Eye. 2014;37(4): 305–13.

21. White CJ, Tieppo A, Byrne ME, Controlled drug release from contact lenses: a comprehensive review from 1965-present. J Drug Deliv Sci Technol. 2011;21(5): 368–84.

22. Mishima S, Gasset A, Klyce SD, Baum JL, Determination of tear volume and tear flow. Invest Ophthalmol Vis Sci. 1966;5: 264–76.

23. Robert L. Stamper, Marc F. Lieberman MVD, Becker-Shaffer's Diagnosis and Therapy of the Glaucomas. In: Elsevier M, editor. Part 5, 8th Edition,.

24. Linsy Farris R, Tear analysis in contact lens wearers. Trans Am Ophthalmol Soc. 1985;83: 501–45.

25. Glasson MJ, Stapleton F, Keay L, Willcox MDP, The effect of short term contact lens wear on the tear film and ocular surface characteristics of tolerant and intolerant wearers. Cont Lens Anterior Eye. 2006;29(1): 41–7.

26. Tieppo A, Pate KM, Byrne ME, In vitro controlled release of an anti-inflammatory from daily disposable therapeutic contact lenses under physiological ocular tear flow. Eur J Pharm Biopharm. 2012;81(1): 170–7.

27. Bajgrowicz M, Phan C-M, Subbaraman L, Jones L, Release of ciprofloxacin and moxifloxacin from daily disposable contact lenses from an in vitro eye model. Invest Ophthalmol Vis Sci. 2015;56(4): 2234–42.

28. Vazquez R, Nogueira R, Orfão M, Mata JL, Saramago B, Stability of triglyceride liquid films on hydrophilic and hydrophobic glasses. J Colloid Interface Sci. 2006;299(1): 274–82.

29. Pishko GL, Lee S, Wanakule P, Sarntinoranont M, Hydraulic Permeability of a Hydrogel-Based Contact Lens Membrane for Low Flow Rates. J Appl Polym Sci. 2007;104: 3730–5.

30. Refojo MF, Permeation of water through some hydrogels. J Appl Polym Sci. 1965;9(10): 3417–26.

31. Diclofenac sodium salt, Material Safety DATA, Product informations by Cayman Chemical Company, 2012.

32. German EJ, Hurst MA, Wood D, Reliability of drop size from multi-dose eye drop bottles: is it cause for concern? Eye. 1999;13: 93–100.