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Assessing the *ex vivo* permeation behaviour of functionalised contact lens coatings engineered using an electrohydrodynamic technique

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Abstract

In vitro testing alone is no longer considered sufficient evidence presented solely with respect to drug release and permeation testing. These studies are thought to be more reliable and representative when using tissue or animal models; as opposed to synthetic membranes. The release of anti-glaucoma drug timolol maleate from electrically atomised coatings was assessed here using freshly excised bovine corneal tissue. Electrohydrodynamic processing was utilised to engineer functionalised fibrous polyvinylpyrrolidone-Poly (N-isopropylacrylamide) coatings on the outer side of commercial silicone contact lenses. Benzalkonium chloride, ethylenediaminetetraacetic acid, Brij® 78 and borneol were employed as permeation enhancers to see their effect on *ex vivo* permeation of timolol maleate through the cornea. Formulations containing permeation enhancers showed a vast improvement with respect to cumulative amount of drug permeating through the cornea as shown by a six fold decrease in lag time compared to enhancer-free formulations. Most drug delivery systems require the drug to pass or permeate through a tissue or biological membrane. This study has shown that to fully appreciate and understand how a novel drug delivery system will behave not only within the device but with the external environment or tissue, it is imperative to have *in vitro* and *ex vivo* data in conjunction.

Introduction

The ability to achieve controlled and/or sustained ocular drug delivery is a constant challenge faced by research scientists [1, 2]. Whilst more conventional dosage forms such as eye drops boast ease of formulation, there is the issue of eye micro-structure serving as a barrier. Due to the complexity of the organ, sufficient therapeutic drug levels are difficult to achieve consequently leading to low bioavailability and frequent administration [1].

Despite efforts to improve drug bioavailability from a formulation view point (e.g. increasing viscosity [3], forming complexes with cyclodextrins [4, 5]), the issue of sustaining drug delivery is still prominent. As a result, novel approaches have been introduced which include the use of ocular devices as drug reservoirs [2]. The most common concept to emerge from this are contact lenses. More commonly used for vision correction, contact lenses have more recently found to act as successful drug delivery devices, achieving controlled and sustained active delivery. The use of these removable implants increases retention time of the drug in the pre-corneal region whilst minimising the amount of drug being excreted or removed by physiological mechanisms such as nasolacrimal drainage.

Table 1. Composition of each electrohydrodynamically processed formulation. Each formulation contained PVP and PNIPAM at a 50:50 ratio to achieve 5%w/v polymeric solutions.

Formulation	Timolol maleate concentration (%w/w of the polymer)	Permeation enhancer	Permeation enhancer concentration (% w/v)
Composite-TM	5	—	—
*F*1	5	BAC	0.01
*F*2	5	EDTA	0.5
*F*3	5	Brij® 78	0.1
*F*4	5	Borneol	1
*F*5	15	BAC	0.01
*F*6	15	EDTA	0.5
*F*7	15	Brij® 78	0.1
*F*8	15	Borneol	1

The aim of this study was to develop and characterise nano-coatings for contact lenses with the view to achieve the sustained release of anti-glaucoma drug timolol maleate (TM). The research and development sector of pharmaceutics is constantly evolving; building on and updating existing methods used in this remit. Whilst there is a focus on *in vitro* testing of ocular formulations with respect to release and permeation, this alone is no longer considered sufficient characterisation [6]. *In vitro* testing involves measuring the release of an active drug from a matrix in an environment simulating physiological conditions (37 °C, pH 7.4). Regardless of the ability to characterise drug release without using animals, the dialysis membrane used *in vitro* release testing may not be an adequate layer to mimic biological tissue. As such, it is vital to conduct *in vitro* drug release and *ex vivo* studies are vital in conjunction in order to arrive at more accurate conclusions. Quantifying the rate of drug permeation through a biological membrane is vital, as its impact is key in the absorption and distribution of the released drug.

Electrohydrodynamic atomisation (EHDA), more specifically electrospinning was utilised in this instance to engineer fibrous coatings for contact lens surfaces. The on-demand, cost effective process has already shown its potential in an array of applications [7] including wound management [8–13], drug delivery [14–22] bioengineering [23–26] and theranostics [27]. Here, electrospun fibrous coatings were engineered to assess the *ex vivo* release and permeation of TM through freshly excised cornea as an extension of previous work [18]. The effectiveness of four different permeation enhancers in increasing TM permeation through bovine cornea was evaluated.

Materials

Polyvinylpyrrolidone (PVP) (4.4×10^4 g mol^{-1}) was obtained from Ashland, Worcestershire, United Kingdom. Poly (N-isopropylacrylamide) (PNIPAM) ($2–4 \times 10^4$ g mol^{-1}), ethanol, TM, benzalkonium chloride (BAC), ethylenediaminetetraacetic acid (EDTA), Brij® 78 and borneol were all purchased from Sigma Aldrich, Dorset, United Kingdom. All reagents used were of analytical grade.

Methods

Solution preparation

Solutions containing PVP and PNIPAM (now referred to as composite) at 50:50 ratio to achieve 5%w/v solutions were prepared by dissolving the polymers in ethanol by mechanical stirring for 30 min at ambient temperature (23 °C). Two different TM concentrations (5%w/w and 15%w/w of the polymer weight) were prepared using this stock solution. These base solutions were then used to prepare further formulations each containing a different permeation enhancer. Table 1 shows the final composition of the 8 solutions prepared for this study.

Coating engineering

These solutions were processed using EHDA, more specifically the electrospinning process. A schematic diagram of the set-up can be seen in figure 1. The solutions were drawn into 5 ml syringes that were attached to a syringe infusion pump. The pump allowed controlled flow of liquid through the electrospinning set-up. The solution was fed through silicone tubing to a conductive stainless steel needle; which was attached to a high power voltage supply. All atomisation processes were carried out in ambient conditions. The resulting coatings were first collected on microscope slides for pilot studies then subsequently onto dehydrated commercial

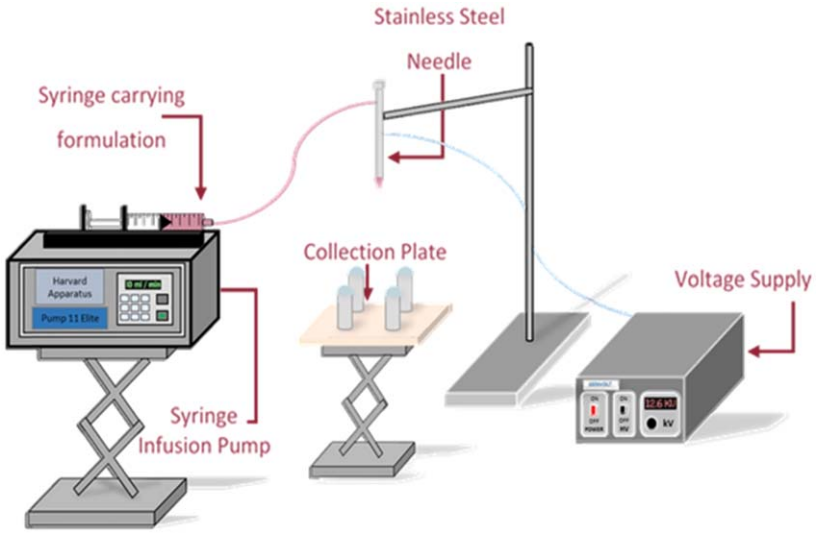

Figure 1. A schematic diagram exhibiting the key components of the electrohydrodynamic atomisation process.

PureVision Balafilcon A silicone contact lenses, sourced from Bausch and Lomb, New York, United States of America. Controlled deposition of the coatings was achieved using a lens holder, which could accommodate up to four lenses. To establish the weight of the coatings, the lenses were weighed before and after deposition. All engineering processes were carried out at ambient temperature (23 °C± 0.5 °C).

Drug encapsulation efficiency (EE)

To determine TM EE, weighed coatings samples were dissolved in ethanol for 1 week. UV spectroscopy ($\lambda = 295$ nm) was used to determine the amount of drug loaded into the coatings. Equation (1) was used to calculate EE

$$\mathrm{EE} = \frac{\text{Drug Added} - \text{Free Drug}}{\text{Drug Added}}. \tag{1}$$

Calculating the amount of drug that is present within the atomised coatings aids the analysis of subsequent *ex vivo* testing.

***Ex vivo* testing**

TM release from the atomised coatings and permeation through freshly excised bovine cornea was studied using vertical diffusion cells. The corneas were excised from fresh bovine eyes and were consequently fixed between the receptor and donor compartment. The eyes were first examined for any corneal damage before dissection to obtain the cornea with a 2 mm sclera border to preserve corneal structure. The cornea-scleral tissue was washed with PBS and mounted in between glass donor compartment (surface area = 1.77 cm^2) and receptor compartment with the corneal endothelium facing the latter. The receptor was filled with 12 ml of PBS and contained a mini magnetic stirrer to ensure constant stirring. The temperature of the glass cells was maintained at 37 °C via a heating block. At pre-determined times, 400 μl of receptor medium was removed from the receptor compartment and replaced with fresh PBS of equal volume. Cumulative drug permeation was analysed using UV spectroscopy ($\lambda = 295$ nm). The cumulative amount of timolol malate permeating through the cornea was plotted as function of time and the linear slope of the resulting plot was used to calculate the steady state flux.

Results and discussion

Coating engineering

Previous work carried out in this area [17, 18] has already showed the novel lens holder used here was able to accommodate up to 4 lenses whilst keeping the lenses stable and stationary during the deposition process. A masked arm was used to ensure only the peripheral regions of the lenses were coated so as not to obstruct vision. Figure 2(a) shows an uncoated dehydrated lens while figure 2(b) shows a model-coated lens with a central region void of the deposited coating. The fine white mist on the latter shows the outer side (pre-corneal region) coated

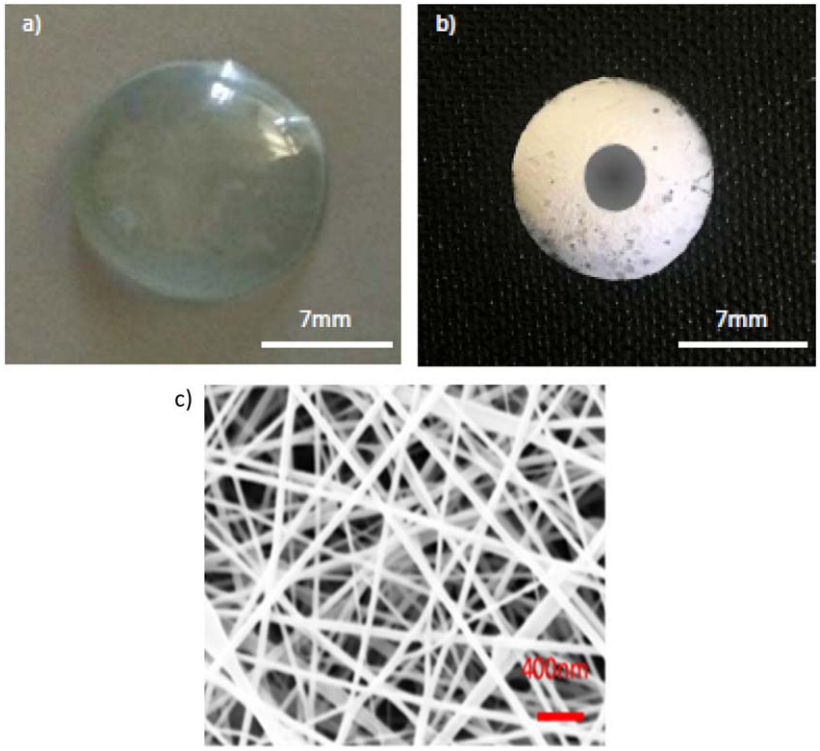

Figure 2. Digital images of (a) uncoated lens, (b) a lens with a typical electrospun coating, (c) SEM image of a typical electrically atomised coating at ×50k magnification.

Table 2. Fibre composition and drug encapsulation efficiency of each electrically atomised coating.

	Theoretical fibre composition			
Formulation	Polymer (%w/w)	Timolol maleate (%w/w)	Permeation enhancer (%w/w)	Drug encapsulation efficiency (%)
F1	95.05	4.75	0.2	92.21
F2	89.96	4.35	8.69	60.92
F3	93.45	4.67	1.88	93.11
F4	80	4	16	51.75
F5	86.81	13.02	0.17	70.74
F6	80	12	8	57.01
F7	85.47	12.82	1.71	99.7
F8	74.1	11.1	14.8	82.45

with the electrospun fibrous matrix. Scanning electron microscopy images showed the coatings were characteristically made up of smooth, non-featured nanofibers (figure 2(c)).

TM EE and fibre composition

Whilst table 1 shows the constituents that make up the formulations and their concentrations, table 2 displays the fibre composition; i.e. what percentage of each coating is taken up by each component, based on drug EE. Evidently, the differences in percentage here are solely due to the EE and the concentration of permeation enhancer. The highest EE was found with *F*7, with almost all of the loaded drug (99.7%) being encapsulated within the matrix and the lowest with *F*4 (51.75%). For both drug loadings (5%w/w and 15%w/w), it seems the highest encapsulation was achieved with formulations containing borneol, as seen in table 2.

Ex vivo permeation testing

Figure 3 shows the permeation of TM through freshly excised bovine cornea following release from the electrospun permeation enhancer-loaded coatings; with table 3 summarising the parameters derived from these *ex vivo* studies. A lag time of 30 min was deciphered for all eight formulations. This temporal measurement is quantified here as the time taken for the drug to diffuse/move through the spun polymer matrix of the coating and through the cornea before released into the release medium in the receptor compartment. This was a six-

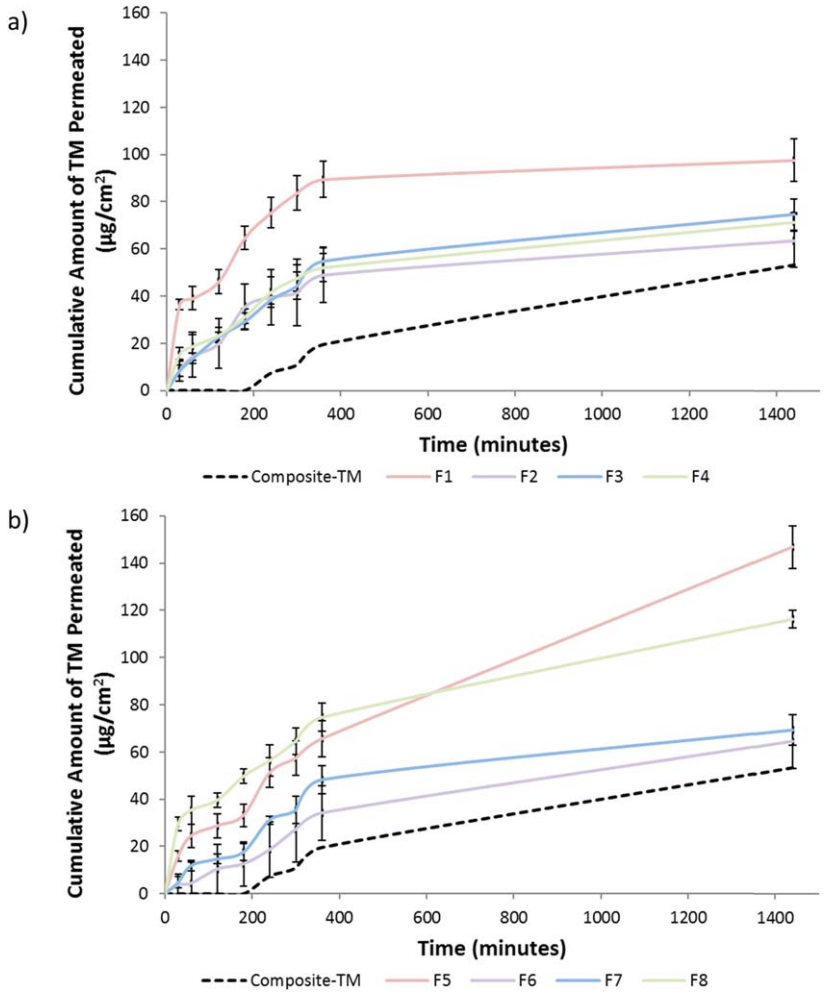

Figure 3. *Ex vivo* cumulative amount of timolol maleate permeated across freshly excised bovine cornea for initial timolol maleate loading of (a) 5%w/w and (b) 15%w/w.

Table 3. Summary of parameters derived from *in vitro* and *ex vivo* studies.

Formulation	*In vitro* release after 24 h (%)[a]	Steady state flux (μg cm^{-2} min^{-1})[a]	Apparent permeability coefficient (cm^2 min^{-1})[a]	Lag time (mins)
Composite-drug	68.25 ± 1.63	0.057 ± 0.017	0.012 ± 0.0038	180
*F*1	84.63 ± 1.99	0.076 12 ± 0.017	0.017 ± 0.0039	30
*F*2	83.92 ± 2.08	0.098 ± 0.010	0.037 ± 0.0038	30
*F*3	81.49 ± 5.38	0.104 ± 0.010	0.038 ± 0.0087	30
*F*4	80.28 ± 1.80	0.101 ± 0.0058	0.049 ± 0.0027	30
*F*5	84.83 ± 3.78	0.057 ± 0.0042	0.0084 ± 0.0027	30
*F*6	81.94 ± 2.68	0.074 ± 0.0044	0.006 13 ± 0.000 37	30
*F*7	86.72 ± 3.09	0.098 ± 0.0088	0.011 ± 0.000 95	30
*F*8	77.33 ± 8.14	0.059 ± 0.0099	0.0048 ± 0.000 81	30

[a] Values are mean ± standard deviation.

fold decrease from the lag time calculated for permeation-free coating; highlighting the fact that the proposed reasoning for incorporating the permeation enhancers was successful. The lag time of drug permeation was reduced; giving more controlled and faster drug permeation.

The cumulative amount of drug permeated achieved with permeation enhancer free coatings was approximately 53.39 ± 3.95 μg cm^{-2} after 24 h, the lowest of all 9 formulations (figure 3). Regardless of specificity of permeation enhancer, the incorporation of the additives increased the total of drug permeated through the cornea. Formulations containing EDTA (*F*2 and *F*6), showed to have the lowest amount of drug permeated per area after 24 h. This could be attributed to these formulations existing as suspensions before

electrohydrodynamic processing. EDTA is not soluble in ethanol and hence stayed particulate in *F*2 and *F*6. These EDTA particles may act as additional diffusional barriers, hindering the movement of TM through the polymeric matrix and the corneal membrane.

A study carried out by Burgalassi *et al* found that BAC, EDTA and Brij® 78 all found to enhance the permeation of timolol by 3.06, 1.63 and 1.16 fold, respectively, with BAC being the most active enhancer [28]. Similar results were found in the present study; with formulations containing BAC achieving highest amount of TM permeated through the cornea per area (5%w/w drug loading: 97.6 μg cm^{-2} and 15%w/w: 146.8 μg cm^{-2}). The influence of borneol has been previously assessed on *in vitro* release and permeation of hydrophilic quinolone antibiotic ofloxacin. It was found the incorporation of the naturally occurring compound resulted in a 2.15 fold increase in the release of the antibiotic [29]. Borneol has also found to enhance the permeability of the blood-ocular barrier to dye Evan's Blue [30] suggesting its use as a useful penetration enhancer in ophthalmic drug delivery. Its use in these electrically atomised coatings also mirror these results: the amount of TM permeating through the cornea is greatly increased compared to enhancer-free coatings. A permeability coefficient higher than 20×10^{-6} cm^2 h^{-1} (as seen here with *F*0–*F*8) is indicative of high/good permeability. The evidence collated from *in vitro* probe release showing the atomised coatings does not detach from the lens shows there is increased contact time with the corneal surface in the pre-corneal region. This along with the hydrophilicity of TM and the excipients used (i.e. the permeation enhancers) aided the release and permeation of TM through the cornea at a much more sustained rate than without the enhancer additives. These values are considerably lower than that of commercial eye drops (20.458 μg cm^{-2} h^{-1}) [31] showing these electrospun coatings on contact lenses delayed TM transport through the corneal membrane. This permits for less frequent dosing and hence reduces the risks of systemic absorption and ocular toxicity associated with high drug loading. As expected, the amount of TM released and permeated from *F*5 to *F*8 was a lot higher than their lower drug loading counterparts. This, however, contradicts the results found with *in vitro* drug release studies (table 3). The drug loading did not affect the cumulative percentage release of TM; however, there is an evident difference with *ex vivo* permeation studies. This could be a direct result of the fact that the cellophane dialysis membrane may not an adequate membrane to mimic biological membrane. It is because of this *in vitro* drug release and *ex vivo* studies are vital to conduct in conjunction to get a more accurate conclusion.

Conclusion

By utilising a specific combination of polymers and permeation enhancers, this study has assessed and shown the potential of using EHDA to engineer robust coatings for contact lenses to increase drug permeation through the cornea and consequently improving ocular drug bioavailability. The *ex vivo* studies showed a vast improvement with respect to timolol maleate permeation upon the addition of permeation enhancers compared to additive-free formulations. This increase in drug permeation over a more appropriate time frame has the potential to minimise ocular toxicity due to less being absorbed systemically. Combining novel engineering techniques like EHDA and an already established drug delivery device has shown great prospects in personalised ocular drug delivery whilst overcoming major disadvantages of more conventional dosage forms.

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