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The contact lens-tear film interface: Investigating the tear envelope

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ABSTRACT

Purpose: To explore the complex interface between the tear film, a unique mucosal fluid which is fundamental to ocular homeostasis and optimal vision, and an in-situ contact lens. This study exploits the use of a unique tear envelope (TE) extraction technique, which harvests the material-influenced layer of tear film that is in intimate contact with the lens during wear, to specifically investigate the influence of contact lens wear on tear film protein dynamics.

Methods: TEs were collected from freshly removed worn lens using a novel microcentrifuge 'piggyback' technique. Two distinct ex vivo studies were performed to investigate the key influencing factors involved. Non lens-wearing tear samples were also collected from all wearers. A compositional protein profile for each TE and tear film (TF) sample was obtained using an Agilent 2100 Bioanalyzer lab-on-a-chip microfluidic assay which detected proteins in a 14–230 kDa range.

Results: The data demonstrated that the TE protein compositional profile was quite distinct from either that of tear components deposited on the lens or those held in the tear menisci. For example, for one of the participant subgroups the tear protein average values in tears (n = 39) were determined at 35.2 ± 2.5 % lysozyme, 17.2 ± 0.6 % lipocalin, 7.3 ± 1.6 % IgA, 20.3 ± 1.3 % lactoferrin and 0.4 ± 0.4 % albumin as a function of total protein detected. In contrast, the average TE values were measured at 49.2 ± 3.7 %, 21.3 ± 3.9 %, 7.8 ± 1.6 % and 10.2 ± 1.7 % and 1.3 ± 2.8 % respectively with omafilcon A wear. In addition, 63 % of all TE samples (n = 180) (wearing lotrafilcon B and omafilcon A lenses) were albumin positive compared with only 19 % of all pre-lens insertion tear film samples (n = 237).

Conclusions: The TE approach not only allows material differentiation, but it can determine changes in the ocular host response that may otherwise be missed by sole non lens-wearing tear film sample analysis.

1. Introduction

The hydrogel contact lens is a longstanding and well-established example of a hydrogel-based biomaterial. From the time of its inception it was recognised as providing greater comfort than its polymethyl methacrylate-based counterparts [1]. Lens wear is, however, still associated with a range of adverse responses encompassing contact lens induced dry eye, contact lens peripheral ulcers, contact lens related acute red eye, superior epithelial arcuate lesions, giant papillary conjunctivitis and corneal infiltrates [2–8]. The behaviour of the lens in the ocular environment is a classic example of the phenomenon sometimes referred to in biomaterials science as a "guest–host interaction." This interaction is complicated by the fact that the contact lens can be ten to 50 times thicker than the tear film (~50–200 μm [9] vs 4 μm [10,11] respectively) and its presence leads to the establishment of a posterior tear film layer. Major advantages of contact lens research are that the ocular environment is accessible; insertion and removal of the lens for investigative purposes does not require surgical intervention or undue patient distress. Removal of the lens for examination is relatively simple as is the direct sampling of the tear film.

One shared feature of all contact lens related adverse events is their unpredictability; therefore, there is a need to develop techniques to investigate the interaction of both existing and new materials with a wide range of wearers. The interaction of the contact lens with the tear film is dependent both on the lens material, and the individual tear chemistry of the wearer. Whilst changes pertaining to the lens are generally investigated by contact lens deposition studies, this study addresses an alternative aspect: the effect of the lens on local tear fluid

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composition at the interface.

Diagnostic analyses of human tears are primarily performed by investigation of the protein components. A great and varying number of proteins has been identified in tears [12–14]; the 2017 DEWS report estimates a number in the region of 1800 [15]. Tear proteins play an important role in ocular homeostasis and immunity and comprise both indigenous proteins [16,17], and those that result from vascular leakage [18,19]. The many functions of tear proteins include essential immunological protection, anti-microbial defence, wound healing, lubrication, free radical scavenging and chemical messenger activity, to name but a few. The predominant indigenous tear proteins are lysozyme, lipocalin, secretory IgA and lactoferrin and they make up around 80 % (~6 mg/ml) of total tear protein [17]. Serum albumin is also found to be abundantly present in tears under certain conditions [19]. It is the most concentrated protein in plasma and its presence in tears is thought to be plasma-derived [20,21]. The concentration and presence of plasma-derived proteins in tears varies depending on the intactness and stability of the blood-tear barrier, which is affected by several factors including inflammation, ocular dysfunction and diurnal variation [19,22–24]. These "major" tear proteins were chosen as the key analytes to be investigated in this study. Their main roles and properties are summarised in Table 1.

The influence of the lens on the tear proteins is complex and forms the basis of this study. Some materials can absorb specific tear film components and thereby reduce the levels of specific components in the interfacial tear film [25,26]. Alternatively, materials can drive the generation or infiltration of new components or augment the level of existing components; in some cases, this increases the number of tear film components [27–29].

The aim of this study was to examine material related changes in the interfacial region lying between the tear film and the in-situ contact lens – which we have termed the "tear envelope" (TE). The tear envelope can be thought of as being the tear film that is held in contact with the lens by surface forces. The specific composition of this film is quite distinct from either that of tear components deposited on the lens or held in the tear menisci. Lens influenced TE samples were measured and compared against non lens-wearing tear film (TF) samples. In addition, the influx of albumin into tears, indicative of plasma leakage, which can serve as a biomarker for vascular permeability and ocular insult, was also evaluated. The aim here was to explore the complex interface between the tear film and an in-situ contact lens.

2. Materials and methods

The studies described herein had received prior ethics approval by the appropriate committee at Aston University and conformed to the tenets of the Declaration of Helsinki. TF and TE collection was carried out as part of a series of clinically managed studies at two established centres of ophthalmic research. Experienced clinically trained researchers were responsible for setting up, managing the clinical studies, participant recruitment and sample collection. Written informed consent of each participant to take part was obtained once the requirements of the study had been explained. None of the participants involved had been previously diagnosed with any eye-related problems and all were previous contact lens wearers.

Table 1
Tear protein analytes investigated in this study (listed by increasing molecular weight).

Protein name	Theoretical size (kDa)	Isoelectric point (pI)	Function
Lysozyme	14.6	11.4	Antibacterial enzyme; destroys the cell walls of certain bacteria. Involved in innate immunity.
Tear lipocalin	16–18	~5	Ability to bind small, hydrophobic molecules such as retinol to transport and protect.
sIgA	160 (x2 dimer)	4.5–6.5	Primary antibody of the mucosal surfaces. Acquired immunity.
Albumin	68	4.7	Transportation of free fatty acids, stabilising the osmotic pressure.
Lactoferrin	82	8.7	Inhibitor of bacterial growth possible anti-inflammatory properties. Innate immunity.

2.1. Contact lens materials

Details pertaining to all the lens materials used in this study are summarised in Table 2.

2.2. In vivo tear and tear envelope sample and participant selection

Two distinct participant populations were used as part of a series of clinically managed studies. The first study was implemented to investigate the interfacial TF/lens wherein protein interaction at the lens surface can be influenced by the properties of the material as determined by TE dynamics. Therefore, two very distinct lens materials were chosen; a charged anionic lens (Etafilcon A) versus a truly neutral lens (nelfilcon A) with the view to examining their interaction with the two prominent cationic proteins in the tear film – lysozyme and lactoferrin which have been widely studied in terms of deposition analysis. A comparison which offered a clear surface differential for TE sampling investigation. Then, applying the principle that a TE sample can be used to ascertain the influence of a lens material on the lens wearing tear composition profile (in this case – the proteinaceous aspect), a second study was performed in which two additional materials were investigated to further understand material differentiation. This study comprised a silicone contact lens material (lotrafilcon B) in comparison with a conventional lens material (omafilcon A) and allowed a larger participant population and a longer wear schedule to be evaluated.

With the first study subset eight participants were enlisted, each participant wore either nelfilcon A (Week 1: n = 4) or etafilcon A (Week 1: n = 4) lenses for 30 mins daily for five days in a row on a random crossover basis (one material one week and the other the following week). Baseline pre-lens wear TF samples were collected from each participant at the start of the study (week 1). TE samples were collected after each 30 mins wear schedule.

With the second study subset TF and TE samples were collected from 39 participants, wearing either lotrafilcon B (n = 21) or omafilcon A (n = 18) material. Two lens care solutions, ReNu Multiplus (B&L) and Clear

Table 2
Contact lens material properties.

USAN Name	Etafilcon A	Omafilcon A	Nelfilcon A	Lotrafilcon B
Commercial Name	Acuvue 1-Day	Proclear	Focus Dailies	Air Optix Aqua
Manufacturer	Vistakon[a]	CooperVision	Alcon	Alcon
Lens Type/ FDA Group	IV (anionic)	II (non-ionic)	II (non-ionic)	SiHy[b] (non-ionic)
Water Content (%)	58	62	69	33
Tensile Modulus (MPa)	0.3	0.3	0.7	1
Principle components	HEMA, MA	HEMA, PC	Modified PVA	DMA, TRIS, Siloxane macromer
Surface treatment	No treatment	No treatment	No treatment	25 nm plasma coating

HEMA, 2- hydroxyethyl methacrylate; MA, methacrylic acid; PC, phosphorylcholine; PVA, polyvinyl alcohol; DMA, N,N-dimethylacrylamide; TRIS, trimethylsiloxy silane.

[a] Division of Johnson & Johnson Vision Care.

[b] Silicone hydrogel (Group V).

Care (AO Sept) (Alcon), were used in a randomised manner, lotrafilcon B-ReNu (n = 11), lotrafilcon B-Clear Care (n = 10), omafilcon A ReNu (n = 8) and omafilcon A-Clear Care (n = 10) for the duration of the study. Participants were assessed, and TF and TE samples were collected at seven scheduled visits over a six-month period. TF samples were initially collected one week prior to lens wear (Visit 1: Baseline) and then upon lens removal at each visit (Visits 2–7). Lenses were worn on a one-month daily wear basis without lens or solution cross-over. The lenses were removed (and a TE sample was collected) at each of these visits and each participant was given a fresh new lens of the same material. Visit 2–7 TF samples were collected as soon as possible (within 5 min) upon lens removal. TE samples were collected at each visit prior to lens removal. A total of 237 and 180 TF and TE samples respectively were collected, which reflects a few missed visits by some participants (however no two visits in a row were ever missed), in addition to the fact that two participants did not complete the study due to personal non-study related issue. The higher number of TF samples is explained by the fact that it incorporates a set of pre-lens wear baseline TF samples.

All TF and TE samples were taken between 10:00 hrs and 16:00 hrs to limit the effects of diurnal variation which are known to influence certain protein concentration levels in tears [19,30].

TF and TE samples were collected from both eyes and upon collection were stored at 4 °C and analysed in a university-laboratory within 72 h.

2.3. Tear sample collection protocol

Narrow bore microcapillary pipettes (Sigma Aldrich, Dorset, UK) were used to collect at least 4 μl of tears from the lateral canthus and/or inferior marginal strip. Care was taken to avoid contact with the ocular surface to avoid reflex tearing, and time taken was recorded for each tear sample to reach the minimum specific volume – measured by the graduated microcapillary pipettes. Samples were either analysed on the day of collection or stored at −80 °C, but not for longer than one week after collection.

2.4. Tear envelope collection protocol

The interfacial tear fluid envelope was collected using an extension of the microcentrifuge 'piggyback' technique described previously [17]. The principle of this technique involves centrifuging off the tear fluid which surrounds and has enveloped the contact lens – there is no chemical extraction or treatment step involved. Each freshly removed lens was placed, using tweezers, into a 0.5 ml microcentrifuge tube, and then 4 μl of deionised water was added to assist removal. The lens-containing microcentrifuge tube was vortexed for 5 s and a small hole was then made in the bottom of the tube. This microcentrifuge tube was placed into a larger, 1.5 ml, microcentrifuge tube. The 'piggyback' pair of microcentrifuge tubes was then centrifuged at 3000 *g* for 5 min. The resulting eluate (≤10 μl) was then collected for immediate analysis or storage at −80 °C. All samples were analysed within a week of collection.

2.5. Tear and tear envelope sample protein profile analysis

TF and TE samples were analysed using an Agilent 2100 Bioanalyzer (Agilent Technologies GmbH Waldbronn, Germany). in combination with Protein 230 Plus LabChip kits (sensitivity 0.005 mg/ml). The 230 Plus LabChip kits enable the separation of proteins by molecular weight in a 14–230 kDa range. Following the manufacturer recommendations 2 μl of sample buffer (with 3.5 % vol β-mercaptoethanol was used as a reducing agent. These micro-fabricated chips enable analysis of ten 4 μl sample wells in less than 30 min. The samples are separated electrophoretically and are detected by fluorescence (670–700 nm). The results from each sample well are translated into individual electropherograms with molecular weight [kDa] (by migration time in seconds [s]) plotted against intensity fluorescence units [FU]. Each assay run includes a standard molecular weight ladder with a lower limit marker at 4.5 kDa and an upper limit marker at 240 kDa to provide an internal calibration. Individual peaks of test samples were thus assigned a molecular weight; the peak area indicates 'relative' concentration. Overall protein profiles are calculated can consequently be presented with each component shown as a function of the total protein detected in that sample, which allows cross correlation of samples. The intra-sample reproducibility of the Bioanalyzer was investigated by assaying individual samples ten times; it is ±4.5 % SD as a function of total protein detected. The molecular weight sizing reproducibility of the Agilent Bioanalyzer determined by the manufacturer is quoted at 5 % or better. In the figures standard deviation indicates the deviation about the mean in that population. The analytical specification sensitivity is given in the region of 10 μg/ml in PBS; concentrations below this are not detected by this method and consequently were deemed negative for the purpose of *this* study.

3. Results

3.1. First study subset: Ear envelope sampling: Sorptive versus non-sorptive behaviour

The ability of the TE sampling technique to reflect the effects of lens chemistry on the composition of the interfacial tear layer that surrounds the lens is usefully illustrated by the study of the interaction with tears of a high-water content anionic FDA Group IV lens (etafilcon A). All Group IV anionic materials show significant ability to interact with cationic tear proteins, particularly lysozyme. Etafilcon A is used to illustrate the way that this selective sorption capability is reflected in the TE. The specific composition of the TE was quite distinct from those held in the tear menisci (and to the widely known levels of tear components deposited on this anionic conventional lens). Fig. 1A shows an electropherogram illustrating the protein profile of a baseline basal TF sample taken from the eye of a non lens-wearing participant. Fig. 1B shows the TE protein profile from the same participant after 30 min of etafilcon A lens wear.

Although the number of protein peaks detected is the same in both samples (Fig. 1A and B) the relative concentration of the lysozyme peak, expressed as a percentage of the total protein in the electropherogram, was significantly different. A secondary control for the same participant was obtained by taking a TE sample after 30 mins wear of a neutral (non-ionic) high water content lens, nelfilcon A. This showed a tear protein profile with no measurable diminution of the lysozyme peak. These experiments were both by repetitive sampling of the same participant (n = 5) and by sampling different participants (n = 8).

Although the number of protein peaks detected in both samples were similar, the relative value of each protein peak detected, expressed as a percentage of the total protein, was very different. This was particularly apparent with the two cationic proteins: lysozyme (~14 kDa) and lactoferrin (~91 kDa). The electropherogram peaks associated with these two proteins (highlighted) were significantly reduced in the TE sample (Fig. 1B) compared with those in the non lens-wearing basal tear fluid (Fig. 1A). The proportion of lysozyme measured in the tears of this participant was ~35 % of the total protein detected, compared with ~10 % measured in the etafilcon A TE sample. Similarly, lactoferrin was reduced from ~20 % to ~12 % in the TE sample. This reduction in lysozyme, and to a lesser extent lactoferrin, concentration may be explained by the well-known interaction, and subsequent deposition of lysozyme, that occurs with the etafilcon A material.

Overall, for all the participants the protein average values in the baseline TF samples (n = 8) were determined at 35.9 ± 2.9 % lysozyme, 16.9 ± 1.7 % lipocalin, 7.1 ± 0.4 % IgA and 20 ± 2.0 % lactoferrin as a function of total protein detected. In contrast, the average TE values were 9.5 ± 1.8 %, 43.7 ± 5.0 %, 6.7 ± 1.5 % and 12.2 ± 1.6 % respectively with etafilcon A wear and 31.9 ± 3.7, 23.0 ± 4.1, 4.5 ± 2.0 and 10.9 ± 1.7 for the nelfilcon A lens-wearing TE sample. Fig. 2

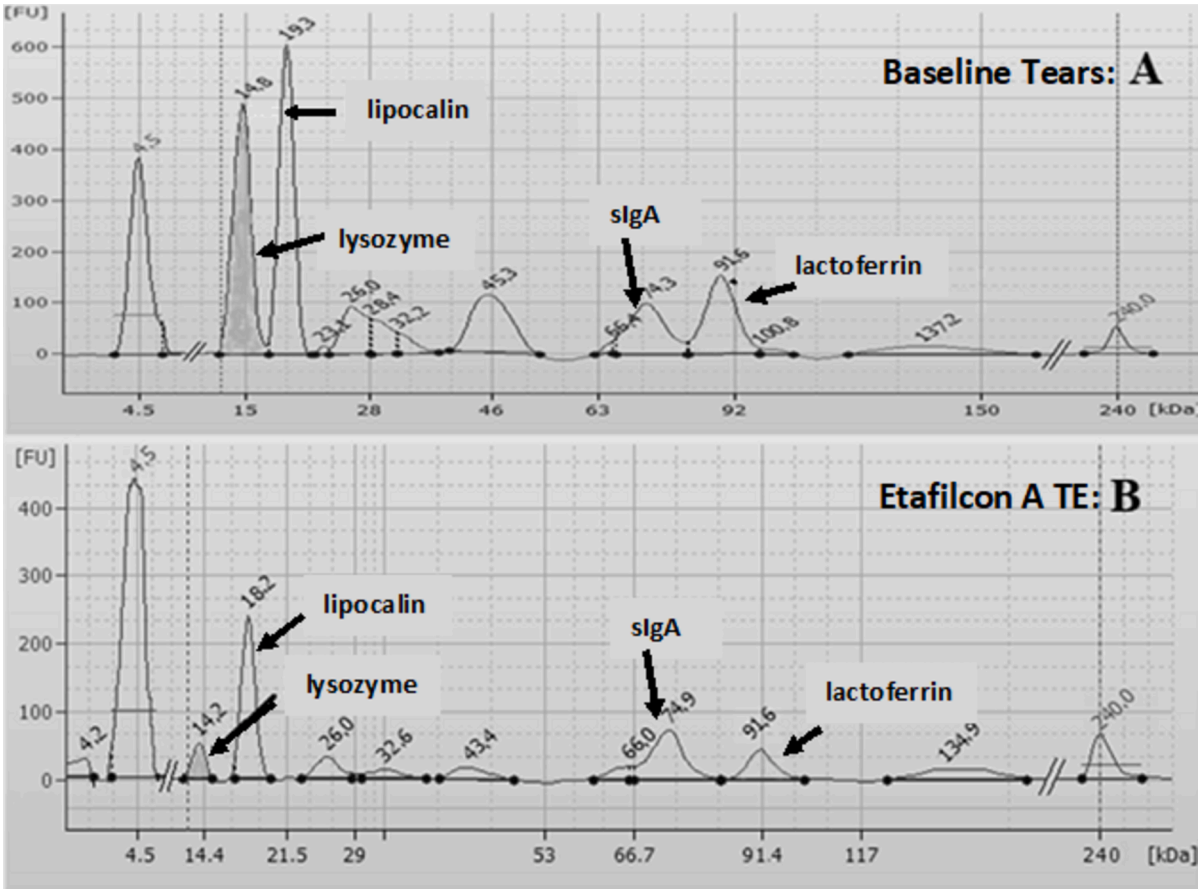
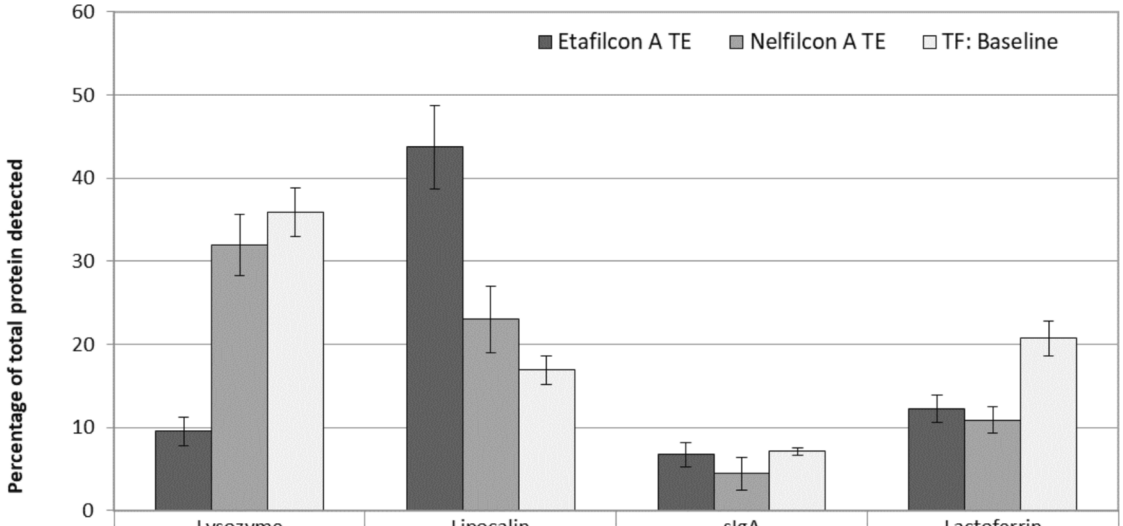


Fig. 1. Protein profile electropherograms showing the difference between a non lens-wearing TF sample (A) and a TE sample from the same participant after 30 mins etafilcon A lens wear (B). Note the clear difference in the lysozyme (~14 kDa) peak between the two samples.



	Lysozyme	Lipocalin	sIgA	Lactoferrin
Etafilcon A TE	9.5	43.7	6.7	12.2
Nelfilcon A TE	31.9	23.0	4.5	10.9
TF: Baseline	35.9	16.9	7.1	20.7

Fig. 2. Mean (±SD) protein percentage as a function of total protein detected in etafilcon A and nelfilcon A tear envelope samples versus baseline tear film sample percentages (n = 8).

summarises the main tear protein data determined in baseline basal tears versus the TE samples obtained during etafilcon A and nelfilcon A contact lens wear.

3.2. Second study subset

3.2.1. Tear sample protein changes with lens wear over time

The variation in TF protein composition during wear and specifically here with lotrafilcon B and omafilcon A materials is presented in Fig. 3, wherein the mean pre-lens wear baseline TF sample protein values are compared against the TF samples collected monthly (upon lens removal) over the course of the six-month study. A not-insignificant level of protein variation was determined where small but subtle changes in tear film protein profiles were observed. Lipocalin and sIgA levels were raised during lens wear with lysozyme and lactoferrin percentages showing a reduction for both lens materials. Interestingly the TF lipocalin levels did change from 16.4 ± 2.2 % to 18.1 ± 2.1 % for the omafilcon A lens wearers over the duration of the study. In terms of albumin, the mean average baseline TF levels at 0.22 ± 0.12 % did not change significantly with the omafilcon A lens wearers, but a slight increase to 0.62 ± 0.7 % was observed with the lotrafilcon B lens material wearers over time (visits 2–7).

3.2.2. Tear film versus tear envelope sample analysis

Mean percentage levels of lysozyme, lipocalin, sIgA, lactoferrin and albumin comparing the average TEs of lotrafilcon B and omafilcon A materials and TF over the six-month wear period are presented in Fig. 4. The TF averages include both baseline and the monthly non lens-wearing samples (Visit 1–7). Distinctive material influenced patterns were evident. For example, the omafilcon A material exhibited a lysozyme rich TE at 49.2 ± 3.7 % of the total protein detected compared with the population average of 35.2 ± 2.5 % in the non lens-wearing TF sample and 33.8 ± 3.6 % in the TE of the lotrafilcon B lens wearing group. Lotrafilcon B material on the other hand revealed a lipocalin rich TE at 25.4 ± 2.6 % compared with an average of 17.2 ± 0.6 % in the TF samples and 21.3 ± 3.9 % in the TE of the omafilcon A wearers.

3.2.3. Albumin and the tear envelope

The material influenced TE samples exhibited contrasting albumin levels (Fig. 4). TE samples of participants wearing the lotrafilcon B material exhibited an overall average of 5.4 ± 9.5 % of the total protein detected compared with average levels of only 1.3 ± 2.8 % for the omafilcon A TE samples. Although both materials demonstrated a propensity for albumin influx when these values are compared with 0.22 ± 0.6 % for the pre-lens wear baseline TF samples and an overall average of 0.4 ± 0.4 % for the TF samples taken over the duration of the six-month study. Significantly, levels of albumin in excess of 20 % of the total protein detected were not uncommon in the TE samples of participants wearing the lotrafilcon B material. The differences in levels of albumin, spread over all visits and participants, in the TEs of the omafilcon A lens wearers compared to the lotrafilcon A lens wearers are illustrated in Fig. 5A and B respectively.

A breakdown of the overall albumin positive TF and TE samples in the lotrafilcon B versus omafilcon A study is provided in Fig. 6. The sensitivity threshold of the Bioanalyzer assay determined that a level of a given analyte below 5 μg would not be detected and thus albumin levels below this would be regarded as negative in this study. In general albumin was detected more frequently, and at higher concentrations in TE samples compared with the non lens-wearing TF samples. 63 % of the TE samples were positive for albumin compared with only 19 % in the non lens-wearing tears. In terms of material differentiation 50 % of the omafilcon TE samples were albumin positive in contrast with 71 % of lotrafilcon B TE samples. An increase in albumin levels in response to lens wear was apparent; a higher incidence of albumin was determined in the TE samples compared with the non lens-wearing TF samples.

Each Visit 2–7 TF samples were taken as soon as possible (within 5

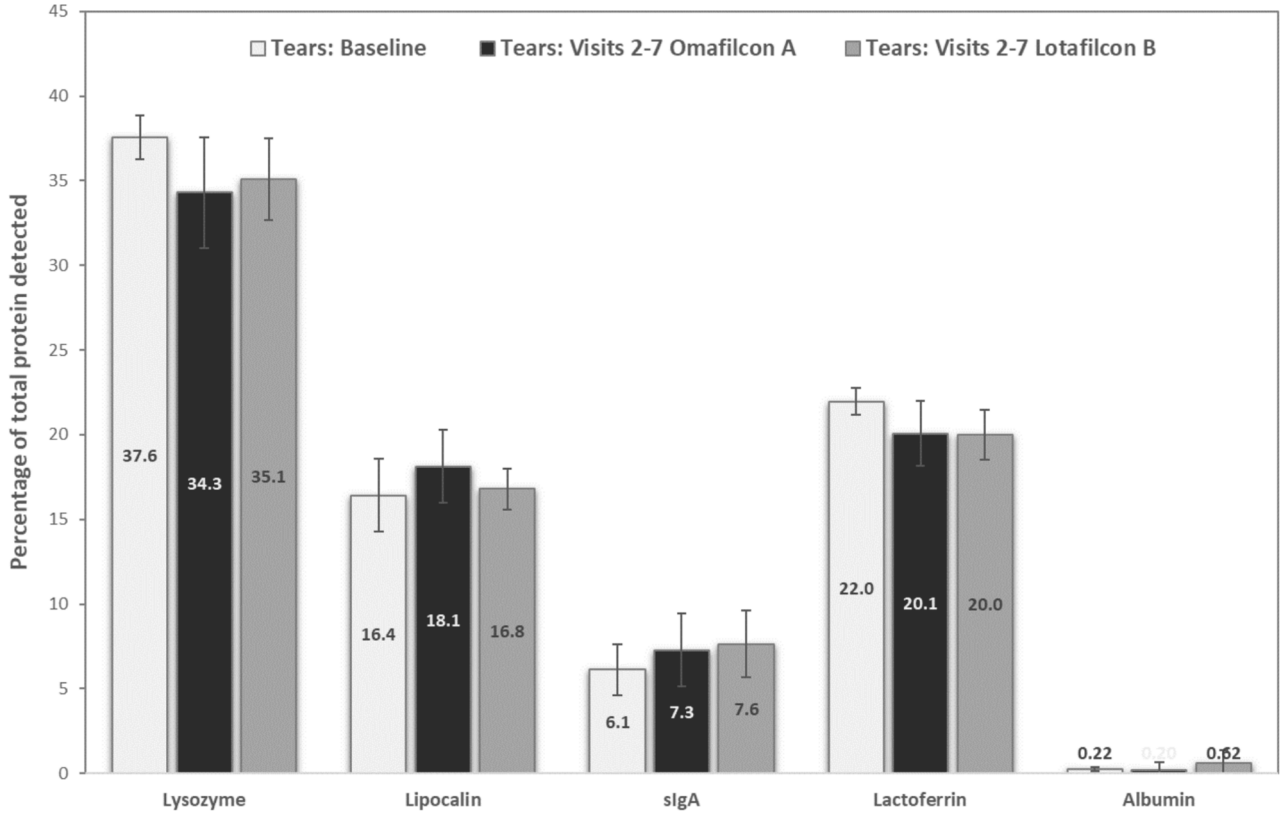
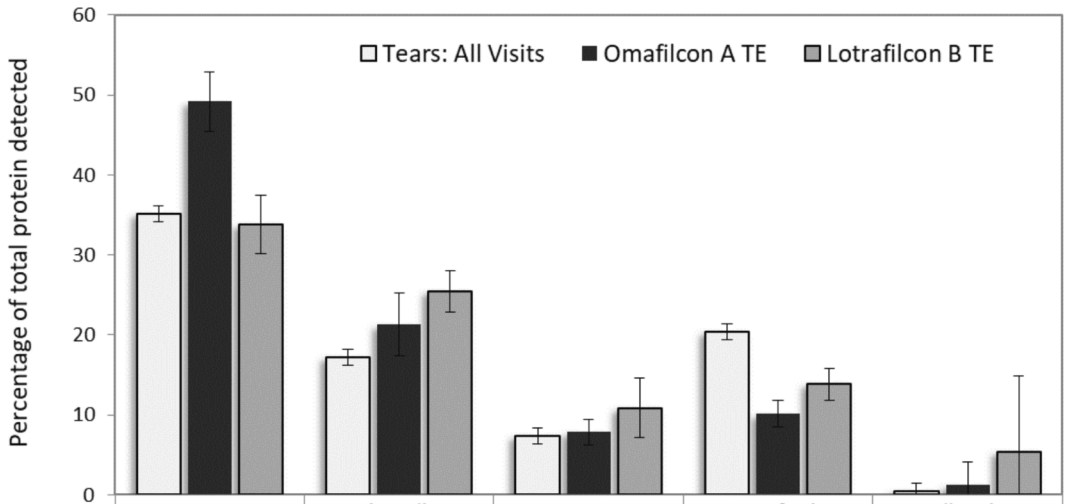


Fig. 3. Mean (±SD) pre-lens wear baseline (Visit 1) TF sample protein percentages compared with the TF sample averages collected upon lens removal at each monthly visit (visits 2–7) wearing either lotrafilcon B or omafilcon A lenses. Results presented as a function of total protein detected.



	Lysozyme	Lipocalin	sIgA	Lactoferrin	Albumin
Tears: All Visits	35.2	17.2	7.3	20.3	0.4
Omafilcon A TE	49.2	21.3	7.8	10.2	1.3
Lotrafilcon B TE	33.8	25.4	10.9	13.8	5.4

Fig. 4. A comparison of the mean (±SD) TE profiles values for lotrafilcon B participants (n = 21) and omafilcon A participants (n = 18) in addition to the overall TF sample average for all participants (n = 39) collected over a six-month, seven-visit schedule (percentage as a function of total protein detected).

min) after lens removal but sampling 4 µl of tears can also take up to 5 min to collect, conceivably enough time for albumin levels to recede in the non lens-wearing TF film. Fig. 7 illustrates this concept further. It shows an overlay of two protein profile electropherograms from the same participant, a lotrafilcon B TE sample electropherogram (light trace) and a corresponding non lens-wearing TF sample electropherogram (dark trace). The TE sample was found to include, amongst other proteins, a large albumin peak (31 % of total protein detected-against only 27 % lysozyme detected). The corresponding TF exhibited a significantly reduced level of albumin (comprising only 5 % of total protein detected). This change in concentration is dramatic with levels falling from 31 % of the total protein detected down to 5 % of total protein composition.

4. Discussion

The TE technique was developed to be able to investigate lens-tear interactions, and to monitor changes in ocular health in response to the in-situ biomaterial. The composition of the interfacial TE is distinct from that of the normal flowing TF. Its isolation and analysis has revealed specific influences of material, contact time and participant-to-participant variation on the composition and dynamics of the tear film in the lens-wearing eye.

Initially the analysis of the contrasting etafilcon A and nelfilcon A TEs offered an insight into interesting lens-influenced dynamics. The reduction of lysozyme levels in the interfacial tear (envelope) sample in contact with the anionic material is likely due to the high rate of absorption of lysozyme on the etafilcon A material. As a result, lysozyme is partially denuded at the interface. However, lysozyme levels were largely unaffected by the neutral lens material, nelfilcon A, the levels of which were on a par with those found in the normal non lens-wearing respective TF. Conversely in the etafilcon A TE sample a significantly prominent lipocalin peak was observed (Fig. 1 B). Tear lipocalins have an acidic pI (and a low molecular weight) [31] and thus are likely exhibit repulsion at the lens interface wherein a build-up at the surface, and thus a substantial peak, will be apparent. These initial experiments validated TE analysis as a key marker of the effects of the lens material on the normal TF fluid dynamics by what might be characterised as "attraction and repulsion" phenomena.

Material differentiation is fundamental to tear compositional dynamics, where protein interaction at the lens surface is greatly influenced by the properties of the material. The aim of the second study was to illustrate this point in more detail; two further materials were investigated to include a silicone contact lens material in comparison with a conventional lens material. And in the TE analyses of conventional versus silicone hydrogel materials, different protein compositional variations were observed. The omafilcon A lens generated a lysozyme-rich interfacial TE; this build-up of lysozyme at the lens-tear interface contrasted those levels found in the basal tear samples. In contrast, the levels of lysozyme in the TE surrounding the lotrafilcon B material were similar to those found in the non lens-wearing TF. The omafilcon A lens is a phosphorylcholine-containing lens. Zwitterionic phosphorylcholine, composed of a negatively charged phosphate bonded to a small, positively charged choline group, is the hydrophilic polar head group of some phospholipids, found in the membrane of cells. The charges at the surface of the lens may attract the cationic lysozyme but the interaction is not strong and thus lysozyme is displaced into the surrounding TE. Omafilcon A is a unique material and investigating the TE has highlighted this fact particularly as exemplified by its interaction with lysozyme. It is the only lens material tested to date that causes this lysozyme build-up at the lens surface.

On the other hand, the lotrafilcon B material demonstrated a distinct lipocalin enriched TE. Hydrophobic-hydrophobic interaction between lipocalin and the hydrophobic segments in the backbone of the lotrafilcon B material (or equally lipoidal species absorbed to its surface) is not unexpected. Lipocalins bind a range of hydrophobic ligands; specific hydrophobic residues involved in interactions have been well established in lipocalins. The levels of lipocalin at the interface with the hydrophilic conventional omafilcon A lens are similar to those established in the non lens-wearing TF samples. It was said that the lotrafilcon coating was designed on the basis of Baer's "Moderate Surface Energy" hypothesis. It is not, strictly speaking, hydrophilic. It has a total surface energy of 48–50 mN/m and in in terms of the present study it has a surface broadly comparable to that of PMMA. A fuller description of the lotrafilcon process is given in the relevant chapters of the 1st and 2nd editions of "Silicone Hydrogels", edited by Debbie Sweeney [32]. The average percentage of lactoferrin detected in the TE samples of all the materials was lower than the levels found in non lens-wearing TF

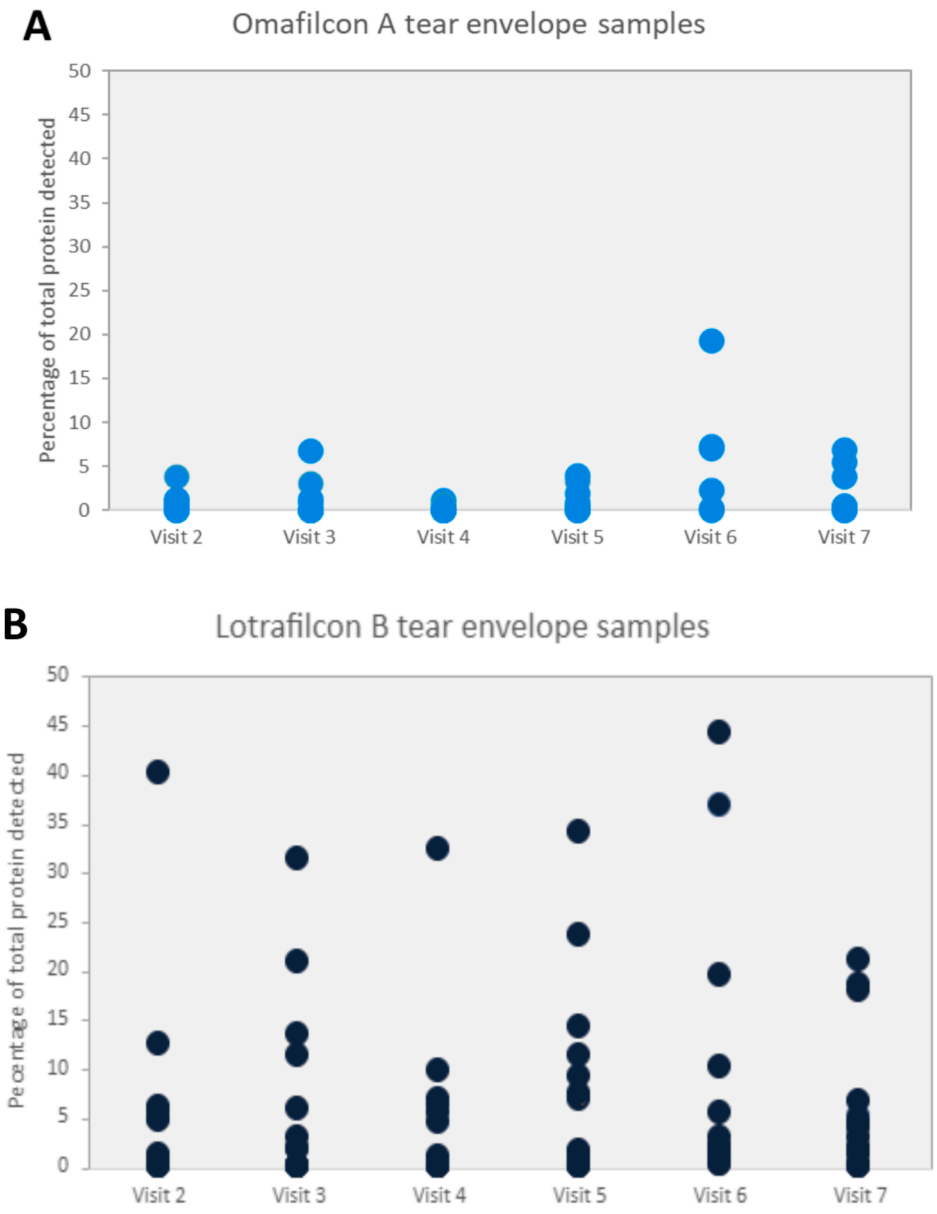


Fig. 5. Percentage albumin levels as a function of total protein detected in the TEs of (A) omafilcon A participants (n = 18) and (B) lotrafilcon B participants (n = 21) collected over a six-visit regime.

samples. This has been observed for all the many materials (both conventional and silicone) tested previously [33,34]. The reason this occurs is unknown but its interaction with different lens materials is distinctive and different from the other cationic protein lysozyme, suggesting a non-charge-related interaction.

The interaction of PVA-based materials with the ocular environment raises an interesting point. The nelfilcon TE results (e.g. Fig. 2) seem to be all subsumed under a single observation – an increase in lens-lipocalin interaction, which in turn suggests the presence of a hydrophobic lens-tear interface. It is widely assumed, or asserted, that polyvinyl alcohol is a biocompatible polymer. Whilst it is true that it causes little disturbance to biological environments when fully hydrated it is probably best regarded as a classical bioinert material. Consideration of the structure shows that is essentially polyethylene with hydroxyl groups on alternate carbon atoms, which is apparent when a PVA contact lens surface is repeatedly exposed to a hydrophobic air interface. The situation with blood contact involves lipoidal components as the predominant hydrophobic components of the environment. In a useful study and summary of the hemocompatibility and biocompatibility of PVA, in vascular grafts, Alexandre et al concluded that "PVA was slightly irritant to the surrounding tissues", a situation that was improved by incorporation of dextran [35].

A similar situation has been observed with the Ciba PVA-based Focus Dailies contact lens (now owned by Alcon). Shortly after the launch of the original material, Maissa et al. [36], noted the presence of PVA in the packing solution. This was puzzling since one of the pillars of the 1998 prize-winning nelfilcon process was the absence of non cross-linked PVA in the product [37,38]. Modified versions of Focus Dailies involved the enhanced elution of nonfunctionalized poly (vinyl alcohol) (PVA) from the lens matrix, which was achieved by addition of linear soluble PVA to the lens matrix for later release into the tears [39,40]. The hydrated emergent PVA chains provide a hydrophilic surface "shield" that mask the hydrophobic aspect of the PVA matrix.

The use of the TE technique as a diagnostic tool can be exemplified by its application in albumin studies. Albumin leakage into the lens-wearing TF appeared to be associated with the stiffer and more hydrophobic lotrafilcon B material compared with the softer zwitterionic omafilcon A material. Mechanical stimulation and irritation are a logical

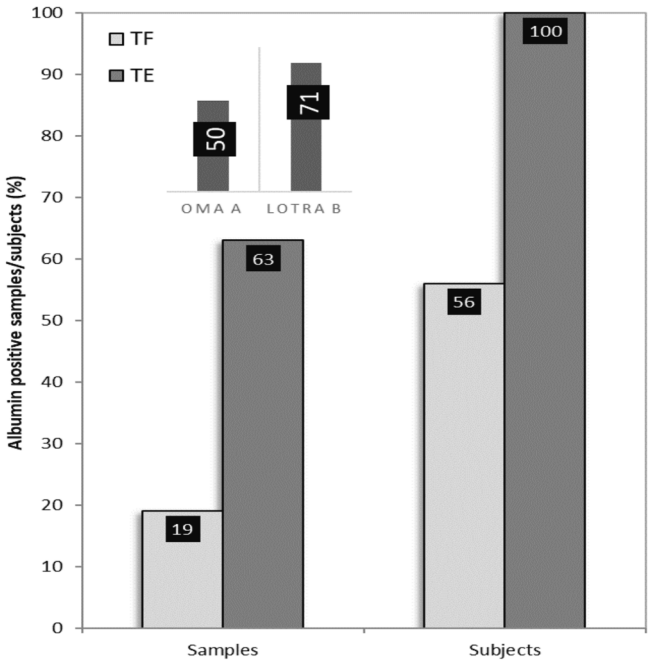


Fig. 6. Percentage incidence of albumin in the non lens-wearing and the lens TE samples for lotrafilcon B participants (n = 21) and omafilcon A participants (n = 18) compared with the percentage number of participants that exhibited at least one positive TE or non lens-wearing albumin positive TF sample over the seven-visit collection schedule. A breakdown of the material-specific TE numbers is also provided.

cause of elevated albumin leakage and the presence of the lens in the eye is stimulating albumin leakage into tears. The fact also that the non lens-wearing TF (collected as quickly as possible after lens removal) of the participants wearing the lotrafilcon B lenses showed very little albumin demonstrated that the presence of albumin may be transient, and thus its occurrence in the TE samples is clearly a response to lens wear. This strongly points to a lens-generated albumin influx. Once the lens stimulus is removed, plasma leakage subsides and thus, in most cases, albumin is no longer detected in the non lens-wearing TF. These results do however bring together three important points, first, the fact that contact lens materials can upregulate the levels of albumin into tears, second, a rapid clearance (within minutes) of albumin from the tear pool upon lens removal occurs, and third, the concentration of albumin in tears is extremely changeable – this is unlike any other prominent protein in tears. Albumin levels are not influenced by duration of wear (Fig. 5), but solely by the presence of a lens in the eye. These levels are thereafter governed by the specific material and its interaction with the ocular environment.

Albumin (a ~ 68 kDa molecular weight protein with a net negative charge at physiological pH) in tears is thought to be plasma derived. In plasma, the role of albumin is varied; its functions include the binding and transportation of a variety of molecules, stabilizing the osmotic potential and increasing the viscosity of blood. However, its role and purpose in tears is unclear, but its presence in tears is deemed indicative of the instability of the blood-tear barrier and has been found to occur in conjunction with a number of ocular disorders [30,41–43]. Overnight levels of albumin in tears are known to be higher than daytime levels [19,30,44]; and occurs in line with the body's daily circadian variations. The response of the wearer to an inserted contact lens material may, in some cases, be associated with a degree of material-induced plasma leakage, vascular permeability and/or changes in the indigenous protein secretion. Although these elevated albumin levels have not effectively been shown to be linked to a specific disease or ocular pathology and as yet albumin is not a biomarker specifically used to monitor any individual ocular pathology. However, one fact remains clear – contact lens wear influences vascular permeability and plasma leakage and tear envelope studies provide the means of monitoring the extent to which this occurs.

The contact lens can be considered an extension of the cornea and its ability to allow some exchange of tear components is paramount [45,46]. Ideally the lens should allow the cornea to respire normally and facilitate a continuous tear film to be maintained on the lens, while minimising the build-up of deposits. What is certain is that each tear interface has a unique compositional profile which is intimately dependent on the material in situ and can be used to ascertain the influence of a lens material on the normal tear (in this case – protein) composition profile. The lens may selectively absorb one or more tear components, or the lens chemistry might cause a specific stimulation resulting in a change in either anterior or posterior tear composition (possibly both). The lens may be inert and merely serve to carry an adherent tear layer; it may also be an inert sampling tool wherein the posterior and anterior tear layers may be separated and be distinctly influenced simply by the presence of the lens (rather than its specific chemistry).

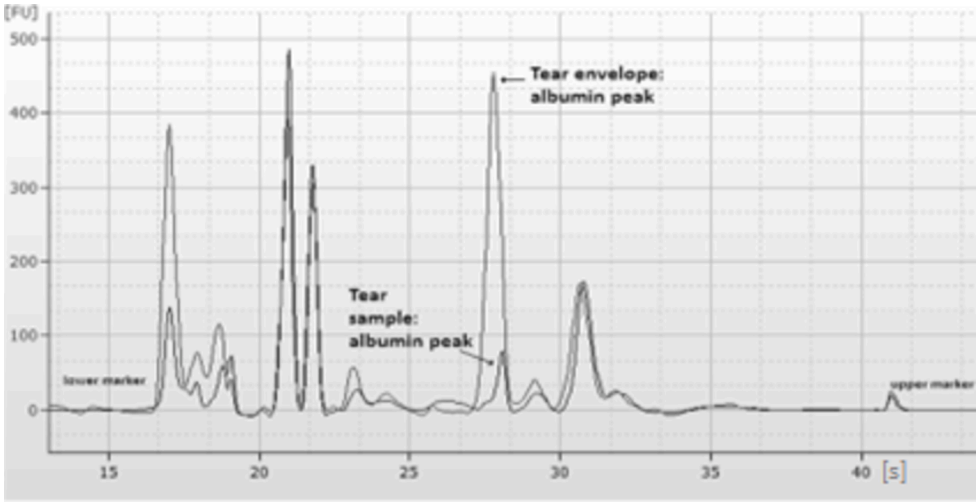


Fig. 7. Comparison of albumin peak profile from a lotrafilcon B TE (light trace) with a TF sample (dark trace) taken immediately after the lens was removed. The albumin peaks detected in both electropherograms are highlighted (at a migration time of ~28 s [s]).

5. Conclusions

The use of a contact lens as a probe to examine material influence on the surrounding tear film has been investigated enabling analysis of the interfacial tear fluid, which we refer to as the 'tear envelope'. The 'tear envelope' has been identified as a unique material-influenced layer of tear film that is in intimate contact with the lens during wear. A technique for its collection and isolation has been developed and adapted for a number of applications in contact lens-host response studies. The tear envelope sampling technique allows us to obtain compositional information regarding the in-situ lens-wearing material-influenced tear sample. It provides evidence pertaining to the influence of the lens material on the tear chemistry, which would be difficult to obtain with a tear sample taken after the lens is removed and when the eye has recovered from the lens-wearing stimulus. TE analysis has been shown to be a preferential means of *in situ* measurement of specific tear components and in particular for plasma leakage proteins. This is exemplified by the detection of albumin influx during contact lens wear in TE samples; levels which decrease rapidly upon lens removal and may not be detected over time in the non lens-wearing TF sample.

The selective sorptive characteristics of anionic lenses are interesting although predictable. They illustrate an important aspect of the capability of the TE technique in the study of the effects of the lens on the ocular environment. The more interesting, and potentially significant capabilities of the technique lie, however, in the ability to detect changes in tear composition resulting from the stimulation of ocular biochemical responses to the presence of the lens. The TE may be used to determine lens-wearing changes in the ocular host response that may otherwise go unnoticed using solely tear sample analysis. The tear sample and tear envelope give complementary information which enables the dynamics of lens-eye interaction to be characterised. In particular the tear envelope shows (a) the influence of the specific lens on albumin leakage into the posterior tear film, and (b) the extent to which the hydrophobic or charged nature of the lens leads to enrichment at the lens interface of lactoferrin or lysozyme, respectively. Tear envelope collection and analysis can enhance the comprehensive platform of techniques currently being used to gain a greater understanding of contact lens behaviour.

Currently there is no answer to the question "which lens is optimal/what is the ideal material", the holy grail of a comfortable, biocompatible adverse event free lens for all has yet to be realised. The lens which can be worn with minimal interruption to the ocular environment has to be the goal. The use of the TE as a probe to investigate the lens-tear interface can aid our understanding of that in eye lens-wearing dynamic.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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