Doctoral thesis

The impact of long-term wear of modern daily disposable contact lenses on ocular physiology

INTERDISCIPLINARY DOCTORAL PROGRAM
IN OPTOMETRY AND VISION SCIENCE
(UNIVERSITY OF VALENCIA)
AND BIOCYBERNETICS AND BIOMEDICAL ENGINEERING
(WROCLAW UNIVERSITY OF SCIENCE AND TECHNOLOGY)

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Wrocław, 2018

Politechnika
Wrocławska

Praca doktorska

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W DYSCYPLINIE BIOCYBERNETYKA
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Wrocław, 2018

Tesis doctoral

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PROGRAMA DE DOCTORADO EN OPTOMETRÍA Y CIENCIAS DE LA VISIÓN

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 642760, called EDEN (European Dry Eye Network), European Joint Doctorate.

DECLARATION

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration, except where specifically indicated in the text. It has not been previously submitted, in part or whole, to any university of institution for any degree, diploma, or other qualification.

Signed: …….

Maryam Mousavi, MOptom

Santiago García Lázaro, PhD from the University of Valencia and **Prof. Daoud Robert Iskander** and **Dorota Szczęsna-Iskander, PhD** from Wroclaw University of Science and Technology, **CERTIFY** that the present report entitled: "The impact of long-term wear of modern daily disposable contact lenses on ocular physiology", summarises the research work carried out under their supervision, by **Maryam Mousavi, MOptom** and constitutes her thesis to apply for the double interdisciplinary degree of Doctor of Philosophy in Optometry and Vision Sciences at the University of Valencia and Biocybernetics and Biomedical Engineering at Wroclaw University of Science and Technology.

And to make it be on record, and complying with current legislation, they sign the present certificate in _____, on the _____ day of _____ of the year_____

_____	_____	_____
Santiago García Lázaro	D. Robert Iskander	Dorota Szczęsna-Iskander

DEDICATION

To the light of my eyes, my remarkable family! Your unconditional love and faith are the reasons to where I am today and what I wished for.

Where there is no vision, there is no hope!

~ George Washington Carver

ACKNOWLEDGEMENT

I would like to start by expressing my sincere gratitude to my supervisor Robert Iskander for the continuous support during my PhD study, for his patience, motivation, and immense knowledge. His guidance has aided me along during the completion of this thesis. I could not have imagined of having a better advisor and mentor for my PhD study. My sincere thanks also, goes to Izabela Garaszczuk, who collaborated with me in this project and supported me all along. I thank her for the stimulating discussions and for all the fun we have had in the last three years. My heartfelt gratitude goes to Danilo Jesus for his encouragement, help and support. I would like to thank Dorota Szczesna-Iskander for her participation in the study. I am profoundly grateful to Richard Armstrong, not only for his support and for input to my thesis but also, for his wise counsel and sympathetic ear.

I would like to thank David Madrid for applying for this grant and to help my dream come true. A deep gratitude to James Wolffsohn for encouraging me to apply for this position and for his support along the way.

I would also, like to thank Alejandro Cervino for his kind support and Santiago García Lázaro for his guidance during my secondment. A heartfelt thank you to Andreas Hartwig for all his support and friendship. My sincere appreciation to Pawel Glowa for all his kindness and support.

I cannot finish without acknowledging my friends and colleagues at the Image Processing Lab and the EDEN network group especially, Clara Llorens Quintana and Laura Rico Del Viejo for all their friendship, fun and support.

I would be remiss if I did not thank Ashruti Patel and Dionysis Skiadopoulos. My interminable gratitude goes to them for all their help and support; your friendships are a gift I will always treasure.

ABSTRACT (ENGLISH)

Numerous studies have shown that contact lens wear disrupts the tear film by separating it into a pre- and post-lens tear film altering its physiological functions. This leads to changes in the tear film compositions: lipids, proteins, mucins, and electrolytes. The pre-ocular tear film is constantly changing by undergoing a formation (build-up) phase directly after a blink, a fairly stable inter-blink phase and ultimately a tear film destabilisation that can lead to tear film break-up in subjects with dry eyes or when the eye is left open for a long period of time. There are differences in the build-up, stability, and thinning phases of tear film dynamics during the contact lens wear. The build-up and stability phase have been observed to be shorter and at times indistinguishable in contact lens wear than in non-contact lens wear. Upon the lens insertion, a thin layer of tears covers the lens. The tear film dynamic is dependent on both the lens material and the individual tear composition. Interaction between protein in the tear film and contact lens material alongside the change in the composition of tears are particularly a key issue for contact lens discomfort during contact lens wear. The lipid layer of the pre-lens tear film during contact lens wear is also much thinner than in the same eye without lens wear. Contact lens wear also has an influence on tear evaporation rate leading to the feeling of dryness and discomfort. Lipid deposition on contact lenses may play a role in disruption of the tear film due to poor surface quality between contact lens material and tear film. This phenomenon is called dewetting, leading to an increased pre-lens tear film evaporation rate and subsequent increase in tear osmolarity.

Many studies have concluded that *all* soft contact lens materials adversely affect tear film physiology. Differences have recently been shown to exist in in-vivo tear film surface quality, depending on lens type. Therefore, better techniques assessing tear film quality and its dynamic changes, enhance understanding of tear film compatibility with various contact lens materials may aid eye care professionals in recognising the nature of tear film behaviour with different lens range. It may also be helpful to practitioners in improving contact lens fitting to reduce contact lens discomfort and dropout. Although non-invasive clinical evaluation of lens-tear interaction makes the assessment of tear film possible, the challenge remains due to tear film dynamics and moulding the dewetting process, diversity

in observer's analysis and software analysis (algorithm) leading to unreliable and at times unrepeatable measurements. In addition, the knowledge on contact lens material and their compatibility with tear film is still confined.

This dissertation is an in-depth work focused on tear film quality during the wear of daily disposable contact lenses. The project was designed in accordance with the European Dry Eye Network (EDEN). The main research goals of this work were to better understand the effect of contact lens wear on tear film physiology, to assess the longitudinal effect of contact lens wear on clinically standard and non-standard ocular surface parameters, and to set guideline for optimised contact lens fitting. The expected outcomes of the study included better understanding of the relationship between ocular physiology and contact lens wear, the clinical utility and efficacy of non-invasive measurements of tear film, and the additional information for an eye care professional regarding contact lens discomfort and dropout.

It was only after careful considerations and contact with eminent researchers in the field from different institutions, over a period of one year, alongside thorough literature reviews that the methodology protocol for a longitudinal study was finally created. The first step was to design and undertake a pilot study to test the sustainability of fitting subjects with two daily disposable contact lenses of different materials: a silicone hydrogel (SiHy) lens and a hydrogel (Hy) lens. Following successful completion of the pilot project, the schedule of the main measurement acquisition part was confirmed.

The main study recruited 60 healthy, young, regular or occasional contact lens wearers (19 males and 41 females), aged (mean ± standard deviation) 25.5 ± 4.3 years, ranging from 20 to 37 by sending emails via university newsletters to inform about the longitudinal research project. The study protocol consisted of a qualifying visit (Baseline), contact lens fitting visit on the following day (Day 2), a control visit at two weeks (to ensure that the participants adhered to the study protocol). The control visit was also included in order to explain any further queries before the follow-up visits at three, six and twelve months. A sub-group of Hy and SiHy fitted subjects were recruited for the final Control Visit after completing the 12-month course of contact lens wear. The following measurements were included: assessing environmental factors by monitoring the laboratory temperature and relative humidity with a thermo-hygrometry device; the scores of Ocular Surface Disease

Index questionnaire and Contact Lens Dry Eye Questinnaire-8 to report the symptoms; meniscus tear height measurement with the Keratograph 5M (K5M); Tear Film Surface Quality (TFSQ) assessment with High-Speed Videokeratoscopy (HSV); tear osmolarity by TearLab Osmolarity System; non-invasive methods were used with K5M to assess break-up time and ocular redness. Additionally, a slit lamp biomicroscope with ×10 magnification, cobalt blue illumination, a Wratten 12 yellow-barrier filter and 1 mg fluorescein sodium ophthalmic sterile strips were used to observe ocular surface staining and the tear film break-up. Lissamine Green strips 1.5 mg were used to assess lid wiper.

The main results of this longitudinal study show clinically insignificant impact of contact lens wear on ocular surface physiology and, interestingly, reduced levels of tear osmolarity during the course of the study. These results are the first of its kind in this field.

Although tear film is affected by contact lens wear, in this study it has been concluded that, in general, there is no statistically significant difference in tear film quality between contact lens wear and non-contact lens wear. However, other studies show that tear film physiology has been adversely affected due to increased evaporation rate and tear film thinning with soft contact lens materials. Studies that are more recent have showed all contact lenses causing a significant reduction in TFSQ compared with bare eye measurements. Wearing soft contact lenses causes tear film instability, a decrease in blinking frequency, and increases symptoms of ocular irritation. Interestingly, in this study the result showsde a reduction in osmolarity, which is different from what the current literature supports; stable or a rise in osmolarity during contact lens wear. The corneal staining also reveals an improvement of ocular surface health at the final Control Visit while those of conjunctival staining showed no significant differences between the Baseline and the final Control Visit. Reduction in osmolarity may be due to the timing of the measurements done (shortly after contact lens removal) or it may be due to corneal desensitisation after prolonged contact lens wear – as the osmolarity values go even smaller during follow up visits. However, modern daily disposable materials and healthier contact lens wearing habits may indorse these improvements.

There is no restriction on contact lens choice in the country, where this research was conducted. Contact lenses are of competitive costs due to the ease of availability without prescription from multiple sources. There are similar contact lens markets in several other

countries. An increased number of contact lens fittings is led due to this, and therefore more affordable options with subjects opting for monthly reusable economic lenses over daily disposable lenses (54% of participants in this study wore monthly and 25% fortnightly soft contact lenses). This behaviour may lead to poor fitting decisions, lack of follow-up by the eye care professionals and perpetuating risky habits. However, a combination of better compliance, more moderate wearing schedule and appropriate contact lens fit and control, may have attributed to a decrease in osmolarity and stable ocular physiology shown in this study. Nevertheless, there is still need for continuous research to further understanding the mechanisms of ocular health with contact lens wear to overcome and improve this barrier.

Summarising, the study provides eye care professionals with new knowledge and guidelines on the mid-term effect of daily disposable soft contact lens wear on ocular surface physiology, provided that an advising optometrist implements a firm controlled regime.

STRESZCZENIE (POLSKI)

Liczne badania pokazały, że noszenie soczewek kontaktowych zaburza film łzowy zmieniając jego fizjologiczne funkcje. Obecność soczewki kontaktowej na oku powoduje zmiany w składzie filmu łzowego, tj. lipidów, białek, mucyn i elektrolitów. Film łzowy na oku nieustannie się zmienia, przechodząc etap stabilizowania się bezpośrednio po mrugnięciu, stabilną fazę między mrugnięciami i ostatecznie fazę destabilizacji, która może prowadzić do przerwania filmu łzowego, gdy oko pozostaje otwarte przez długi czas lub wcześniej u osób z zespołem suchego oka. Na soczewce kontaktowej dynamika filmu łzowego jest inna, istnieją różnice w fazie stabilizowania, stabilności i ścieniania filmu łzowego. Zauważono, że w przypadku soczewek kontaktowych faza stabilizowania i stabilności jest krótsza, a niekiedy nieodróżnialna w porównaniu do dynamiki filmu łzowego na oku bez soczewki.

Po założeniu soczewki kontaktowej, film łzowy jest rozdzielony na dwie części. Cienka warstwa łez pokrywa soczewkę, a jej dynamika zależy zarówno od materiału soczewki, jak i indywidualnego składu łez. Interakcja między białkami filmu łzowego a materiałem soczewki kontaktowej, obok zmian składu łez, ma kluczowy związek z problemem dyskomfortu odczuwanym podczas noszenia soczewek kontaktowych. Warstwa lipidowa filmu łzowego jest również znacznie cieńsza na soczewce kontaktowej, niż w tym samym oku bez obecności soczewki. Noszenie soczewek kontaktowych ma również wpływ na szybkość parowania łez, prowadząc do uczucia suchości i dyskomfortu. Odkładające się na powierzchni soczewki osady lipidowe i białkowe wpływają na szybsze przerwanie filmu łzowego. Na soczewkach kontaktowych można zaobserwować zjawisko zmiany zwilżalności powierzchni (z ang. dewetting), które prowadzi do nagłego przerwania filmu łzowego i szybko postępującego wysuszania powierzchni soczewki. Zwiększone parowanie filmu łzowego z powierzchni soczewki i destabilizacja filmu łzowego prowadzą do zwiększenia osmolarności łez.

Według wielu badań wszystkie miękkie soczewki kontaktowe mają niekorzystny wpływ na fizjologię filmu łzowego. Jednak istnieją przesłanki, że jakość filmu łzowego badanego in vivo zależy od rodzaju soczewki kontaktowej. Wykorzystanie zaawansowanych technik pomiarowych jakości i dynamiki filmu łzowego może pozwolić na dogłębne zrozumienie

różnic w biokompatybilności różnych materiałów soczewek kontaktowych i filmem łzowym. Tym samym może pomóc specjalistom od oczu w rozpoznaniu charakteru zachowania filmu łzowego na różnych rodzajach soczewek oraz pomóc w doborze właściwego materiału soczewki kontaktowej, co zmniejszy częstość występowania dyskomfortu i porzucania soczewek kontaktowych.

Wiedza na temat materiałów soczewek kontaktowych i ich kompatybilność z filmem łzowym jest nadal ograniczona. Pomimo, że nieinwazyjna kliniczna ocena interakcji soczewki i filmu łzowego jest możliwa, wyzwaniem pozostaje ilościowa ocena dynamicznych procesów filmu łzowego na oku i soczewce oraz oprogramowanie (algorytm) prowadzące do rzetelnej i powtarzalnej oceny.

Niniejsza rozprawa doktorska jest dogłębną pracą koncentrującą się na jakości filmu łzowego podczas noszenia jednodniowych soczewek kontaktowych. Badanie zostało przeprowadzone zgodnie z projektem badawczym Europejskiej Sieci Suchego Oka (z ang. European Dry Eye Network – EDEN), a główne cele badawcze tej pracy to: lepsze zrozumienie wpływu soczewek kontaktowych na fizjologię filmu łzowego; zbadanie wpływu noszenia soczewek kontaktowych na standardowe i niestandardowe kliniczne parametry powierzchni oka oraz zaproponowanie nowych wytycznych dla optymalnego dopasowania soczewek kontaktowych. Spodziewane wyniki badania obejmują lepsze zrozumienie zależności między fizjologią oka a noszeniem soczewek kontaktowych, określenie klinicznej użyteczności i skuteczności nieinwazyjnych pomiarów filmu łzowego oraz znalezienie dodatkowych informacji dla specjalisty badań oka dotyczących powodów dyskomfortu i porzuceń soczewek kontaktowych.

Ostateczny protokół badania podłużnego został opracowany po trwającym rok dokładnym studiowaniu literatury naukowej i wnikliwych rozważaniach i konsultacjach z wybitnymi badaczami z dziedziny badań nad filmem łzowym i soczewkami kontaktowymi z różnych instytucji. Pierwszym krokiem było zaplanowanie i przeprowadzenie badania pilotażowego w celu dopasowania ochotnikom soczewek kontaktowych wybierając jeden z dwóch rodzajów materiałów: soczewki silikonowo-hydrożelowej (SiHy) i soczewki hydrożelowej (Hy). Po pomyślnym zakończeniu projektu pilotażowego zatwierdzono harmonogram głównej części badań.

Do głównego badania zrekrutowano ostatecznie 60 zdrowych, młodych, regularnych lub okazjonalnych użytkowników soczewek kontaktowych (19 mężczyzn i 41 kobiet) w wieku (średnia ± odchylenie standardowe) 25,5 ± 4,3 lat, w zakresie od 20 do 37 lat. Informację o badaniu przekazywano za pośrednictwem uczelnianego biuletynu informacyjnego. Protokół badania składał się z wizyty kwalifikacyjnej (badanie bazowe), wizyty dopasowania soczewki kontaktowej odbywającej się następnego dnia (dzień 2), wizyty kontrolnej po dwóch tygodniach (w celu potwierdzenia przestrzegania protokołu badania przez ochotników) oraz kolejnych wizyt kontrolnych odbywających się po trzech, sześciu i dwunastu miesiącach. Podgrupa osób, którym dopasowano soczewki Hy i SiHy została dodatkowo poproszona przyjście na serię pomiarów po ukończeniu dwunastomiesięcznego cyklu noszenia soczewek kontaktowych. Uwzględniono następujące pomiary: ocenę czynników środowiskowych poprzez monitorowanie temperatury laboratoryjnej i wilgotności względnej za pomocą termo-higrometru; ocenę symptomów wykorzystując kwestionariusz Wskaźnik Choroby Powierzchni Oka (z ang. Ocular Surface Disease Index – OSDI) i kwestionariusz Suchego Oka wywołanego Soczewkami Kontaktowymi (z ang. Contat Lens Dry Eye Questinnaire – CLDEQ-8); pomiar wysokości menisku łzowego przy użyciu Keratografu 5M (K5M); cenę jakości powierzchni filmu łzowego (z ang. Tear Film Surface Quality – TFSQ) za pomocą szybkiej wideokeratoskopii (z ang. High-Speed Videokeratoscopy – HSV); osmolarność łez za pomocą systemu TearLab; nieinwazyjne metody oceny czasu przerwania filmu łzowego i zaczerwienienia spojówki za pomocą K5M. Dodatkowo zastosowano biomikroskop z lampą szczelinową z powiększeniem X 10, filtr kobaltowy, filtr żółty Wratten 12 i sterylne oftalmiczne paski nasączone 1 mg NaFl, w celu zaobserwowania barwienia powierzchni oka i przeprowadzenia testu czasu przerwania filmu łzowego. Paski z zielenią lizaminy (1,5 mg) zostały użyte do oceny krawędzi brzegu powieki.

Główne wyniki badania podłużnego wykazały klinicznie nieistotny wpływ noszenia soczewek kontaktowych na fizjologię powierzchni oka i, co ciekawe, obniżanie poziomu osmolarności łez w trakcie badania. Jest to pierwszy tego rodzaju wynik zaobserwowany podczas noszenia soczewek kontaktowych.

Mimo, że noszenie soczewek kontaktowych ma bezwzględnie wpływ na film łzowy, w tym badaniu nie stwierdzono, statystycznie istotnej różnicy w jakości filmu łzowego między kolejnymi wizytami a pierwszą wizytą bez soczewki. Większość badań pokazuje

niekorzystny wpływ na fizjologię filmu łzowego spowodowany zwiększonym parowaniem i szybszym ścienianiem filmu łzowego na powierzchni miękkich soczewek kontaktowych. Nowsze badania wykazały, że wszystkie soczewki kontaktowe powodują znaczne pogorszenie TFSQ w porównaniu z pomiarami na oku bez soczewki. Noszenie miękkich soczewek kontaktowych powoduje niestabilność filmu łzowego, zmniejsza częstotliwość mrugania i nasila objawy podrażnienia oczu. Co ciekawe, w tym badaniu wyniki wykazały zmniejszenie osmolarności, co jest przeciwstawne do doniesień literaturowych wykazujących zwiększanie lub utrzymywanie stabilnego poziomu osmolarności łez podczas noszenia soczewek kontaktowych. Barwienie rogówki również wykazało poprawę stanu zdrowia powierzchni oka na ostatniej wizycie kontrolnej, podczas gdy barwienie spojówek nie wykazało istotnych różnic między badaniami bazowymi a końcową wizytą kontrolną. Zmniejszenie osmolarności mogło być spowodowane wykonaniem pomiaru krótko po zdjęciu soczewek kontaktowych lub przyzwyczajeniem rogówki do długotrwało noszenia soczewek kontaktowych, ponieważ wartości osmolarności zmniejszały się w kolejnych wizytach kontrolnych. Jednakże, poprawa stanu zdrowia powierzchni oka mogła być również związana z noszeniem soczewek jednorazowego użytku wykonanych z nowoczesnych materiałów, większą kontrolą użytkowników i zdrowszymi nawykami noszenia soczewek podczas udziału w projekcie.

W kraju, w którym przeprowadzono badania, nie ma ograniczeń dotyczących wyboru soczewek kontaktowych. Soczewki kontaktowe są łatwo dostępne z wielu źródeł bez recepty. W kilku innych krajach istnieje podobny rynek soczewek kontaktowych. Wielu użytkowników decyduje się na miesięczny tryb wymiany soczewek ze względów ekonomicznych (54% uczestników tego badania nosiło wcześniej soczewki miesięczne, a 25% uczestników soczewki dwutygodniowe). Wolny rynek soczewek może prowadzić do nieodpowiednich decyzji użytkowników, braku regularnych wizyt u specjalistów oczu i utrwalania niewłaściwych nawyków. Połączenie lepszego podporządkowania pacjenta, bardziej umiarkowanego noszenia soczewek i odpowiedniego dopasowania oraz kontroli soczewek kontaktowych, może być przyczynić się do zmniejszenia osmolarności i stabilnej fizjologii oka pokazanej w tym badaniu. Jednakże nadal istnieje potrzeba dalszych badań, aby lepiej zrozumieć mechanizmy zachodzące na powierzchni oka podczas noszenia soczewek kontaktowych.

Podsumowując, przeprowadzone badanie dostarcza specjalistom optometrystom i okulistom nowej wiedzy i wytycznych na temat wpływu codziennego noszenia jednorazowych soczewek kontaktowych na fizjologię powierzchni oka.

RESUMEN (ESPAÑOL)

Numerosos estudios han mostrado que las lentes de contacto disrumpen la película lagrimal debido a la creación de una capa pre y post lente produciendo una alteración de sus funciones fisiológicas. Esto conduce a cambio en la composición de la película lagrimal: lípidos, proteínas, mucina y electrolitos. La película lagrimal pre-ocular varía constantemente tras el parpadeo habiendo una primera fase de formación, inmediatamente tras el parpadeo, una fase más o menos estable entre parpadeos, y una última fase de adelgazamiento y posterior desestabilización de la película lagrimal que puede desencadenar la ruptura de la película lagrimal en sujetos con síndrome de ojo seco o cuando el ojo permanece abierto un periodo prolongado de tiempo. Existen diferencias en la dinámica de la película lagrimal entre las fases de formación, estabilidad y adelgazamiento con el porte de lentes de contacto. Las fases de formación y estabilidad se han observado más cortas y en ocasiones indistinguibles en usuarios de lentes de contacto respecto a no usuarios. Tras la inserción, una fina capa de lágrima cubre la lente siendo la dinámica de la película lagrimal dependiente del material de la lente y de la composición particular de la lágrima del usuario. La interacción entre la proteína de la película lagrimal y el material de la lente de contacto junto al cambio en la composición de la lágrima son particularmente claves para el discomfort del porte de lentes de contacto. La capa lipídica de la película lagrimal pre-lente durante el porte de la lente de contacto es más fina que sin la lente en un mismo ojo. Las lentes de contacto también tienen influencia en el ratio de evaporación de la película lagrimal provocando un aumento de la sequedad ocular e incomodidad. La deposición de lípidos sobre la lente de contacto puede jugar un papel en la disrupción de la película lagrimal debido a la pobre calidad de la superficie que se sitúa entre el material de la lente de contacto y la película lagrimal. Este fenómeno se conoce como deshidratación y conduce a un aumento del ratio de evaporación de la película lagrimal pre-lente y el consiguiente aumento de la osmolaridad.

Varios estudios han concluido que todos los materiales de lentes de contacto afectan negativamente en la fisiología de la película lagrimal. Recientemente se han observado diferencias en la calidad de la superficie de la película lagrimal en observaciones in-vivo, dependiendo del tipo de lente de contacto. Por lo tanto, mejores técnicas para evaluar la calidad de la película lagrimal y su dinámica pueden ayudar a entender mejor la

compatibilidad de la película lagrimal con los materiales de lentes de contacto permitiendo a los profesionales de la visión y de la salud ocular reconocer la naturaleza del comportamiento de la película lagrimal con diferentes lentes de contacto. También puede servir de ayuda a los profesionales en mejorar las guías de adaptación con el fin de reducir el discomfort y la tasa de abandono. Aunque la evaluación clínica no invasiva de la interacción lente de contacto-lágrima hace posible la valoración de la película lagrimal, el desafío sigue siendo la dinámica de la película lagrimal y el proceso de deshidratación, ya que la diversidad en el análisis del observador y en el análisis informático (algoritmo), conducen a medidas poco fiables y de baja repetitibilidad. Además, el conocimiento de los materiales de las lentes de contacto y su compatibilidad con la película lagrimal aun es limitado.

El presente estudio es un trabajo en profundidad enfocado en la valoración de la calidad de la película lagrimal durante el porte de lentes de contacto desechables de uso diario. El proyecto fue diseñado de acuerdo con el European Dry Eye Network (EDEN) y los principales objetivos fueron: entender mejor el efecto del porte de las lentes de contacto sobre la fisiología de la película lagrimal; valorar el efecto longitudinal del porte de la lente de contacto en los parámetros de la superficie ocular clínicamente estándar y no estándar, y establecer una guía optimizada de adaptación de lentes de contacto. Los resultados esperados del estudio incluyeron una mejor comprensión de la relación entre la fisiología ocular y el porte de la lente de contacto, la utilidad clínica y eficacia de las medidas no invasivas de la película lagrimal, y la información adicional para un profesional de la visión respecto a la incomodidad y abandono del uso de lentes de contacto.

Después de consideraciones cuidadosas y contactos con eminentes investigadores en el campo de las lentes de contacto de diferentes instituciones durante un año, así como de una exhaustiva revisión de la literatura específica, se desarrolló el protocolo metodológico para el estudio longitudinal. El primer paso fue diseñar y llevar a cabo un estudio piloto para testear sujetos adaptados con dos lentes de contacto desechables diarias de diferente material: una lente de hidrogel de silicona (HiSi) y una de hidrogel (Hi). Tras la finalización exitosa del proyecto piloto, se concretó el cronograma del apartado de medidas del estudio principal. En el estudio principal se reclutaron 60 pacientes jóvenes, portadores regular u ocasionalmente de lentes de contacto (19 hombres y 41 mujeres), con una edad de

25.5 ± 4.3 años (media ± desviación estándar), comprendidas entre los 20 y 37 años, con un envío por correo electrónico informando de la naturaleza del proyecto. El protocolo consistió en una visita inicial donde se recogieron los datos iniciales (Baseline), vista para la adaptación de la lentes en el siguiente día (Día 2) y visita de control a las 2 semanas (con el fin de asegurar que los participantes se adherían al protocolo del estudio). La visita de control fue también incluida con el fin de dar respuesta a cualquier duda que tuviese el participante en el estudio antes de las visitas de seguimiento a 3, 6 y 12 meses. Un subgrupo de sujetos adaptados con Hi y HiSi fueron reclutados para la Visita Control final después de completar el ciclo de 12 meses de porte. Las medidas que fueron realizadas en el estudio fueron: valoración de los factores medioambientales por monitorización de la temperatura del laboratorio y humedad relativa con termo-higrómetro; cuestionarios de sintomatología Ocular Surface Disease Index y Contact Lens Dry Eye Questinnaire-8; medida del volumen del menisco lagrimal mediante el topógrafo Keratograph 5M (K5M); evaluación de la calidad de la superficie de la película lagrimal (TFSQ) mediante videoqueratoscopia de alta velocidad; osmolaridad lagrimal utilizando el sistema TearLab; métodos no invasvos fueron utilizados con K5M para valorar el tiempo de ruptura lagrimal y el ojo rojo. Además, para valorar tinciones de la superficie ocular y la ruptura de la película lagrimal se utilizó una lámpara de hendidura con una magnificación X10, iluminación azul cobalto, un filtro amarillo Wratten 12 y tiras esterilizadas de fluoresceína sódica de 1 mg. Verde de lisamina en tiras de 1.5 mg también fue utilizada para valorar la conjuntiva tarsal.

Los principales resultados obtenidos en este estudio longitudinal muestran un impacto insignificante del porte de lentes de contacto sobre la fisiología de la superficie ocular e, interesantemente, una reducción de los niveles de osmolaridad durante el curso del estudio. Este es el primer estudio en este campo que apunta en esta dirección.

Aunque la película lagrimal esta afectada por el uso de lentes de contacto, en este estudio se concluye, que en general, no existen diferencias estadísticamente significativas en la calidad de la película lagrimal entre llevar y no llevar lentes de contacto. Sin embargo, otros estudios muestran que la fisiología de la película lagrimal ha sido deteriorada debido al aumento del ratio de evaporación y al adelgazamiento de la película lagrimal con materiales blandos. Estudios más recientes han mostrado que todas las lentes de contacto causan una significante reducción del TFSQ comparado con medidas en ojo desnudo. El porte de lentes de contacto blandas causan inestabilidad de la película lagrimal, disminución de la

frecuencia de parpadeo, y aumento de los síntomas de irritación ocular. En nuestro estudio, los resultados mostraron una reducción de la osmolaridad, que está en desacuerdo con lo que sostiene la bibliografía actual, que muestran valores estables o mayores. La tinción corneal también presentó una mejora de la salud de la superficie ocular en la Visita Control final, mientras que la tinción conjuntival mostró que no existían diferencias entre el Baseline y la Visita Control final. La reducción de la osmolaridad puede ser debida a que la medida se realizó muy poco tiempo después de retirar la lente de contacto o por la insensibilización corneal producida por un prolongado uso de la lente de contacto - ya que los valores de osmolaridad son incluso más bajos en las visitas de seguimiento-. Estas mejoras pueden estar respaldadas por los materiales desechables diarios modernos y los hábitos de porte de lentes de contacto más saludables.

No hubo restricción sobre la elección de la lente de contacto en el país donde se realizó la investigación. Las lentes de contacto tienen un precio competitivo debido a que no se necesita prescripción y se pueden comprar por diferentes vías, siendo además, el mercado de las lentes de contacto similar al de varios otros países. Debido a estos factores, se consigue un mayor número de adaptaciones, pero muchos de los sujetos optan por lentes mensuales que son más asequibles que las lentes diarias (el 54% de los participantes en este estudio usaban lentes blandas de reemplazo mensual y el 15% de reemplazo quincenal). Este cambio en el tipo de porte de las lentes de contacto puede llevar a tomar decisiones inadecuadas, falta de seguimiento por parte de los profesionales de la visión y perpetuación de los hábitos de riesgo. Sin embargo, una combinación de un mejor cumplimiento de las normas de uso, un calendario más moderado del porte y una apropiada adaptación y un mayor control pueden proporcionar una disminución de la osmolaridad y una fisiología ocular estable como se ha mostrado en este estudio. Sin embargo, todavía existe la necesidad de continuar investigando para comprender mejor que ocurre con los mecanismos de salud ocular cuando existe una lente de contacto adaptada.

Resumiendo, este estudio proporciona a los profesionales del cuidado de la visión nuevos conocimientos y directrices del efecto a medio plazo del uso de lentes de contacto blandas de reemplazo diario sobre la fisiología de la superficie ocular, siempre que se siga un régimen de uso firme y controlado por un optometrista.

LIST OF ABBREVIATIONS AND ACRONYMS

CLD	Contact Lens Discomfort
CLDEQ-8	Contact Lens Dry Eye Questionnaire-8
CNV	Trigeminal Nerve
CV	Control Visit
FBUT	Fluorescein Tear Film Break-Up Time
FNIKBUT	First-NIKBUT
HSV	High-Speed Videokeratoscopy
Hy	Hydrogel
K5M	Keratograph 5M
Lf	Lactoferrin
LWE	Lid Wiper Epitheliopathy
MGD	Meibomian Gland Dysfunction
MNIKBUT	Mean-NIKBUT
NBC	Natural Blinking Conditions
NIKBUT	Non-Invasive Keratograph Break-Up Time
OSDI	Ocular Surface Disease Index
SBC	Suppressed Blinking Conditions
SiHy	Silicone Hydrogel
TBUT	Tear Break-Up Time
TFSQ	Tear Film Surface Quality
TMH	Tear Meniscus Height
NIBUT	Non-Invasive Break Up Time
sIgA	Immunoglobulin A
LTB4	Leukotriene B4

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I. INTRODUCTION

Vision is one of the five human senses that allows us to perceive the world around us. Although one might think that senses operate independently, they interact closely via complex neuronal circuits to enable the brain process stimuli and respond with appropriate commands. This phenomenal aesthesis has always intrigued me to the point where I decided to follow optometry as a career with specific interest in research.

My work experience as a qualified optometrist gave me an insight into the field of contact lens wear and its effect on ocular surface quality. Indeed, I have encountered many patients who suffered contact lens-related problems, aggravated by ongoing poor ocular surface quality. Many studies have investigated the prevalence of contact lens wear dropouts relevant to a poor ocular surface quality and they have estimated them to range between 12% and 43% (Efron et al., 2010, Muselier-Mathieu et al., 2014, Nichols et al., 2017, Sulley et al., 2017). This is primarily why I have chosen this field of research, to answer unsolved questions and possibly improve our understanding of the impact of contact lens materials on anterior eye disease. Specifically, I aim to investigate the impact of long-term modern daily disposable contact lens wear on ocular physiology with reference to tear film surface quality and contact lens materials. This study focused on modern daily disposable contact lenses, as they may have a lesser effect on the ocular physiology. Parameters considered to be measured, such as osmolarity and tear film surface quality, which are not being measured routinely in clinical practice may provide a predictor that can be introduced in clinic as an additional guide for a successful outcome in contact lens wear. The ultimate objective is to use this data to improve our knowledge on how to reduce the number of contact lens wear dropouts and contact lens-related complications, such as contact lens-related

dry eye. The refractive surface of the eye relies on a dynamic equilibrium between the ocular surface, tear film, eyelids and lacrimal glands to maintain homeostasis, which is the regulation and maintenance of a constant internal environment, whereby conditions are controlled in the human body to be in a stable state. An insult to any of those structures results in ocular surface disease. The tear film is the major contributor to this balance, and its quality is the determinant of ocular surface health.

1.1 Ocular Surface

The ocular surface is continuously challenged by environmental parameters, pathogens, as well as its own component quality disturbance. These components act as a single unit to guarantee its homeostasis and respond to feedback mechanisms resulting in coordinated reactions to various stimuli. This functional unit is innervated by the ophthalmic division of the trigeminal nerve (V1) and the facial nerve and it is rich in lymphocytes, hormones and several other cytokines in order to ensure optimal protection and function of the ocular system (Rolando and Zierhut, 2001).

The components of the ocular surface include the tear film, cornea, conjunctiva, Meibomian and lacrimal glands and the muco-epidermal junction of the lid, of which the tear film is certainly the most dynamic element (DelMonte and Kim, 2011).

1.2 Ocular Glands

It is important to understand the immunological cooperation between the lacrimal gland, cornea, and conjunctiva for the maintenance of the ocular surface homeostasis. The lacrimal gland is part of this immunological functional unit, which predominantly serves as the source of innate immune cells for the defence against endogenous and

exogenous factors that can lead to infection and/or inflammatory response (Zierhut et al., 2002). More specifically, the lacrimal gland secretes a large array of lymphocytes, natural killer cells and immunoglobulins. Furthermore, the epithelial cells of this gland secrete a number of proteins, which are necessary for an appropriate immune response (Zierhut et al., 2002). Anatomically, the lacrimal gland consists of a large orbital part and a smaller palpebral part, separated by the lateral expansion of the levator palpebral superioris muscle. The orbital part has a superior surface within the frontal bone and an inferior surface marked by the levator palpebral superioris muscle and lateral rectus, while the palpebral part is in the superior fornix and situated upon the course of lacrimal ducts. Histologically, it is a mixed gland containing mucoacinar units surrounded by connective tissue rich in blood vessels and excretory ducts. Moreover, goblet cells are found within the cuboidal epithelia of the gland.

The conjunctiva and cornea also have unique structural and functional properties, which play a crucial role in the defence of the ocular surface. These highly specialised tissues contain different cells against antigenic challenge; myeloid antigen presenting cells activate chemokine receptors to gather around lymphatic pathways. It is important to note the lack of class II major histocompatibility complex (MHC), a phenomenon that might explain the state of 'ocular immune privilege' of the ocular tissues in their healthy condition (Hori, 2008), (Zhou and Caspi, 2010).

1.3 Tear Film and Corneal Interface

The corneal epithelium is composed of non-keratinised, stratified squamous cell layers. The corneal epithelium along with the tear film interface are vital for the refractive power of the eye. The innermost layer of the tear film, the mucinous layer, interacts with the epithelium to aid homogeneous spreading of tears with each blink.

The middle layer, known as the aqueous layer, constitutes the majority of the tear film thickness. The major and accessory lacrimal glands of Krause and Wolfring secrete its contents. This layer's function includes removal of debris, oxygen diffusion, antibacterial activity and buffering capacity. The outermost layer, the lipid layer, mainly prevents evaporation of the tear film and it is formed from the secretion of the Meibomian, Zeiss and Moll glands (McCulley and Shine, 2003; DelMonte and Kim, 2011).

Even though the model described above assumes three separate layers of the tear film with different thicknesses, it is important to emphasise that, in reality, the tear film is dynamic and depends on a balanced equilibrium of the different constituents (Bron et al., 2015).

Tear film lipids have been shown to be moderately stable from blink to blink, a finding that signifies the need to analyse tear fluid dynamics during the complete blink cycle (Braun et al., 2015). This cycle is thought to comprise of four parts:

1. The downstroke during which the superior lid moves inferiorly and stops;
2. The stopping point of the downstroke marks the turning point;
3. The upstroke during which the superior lid moves superiorly;
4. The interblink lasts until the next downstroke begins.

During this cycle, the tear film is disturbed deviating from the ideal perfect model. In fact, the lipid layer is not uniformly spread over the aqueous layer. One reason for this is the fact that the Meibomian orifices, which secrete the lipids, are spaced approximately one millimetre from one another, leading to an 'imperfect' lipid layer. It has been observed that, during the downstroke of the blink, there are transient ripple

patterns, which can be defined as small waves of the tear film surface. Moreover, recent studies have shown that the ripples created during the blink cycle may be independent of the tear film composition and lid motion (Pult et al., 2015). Experimental images show that the ripples remain static with respect to the corneal surface and their wavelength follows the spacing of epithelial squamous cells, indicating the association between tear film lipid distribution and corneal surface. Another factor to consider relates to the turning point of the blink. Many blinks are incomplete and hence, the quantity of lipid can significantly increase at the beginning of the upstroke phase. This sequence of events can clearly affect the positioning and distribution of the tear film ripples during the blink cycle (Braun et al., 2015).

Studies indicate that the thin aqueous film can form because of the conjunction of the Meibomian lipid layer and the mucin layer. It is estimated that the thickness of this thin film is approximately several micrometres (King-Smith et al., 2004). What enables this very thin film to remain stable on the ocular surface is, first, the hydrophilic property of the ocular surface and, second, the reduction of surface tension of water molecules to spread evenly rather than form a drop. The first condition is met by mucins attached on the corneal epithelium. A healthy tear film facilitates the spreading of the mucin by means of a negatively charged epithelial surface induced by the glycocalyx on its surface; tear volume loss affects the polarisation of the glycocalyx envelope, eventually leading to irregularities in the mucus gel with subsequent keratinisation and dry spot formation (Argueso and Gipson, 2001). Figure 1 schematically represents the hypothesis on the separate layers of the tear film of a healthy subject.

As for the surface tension of the aqueous, it is achieved by surfactant molecules within the lipid layer. The actual composition of the surfactants is very precisely described: fatty acids, phospholipids, and meibomian lipids (Millar and Schuett 2015). It is believed that the meibomian lipids play the most significant role in the prevention of the aqueous film collapse because they have viscous and elastic properties combined (Arita et al., 2017). In other words, meibomian lipids can be liquid enough to ensure even spreading, but also solid enough to prevent aqueous film collapse. The common belief that supports tear film evaporation is merely due to a defective lipid layer contradicts evidence, which shows great variability within blink cycles or with variable physical parameters. Furthermore, in vitro studies indicate that the lipid layer does not play a role in the evaporation of the tear film and it is the composition of meibum that correlates with this phenomenon (Millar and Schuett, 2015).

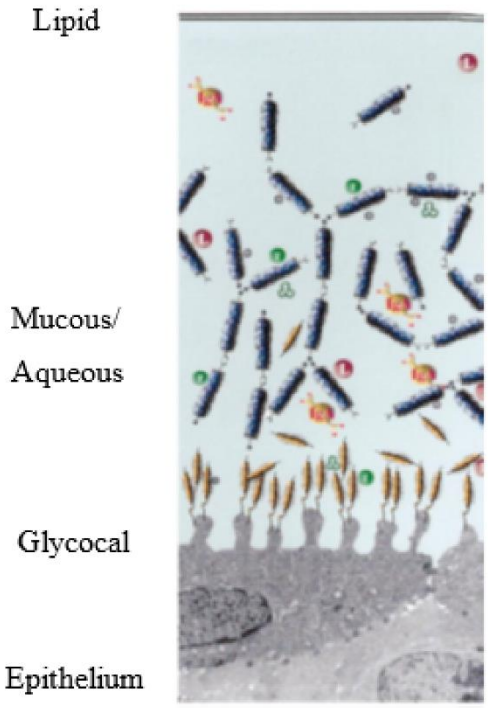

Figure 1. The hypothesis on the separate layers of the tear film. Mucin release from conjunctival goblet cells; upon secretion, mucin molecules are free to scatter into the tear fluid and offer an

attachment for other constituents of the tears, such as immunoglobulins, lysozyme and other proteins (Argueso and Gipson, 2001). Permission to use the image in this thesis was granted.

Optical techniques that are now experimentally used to measure tear film thickness are non-invasive with extremely high axial resolution (micrometer), smaller than the actual thickness of the tear film. Moreover, it is now possible to non-invasively visualise the dynamics of the tear film, such as higher resolution microscopy, wavelength dependent fringes and thickness or angle dependent fringes. Specifically, these high-resolution systems identified abnormal lipid shapes called "islands" and "lenses", which may represent lipid droplets over the aqueous film. As discussed earlier, this observation may be due to lack of surfactant with subsequent rupture of the tear film and it is consistent with findings of other studies, which signified the involvement of meibomian lipid in a defective evaporation profile (Bai and Nichols, 2017).

Thickness dependent fringes have the advantage of allowing the generation of a two-dimensional map of the thickness of the tear film. In addition, confocal microscopy studies show different thickness peaks one of which is 3 μm. This layer may represent the full thickness of the tear film, since it is too thick to only correspond to the lipid layer. Moreover, the 3 μm peak shows strong reflection and that is because of the higher difference between the refractive index of the tear film and the corneal epithelium. As a result, we can safely assume that the normal tear film thickness is about 3 μm, as King-Smith (2004) remarked, a value comparable to that of the pre-lens tear film, however, reflex tearing can of course alter this value.

The tears, like any other body fluid, represent an easily accessible marker for the evaluation of the health of the underline cornea. This ocular body fluid contains, apart from the lipids discussed above, proteins, electrolytes, small molecule metabolites,

which render the tear film an effective protective layer against the outside environment.

Tear proteins are perhaps the most important molecules regarding antimicrobial activity against pathogens. Such proteins include lysozyme, immunoglobulins defensins, lactoferrin and mucins. While most of them are involved with the direct killing of microorganisms, others play a role in the aggregation of pathogens prior to being phagocytosed. A study by Zhou et al (2012) identified 1543 tear proteins, found in normal subjects and it represents the largest study to date to analyse the human tear proteome. This data can be used as a reference list for biomarker assay to identify ocular surface changes related to either disease, tissue dysfunction or adverse reaction to contact lenses (Wizert et al., 2017).

One of the proteins of the tears that has been extensively studied is lactoferrin (Lf). Lf has both antibacterial and anti-inflammatory properties. It is mainly produced by the lacrimal glands, however, Santagati et al (2005) reported production of Lf by the ocular surface epithelial cells, particularly in the conjunctiva. Furthermore, there have been recent reports of meibomian glands Lf secretion (Flanagan and Willcox, 2009). The normal range of Lf in the non-contact lens wearing human subjects ranges between 0.63 and 2.9 gr/l. Even though many studies have confirmed that Lf concentration does not differ between contact lens and non-contact lens wearers, Lf has been reported to be a commonly found deposit on contact lenses and, therefore, may be implicated in discomfort, irritation, microbial contamination, infection and dropout. Ionic and higher water content contact lens material tend to show more Lf deposition through electrostatic bonding. Lysozyme is another major synthesized tear protein, which secrete the mucous and also provide anatomical barriers against

antimicrobial factors (Flanagan and Willcox, 2009). These two proteins are protective elements playing a role in non-specific immunity of the ocular surface (Pinard et al.,2003).

Tear osmolarity, which reflects the ionic environment of the tear fluid, is defined as the number of osmoles per litter of solution (Stahl and Jalbert, 2018). This variable is useful in order to understand the delicate balance between tear production, evaporation and drainage and its normal values vary from 270 to 315 mOsm/L (Tomlinson et al., 2006). Normal osmolarity is determined by the concentration of the electrolytes in the muco-aqueous component. It is important to note that osmolarity values differ between the tear meniscus and across the ocular surface. During tear film break up, osmolarity has been found to rise to approximately 1900 mOsm/L, a value that is significantly higher than the one in the tear meniscus (García-Resúa et al., 2014). This is because of the blending of tears over the ocular surface with those in the meniscus. The clinical relevance of this observation lies with the fact that the osmolarity difference between the ocular surface tear film and that in the meniscus will be greater in the case of tear film abnormalities compared to that of normal eyes. Tear film osmolarity, thinning and evaporation constitute reliable measures of tear film quality and stability, which may also be useful tools when assessing not only ocular conditions such as dry eye disease but also contact lens tolerance and dropout. (Wolffsohn et al., 2017).

1.4 Ocular surface sensation and neural regulation of tear production

The ocular surface is densely innervated by sensory fibres of the ophthalmic division (V1) of the trigeminal nerve, with the cornea showing the densest neural projections. The corneal nerves, with their unmyelinated and thinly myelinated fibres, enter the corneal stroma while losing their myelin sheath and form a plexus located in the anterior one-third of the stroma. From there, the majority of the nerve pierce Bowman's layer to terminate in the corneal epithelium (Figure 2).

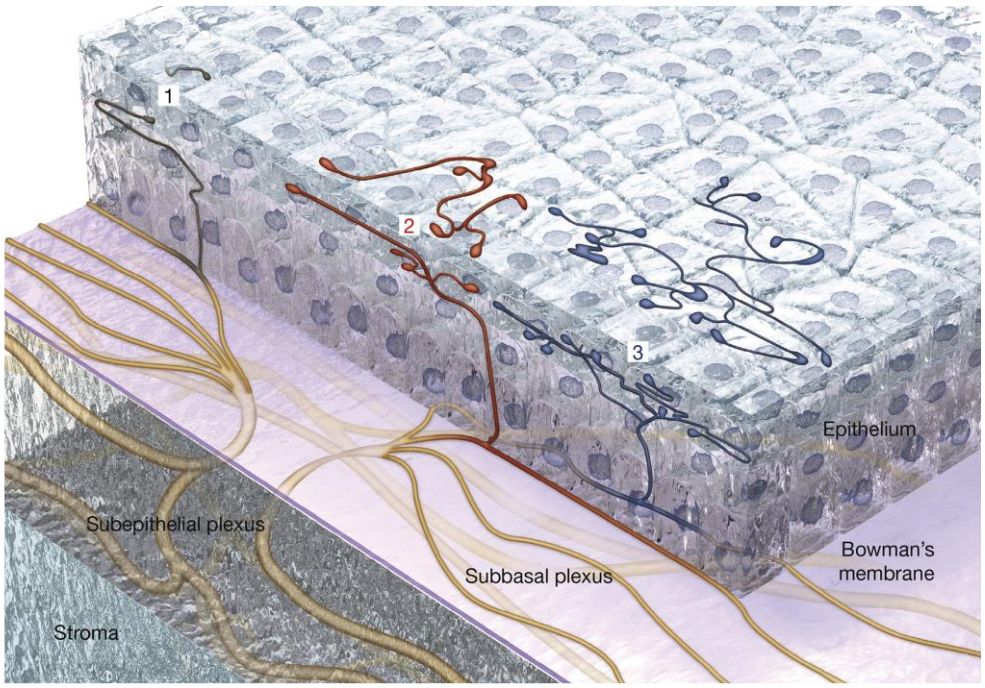

Figure 2. Model of superficial nerve terminals in the mouse corneal epithelium demonstrating examples of simple (1, black), ramifying (2, red) and complex (3, blue) nerve terminals (Belmonte et al., 2017)

Electrophysiological studies have shown that sensory corneal nerve fibres are classified as either poly modal nociceptor, cold thermoreceptor or mechano-nociceptor neurons. (DelMonte and Kim, 2011 and Belmonte et al., 2017). Polymodal nociceptors

are activated with noxious stimuli, heat and chemical (endogenous or exogenous) irritants. Mechano-nociceptors respond only to pure mechanical stimuli that signal a force of magnitude high enough to cause damage to the corneal epithelium. Cold thermoreceptors are involved in regulating tear production to ensure sufficient moistness of the ocular surface and in signalling the sense of irritation caused by acute dryness of the cornea. These fibres are also able to detect slight changes in tear osmolarity and therefore tear film break up (Belmonte et al., 2017).

The ocular glands that secrete the component of the tear film have parasympathetic and sympathetic fibres, which originate from the pterygopalatine/ ciliary and superior cervical ganglia, respectively. Sympathetic fibres can regulate lacrimal gland secretion in two ways. First, through vasodilation or vasoconstriction, they can change blood flow and therefore electrolyte and water secretion. Second, α1- and β – adrenoreceptor activation can directly cause protein, electrolyte and water secretion. Moreover, the meibomian gland has been shown to possess M1, M2, M3, M4 and M5 muscarinic receptors within the acini, ducts and basal epithelium, which are related to cell proliferation stimulation (Belmonte et al., 2017).

Sympathetic nerve fibres are found in the meibomian gland acini, which regulate vascular secretion. As for the innervation of the conjunctiva goblet cells, which secret the gel required to form mucin, electrolyte and water, it is mediated via M1, M2 and M3 muscarinic receptors located near the secretory granules and α1A- and β3-adrenoreceptors.

When tear secretion is reduced the corneal epithelium is left exposed to the outside environment. Stress to the ocular surface epithelium can then be caused by the tear hyper-osmolarity and increased evaporation. This series of events may lead to a local

inflammatory response with subsequent ocular surface nerve damage. If this insult to the ocular surface is prolonged, it will result in abnormal peripheral and central nerve terminal sprouting, with alteration in synaptic transmission, finally resulting in chronic pain (Belmonte et al., 2017).

Noxious stimulation of afferent neurons of the ocular surface contributes to the sensation of discomfort, dryness and pain and it is thought that tear film break-up results in increased sensation, irritation, allodynia and hyperalgesia. If the noxious stimuli are repetitive, the level of pain or sensitization may display higher temporal summation and reduced descending control. In other words, an insult to the ocular surface homeostasis may lead to anatomical and functional changes at higher neural pain pathways. This is of relevance when considering tear film quality after prolonged contact lens wear and it may explain the different levels of tolerance amongst contact lens patients (Murphy et al., 2001; Belmonte et al., 2017).

1.5 Effect of Contact Lenses on the Ocular Surface

Soft contact lenses diameter ranges from 2 to 3 mm beyond the corneal edge; thus, they have an impact on the limbus and the underlying bulbar conjunctiva. Since the cornea is mainly receiving oxygen by diffusion from the external environment, the ocular environment is under stress due to subclinical inflammation in closed eye conditions or in the presence of a contact lens and therefore, oxygen supply is restricted to the conjunctival arterioles. Importantly, contact lens wear may affect the regenerative capacity of limbal epithelial stem cells, making the cornea prone to defective epithelialisation and subsequent recurrent erosions and corneal neovascularisation. This latter complication of contact lens wear is a result of a noxious stimulus that disrupts the dynamic equilibrium of vascular endothelium growth factors,

which normally maintain corneal avascularity (Stapleton et al., 2006). Apart from these complications, contact lenses further interrupt corneal physiology in several ways; significant corneal thinning has been reported both at an epithelial and stromal level (Liu and Pflugfelder, 2000). Although the exact mechanism of thinning is not fully understood, chronic corneal oedema and enhanced keratocyte apoptosis have been implicated. Corneal thinning with induced ectasia, induced curvature and even keratoconus have also been associated with soft contact lens wear after as little as 18 months (Liu and Pflugfelder, 2000).

1.6 Contact Lens and Tear Film

Contact lens history dates back to the sixteenth century when Leonardo Da Vinci and Rene Descartes devised a glass tube filled with liquid to be placed directly in contact with the cornea. However, it was in early 1880s that the technology of contact lenses was revolutionised with the first contact lens of a convex shape and with refractive power. About fifty years later Dr Dallos and Istvan Komaromy created contact lenses based on moulds of the living cornea. It was only in 1948, though, that the first corneal lens was invented by Keven Touhy. In 1958, a chemist, Otto Wichterele developed a new material called hydrogel, which was then introduced in the contact lens manufacture industry (Nicolson and Vogt, 2001). Over the next 40 years contact lens materials continued to improve in order to allow greater oxygen permeability and patient comfort (Murphy et al, 2001).

Contact lenses interact with the ocular surface at the level of the corneal and conjunctival epithelia and the tear film. Contact lenses are medical devices that must allow sufficient oxygenation on the corneal tissue to support aerobic metabolism and

prevent hypoxia. Their material must also conserve normal tear film integrity and prevent adhesion of bacteria and debris (Stapleton et al., 2006).

Hydrogel material is formed by the polymerisation and cross-linking of monomers and the main constituent is the hydrophilic poly 2-hydroxyethyl methacrylate (HEMA). Oxygen permeability depends on the water content of the material, which means that it is reduced by the solubility of oxygen in water. Silicone hydrogel materials have been introduced to offer significantly higher oxygen permeability, since there is a better solubility of oxygen in silicone rather than water (Stapleton et al., 2006). Moreover, the hydrophobic property of silicone has been controlled with surface treatment and the integration of internal wetting polymers. Current literature supports that silicone hydrogel lenses have a higher clinical performance compare to hydrogel materials based on markers such as limbal and bulbar hyperaemia, epithelial microcysts and staining (Brennan et al, 2002). Microcysts that result from contact lens wear are regarded as the most reliable marker of hypoxic stress. Generally, less than 10 microcysts indicate no lens wear or daily wear, whereas more than 50 can be a sign of severe chronic hypoxia. Limbal capillaries may be engorged even after as little as four hours of hydrogel lens wear. However, silicone hydrogel lenses show no or minimal limbal hyperaemia even after nine months of extended wear (Brennan et al, 2002). This may be due to their increased oxygen permeability compare to hydrogel. Prolonged limbal hyperaemia can lead to up-regulation of some molecules such vascular endothelial growth factor and basic fibroblast growth factor, both of which induce proliferation of the vascular endothelium and neovascularization (Murphy et al, 2001; Papas et al., 2014).

Regardless of material, contact lenses need a stable tear film for optimal wear. These medical devices can potentially affect the stability of the tear film via six different mechanisms (Papas, 2014):

1. Alteration the structure and function of the tear film;
2. Influencing the lid/cornea/tear interface;
3. Compartmentalisation the tear film;
4. Changing pre- and post- lens tear exchange;
5. Increasing tear instability
6. Altering normal defence mechanisms of the ocular surface, such as phagocytosis.

1.6.1. Pre-lens Tear Film

Contact lenses affect the integrity of the pre-corneal tear film, essentially dividing it into two sections; one on the front surface of the contact lens, known as pre-lens tear film and two, on the posterior surface of the lens, the post lens tear film (Figure 3). Evidently, the pre-lens tear film is an important factor for the clinical performance of lenses in many ways: minimising friction between the tarsal conjunctiva and the anterior lens surface; providing a smooth optical surface; preventing surface drying and debris deposition; and maintaining ocular surface homeostasis (Young, 1991; Craig et al., 2017a).

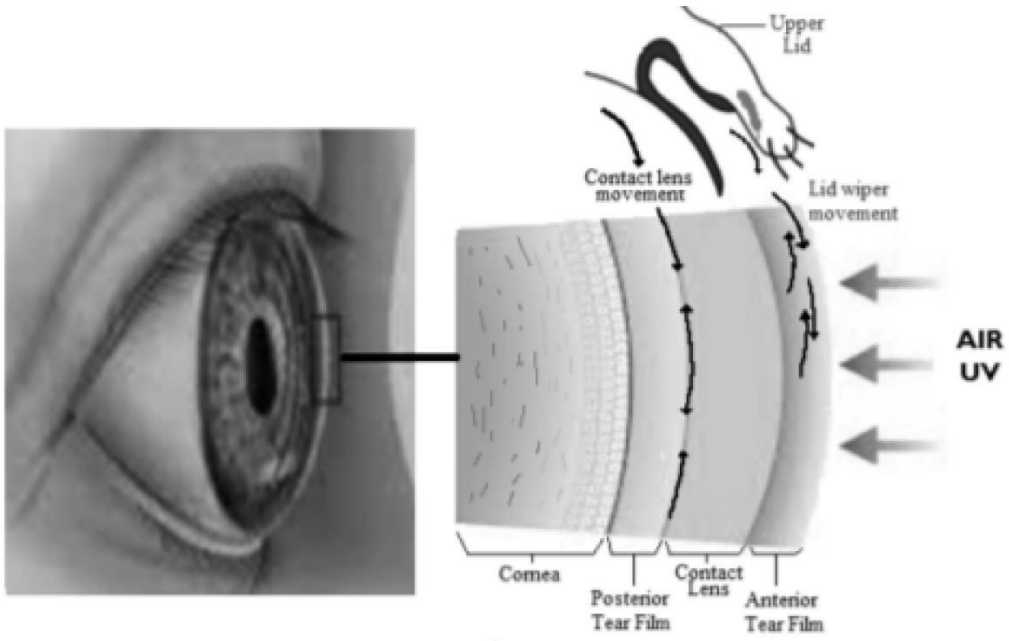

Figure 3. Diagram illustrating the compartmentalisation of the tear film after contact lens insertion (Mann and Tighe, 2013)

The pre-lens tear film is generally less stable than the pre-corneal because of the discontinuation of the ocular surface, associated with surface tension and tear evaporation. Upon insertion, the contact lens disturbs the tear film thickness, causing reflex tearing and an initial hypo-osmolar and thick tear fluid. However, once the lens has settled the pre-lens tear film thins back to approximately 3 µm (Mann and Tighe, 2013 and Craig et al., 2017b).

Even though studies have agreed that different hydrogel lens (Hy) types have little effect on the stability of the pre-lens film, it has also been reported that the thicker the lens the thicker the pre-lens film will be. As for the contact lens water content, the current evidence is controversial as to whether this influences pre-lens tear film quality. Finally, silicone hydrogel (SiHy) lenses are no different to their hydrogel counterparts regarding the thickness of the pre-lens tear film (Mann and Tighe, 2013).

1.6.2. Tear Evaporation

It is well known that tear evaporation is higher in contact lens wearers compared to non-lens wearers but there is no evidence to suggest the correlation between lens material and evaporation rates. As discussed earlier, tear films with a thinner lipid layer may result in greater evaporation. Even though the lipid layer thickness cannot directly predict contact lens tolerance, it was found that eyes intolerant to lens wear exhibited an increased level of phospholipase A2, degraded tear lipid and lipocalins. This finding suggests that lipid degradation is associated with pre-lens tear film instability (Mann and Tighe, 2013; Glasson et al., 2003).

1.6.3. Post-lens Tear Film

Contact lenses disrupt tear exchange in the post-lens tear film due to increased deposition of debris, antigens and/or toxins. Prolonged accumulation of these elements has been linked to various inflammatory and infectious responses. The thickness of the post-lens tear film seems to be less stable compared to that of the pre-lens film mainly because of parameters such as eyelid pressure and contact lens curvature variability (Fornasiero et al, 2006). This thesis concerns the wear of soft contact lenses only, in which, post-lens tear film thickness has little or no clinical significance in terms of vision enhancement.

The environment of the ocular surface is significantly influenced by contact lens wear, however, the changes incurred are subject-dependant. These changes can be divided in two categories; first, a contact lens can eliminate or reduce certain components of the tear film, and second, the lens can induce an increase of existing components and/or trigger the influx of new elements (Mann and Tighe, 2013).

1.7 Tear film physiology and contact lenses

The most relevant constituents of the tear film in the context of interaction between tears and contact lenses are proteins, lipids, electrolytes and mucin. When examining this interaction, it is important to consider that a lens is significantly thicker than the pre-corneal tear film and therefore, many tear fluid elements will be exposed to the environment at the presence of a lens. Moreover, a contact lens can be a means of deposition of other substances such as make up and skin lipids, which can disrupt the ocular surface homeostasis. There has to be a balance between the hydrophilic and hydrophobic properties of a lens and the microenvironment of the ocular surface, and this is a great challenge in contact lens research and manufacture (Zhou et al., 2012). Specifically, the lens-tear interaction must provide a smooth refractive surface and, at the same time, maintain an environment of metabolic balance, where sufficient lubrication and antibacterial defence is optimal. This interaction is far from static and it involves sliding and shearing forces and friction induced by the eyelids. There is well documented evidence that ocular comfort may be compromised during contact lens wear (Fonn, 2007., Dumbleton et al., 2008) and this discomfort can subsequently lead to discontinuation of contact lens wear (Yong et al., 2002). Factors associated with contact lens-related ocular discomfort include itchiness, dryness, irritation, scratchiness and redness signs (Glasson et el., 2003). These sensations have also been associated with dry eye conditions in non-lens wearers (Fonn, 2007). However, contact lens wearers are more likely than non-lens wearers to experience increased symptoms of dryness especially toward the end of the day. Contact lens–induced discomfort can be improved (but not always eliminated) by removing the lenses and replacing them with new ones during the day (Nichols et al., 2005; Young et al., 2007). This indicates

that contact lens-induced discomfort may be mediated by components present or released into the tears during the day, and the levels of these components may vary in tears at the end of the day, when discomfort is most intense (Sack et al., 1996).

Immunologically, complement system activation factors can be found on contact lenses after 8 hours of wear; leukotriene B4 (LTB4) can be found in increased concentrations in tears during an acute inflammatory event that occurs within 18 hour of lens wear. (Thakur et al., 1998). As part of a complex inflammatory response, lipid inflammatory mediators participate in corneal responses to injury and infection (Gronert, 2008). The lipid-related protein secretory phospholipase A2 is an enzyme secreted by the lacrimal glands and conjunctival goblet cells. It is present in high concentration in tears of normal subjects (Nevalainen et al., 1994; Sarri et al., 2001). Elevated levels of phospholipase A2 have been reported in tears of patients with external inflammatory disease, dry eye (Aho et al., 2002) and in the tears of contact lens intolerant individuals (Glasson et al., 2002). Contact lens wear may also affect the level of antibodies in tears. The principal function of antibodies such as Immunoglobulin A (IgA) appears to be in the prevention of the adhesion of microorganisms to ocular surfaces (Wilcox and Lan, 1999). Thus, IgA aids in removing bacteria by stimulating phagocytosis and decreasing the ability of bacteria to adhere. However, the effect of contact lens wear on levels of lgA is controversial, with some studies finding no effect, (Balasubramanian et al., 2012; McClellan et al., 1998) and other studies showing a reduction in its concentration during contact lens wear (Pearce et al., 1999; Vinding and Nielsen, 1987).

A study by Masoudi et al., (2016) showed a decrease in comfort from morning to evening, more noticeable with contact lens wear. Physiological properties of the tear

film including pH level, osmotic pressure and inflammatory mediator concentration such as Leukotriene B4 (LTB4) vary diurnally and the reduction in ocular comfort in the evening may correlate with measurable increase in the concentration of various mediators in the tear film (Terry and Hill, 1978; Uchino et al., 2006). The arachidonic acid metabolite LTB4 may be one such discomfort mediator and contact lens wear may influence the LTB4 concentration. LTB4 is higher after 8 hours of sleep in regular contact lens wearer compared to non-lens wearers (Thakur and Willcox, 1998). The absolute level of the mediators in tears was not found, however, to be associated with the decrease in comfort seen during contact lens wear, but there is a possibility that levels of LTB4 may be associated with ocular comfort during lens wear (Masoudi et al., 2016). Furthermore, the concentration of LTB4 is found to be higher (1.4 times) in neophyte lens wearers when compared to regular lens wearers (Thakur and Willcox, 2000). Measurement of LTB4 concentrations over consecutive days does not have significant difference, suggesting that no biological diurnal variations exist for this mediator. This may indicate activation of the arachidonic acid metabolic pathway during adaptation to contact lens wear, requiring further investigation of tear levels of other metabolites of this pathway under similar experimental conditions (Masoudi et al., 2016).

The discomfort is believed to be a response to variable factors that stimulate nerve endings on the ocular surface. As this discomfort increases with contact lens wear, stimulation of reflex tearing and inflammatory factors diluting is the natural response to fight this response. Papas et al., (2015) have shown that comfort during contact lens wear is significantly lower at times after 6 hrs of lens wear, but that lens wear for only 4 hours at any time during the day does not impact comfort during wear. This may suggest that mediators of discomfort need to accumulate over time to stimulate the

ocular surface nerves and produce the discomfort response. We can, therefore, assume that the production of inflammatory mediators leads to a discomfort response and that these mediators would most likely appear in tears over the course of a day of lens wear.

1.7.1. Lipoidal Interactions

The role of lipids in contact lens wear is based on three observations. First, the stability of the lipid layer is continuously disturbed to a higher or a lesser degree in the presence of a lens. Second, silicone hydrogel materials attract more lipid deposits. Third, lipid degradation depends on each moiety structure, where the fatty acid chain susceptibility drives these interactions. Contact lens-related degradation can be either enzymatic or oxidative. For example, Glasson et al., (2003) identified higher levels of phospholipase A2 in tear fluid of lens intolerant subjects. This enzyme may cause decrease of the phospholipids by promoting their hydrolysis leading to tear film instability and further to contact lens intolerance (Yamada et al., 2006). Lower concentration of phospholipids was also observed in the tears of dry eye patients comparing to healthy subjects. As for oxidative reactions, the process leads to the production of peroxide and hydroperoxide intermediates, which are found in higher concentration in contact lens wearers. It has been established that malondialdehyde is a reliable marker of oxidation, which has been isolated from ex-vivo contact lenses (Georgakopoulos et al., 2009).

1.7.2. Protein Interaction

Contact lens-related protein denaturation may be induced by different mechanisms, such as lens deposition with protein, lens drying and reaction to the blister solution. However, these results are not reproducible because there is high variability between

materials, patients and studies. Moreover, daily disposable contact lenses eliminate or reduce the significance of protein deposition or denaturation (Glasson et al., 2003; Zhou et al., 2012). Nevertheless, several studies have confirmed the presence of increased levels of tear plasmin, a protein that breaks down fibrin as part of the clotting cascade, even in daily disposable contact lens wear, regardless of the material type (Mann and Tighe, 2013; Masoudi et al., 2016). This phenomenon has been attributed to the selective absorption of vitronectin, a protein involved in haemostasis and upregulation of plasmin production, by the posterior surface of the lens (Zhou et al., 2012).

1.7.3. Mucin Interactions

The interaction of mucin with contact lenses is not well studied, however, there are reports of the presence of mucin balls, which are associated with the mechanical friction and disturbance of the ocular surface. It has been found that mucin levels are inversely proportional to lid wiper epitheliopathy (LWE) severity, however, there is no evidence that mucin interactions have any clinical significance in daily disposable wear regimens (Efron et al., 2016).

1.7.4. Electrolyte Balance

The important question to be answered when researching the electrolyte composition of the tear fluid in contact lens wear is whether a contact lens can directly affect the concentration of different electrolytes or indirectly through tear evaporation. Before answering it is important to note that tear electrolyte composition is not affected by the ionic exchanges via the endothelial pump leak because the volume of the corneal stroma is negligible compared to the volume of the tears and aqueous humour

(Murube, 2006). Even though tear electrolytes mainly originate from the lacrimal gland, plasma leakage is an additional source of ions entering the tears. This is supported by the fact that the osmolarity of blood serum is like that of the tears. However, there are some interesting differences; sodium (Na+) makes up 80% of the tear cations and shows significantly less variation than potassium (K+), calcium (Ca2+) and magnesium (Mg2+); potassium concentration is much higher in tears than blood serum; calcium and magnesium concentrations are much lower than that of blood serum (Stahl et al., 2011).

Contact lenses can either increase tear volume, upon insertion, with subsequent reduction in osmolarity, or lead to an increased evaporation rate hence, increasing osmolarity. Furthermore, factors related to the lens, such as ion permeability, partition coefficient effects and polarisation of ions could all contribute to ion-specific tear concentration changes (Braun et al., 2014; Mann and Tighe, 2013).

1.7.5. Lid Wiper Epitheliopathy

Recent evidence has shown that there is a region in the upper lid responsible for scattering tears with every blink (Figure 4). This area spreads out at about 0.6 mm from the crest of the inner lid margin (mucocutaneous junction) to the subtarsal fold vertically and from the medial superior punctum to the lateral canthus on the horizontal plane (Efron et al., 2016).

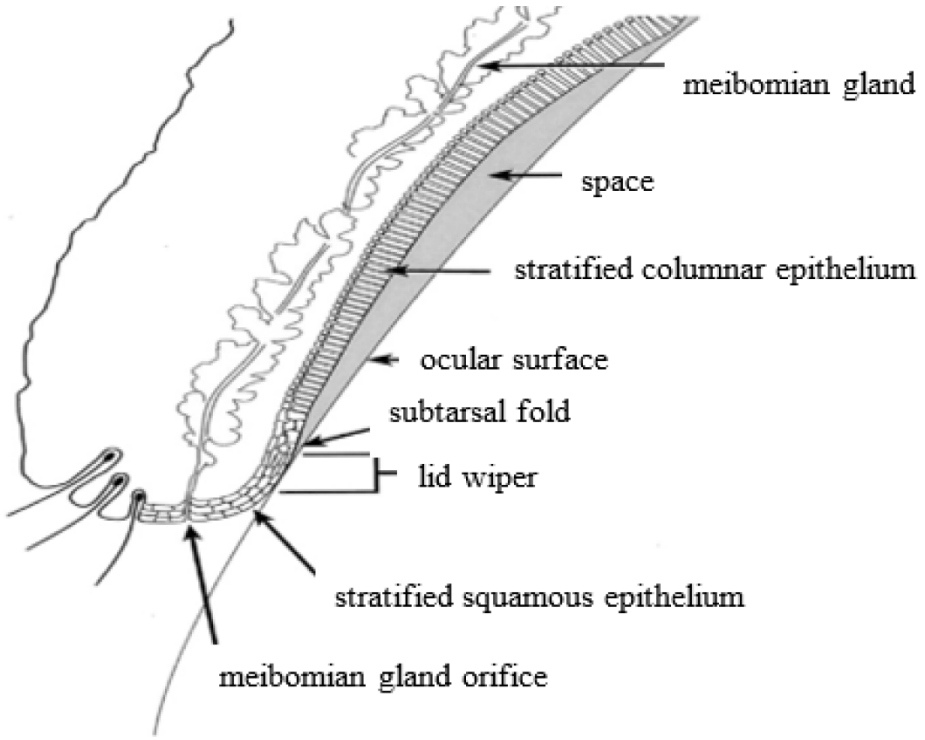

Figure 4. Diagrammatic illustration of the lid wiper as a part of the upper eyelid; the stratified squamous epithelium progresses to become keratinised in this region (Efron et al., 2016)

LWE is a term that describes an insult to the lid wiper epithelia accompanied by sub-clinical inflammation. This condition is believed to be caused by increased friction between this region and the ocular surface or contact lens anterior surface due to poor lubrication. LWE cannot be identified using white light, therefore, staining techniques using lissamine green and fluorescein need to be applied (see Figure 5). It is still debatable whether LWE is a result of contact lens wear, by means of increased friction, due to the fact that contact lens wearers who present with discomfort describe dryness as their primary symptom. What is interesting here is that both contact lens wearers with discomfort and dry eye sufferers have been found to have LWE, signifying the possibility of the same mechanism to explain the symptoms in these two groups (Efron et al., 2016).

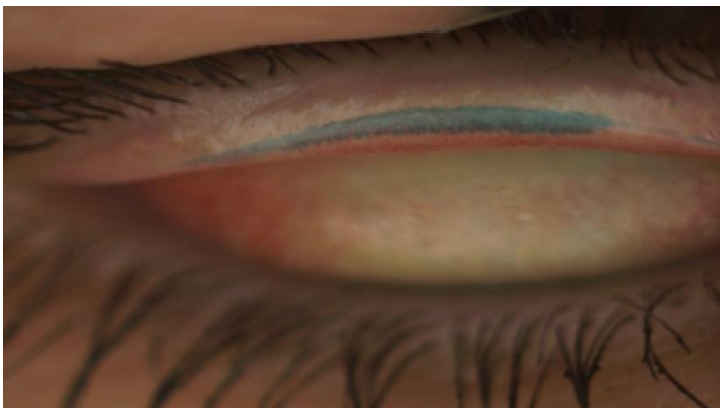

Figure 5. Lid wiper epitheliopathy, seen with lissamine green stain (own image)

An increasing number of studies are incorporating LWE assessment as one of the clinical test performed to evaluate contact lens-related dry eye and dry eye disease in non-contact lens wearers.

1.8 Methods for the Assessment of Tear Film Surface Quality

The surface quality of the tear film can be evaluated with several invasive and non-invasive methods. Traditionally, the most commonly used invasive procedure is tear break up time with the instillation of fluorescein, however, this can cause destabilisation of the tear film since it can alter tear fluid volume. It is therefore essential to include non-invasive techniques for a more accurate assessment. Such methods include interferometry, wavefront and curvature sensing or even methods that involve direct video recording. Such videokeratoscopes use the Placido disc principle, which employs the pre-corneal or pre-lens tear film as a convex mirror to reflect a pattern (i.e., specular reflection), which then can be assessed. This technique has the advantage of measuring the dynamics of tears over time, using a series of video-recorded images (high speed videokeratoscopy – HSV), (Iskander and Collins, 2005).

HSV confirms that tears exist in a dynamic equilibrium, which changes in between blinks. The clinical use of HSV is based on two different indicators, which allow for the assessment of tear film stability; tear film build-up and break-up times (Alonso-Caneiro et al., 2009). In the context of videokeratoscopy, tear break-up time (TBUT) is defined as the time from the last blink to the first appearance of certain tear film instabilities, such as a distortion of the reflected ring pattern (Iskander et al., 2005). Additionally, tear film build-up time is a new clinical indicator used to assess the stability of tears but its clinical significance remains unclear. As discussed earlier, tears spread over the corneal or the anterior contact lens surface in two steps:

1. Elevation of the upper lid distributes the mucus and aqueous layers;
2. The outer lipid layer moves from the inferior to the superior part of the ocular surface, bringing with it more water and making the tear film thicker.

It has been hypothesised that build-up times reflect the distribution of lipid layer from the droplet secreted by the meibomian glands. The mechanism suggested to support this hypothesis involves the movement of the lipid layer over the aqueous and mucus layers to compensate for irregularities of the corneal surface. It is important to note that all studies to date have mainly assessed tear build-up time qualitatively rather than quantitatively, in the context of tear film regularity and stability (Németh et al., 2002; Iskander et al., 2005; Kopf et al., 2008; Best et al., 2013).

1.8.1. Contact Lens-related Discomfort

The Tear Film and Ocular Surface Society (TFOS) conducted a workshop, lasting for about eighteen months, in order to evaluate contact lens discomfort (CLD) using an evidence-based approach. Typically, patients with CLD report a variety of symptoms, such as dryness, foreign body sensation and irritation, all of which worsen over the

day while wearing contact lenses. TFOS established a definition for CLD as follows (Craig et al., 2013):

> *"Contact lens discomfort is a condition characterised by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment, which can lead to decreased wearing time and discontinuation of contact lens wear".*

It is important to emphasise that the definition of CLD assumes that symptoms are present during contact lens wear, and they are resolved upon contact lens removal. Furthermore, this condition arises after the initial adaptation to lens wear and there may be a mismatch between clinical signs and symptoms (Craig et al., 2013). In other words, the severity of symptoms described by the patient may not correlate with the signs observed by the clinician. Finally, the term CLD should not be confused with contact lens-related dry eye, describing a pre-existing dry eye condition, which may or may not be aggravated by the presence of a contact lens. TFOS clinicians have also classified CLD based on aetiology; contact lens-related (material, design, fit, lens care regimen) and environment-related (ocular or external).

The epidemiology data of CLD is rather variable, ranging between 12% and 51%. This is because this data is drawn from patient experienced questionnaires, since there is a poor understanding of the correlation between symptoms and signs, hence making it difficult to diagnose CLD (Craig et al., 2013).

Optimal contact lens fitting is an essential process to establish comfort and good vision, however, in clinical practice, the tools necessary to select and optimally fit a contact lens are limited. In fact, selection of the best fitting contact lens is made almost at random. In other words, it would be more accurate to say that contact lens practice

is currently based on identifying suitable eyes that fit the available lenses than fitting a bespoke lens to individual eyes (Van der Worp and Mertz, 2015). Currently available daily disposable contact lenses vary based on material composition and shape and there is poor correlation in lens fit among different brands, which suggest that there is patient intervariability in terms of the behaviour of lenses of the same material and shape. Another important factor to consider is that the evaluation of lens fit generally occurs a few minutes after insertion, however, the mean duration of lens wear is typically 13-14 hours daily (Wolffsohn et al., 2015). As a result, it is crucial to assess the impact of the-end-of-day lens wear on different patients, since end-of-day discomfort is a major reason for contact lens dropout (Murphy et al., 2001). A study by Wolffsohn et al. (2015) has shown that lens fit differs between 8 and 16 hours; movement on blink was constant; lens lag was reduced by 10%; and the push-up recovery increased by 20%. This study highlighted two key concepts related to diurnal variation in contact lens fitting. First, movement on blink, push up and lens lag only evaluate the wettability of the anterior surface of the lens, hence, indicating that end-of-day changes may be a result of the interaction between the posterior surface of the lens and the ocular surface due to tear composition modification. Second, ocular comfort consistently correlates with lens brand, which suggests that it is not the lens design or material that might determine comfort. Other scientists have rigorously attempted to evaluate the impact of contact lens material on the incidence of CLD. The material characteristics that have been included in this evaluation include ionicity, water content, oxygen transmissibility, surface enhancement, modulus and friction. However, the results are inconclusive due to several confounding factors such as lens design variability and lack of a global consensus for a relevant definition (Craig et al., 2013; Willcox et al., 2017).

In contrast, some studies provide evidence that the water content and the refractive index of the contact lens can influence the progression of contact lens-related dry eye. Specifically, lower water content hydrogel materials (higher refractive index) are less likely to exacerbate dry eye (Nichols and Sinnott, 2006). A possible explanation for this may be that higher water content lenses attract the polar head groups of the tear film lipids, exposing their non-polar tails and resulting in non-invasive Keratograph dry-up time, which is defined as the time period from lens placement until the first observed distortion of the Placido ring pattern (Marx and Sickenberger, 2017).

A study by Szczesna-Iskander and Iskander (2014) analysed the dynamics of tear film surface quality before and during lens wear in subjects with dry eyes and healthy controls. After the initial smoothing of the pre-lens tear film upon contact lens insertion there is a point of sudden deterioration, which corresponds with the process of dewetting. Dewetting is a process where a thin film retracts from a solid to form a bead-shaped drop leading to droplet formation and it is opposite of the spreading process.

This phenomenon is less obvious in the case of pre-corneal tear film assessment, attributed primarily to the evaporation of tears and the gradient of surface tension observed in the different fluid components of the tear film (Marangoni effect). This effect implies that, normally, as the evaporation rate increases, the non-polar lipids spreading also increases with subsequent increase in the thickness of the aqueous layer (Rantamaki et al., 2012; Arita et al., 2017).

Importantly, once the process of dewetting begins, the influence of the contact lens material type is limited. Therefore, it is vital to identify materials that delay the process

of dewetting in order to improve pre-lens tear film surface quality (Szczesna-Iskander and Iskander, 2014).

Papas et al. (2014) conducted a study to assess whether a greater end-of-day CLD correlates with changes of the lens itself. In this study, where daily disposable hydrogel lenses were worn bilaterally for 10 hours by 27 patients, the results were rather interesting; end-of-day comfort was not affected by removing and replacing the lens in the middle of the wearing periods; comfort was also not affected by whether the replacement lens was a new or the pre-inserted. This data shows that short-term changes to the lens during daily wear are not the driving forces of CLD. If that is the case, an alternative explanation for CLD may be the changes in ocular tissue after contact lens insertion, a phenomenon known as fatigue. It would be reasonable to assume that a candidate ocular structure responsible for this could be the upper eyelid, considering its repeated motion onto the contact lens. Another possible explanation for CLD and fatigue could be the dryness sensation mediated by the cold nociceptors of the ocular surface, particularly at the point of edge motion around the periphery of the contact lens, where a discontinuous surface increases evaporative loss.

1.9 Aim and Hypotheses

The aim set for this study was to assess the mid-term effect (12 months) of modern contact lenses on ocular physiology using a battery of standard and non-standard measuring techniques.

In order to conduct this study three hypotheses were considered. First, different contact lens materials affect ocular physiology in different ways. Second, there is no ideal contact lens material that would fit all eyes, considering that not every material has an

identical impact on the ocular physiology. Third, modern contact lenses have a higher degree of biocompatibility with the ocular surface. For ethical reasons no control group (i.e., subjects wearing non-modern contact lenses) was set to test the third hypothesis.

By the end of this project it was expected to achieve certain outcomes: to have a better understanding of the relation between tear film and lens material; to provide an insight on tear film behaviour during contact lens wear; to identify additional measurements as predictors of contact lens discomfort and; to provide practitioners with additional non-invasive techniques that could be introduced in clinical practice in order to assess tear film stability during contact lens wear with the ultimate goal of maintaining good ocular surface health and quality life of contact lens wearer.

II. METHODOLOGY

The agreed methodology for this project is a result of long meetings and discussions to provide a solid backbone for the study. It was early in the discussion process that a longitudinal format was decided to be followed because there are not many such studies undertaken on this topic to date. This study is unique in that the grant sponsoring it allowed participants to receive free contact lenses for the entire duration with subsequent very low dropout rate. Another advantage of the received finance was that there were no restrictions imposed by the sponsor and as a result, publication bias was avoided.

It was only after careful considerations and contact with eminent researchers in the field from different institutions, over a period of one year, alongside thorough literature reviews that the methodology protocol was finally created. The first step was to design and undertake a pilot study to test the sustainability of the design.

Following successful completion of the pilot project, the schedule of the measurement acquisition was confirmed. Specifically, it was particularly important for the measurements to be taken at the time and appropriate intervals so as to minimally affect tear film surface quality considering the large number of measurements required for each patient. Furthermore, the sample size was carefully considered given the longitudinal aspect of the study design, considering the possibility that some participants may withdraw prior to the completion date.

The study recruited 60 healthy, young, regular or occasional contact lens wearers (19 M and 41 F), aged (mean ± standard deviation) 25.5 ± 4.3 years, ranging from 20 to 37 by sending emails via university newsletters to inform about the longitudinal

research project. All subjects were advised to stop wearing their habitual contact lenses or to use any ophthalmic solutions at least three days prior to commencing the study. This was to ensure subjects previously wearing different types of contact lenses achieved a consistent baseline (bare eye) result (Thai et al., 2004). Subjects were also requested to present an up-to-date (within 12 months) optical prescription. Exclusion criteria were signs and symptoms of dry eye, inflammation or tear flow impairment and any systemic disorders known to compromise the ocular surface. Moreover, subjects were excluded if they demonstrated at least two of the following: Ocular Surface Disease Index (OSDI) ≥ 27, conjunctival staining ≥ 2, corneal staining ≥ 2 (Efron grading scale) and fluorescein tear film break-up time ≤ 7 seconds (Villani et al., 2011 and Nichols and Sinnott, 2006). The refractive error was limited to ±5.00 spherical and ±0.75 cylindrical diopters.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Valencia and has been registered as a ClinicalTrials.gov (NCT03531346). The data was collected at Wroclaw University of Science and Technology in Poland. Verbal and written informed consent was obtained from each subject after the nature and possible adverse consequences of the trial were explained.

2.1. Study protocol and techniques

The study protocol consisted of a qualifying visit (Baseline, for the detailed recording sheet see Appendix 1), contact lens fitting visit on the following day (Day 2, Appendix 2), a control visit at two weeks (see Appendix 3) (to ensure that the participants adhered to the study protocol). The control visit was also included in order to explain any further queries before the follow-up visits at three, six and twelve months. Another

sets of measurements were taken after the 12-month visit at the Control Visit (CV) to compare the results with the Baseline visit. At the Control Visit only one eye was assessed, which was based on the type of contact lens that subject was originally fitted at the Day 2 visit. Before the Baseline qualifying visit, a meeting was arranged with all participants, where the study was explained in detail and a consent form was signed. On the Baseline qualifying visit participants handed in two questionnaires, which had been e-mailed to them prior to the study. The Baseline evaluation, performed on the first day, included review of OSDI and the Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8) questionnaires scores (Chalmers et al., 2012, Appendix 4), medical history (Appendix 5), tear film surface quality assessment, osmolarity and non-invasive keratograph break-up time measurements. The anterior surface was examined with a slit lamp biomicroscope and included the assessment of the central lower tear meniscus, the lid margins and meibomian glands to ensure there is no obstruction, while carefully observing if there is excess dryness or tearing (see Appendix 6). Fluorescein strips used to assess the stability of tears and estimate tear break up time (FBUT) and to evaluate conjunctival and corneal staining, prior to that, cornea and conjunctiva were assessed without any dyes (Efron et al., 2003). The examination was based on Efron's grading scale. Lissamine green strips was used to evaluate lid wiper epitheliopathy and Meibo- Scan feature of K5M utilised for meibography assessment.

Laboratory temperature [°C] and relative humidity [%RH] were monitored at the start of the measurements with a thermo-hygrometry device (C3121, Comet, Czech Republic). Effort has been made to maintain the environmental factors during the measurements. A previous study has reported that tear film evaporation rates are higher in dry [30% humidity] conditions compared to normal [40% humidity] in contact lens wearers (Guillon and Maissa, 2008). Another study confirmed that a

decrease in RH and temperature can increase dryness (Maruyama et al., 2004). Subjects were asked to have an environmental adjusting period, had they arrived to the laboratory directly from the outdoors. The study protocol is summarised in Table 1.

Table 1. The schedule of the study protocol

Task	Baseline	Day 2	2-week	3-month	6-month	12-month	CV
OSDI	✓	×	✓	✓	✓	✓	✓
CLDEQ-8	✓	×	✓	✓	✓	✓	✓
Medical History	✓	×	×	×	×	×	✓
TFSQ-NIBUT	✓(PC)	✓(PL)	✓(PL)	✓(PL)	✓(PL)	✓(PL)	✓(PC)
Osmolarity	✓	×	×	✓	✓	✓	✓
NIKBUT*	✓(PC)	✓(PL)	×	✓(PL)	✓(PL)	✓(PL)	✓(PC)
Anterior eye check	✓	✓	✓	✓	✓	✓	×
Contact lens fit assessment	×	✓	×	×	×	×	×
FBUT	✓	✓	✓	✓	✓	✓	✓
Corneal staining	✓	✓	✓	✓	✓	✓	✓
Conjunctival staining	✓	✓	✓	✓	✓	✓	✓
Lid wiper assessment	✓	×	×	✓	✓	✓	✓
Meibography	✓	×	×	✓	✓	✓	✓

OSDI – Ocular surface disease index; CLDEQ-8 – contact lens dry eye questionnaire-8; NIKBUT – non-invasive Keratograph break-up time; TFSQ-NIBUT– tear film surface quality; FBUT – fluorescein tear film break-up time; *PC – pre-corneal tear film; PL – pre-lens tear film

OSDI was used to assess dry eye symptoms reported by the subject during the week before commencing the study (Schiffman, 2000; Özcura et al., 2007). To assess the hitherto habitual contact lens performance, CLDEQ-8) was filled in by habitual contact lens wearers (Chalmers et al., 2012).

Both questionnaires were adapted to the Polish language (Nichols, 2006). A review of medical history was first performed, including general and ocular health, refractive correction, vision (distance and near), last eye examination, last medical examination, previous contact lens wear, family ocular history, allergies, medication, occupation, driving, visual display unit use, smoking and hobbies. This was followed by tear

osmolarity measurements with the TearLab Osmolarity System (TearLab Corp, San Diego, CA) and tear meniscus height with the Keratograph 5M (K5M, Oculus Optikgeräte GmbH, Wetzlar, Germany). Subsequently, non-invasive methods were used to assess Tear Film Surface Quality (TFSQ) with High-Speed Videokeratoscopy (HSV) Medmont E300 (Medmont Pty., Ltd, Melbourne, Australia) and Non-Invasive Keratograph Break-Up Time (NIKBUT) with K5M. Slit lamp ocular surface examination, including lid margins and meibomian glands assessment, corneal and conjunctival assessment. For the corneal and conjunctival staining and FBUT assessment, a drop of 0.9% saline solution was used to moisten 1 mg fluorescein sodium ophthalmic sterile strips (BioGlo, HUB Pharmaceuticals). Meibography images were captured with KM5. Lid Wiper Epitheliopathay (LWE) was assessed using lissamine green strips 1.5mg (HUB Pharmaceuticals, LLC). Measurements performed at the Baseline visit were used for qualifying subjects and formed the baseline database for comparative analyses with the measurements performed at follow-up visits.

On the following day (Day 2, see Appendix 2), there was a morning session and an afternoon session. During the morning session, subjects were fitted with two daily disposable contact lenses of different materials: SiHy lens (Delefilcon A, BC: 8.5mm Diameter:14.2mm) on the right eye and a Hy lens (Omafilcon A, BC: 8.7mm Diameter: 14.2mm) on the left eye. Subjects were blinded with respect to the lens type. For consistency, the examiner kept the same order. Thirty minutes after contact lens application the contact lens fit was evaluated, including contact lens centration, corneal coverage, horizontal lag, blink movement, push-up test and binocular visual acuity (VA) measurement for distance and near (Thai et al., 2004; Nichols and King-Smith, 2004; Wolffsohn et al., 2009).

Subjects returned after four hours of contact lens wear for the afternoon session to undergo contact lens fit reassessment, VA and comfort evaluation, followed by pre-lens TFSQ and NIKBUT measurements. Several factors were taken into consideration while choosing the most suitable lens for the subjects, of which contact lens fit, visual acuity (logMAR 0.00) and reported comfort being the primary factors. If the lenses were equally comfortable and well-fitted, the lens with better pre-lens TFSQ and higher NIKBUT was prescribed. Out of 60 subjects recruited for the study (19 M and 41 F), aged (mean ± standard deviation) 25 ± 4 years, ranging from 20 to 37 years old, four subjects did not fulfil the study criteria. Thirty-nine subjects (27 F and 12 M) were fitted with Silicone-Hydrogel and 17 subjects (11 F and 6 M) with Hydrogel daily disposable contact lenses. There was no statistically significant difference between the SiHy and Hy group in age (Mann-Whitney test, $P = 0.969$) nor gender distribution ($P = 0.797$) at the start of the study. Corneal and conjunctival staining assessment was performed after lens removal. Although post-lens tear film has little or no clinical significance in terms of vision enhancement, however, the fit has been assessed to ensure optimal vertical and transverse lens movement, which would maximise tear exchange and debris elimination. Eye surface staining was photographed with K5M for comparison. Out of 60 subjects recruited for the study, Among the remaining 56 subjects, five were contact lens novices and 12 described their use of habitual contact lenses as occasional (less than three times per week). A two-week supply of the selected contact lenses was provided for each subject, and subjects were then instructed to wear them for any five days per week, as that would normally coincide with their working hours' schedule, minimum eight hours per day and for up to 12 hours each day. The same wearing schedule was followed for the whole duration of the study. All subjects were fully instructed on insertion, removal, lens care and were

given written information, which they were required to follow throughout the study (Appendix 7).

The control visit at two weeks was performed in the afternoon, when subjects wore their contact lenses for at least five hours on the day of the visit. OSDI and CLDEQ-8 were completed at the visit. TFSQ was measured using HSV and the quality of the lens fit was assessed with slit-lamp examination. Subjects then removed their lenses for performing FBUT measurement and ocular health examination, assessing corneal and conjunctival staining. The purpose of the two-week visit was to ensure good contact lens performance, comfort and fit to qualify subjects for frequent contact lens wear for a 12-month period.

At the three, six, twelve-month visits, subjects were advised to wear their contact lenses at least five hours prior to attending. The protocol for these three follow-up visits was kept identical to that of the baseline visit, with the only difference being that the NIKBUT assessment was not performed at the two-week visit, since tear film surface quality TFSQ was assessed with Medmont instead. In the following three visits the pre-lens tear film surface quality was assessed and for the CV the pre-cornea tear film surface quality was assessed.

2.2. Tear osmolarity

Tear osmolarity was measured from the inferior lateral tear meniscus, for both eyes. We used the TearLab Osmolarity System, which allows collection of a relatively small sample of 50nL from the inferior tear meniscus. Consequently, the system automatically measures tear osmolarity, based on the sample's electrical impedance.

Calibration of the instrument was performed on the day of the visit, according to the manufacturer's guidelines. Except for Baseline, when baseline measurements were taken without contact lenses, all other measurements were conducted 10 minutes after lens removal. The same TearLab diagnostic pen was used for all the assessments, always starting with the right eye. Three measurements for each eye were performed by the same practitioner and the results were averaged (Szczesna-Iskander, 2016).

2.3. Tear film surface quality

The principles of non-invasive tear film assessment were first conceptualised by Mengher et al., (1985). This was further refined by Brown, Cho and Guillon, to measure tear dynamics over time, using video recording to capture a series of images with the videokeratoscope (Brown and Cho, 1994; Guillon, 1998; Kopf et al., 2008). Recent technological advancements introduced digital videokeratoscopes, which now allow the clinicians to use the Placido disc pattern to assess the tear film surface. Non-invasive methods are preferred for the evaluation of TFSQ and break-up, since invasive procedures involving fluorescein may destabilise the tear film (Kopf et al., 2008). HSV is used to assess the dynamics of the tear film surface quality with or without contact lenses. The reflected image indicates the quality of the tear film surface over time. A uniform pattern is observed on a healthy, regular tear film, whereas an irregular pattern is seen when there is tear film thinning and/or break up (Szczesna-Iskander et al., 2012; Alonso-Caneiro et al., 2013). The acquisition of tear film dynamics has been performed using the dynamic topography module of the Medmont E-300 available in the Studio 6 software rather than using its dedicated tear film analysis module. The reasons for that are that the tear film module of E-300 does not allow analysing raw videokeratoscopy images and that the internally estimated

parameters of tear film quality has not been validated. Additionally, in the tear film module the sample frequency is 4Hz while in the dynamic topography module it can reach up to 25Hz. TFSQ undergoes certain stages after the blink cycle. Upon opening the eye, the healthy tear film increases in volume, known as the 'build up time' after which, the tears show a phenomenon called smoothing or levelling (Nemeth et al.,2002; Braun et al., 2015). In a stable tear film the levelling phase will last until the next blink, otherwise the tear film is classed as thin. Nichols et al (2005) suggested that this observed thinning may be a result of dewetting, rather than evaporation.

HSV can show the behaviour and dynamics of the tear film between pre-lens at baseline and pre-cornea at subsequent visits. The Placido rings refection change can be observed and assessed illustrating the lens surface dewetting, as it is dewetting that dignifies the change in stability of the pre-lens tear film. Indeed, there is a correlation between dewetting and a poor visual effect (Szczesna-Iskander et al., 2016). As in other measurement of tear film (TF) dynamics blinking has a crucial role in the results derived with HSV. For example, forceful blinking can exert more lipid, which could affect the duration of the levelling phase and therefore, the dynamic of the tear film. Figure 6 shows examples of HSV video frames from E300 videokeratoscope for relatively good and relatively poor pre-lens tear film quality. In other words, a tear film surface well covered with a lipid is ideal, but its dynamic is slow. On the other hand, a surface that is not smooth will render a quick levelling at the expense of the stability (Szczesna-Iskander, 2018). Therefore, lipid layer imaging and then plotting its parameterised characteristics (such as the TFSQ) as functions of time, is important when assessing tear film stability. HSV measures the property of tear film lipid layer in order to evaluate TFSQ. We used a custom design program written in the Matlab environment for objective evaluation of the Placido rings images recorded in Medmont

videokeratoscope. The output plots of this program describe pre-corneal or pre-lens TFSQ as functions of time measured during natural and suppressed blinking conditions. The algorithm, described in detail in the works of Alonso-Caneiro et al (2009, 2013), calculates the textural characteristics of the Placido disk image and relates them to the quality of the tear film surface. From those output plots, non-invasive break up time (NIBUT) is estimated as the time taken from the blink to the first minimum of the smoothed TFSQ time-series (Szczesna-Iskander and Iskander., 2012). Smoothing was achieved by applying the Savitzky-Golay filter. From this point on, this estimator will be denoted in shortas TFSQ-NIBUT.

At the Baseline visit and CV visit, TFSQ-NIBUT was measured on the pre-corneal tear film. Thereafter, on the Day 2, 2-week, 3-month, 6-month and 12-month visits, pre-lens TFSQ-NIBUT was assessed. The protocol involved subjects placing their forehead against the headrest and chin on the chin rest in front of the Medmont instrument in dim light conditions. Following this, they were instructed to fixate centrally at the green target.

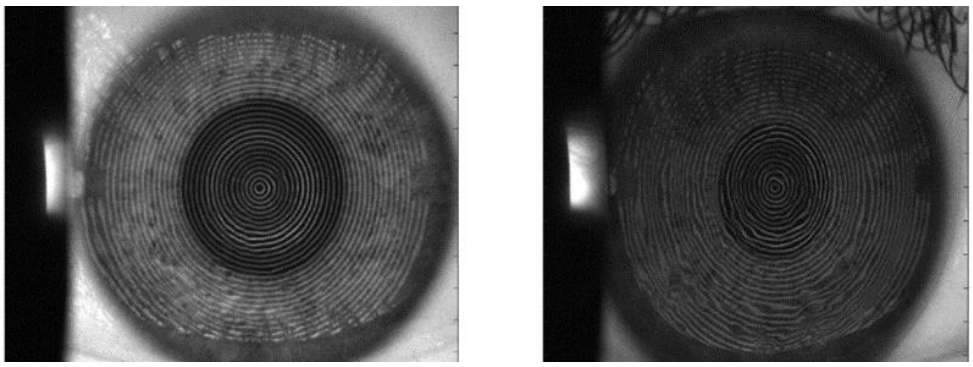

Figure 6. Examples of HSV video frames from E300 videokeratoscope captured on a bare eye for one of the subjects. The image on the left side represents a relatively stable tear film, while the image on the right side represents unstable tear film

Measurements of the right eye were taken first while having the left eye occluded and the process was repeated for the left eye. Two sets of data were recorded, first under natural blinking conditions (NBC), where subjects were requested to blink naturally without intentionally keeping their eyes open for 32 seconds. Secondly, under suppressed blinking conditions (SBC), where subjects were asked to blink twice naturally and then keep their eyes open for 24 seconds. Throughout both procedures, subjects continued to fixate at the central target and measurements were repeated three times to observe consistency, acquiring an average for further comparisons (Guillon, 1998). Both conditions were recorded at 13 Hz and there was a three-minute interval between each measurement to allow sufficient time for tear film recovery (Szczesna-Iskander et al., 2012).

For subjects who showed poor fixation, eyelash interference and eyelid obstruction, measurements were not included and repeated again asking them to fixate correctly and while keeping their eyes open. In the case that data was still unacceptable, these measurements were disregarded. Additionally, SBC measurements were repeated if subjects blinked prior to 24 seconds.

2.4. Non-invasive Keratograph® Tear Film Break-up Time

Non-invasive break-up time was measured with K5M, which can identify localised breaks and disturbances in the Placido disk pattern, projected in infra-red, related to changes in tear film surface quality. Measurements of NIKBUT were performed according to the manufacturer's instructions. Subjects were seated at K5M in a dim room with the eye focused on the central target and were asked to blink twice for the tear film to recover, fixate on the central light source and keep their eyes open for as long as possible. During the measurement time, a video was recorded and real-time

detection and localisation of breaks in the tear film was performed. During the assessment, 22 rings are projected onto the cornea. Points of break-ups appear on a grid mapping the corneal surface. The video recording at 24 frames per second lasted up to a maximum of 25 seconds, or until the patient's next blink, whichever occurred first. The K5M algorithm for estimating tear film quality is proprietary. Hence, in the following, only the results of two tear film estimators were acquired at the end of every assessment: the First-NIKBUT (FNIKBUT), which is the time taken from a blink to the first appearance of a substantial deformation of the Placido disk rings and the Mean-NIKBUT (MNIKBUT), which is the average of the time taken from the blink to the ring deformations in all the regions monitored over the duration of the recording. Assessment was performed three times per eye alternately with one-minute break between measurements. The build-in software was used for the NIKBUT assessment (Figure 7).

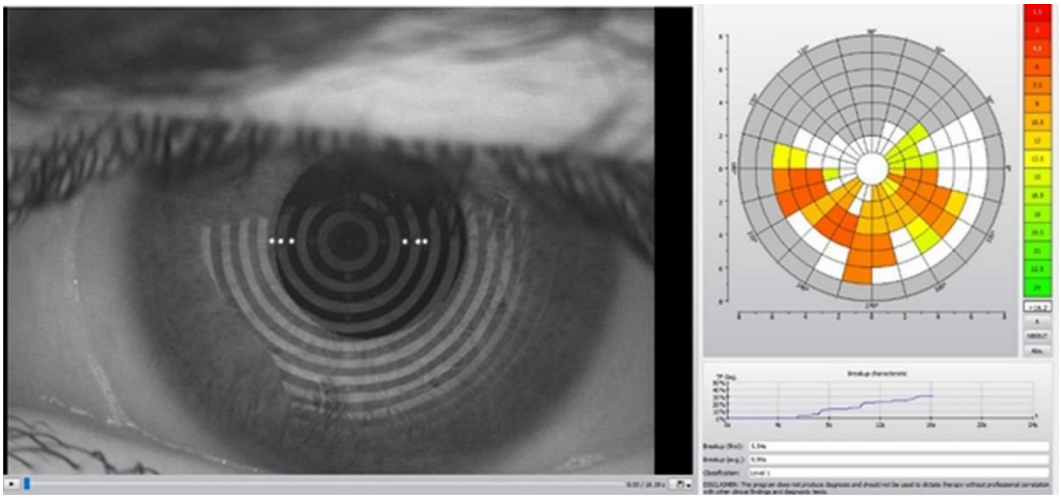

Figure 7. The graphic User Interface of the K5M after the measurement of NIKBUT was estimated. NIKBUT assessment was performed three times per eye alternately with one-minute break between measurements

2.5. FBUT, ocular surface and eyelids assessment

For the measurement of FBUT, a drop of 0.9% saline solution was used to moisten 1 mg fluorescein sodium ophthalmic sterile strips (Bioglo, HUB Pharmaceuticals, CA) and applied onto the inferior bulbar conjunctiva. Subjects were asked to blink twice and keep their eyes open for as long as possible. A slit lamp biomicroscope with ×10 magnification, cobalt blue illumination and a Wratten 12 yellow-barrier filter were used to observe the tear film break-up. FBUT was defined as the time taken from the first blink to the moment when the first tear break-up was observed. The average of three consecutive observations on each eye was recorded to improve accuracy. Corneal, nasal and temporal conjunctival fluorescein staining was also examined (Korb et al., 2001).

The image of the everted upper and lower eyelid of each eye was captured with K5M with white light to examine the lid wiper staining with lissamine green and, subsequently, the Meibo-scan feature of K5M was used to capture the infra-red meibography images. The LWE measurements were grading subjectively using a digital picture and grading scale, including the average between the height and the width grade of the stain on the eyelid margin (Korb et al., 2002). Grading and analyses were done independently by two examiners and the results compared afterward, which showed a high agreement (Figure 8).

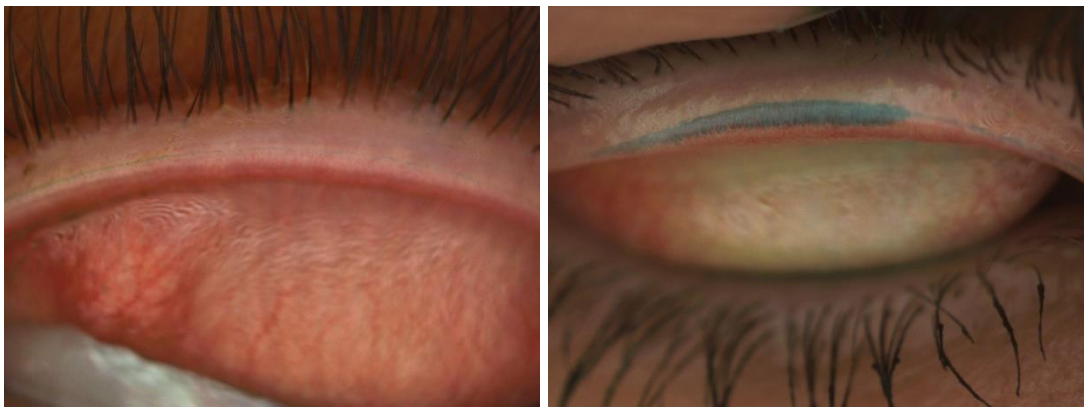

Figure 8. LWE scoring. Left: an image of Marx's line (grade 0); Right: an image of the lid wiper staining (grade 3) acquired for two of the subjects with K5M

2.6. Infrared Meibography

The Meibography images were analysed with ImageJ software by blinded practitioner, using the *Polygon selection* tool. The Meibography score was calculated as the fraction of the area devoid of meibomian glands compared with the entire eyelid area (Figure 9). The evaluation sheet summarising the measurements performed at 3-month, 6-month and 12-month follow-up visit was displayed in the Appendix 8. The corresponding evaluation sheet for Control Visit was attached as Appendix 9. The sheets are presenting the chronological order in which the measurements were performed.

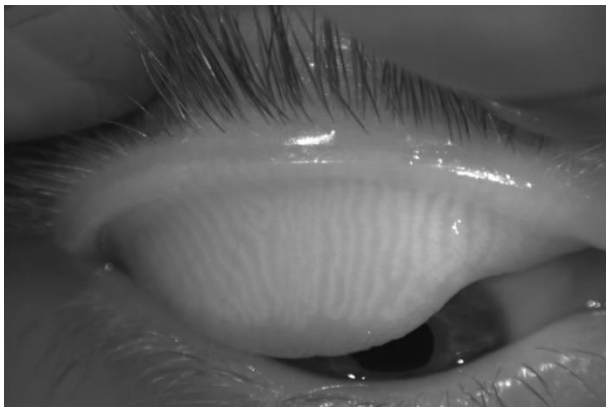

Figure 9. Infrared meibography image of the upper eyelid acquired for one of the subjects with K5M, with 18% score

2.7. Statistical analysis

Each variable was initially analysed using a two-factor repeated measures analysis of variance (ANOVA) which uses a 'sigma-restricted parameterisation, i.e., the effects for categorical predictor variables are represented by codes which sum to zero to compare the effects of lens type and occasion. Different occasions are represented by an initial baseline measure followed by measurements at various monthly intervals and then a final control visit (CV). For some variables, CV was also measured on one type of lens and as a consequence, CV has been omitted from the graph. The reasons for choosing this specific analysis were as follows: (1) it is the most powerful analysis available for analysing repeated-measures designs as it incorporates a large amount of data from several occasions, (2) when more than one variable is present it enables the interactions between variables to be tested, in the present case the interaction between lens type and occasion, i.e., if a trend in the variable with occasion is present whether it is consistent for both lens types, (3) ANOVA is not particularly sensitive to moderate departures from normality (Armstrong et al., 2011), and (4) there are no non-parametric alternatives of a factorial design. Nevertheless, there are some concerns regarding the degree to which some of the variables departed from normality, especially when the variable is a score on a limited scale. In these circumstances, the data were also transformed to square roots (Snedecor and Cochran 1980) prior to analysis but this transformation produced similar results to the untransformed data. In addition, Friedman's ANOVA and the Wilcoxon signed rank test were also applied to subsections of the data where appropriate. A final concern regarding the repeated-measures design is lack of 'sphericity' in which the variances of the differences among all possible pairs of within-subject means are assumed to be equal (Howell 2002). As

a result, the data were corrected for sphericity to examine the effect of this factor but this correction had little effect on the overall conclusions.

There is also the question of whether to correct the 'P' values obtained for the number of tests made ('Bonferroni correction'). Armstrong (2014) argued that the use of the Bonferroni correction should depend on the circumstances of the study. It should not be used routinely and should be considered if: (1) a single test of the 'universal null hypothesis' (H_O) that all tests are not significant is required, (2) it is imperative to avoid a type I error, i.e., of claiming a significant result when it is absent, and (3) a large number of tests are carried out without pre-planned hypotheses. None of these circumstances were relevant to the present study. Pearson's 'r' correlation was used to show the correlation between two different methods of tear film quality assessment (Oculus FNIKBUT and Medmont TFSQ-NIBUT).

III. RESULTS

In this chapter the summary of the results for the study is concluded, and compares three major differences:

- The lens type in each occasion;
- The occasions regardless of the lens type;
- The difference between the lenses on one or more occasion

The first set of graphs presents the means and confidence intervals.

3.1. Environmental Factors

Laboratory temperature, relative humidity and time of contact lens wear at the different occasions are shown in

Figure 10, Figure 11 and Figure 12, respectively.

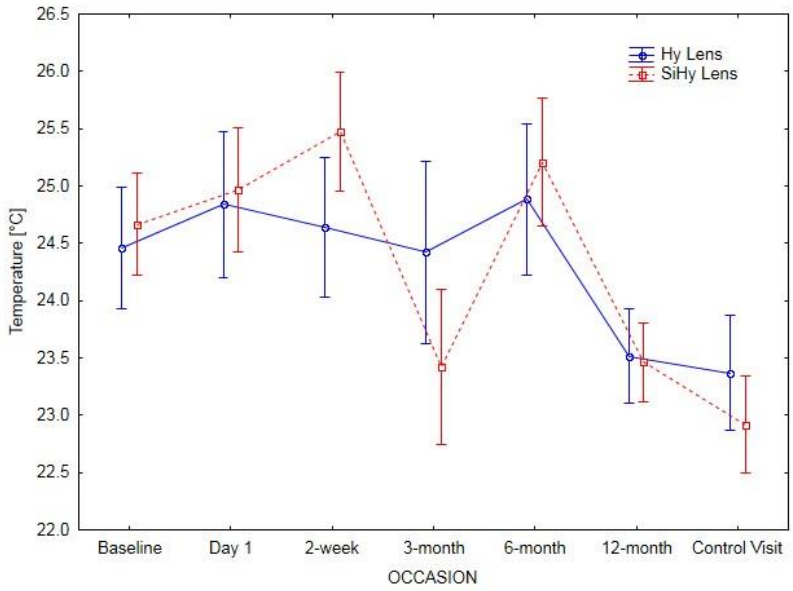

Figure 10. Temperature reported in the time-course of the study

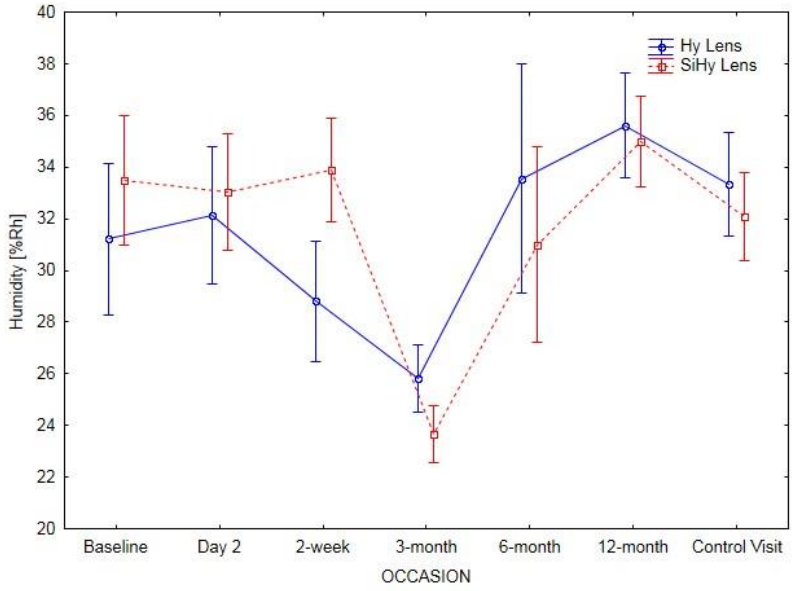

Figure 11. Relative humidity reported in the time-course of the study

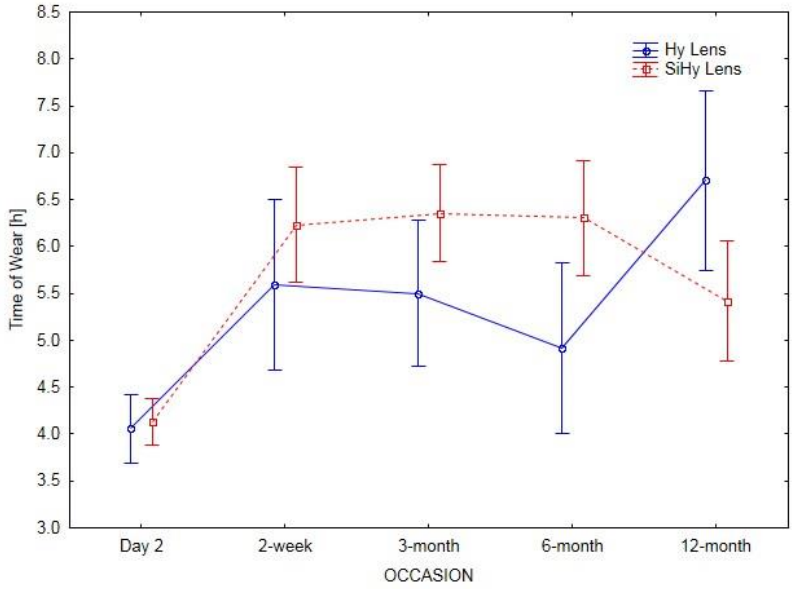

Figure 12. Time of contact lens wear before taking measurements reported during the study course

There were no significant overall differences in these variables with lens type ($F = 1.082$, $P = 0.303$ for time of wear), ($F = 0.37$, $P = 0.547$ for temperature), and ($F = 0.15$, $P = 0.699$ for humidity). There were overall significant differences among occasions for time of wear ($F = 15.750$, $P < 0.001$) largely determined by the difference between time of wear at Day 2 and subsequent occasions, as evidenced in post-hoc

analysis (t = 6.09, P<0.001) for differences between Day 2 and 2-week visit) and between temperature (F = 1765.19, P < 0.001), and relative humidity (F = 14.17, P < 0.001). There were also significant interactions between lens and occasions for these conditions: (F = 6.230, P < 0.001 for time of wear), (F = 2.95, P = 0.009 for temperature), and (F = 2.45, P = 0.027 for relative humidity). In addition, estimated time from waking up, temperature and humidity were compared at the two baselines suggesting a longer time at the second baseline (t = 6.09, P < 0.001), and lower temperatures at the second baseline (1.76 hours, t = 7.07, P < 0.001), and no significant differences in humidity (t = 0.08, P = 0.934).

3.2. Reported symptoms

Values of OSDI and CLDEQ-8 reported in the time-course of the study are displayed in Table 2. Changes in the subjective evaluation such as the OSDI and CLDEQ-8 questionnaires are shown in Figures 13 and 14, respectively.

Table 2. Symptoms assessment throughout the study and statistical changes in reported OSDI and CLDEQ-8 questionnaire scores. Mean ± standard deviation (SD), median values and ranges (in brackets)

SiHy-fitted OSDI Score [-]	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**CV**
Mean ± SD	14.2 ± 12.0	9.9 ± 11.6	11.7 ± 10.4	12.0 ± 8.1	12.8 ± 11.2	4.7 ± 6.8
Range	[0.0, 47.7]	[0.0, 61.1]	[0.0, 52.1]	[0.0, 37.5]	[0.0, 59.1]	[0.0, 22.3]
Median	10.4	5.0	8.3	10.4	10.0	1.0
SiHy-fitted CLDEQ-8 Score [-]	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**CV**
Mean ± SD	8.3 ± 5.9	6.2 ± 3.5	6.4 ± 3.8	6.8 ± 4.0	6.2 ± 3.1	
Range	[0, 22]	[0, 13]	[0, 17]	[0, 18]	[0.0, 15.0]	-
Median	7.0	6.0	6.0	6.0	6.0	
Hy-fitted OSDI Score [-]	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**CV**
Mean ± SD	14.0 ± 12.0	11.0 ± 9.0	10.9 ± 11.7	12.4 ± 11.2	12.1 ± 11.7	8.2 ± 12.3
Range	[0.0, 43.8]	[2.0, 33.3]	[0.0, 35.4]	[2.1, 35.4]	[0.0, 43.8]	[0.0, 37.5]
Median	14.6	7.5	6.3	6.8	8.3	4.2
Hy-fitted CLDEQ-8 Score [-]	**Baseline**	**2-week**	**3-month**	**6-month**	**12-month**	**CV**
Mean ± SD	7.1 ± 5.3	6.3 ± 4.0	6.9 ± 3.9	6.9 ± 5.3	7.1 ± 5.0	
Range	[0, 18]	[0, 15]	[0, 14]	[2, 21]	[0.0, 19.0]	-
Median	6.0	6.0	8.0	4.0	7.0	

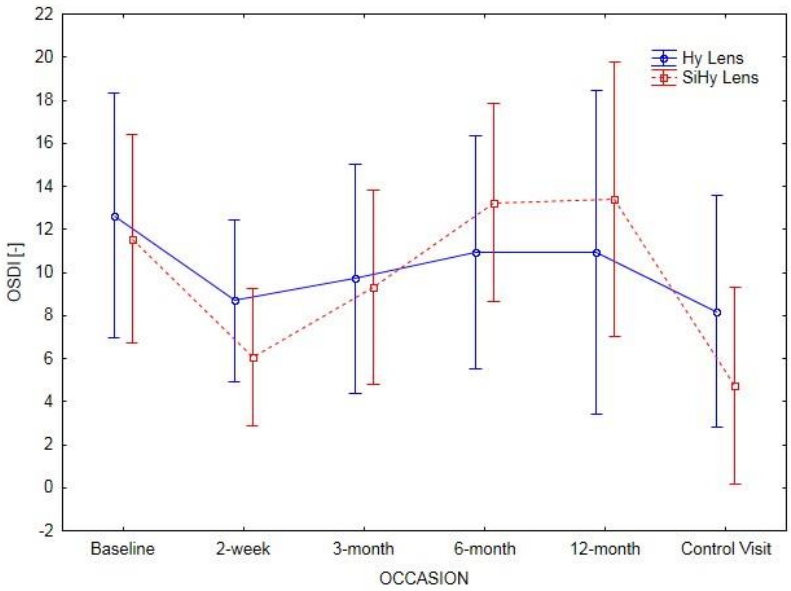

Figure 13. Ocular Surface Disease Index scores reported in the time-course of the study

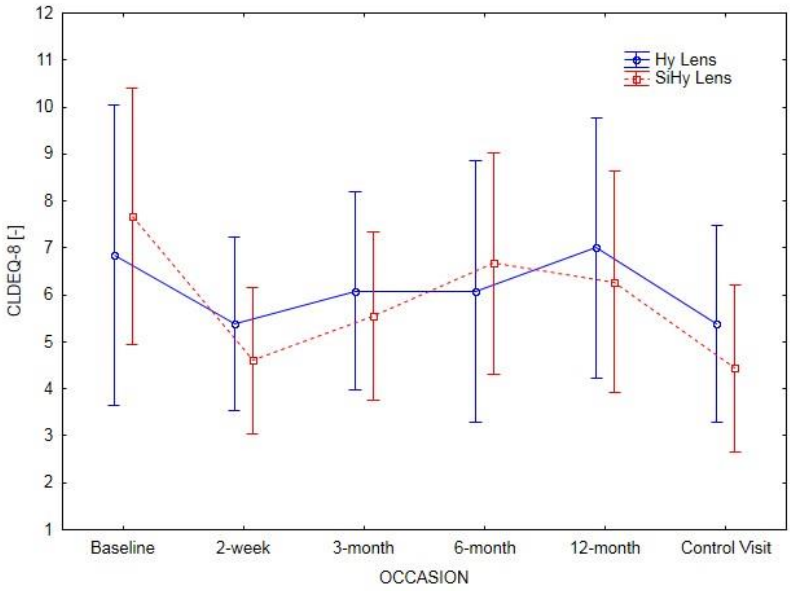

Figure 14. 8-item Contact Lens-related Dry Eye Questionnaire scores reported in the time-course of the study

There was no significant overall difference in the scores associated with the two lens types (F = 0.030, P = 0.864 for OSDI and F = 0.049, P = 0.827 for DEQ-8) but there was an overall significant difference in scores among occasions (F = 3.781, P = 0.003

for OSDI and ($F = 2.345$, $P = 0.044$ for DEQ-8) attributable to the lowest scores at the second baseline. There were no significant interactions between lens and occasions ($F = 0.852$, $P > 0.515$ for OSDI and $F = 0.391$, $P > 0.854$ for DEQ-8). Furthermore, OSDI and DEQ-8 scores were compared at the two baselines suggesting higher scores at the first baseline ($t = 2.95$, $P = 0.006$), ($t = 2.28$, $P = 0.030$).

3.3. Tear volume

Changes in normal tear volume, i.e., TMH for OD and OS are summarised in the Figure 15.

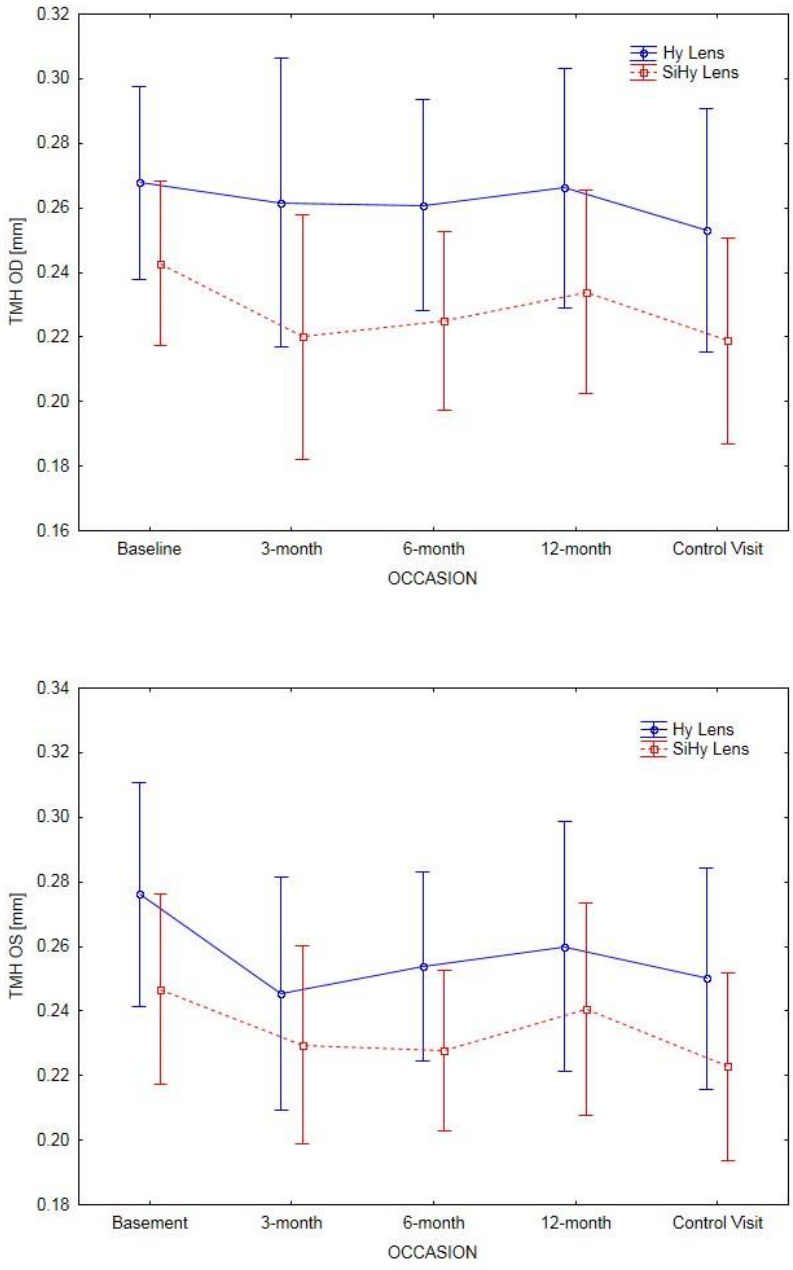

Figure 15. Top – right eye, Bottom – left eye tear meniscus height measures reported in the time-course of the study

There was no significant overall difference in tear meniscus height (TMH) associated with the two lens types (F = 3.701, P = 0.064 for TMH OD), (F = 1.665, P = 0.207 for

TMH OS). There was significant difference in TMH score among occasions for OD ($F = 0.751$, $P = 0.560$) but no significant difference for OS ($F = 2.217$, $P = 0.071$). There was no significant interaction between lens and occasions for OD and OS respectively ($F = 0.117$, $P = 0.976$ for TMH OD), ($F = 0.161$, $P = 0.957$ for TMH OS). In addition, TMH measurements were compared at the two baselines using the t-test suggesting no significant differences ($t = 1.73$, $P = 0.095$) for OD and a higher score at the first baseline for OS ($t = 2.27$, $P = 0.031$).

3.4. TFSQ-based NIBUT

There was no significant overall effect of lens type for OD ($F = 1.55$, $P = 0.219$) but there is an overall significant difference among occasions ($F = 7.92$, $P = 0.001$) determined by a decline in scores over the course of the trial and especially between Baseline and Day 2. There was no significant interaction between lens and occasions ($F = 0.44$, $P = 0.821$) indicating that the time trend was similar for each lens type. In addition, there was no significant difference in the score at the baseline compared with the CV ($t = 0.23$, $P = 0.818$).

There was no significant overall effect of lens type for OS ($F = 0.83$, $P = 0.365$) but there is an overall significant difference among occasions ($F = 8.62$, $P < 0.001$) determined by a decline in scores over the course of the trial especially between Baseline and Day 2. There was no significant interaction between lens and occasions ($F = 0.13$, $P = 0.985$) indicating that the time trend was similar for each lens type. In addition, there was no significant difference in the score at the CV compared with the baseline ($t = 0.87$, $P = 0.401$). TFSQ-NIBUT measures reported in the time-course of the study are displayed in Figure 16.

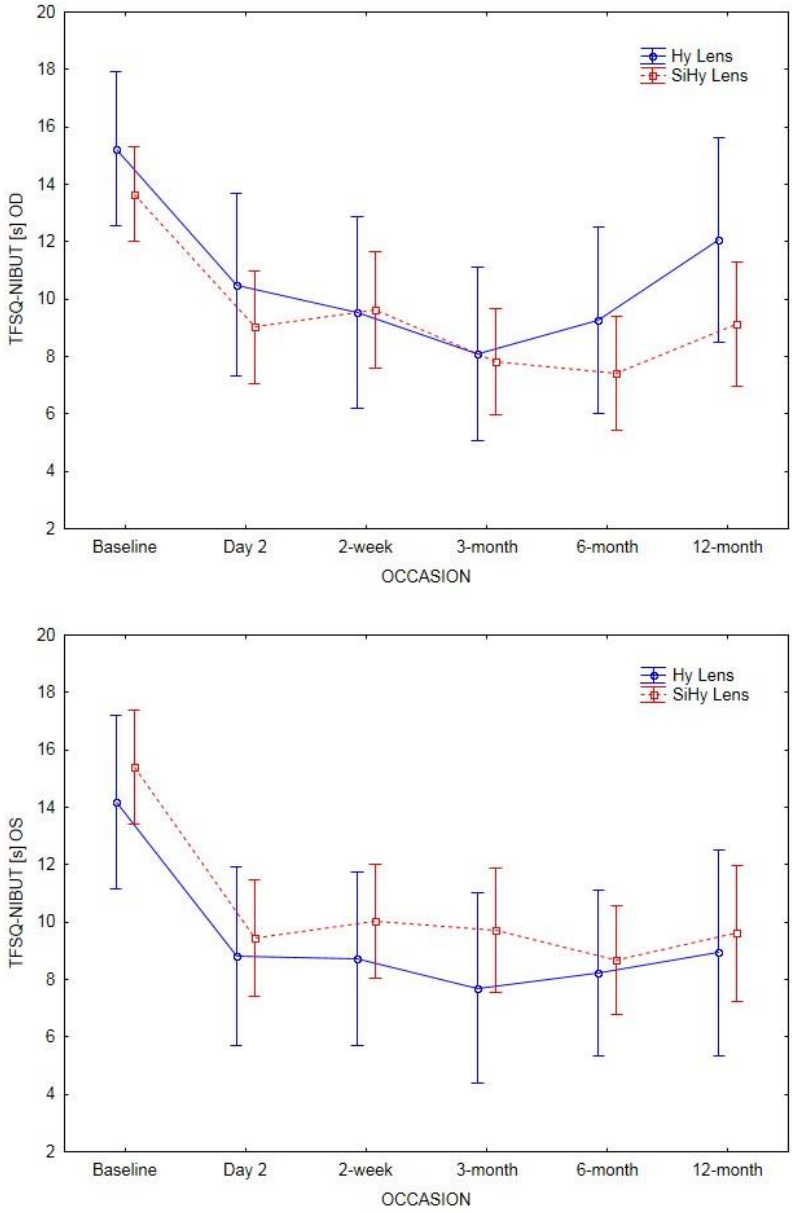

Figure 16. Top - OD, Bottom - OS tear film surface quality reported in the time-course of the study. Note that low TFSQ-NIBUT values (seconds) correspond to good tear film quality and vice versa.

3.5. Tear osmolarity

Changes in the diagnostic objective method of measuring hyperosmolarity are shown in the figures below.

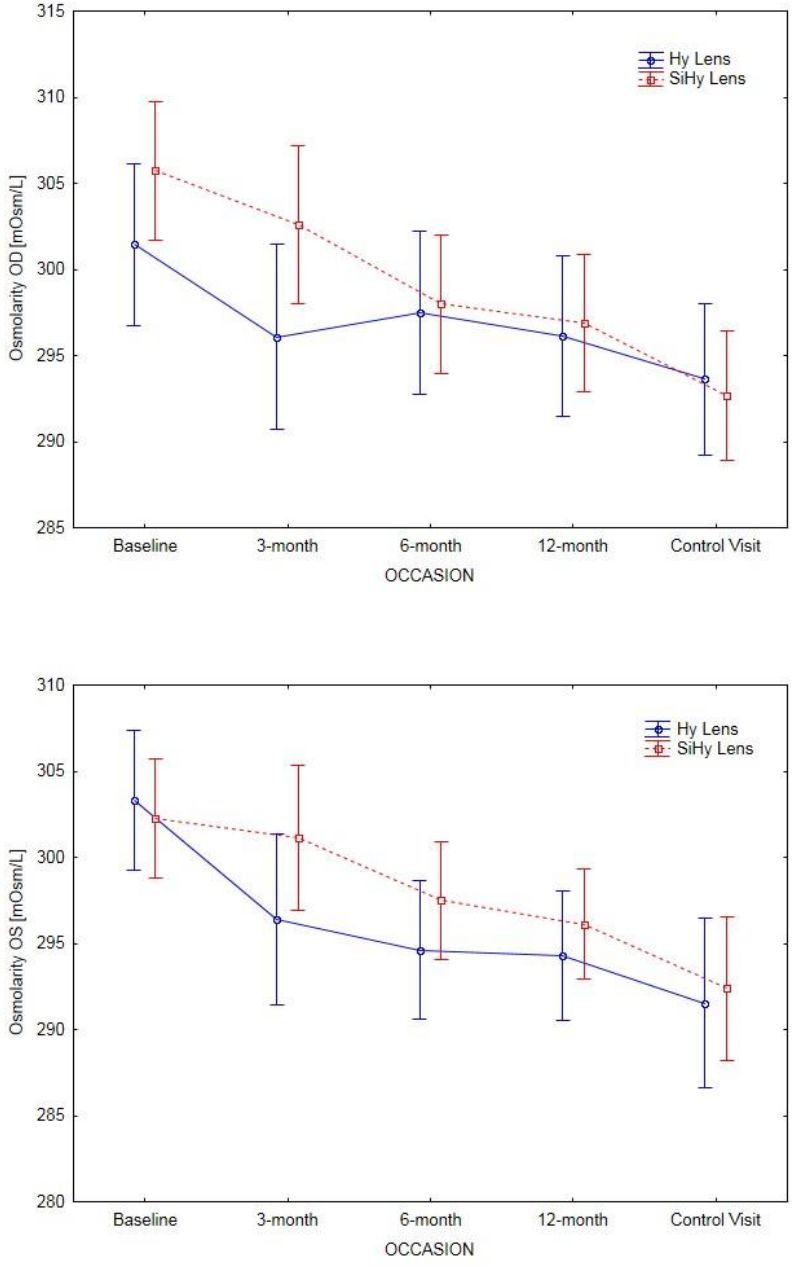

Figure 17.Top – right, Bottom - left eye tear osmolarity reported in the time-course of the study

There were no significant overall differences in osmolarity associated with both lens types (F = 1.50, P = 0.226 for OD), (F = 1.20, P = 0.274 for OS) but there was an

overall significant difference in osmolarity among occasions (F = 7.60, P < 0.001 for OD), (F = 10.50, P < 0.001 for OS) largely attributable to the gradual reduction in osmolarity over the course of the study. There were no significant interactions between lens and occasions (F = 1.2, P = 0.306 for OD), (F = 0.8, P = 0.559 for OS). In addition, osmolarity was compared at the two baselines using the t-test suggesting higher scores at the first baseline (t = 5.00, P < 0.001 for OD), (t =5.35, P < 0.001 for OS). The results of measuring interocular difference in tear osmolarity are shown in Figure 18.

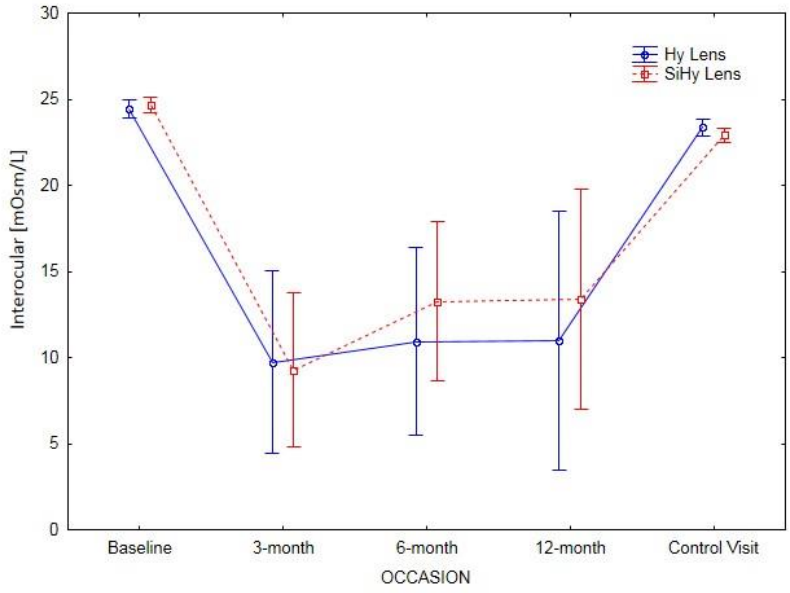

Figure 18. Difference in tear osmolarity between eyes reported in the time-course of the study

There was no significant overall difference in osmolarity associated with both lens types (F = 2.52, P > 0.123) and no overall significant difference in intraocular osmolarity among occasions (F = 0.27, P > 0.90). There was also no significant interaction between lens and occasions (F = 1.22, P > 0.30). Osmolarity was compared at the two baselines using the t-test suggesting no difference between the first and second baseline when both eyes were considered (t = 0.34, P = 0.740).

3.6. Tear film break-up time

Measures of NIKBUT (M-mean and F-first) reported in the time-course of the study are displayed in Table 3.

Table 3. MNIKBUT and FNIKBUT values reported during the study

SiHy-fitted MNIKBUT[s]	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	17.8 ± 4.1	15.6 ± 3.1	15.0 ± 2.7	14.5 ± 3.2	14.5 ± 4.1	15.1 ± 5.1
Range	[8.8, 24.9]	[11.3, 24.5]	[5.0, 20.9]	[8.6, 21.4]	[7.2, 23.1]	[7.4, 24.9]
Median	18.6	14.9	15.2	14.8	14.7	15.9
Hy-fitted MNIKBUT[s]	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	16.9 ± 4.9	15.9 ± 2.6	14.9 ± 2.6	14.5 ± 3.0	15.4 ± 2.5	14.7 ± 4.7
Range	[9.5, 24.9]	[11.3, 19.7]	[11.5, 20.0]	[10.1, 21.5]	[11.6, 21.0]	[8.9, 24.9]
Median	16.8	16.3	14.4	13.9	15.4	12.6
SiHy-fitted FNIKBUT[s]	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	15.4 ± 5.1	9.5 ± 4.6	8.4 ± 4.0	8.5 ± 3.7	8.8 ± 4.3	12.4 ± 5.3
Range	[5.2, 24.9]	[4.5, 24.5]	[2.1, 16.5]	[3.4, 18.9]	[3.1, 23.0]	[5.6, 24.9]
Median	15.6	8.2	7.5	8.1	7.4	12.3
Hy-fitted FNIKBUT[s]	**Baseline (PC)**	**Day 2 (PL)**	**3-month (PL)**	**6-month (PL)**	**12-month (PL)**	**Control (PC)**
Mean ± SD	14.3 ± 5.1	8.4 ± 4.6	9.4 ± 4.0	8.6 ± 3.7	7.6 ± 6.1	12.2 ± 6.0
Range	[5.2,24.9]	[4.5, 24.5]	[2.1, 16.5]	[3.4, 18.9]	[4.4, 20.1]	[5.7, 24.9]
Median	15.6	8.2	7.5	8.1	6.1	9.9

PC - pre-corneal tear film; PL - pre-lens tear

Non-invasive keratography tear film break-up time, i.e., the mean and the first NIKBUT for OD and OS respectively are summarised in the Figure 19.

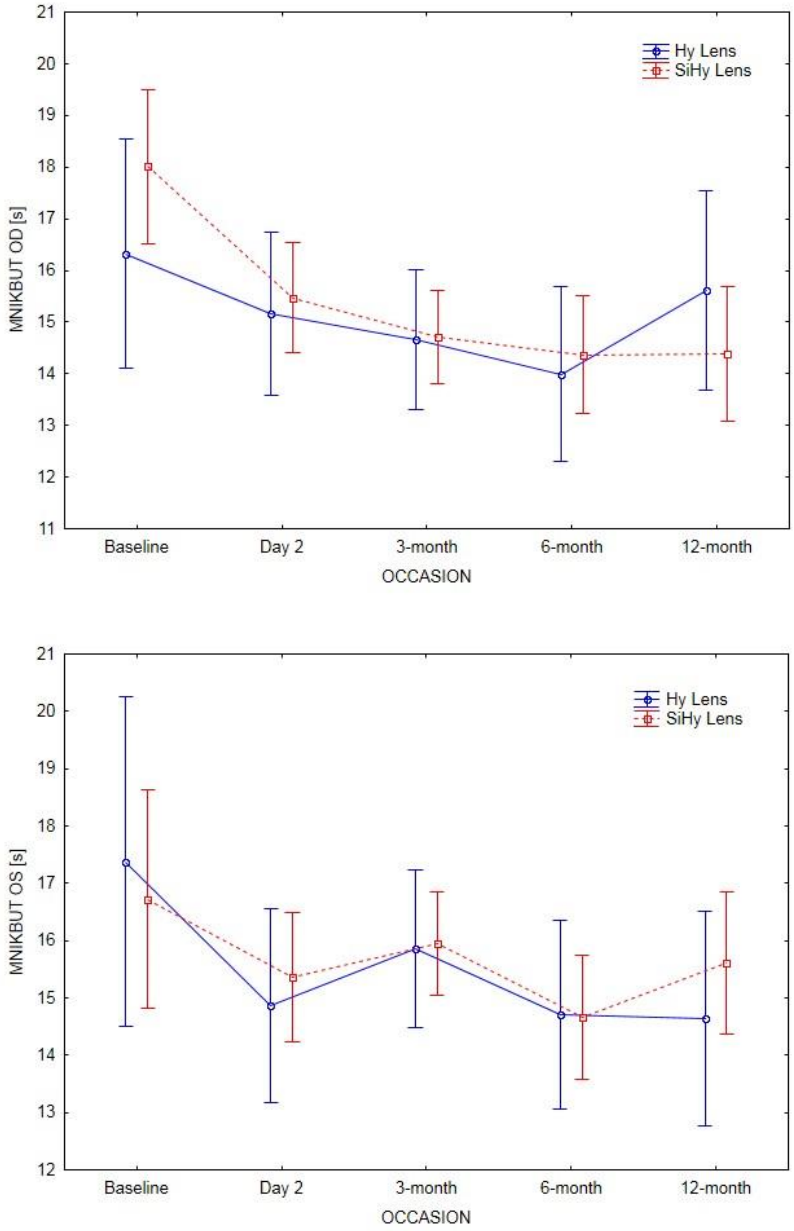

Figure 19. Top - Right eye, Bottom - left eye mean-NIKBUT values reported in the time-course of the study

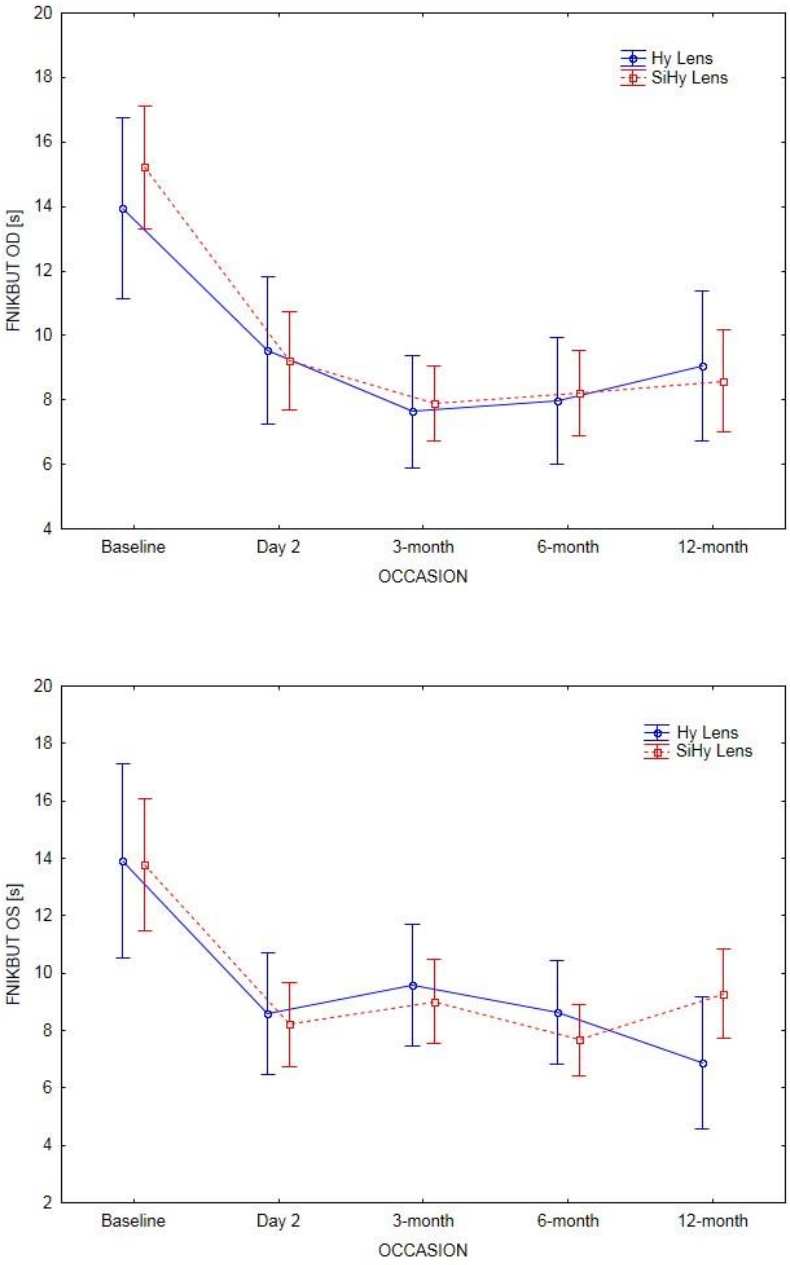

Figure 20. Top - Right eye, Bottom - left eye First-NIKBUT values reported in the time-course of the study

There were no significant overall difference in NIKBUT associated with the two lens types (F = 0.218, P = 0.642 for NIKBUT OD), (F = 0.074, P = 0.787 for NIKBUT OS), (F = 0.007, P = 0.935 for FNIKBUT OD), (F = 0.077, P = 0.783 for FNIKBUT OS) but there were an overall significant differences in NIKBUT among occasions (F = 4.789, P < 0.001 for NIKBUT OD), (F = 2.924, P < 0.225 for NIKBUT OS), (F = 14.491,

P < 0.001 for FNIKBUT OD), (F = 27.860, P < 0.001 for FNIKBUT OS) attributable to the gradual decline from the first baseline to the 6-month measurements for NIKBUT and the drop from the first baseline to the Day 2 visit measurements for FNIKBUT. Also, there were no significant interaction between lens and occasions (F = 0.991, P = 0.414 for NIKBUT OD), (F = 0.310, P = 0.871 for NIKBUT OS), (F = 1.080, P = 0.368 for FNIKBUT OD), (F = 0.039, P = 0.997 for FNIKBUT OS). In addition, mean NIKBUT and FNIKBUT were compared at the two baselines using the t-test suggesting higher scores at the first baseline (t = 3.43, P = 0.003) for OD and no significant differences (t = 1.73, P = 0.111) for OS and there were no significant differences for FNIKBUT OD and OS (t = 1.88, P = 0.078 for FNIKBUT OD), (t = 0.66, P = 0.522 for FNIKBUT OS). Figure 20 shows the FNIKBUT reported over the time-course of the study. Table 4 shows the 'r' Pearson correlation between FNIKNUT and TFSQ-NIBUT.

Table 4. Pearson's correlation between FNIKBUT and TFSQ NIBUT at each occasion

	Baseline	**Day 2**	**3-month**	**6-month**	**12-month**	**CV**
OD	-0.05	0.35*	-0.09	0.21	0.21	0.13
OS	0.13	0.25	0.28*	0.51***	0.07	-0.17

Statistical significance: *: p<0.05; ***: p< 0.001;

Non-significant or low degree of correlation was shown between FNIKBUT and TFSQ-NIBUT on most occasions with exception of OS at 6-month visit. The correlation between MNIBUT and FNIKBUT increases (is artificially inflated) for pre-lens TF due to multi-modality of the data, that result in distributing the estimates of TFBUT into two or more sub-groups.

3.7. Ocular redness

Changes related to the score of clinical evaluation of appearance of a healthy eye including bulbar and limbal redness for OD and OS are shown in Figure 21 to Figure 22. There was no significant overall difference in bulbar redness and limbal redness score associated with the two lens types ($F = 0.258$, $P > 0.614$ for bulbar OD), ($F = 0.455$, $P > 0.503$ for bulbar OS), ($F = 0.794$, $P > 0.377$ for limbal OD), ($F = 0.003$, $P > 0.959$ for limbal OS).

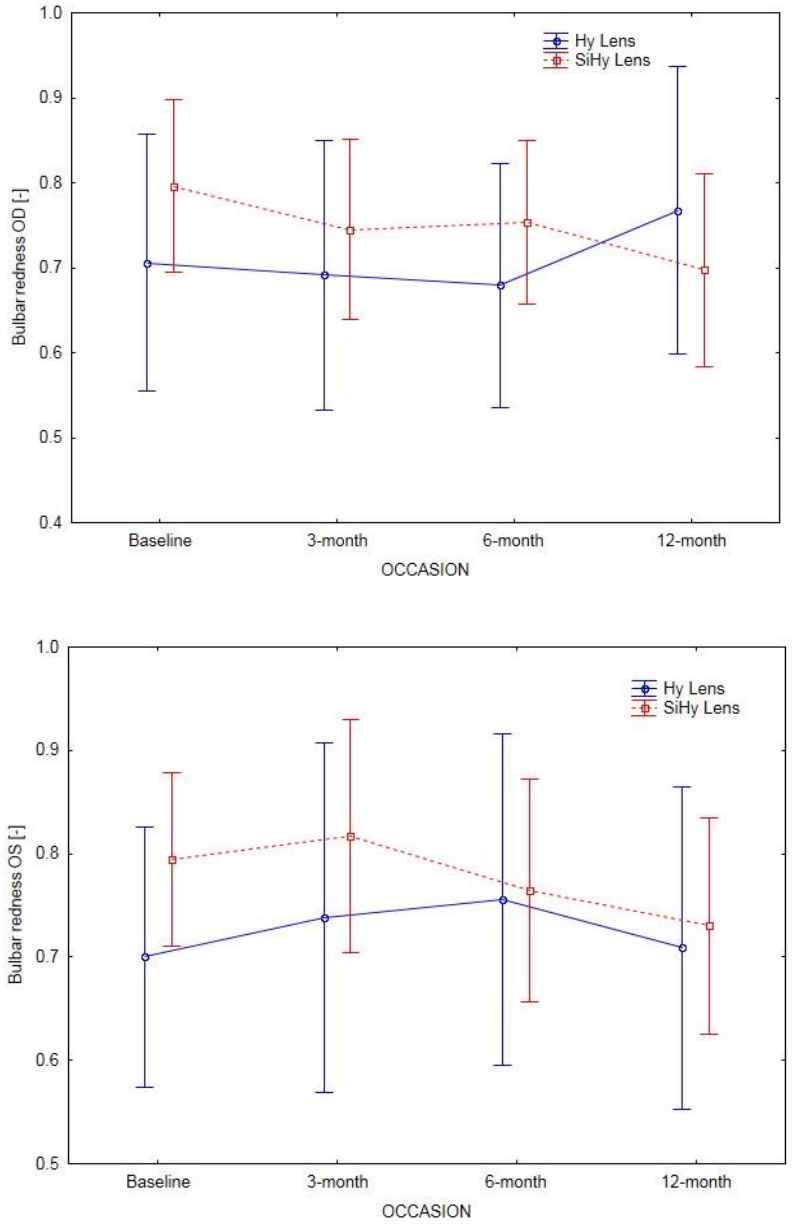

Figure 21. Top – right eye, Bottom - left eye bulbar redness reported in the time-course of the study

There was also no significant difference in bulbar redness score among occasions (F = 0.222, P > 0.881) for OD but there was significant difference in bulbar redness score among occasions (F = 0.640, P > 0.590) for OS. There was no significant interaction between lens and occasions (F = 1.157, P > 0.328). In addition, bulbar and limbal redness score were compared at the two baselines using the t-test suggesting no significant differences (t = 1.27, P = 0.220), (t = 0.35, P > 0.731).

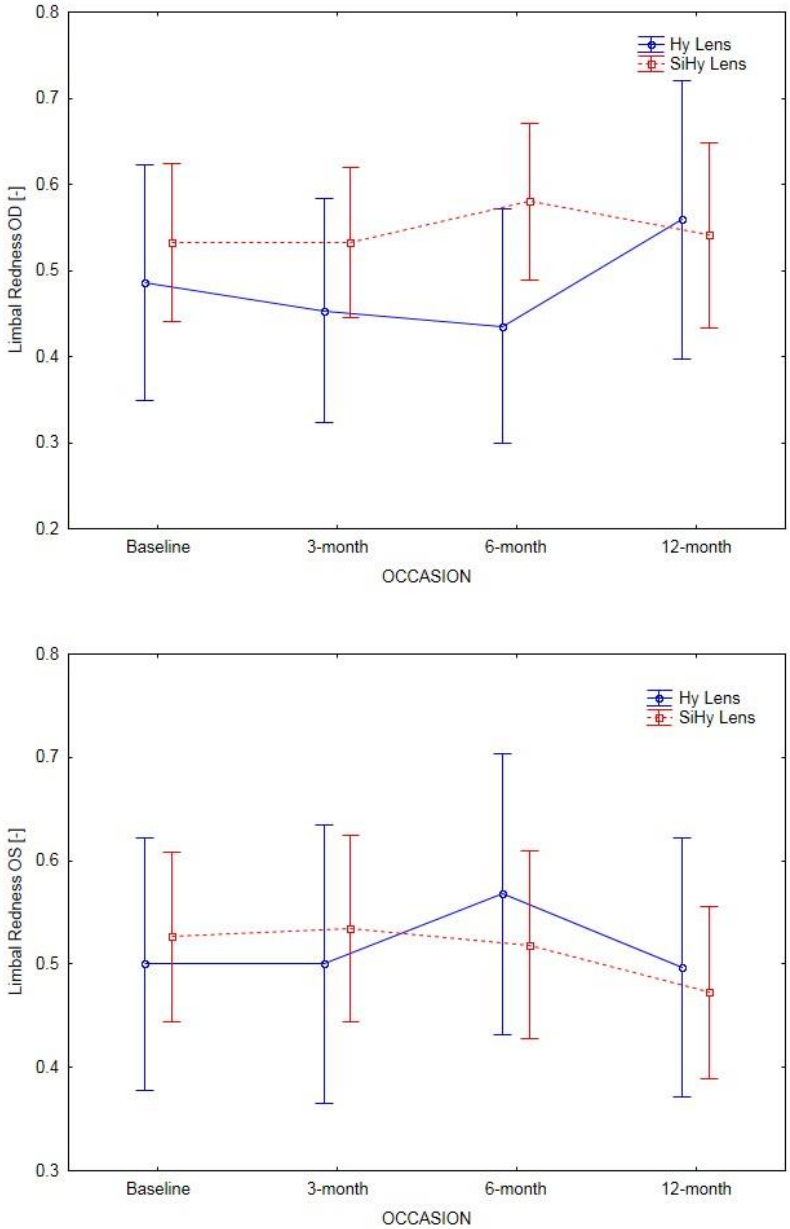

Figure 22.Top – right, Bottom - left eye limbal redness reported in the time-course of the study

3.8. Tear film stability and ocular surface staining

The stability of tears and pathophysiological changes in the anterior eye were assessed using methods such as FBUT, corneal and conjunctival staining. The results are shown in the Figure 20, 21 and 22, respectively.

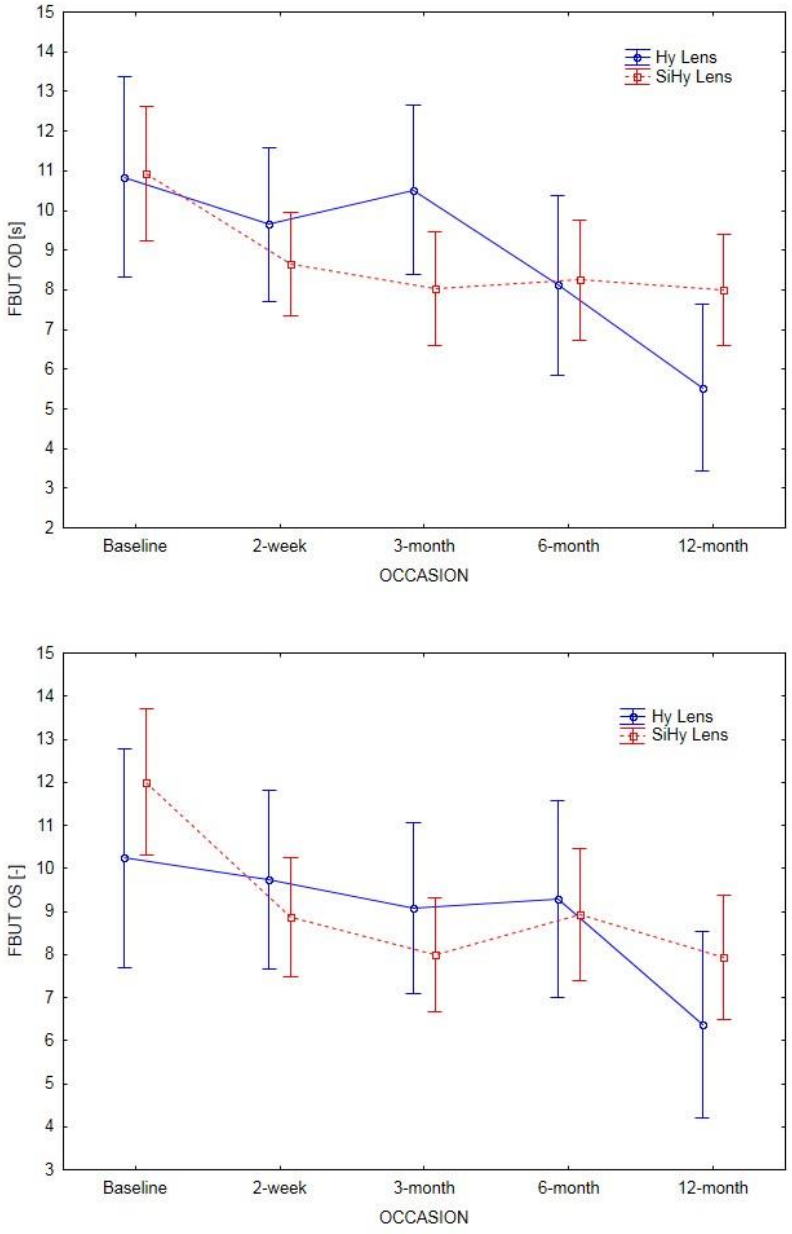

Figure 23. Top - right eye, Bottom –left eye fluorescein tear film break-up time reported in the time-course of the study

There was no significant overall difference in the measurements associated with both lens types for OD and OS respectively ($F = 0.042$, $P > 0.839$ for FBUT OD), ($F = 0.064$, $P > 0.802$ for FBUT OS), ($F = 0.093$, $P > 0.761$ for corneal stain OD), ($F = 0.100$, $P > 0.753$ for corneal stain OS), ($F = 0.202$, $P > 0.655$ for conjunctival stain OD), ($F = 0.075$, $P > 0.785$ for conjunctival stain) but there was an overall significant difference among occasions with the exception of corneal staining OS, conjunctival staining OD, and conjunctival staining OS ($F = 6.284$, $P < 0.001$ for FBUT OD), ($F = 5.860$, $P < 0.001$ for FBUT OS), ($F = 3.408$, $P < 0.010$ for corneal stain OD), ($F = 1.747$, $P > 0.141$ for corneal stain OS), ($F = 2.067$, $P > 0.086$ for conjunctival stain OD), ($F = 1.178$, $P > 0.321$ for conjunctival stain OS).

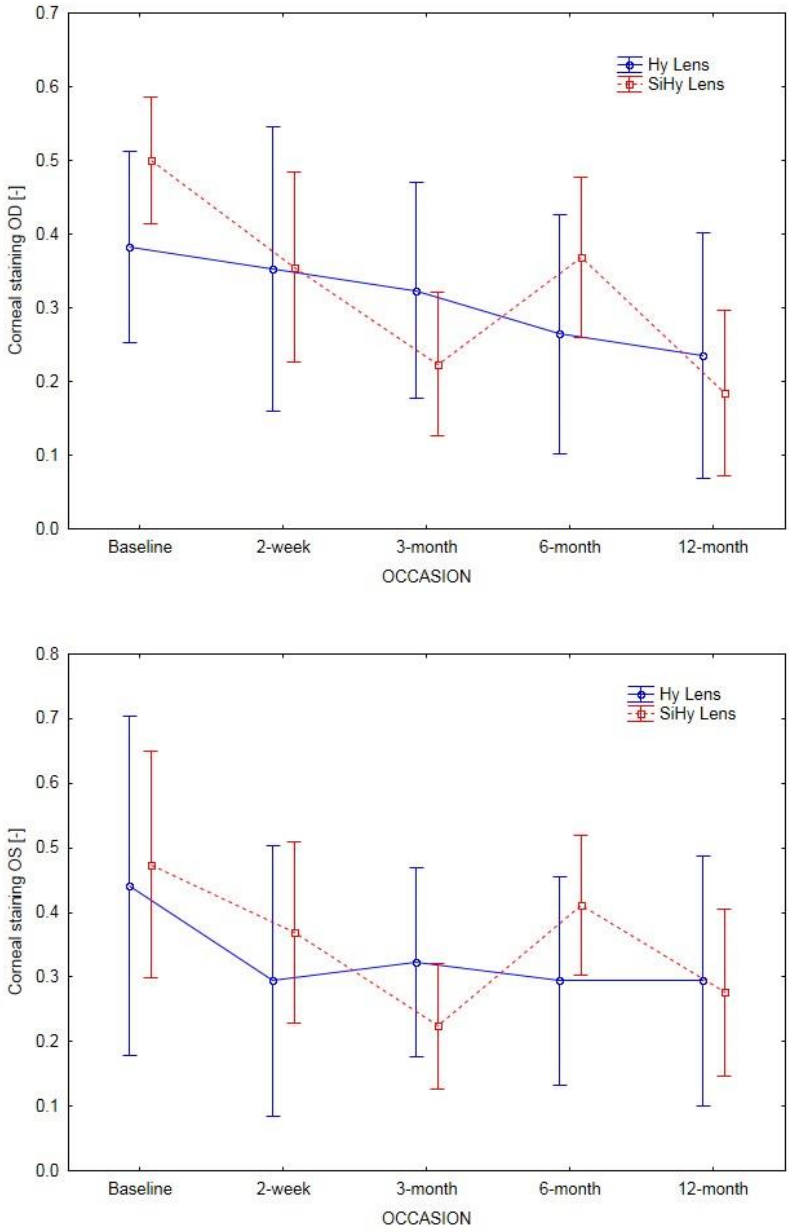

Figure 24. Top - right eye, Bottom –left eye corneal staining scores reported in the time-course of the study

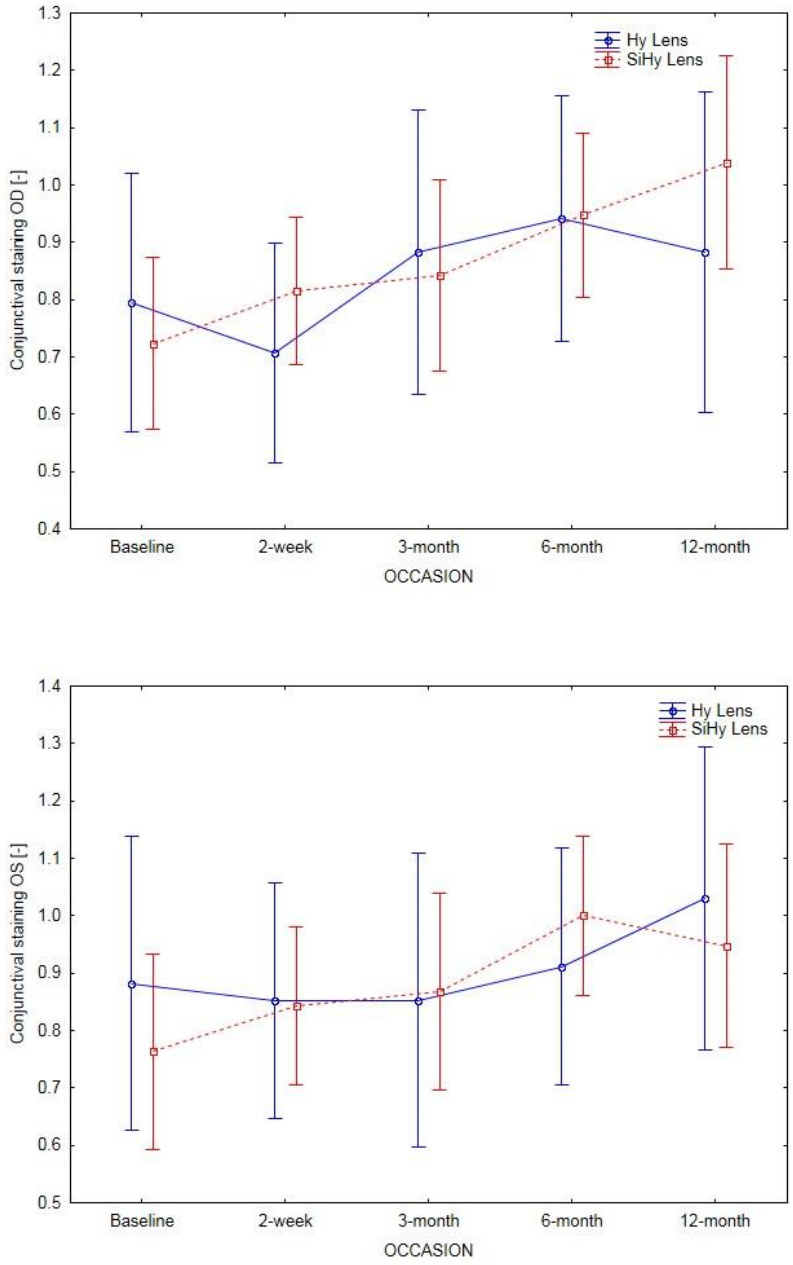

Figure 25. Top - right eye, Bottom –left eye conjunctival staining score reported in the time-course of the study

There was no significant interaction between lens and occasions for OD and OS respectively ($F = 2.230$, $P > 0.067$ for FBUT OD), ($F = 1.32$, $P > 0.263$), ($F = 1.020$, $P > 0.398$ for corneal stain OD), ($F = 0.574$, $P > 0.681$ for corneal stain OS), ($F = 0.528$, $P > 0.716$) for conjunctival stain OD, ($F = 0.374$, $P > 0.827$ for conjunctival stain OS). In addition, the scores were compared at the two baselines using the t-test suggesting a

higher score at the first baseline ($t = 2.51$, $P = 0.022$) and no significant differences for OS ($t = 1.23$, $P < 0.05$). Also, a higher score was observed at the first baseline for corneal staining OD and OS ($t = 9.22$, $P< 0.001$), ($t = 2.55$, $P = 0.025$), but no significant differences for conjunctival staining OD and OS were found ($t = 0.14$, $P = 0.889$), ($t = 1.34$, $P = 0.205$).

3.9. Meibomian glands function

The results of subjective evaluation of Meibomian gland morphology are summarised below. Right eye Meibography scores were displayed in Figure 26, for the upper and lower eyelid, respectively. Meibomian gland dysfunction (MGD) scores for left eye are displayed in Figure 26 and Figure 27, respectively.

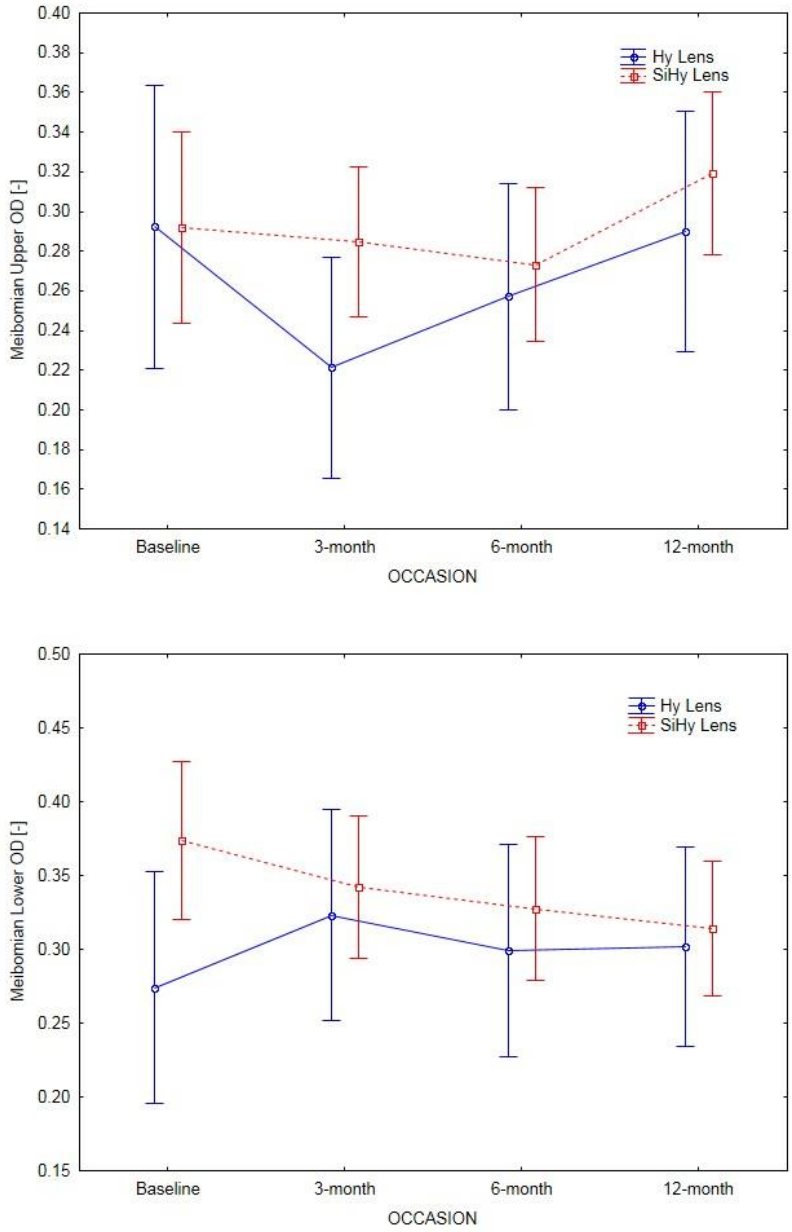

Figure 26. Right eye: top – upper lid, bottom – lower lid Meibo score reported in the time-course of the study

Significant difference in the OS MGD upper lid score (F = 0.312, P = 0.816) was not reported, but there was a significant difference in lower lid score (F = 3.310, P = 0.022). There was no significant interaction between lens and occasions OD upper lid (F = 0.970, P = 0.408), but there was a significant interaction in lower lid (F = 3.149, P = 0.027) probably attributable to the larger difference between lenses at the first baseline. There was

no significant interaction between lens and occasions for OS upper and lower lid (F = 0.875, P = 0.456), (F = 0.636, P = 0.593). Additionally, the MGD OD upper and lower lid and OS upper and lower lids scores were compared at the two baselines using the t-test suggesting no significant differences (t = 0.44, P > 0.05 for OD upper lid), (t = 2.03, P > 0.05 for OD lower lid), (t = 1.67, P > 0.05 for OS upper lid), (t = 0.68, P > 0.05 for OS lower lid).

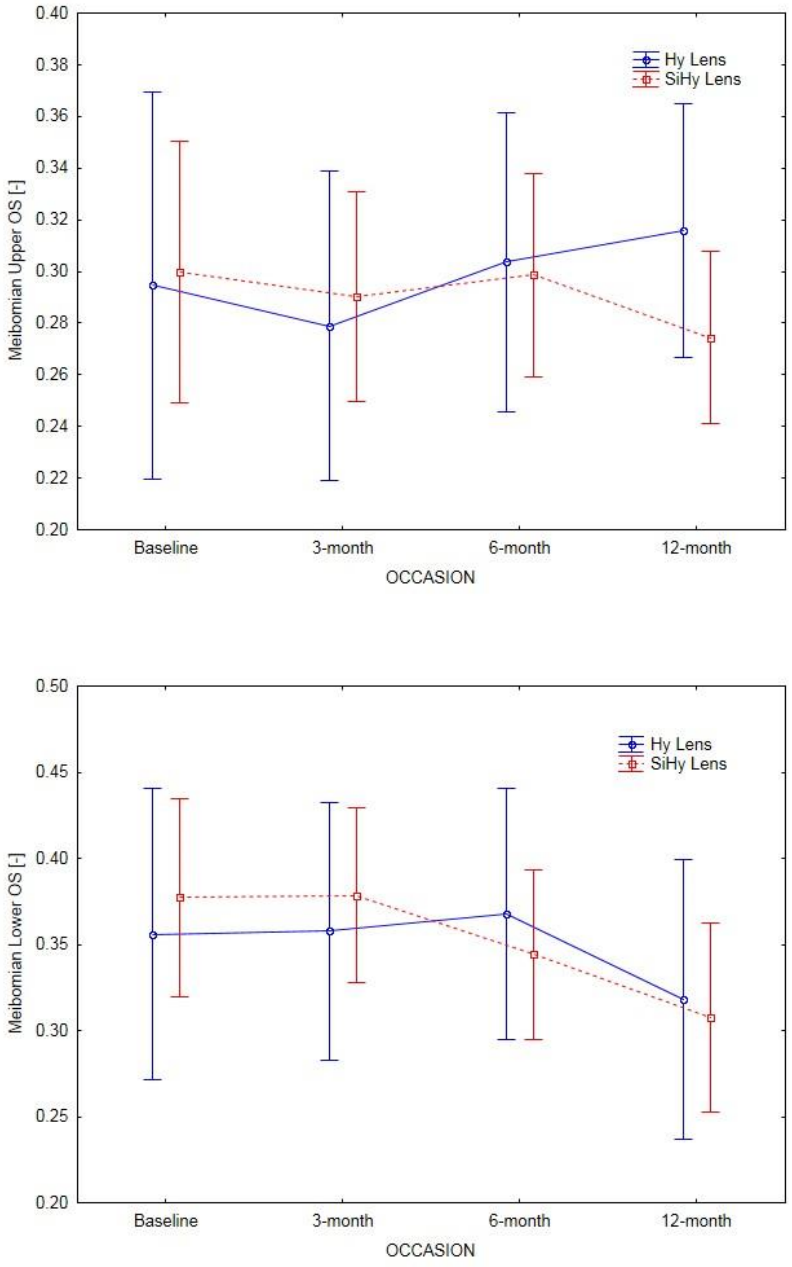

Figure 27. Left eye: top – upper lid, bottom – lower lid Meibo score reported in the time-course of the study

3.10. Lid wiper epitheliopathy

Lissamine green evaluation was used to grade the staining of upper and lower lid wiper for OD and OS. For the right eye results are shown in the Figure 28 and for the left eye in Figure 29 for the upper and lower eyelid, respectively.

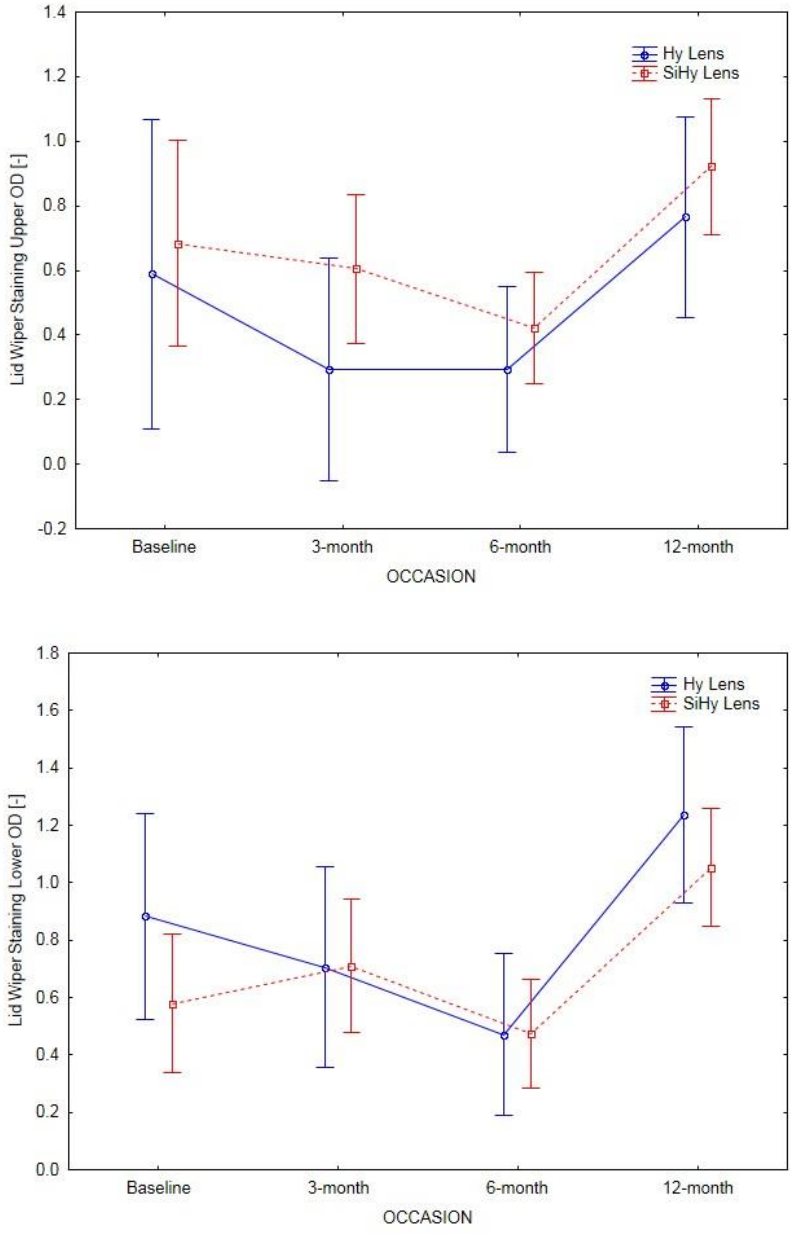

Figure 28. Right eye: top – upper lid, bottom – lower lid wiper staining score (in the range from 1 to 4) reported in the time-course of the study

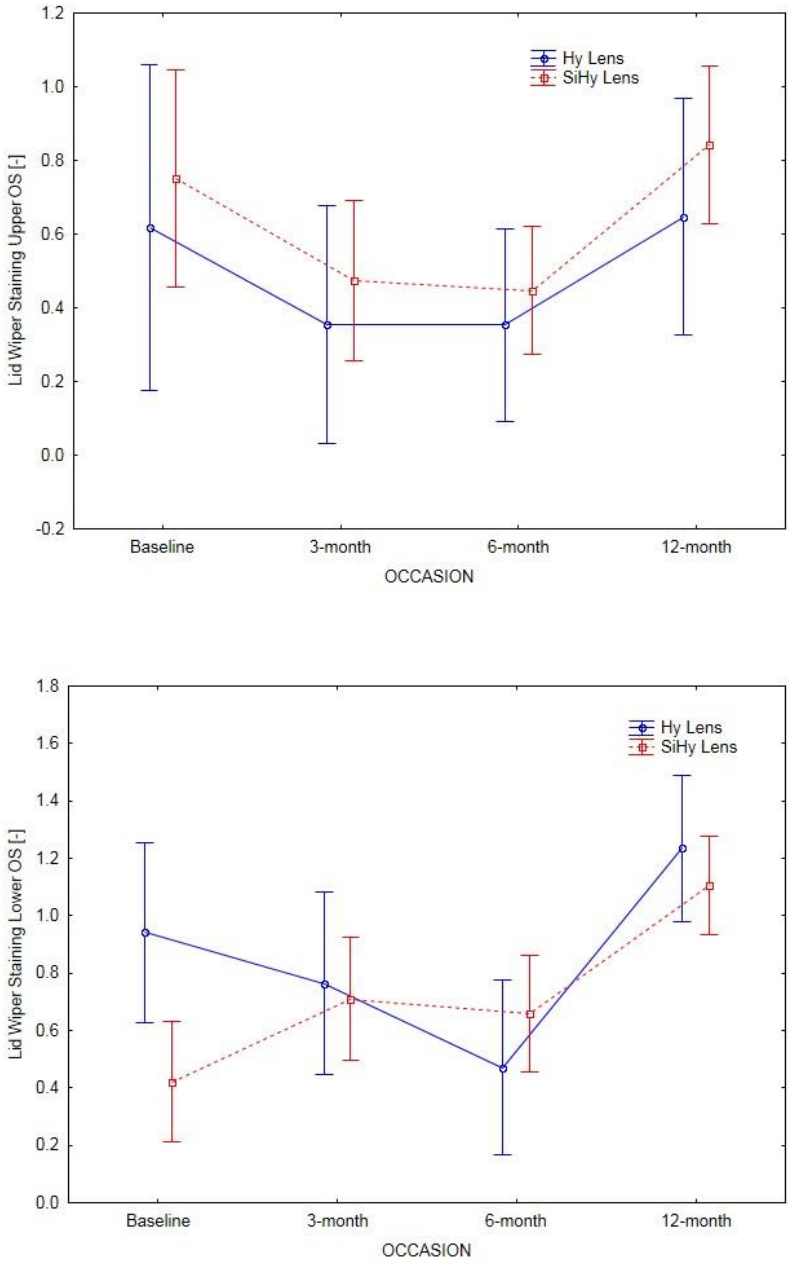

Figure 29. Left eye: top – upper lid, bottom – lower lid wiper staining score (in the range from 1 to 4) reported in the time-course of the study

There was no significant overall difference in the score associated with the two lens types ($F = 1.678$, $P > 0.201$ for OD upper lid), ($F = 0.929$, $P > 0.340$ for OD lower lid), ($F = 1.194$, $P > 0.279$ for OS upper lid), ($F = 1.464$, $P > 0.232$ for OS lower lid) but there was a significant difference in the LWE scores among occasions ($F = 4.899$, $P < 0.003$ for OD upper lid), ($F = 10.262$, $P < 0.000$ for OD lower lid), ($F = 3.593$, $P < 0.015$ for OS

upper lid), ($F = 10.048$, $P < 0.000$ for OS lower lid). There were no significant interactions between lens and occasions ($F = 0.243$, $P > 0.867$ for OD upper lid), ($F = 10.048$, $P < 0.000$ for OD lower lid), ($F = 0.051$, $P > 0.985$ for OS upper lid), with the exception of LWE OS lower lid ($F = 3.091$, $P < 0.029$) suggesting differences between lenses at the first baseline. Moreover, the LWE scores were compared at the two baselines using the t-test suggesting no significant differences ($t = 0.85$, $P > 0.05$ for OD upper lid), ($t = 1.67$, $P > 0.05$ for OD lower lid), ($t = 0.20$, $P > 0.05$ for OS upper lid), ($t = 0.56$, $P > 0.05$ for OS lower lid).

Table 5 shows the results of measurements conducted during the baseline visit for both right and left eyes. It is evident that none of the considered parameters, where applicable, showed statistically significant differences between the fellow eyes. Therefore, in further analysis only the eye with the originally prescribed lens was considered. This arrangement of data was necessary for the Day 2 measurements, where subjects wore two different lenses, to be included in the analysis.

The results of statistical analysis, performed with repeated measures 2-factor analysis ANOVA for each variable as well as the comparison between baseline and the final control visit measurements with a paired t-test, are collected in Table 6.

Table 5. Tear film measures assessed during the baseline visit

	OD	OS	P-value (paired t-test)
OSDI			
Mean ± SD	14.1 ± 11.5		[-]
Range	[0.0, 47.7]		
Median	11.5		
CLDEQ-8			
Mean ± SD	7.7 ± 5.7		[-]
Range	[0, 22]		
Median	7.0		
TFSQ-NIBUT*			
Mean ± SD	12.4 ± 4.9	11.5 ± 6.2	P = 0.373
Range	[2.5, 23.9]	[2.81, 25.74]	
Median	13.4	12.8	
MNIBUT*			
Mean ± SD	13.95 ± 4.80	14.29 ± 5.81	P = 0.698
Range	[2.58, 23.63]	[2.81, 25.74]	
Median	14.06	14.09	
Tear Osmolarity			
Mean ± SD	301 ± 9	300 ± 7	P = 0.488
Range	[289, 333]	[291, 322]	
Median	302	300	
MNIKBUT*			
Mean ± SD	17.49 ± 4.40	16.87 ± 5.24	P = 0.408
Range	[6.81, 24.92]	[6.47, 24.98]	
Median	18.28	17.15	
FNIKBUT*			
Mean ± SD	14.59 ± 5.51	13.87 ± 6.49	P = 0.225
Range	[2.48, 24.92]	[3.31, 24.98]	
Median	14.07	11.93	
Corneal staining			
Mean ± SD	0.3 ± 0.4	0.4 ± 0.5	P = 0.272
Range	[0.0, 1.5]	[0.0, 2.5]	
Median	0.0	0.5	
Conjunctival staining			
Mean ± SD	0.7 ± 0.5	0.8 ± 0.6	P = 0.738
Range	[0,0, 1.5]	[0.0, 2.5]	
Median	1.0	1.0	
FBUT*			
Mean ± SD	10.6 ± 5.1	11.1 ± 5.3	P = 0.495
Range	[1.7, 23.3]	[2.0, 21.3]	
Median	8.5	9.7	

*Pre-corneal, SD – Standard deviation

Table 6. Summary of statistical analysis (ANOVA, 2-factor, repeated-measures) for each variable and comparison of baseline and final control measurements

Variable	Effect			
	Lens (F)	Occasion (F)	Lens × Occasion (F)	Baseline vs Control (t)
Temperature	0.37 ns	1765.2***	2.95 **	7.07 *** B>C
Humidity	0.15 ns	14.17 ***	2.45 *	0.08 ns
Time of wear	1.08 ns	15.75***	6.23 ***	Not applicable
OSDI	0.03 ns	3.78 **	0.85 ns	2.95** B>C
CLDEQ-8	0.05 ns	2.34 *	0.39 ns	2.28 * B>C
TMH OD	3.70 ns	0.75**	0.12 ns	1.73 ns
TMH OS	1.67 ns	2.22 ns	0.16 ns	2.27 ns
TFSQ-NIBUT OD	1.55 ns	7.92***	0.44 ns	0.23 ns
TFSQ-NIBUT OS	0.83 ns	8.62 ***	0.13 ns	0.87 ns
Osmolarity OD	1.50 ns	7.60 ***	1.20 ns	5.00 *** B>C
Osmolarity OS	1.20 ns	10.50 ***	0.80 ns	5.35 ** B>C
Osmolarity IOD	2.52 ns	0.27 ns	1.22 ns	0.34 ns
MNIKBUT OD	0.22 ns	4.79 **	0.99 ns	3.43 ** C<B
MNIKBUT OS	0.07 ns	2.92 *	0.31 ns	1.73 ns
FNIKBUT OD	0.08 ns	19.40 ***	0.28 ns	1.88 ns
FNIKBUT OS	0.01 ns	14.49 **	1.07 ns	0.66 ns
Bulbar OD	0.26 ns	0.22 ns	1.16 ns	1.27 ns
Limbal OD	0.79 ns	0.84 ns	1.63 ns	0.61 ns
Bulbar OS	0.45 ns	0.64 ns	0.48 ns	0.61 ns
Limbal OS	0.01 ns	0.93 ns	0.66 ns	0.35 ns
FBUT OD	0.04 ns	6.28 ***	2.23 ns	2.51 * B>C
FBUT OS	0.06 ns	5.85 ***	1.32 ns	1.23 ns
Corneal Staining OD	0.09 ns	3.41 *	1.02 ns	9.21 *** B>C
Conjunctival Staining OD	0.20 ns	2.07 ns	0.53 ns	0.14 ns
Corneal Staining OS	0.10 ns	1.75 ns	0.57 ns	2.55* B>C
Conjunctival Staining OS	0.79 ns	1.18 ns	0.37 ns	1.34 ns
Meibomian OD Upper	0.91 ns	3.01 *	0.97 ns	0.44 ns
Meibomian OS Upper	0.06 ns	0.31 ns	0.87 ns	1.67 ns
Meibomian OD Lower	1.06 ns	0.53 ns	3.15 *	2.03 ns
Meibomian OS Lower	0.01 ns	3.31 *	0.64 ns	0.68 ns
LWE OD Upper	1.68 ns	4.90 **	0.24 ns	0.85 ns
LWE OS Upper	1.19 ns	3.59 *	0.05 ns	0.2 ns
LWE OD Lower	0.93 ns	10.26 ***	0.75 ns	1.67 ns
LWE OS Lower	1.46 ns	10.04 ***	3.09 *	0.56 ns

OD - right eye; OS - left eye; IOD – Interocular difference; F – the Fisher statistic, t – the Student statistic; ns – not significant P>0.05; * – p<0.05; **: p< 0.01; *** – p< 0.001; B – baseline; CV – final control, LWE - lid wiper epitheliopathy

3.11. Utility of measuring TBUT for prescribing Contact Lenses

Before the longitudinal study was concluded, it was of interest to find out whether routinely measuring tear film break-up time would add clinical value for prescribing contact lenses.

A sub-group of 46 subjects were included in this part of the study. Data from Baseline and Day 2 visits were taken into account, additionally subjective comfort was assessed for subjects to differentiate between two lenses (which lens was better if any). Thirty-four subjects were fitted with SiHy and 12 with Hy contact lenses. Figure 30 describes the preference in terms of comfort between the lenses.

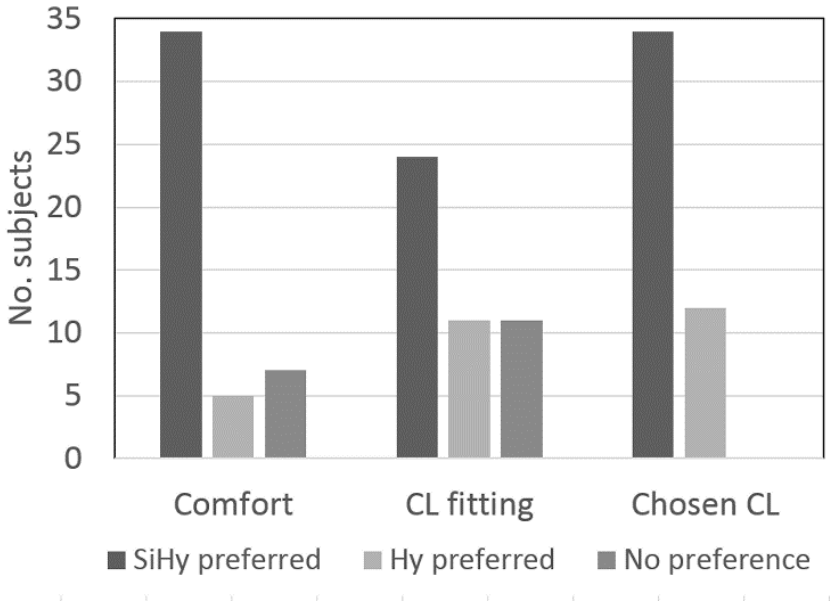

Figure 30. Number of subjects fitted with SiHy and Hy contact lens based on comfort and best fit

Figures 31 and 32 show the box plots corresponding to the FNIKBUT and MNIKBUT values of OD and OS before the contact lens insertion (Baseline) and after four hours of wearing CLs, respectively. At the Baseline, there were no statistically significant differences between the eyes (P =0.37 and P = 0.59 for FNIKBUT and MNIKBUT, respectively). Similarly, no statistically significant differences between the eyes wearing

two different CLs were obtained (P =0.19 and P =0.98 for the first and the mean NIKBUT, respectively). Furthermore, statistically significant differences in tear film quality were found between the baseline condition and the contact lens wear condition for SiHy CL (P < 0.001 and P = 0.006 for the first and the mean NIKBUT, respectively) and for the Hy CL (P < 0.001 for the first NIKBUT). However, no statistically significant difference was found between the two conditions for the Hy CL tear film quality measured with the mean NIKBUT (P = 0.13).

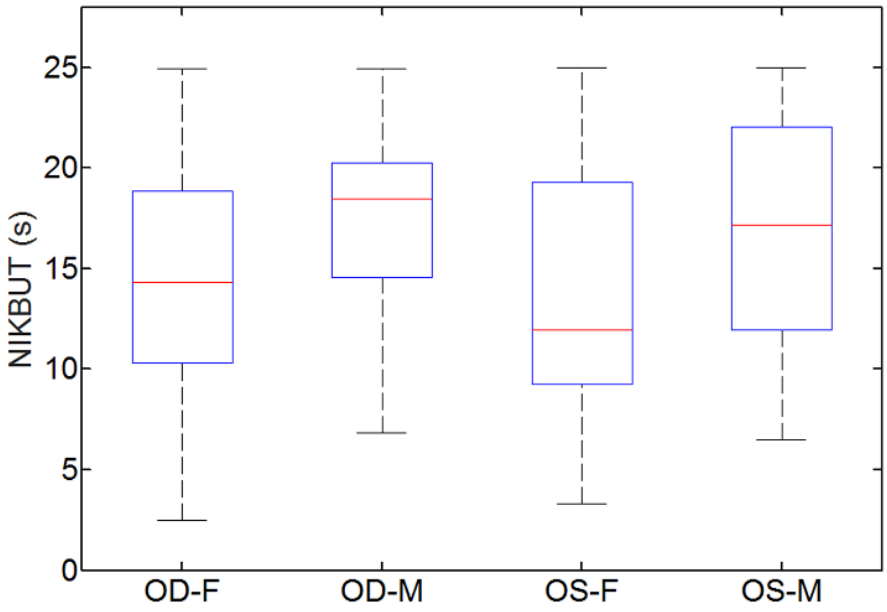

Figure 31. FNIKBUT (F) and MNIKBUT (M) after four hours of contact lens wear for SiHy contact lens on the right eye (OD) and the Hy contact lens on the left eye (OS)

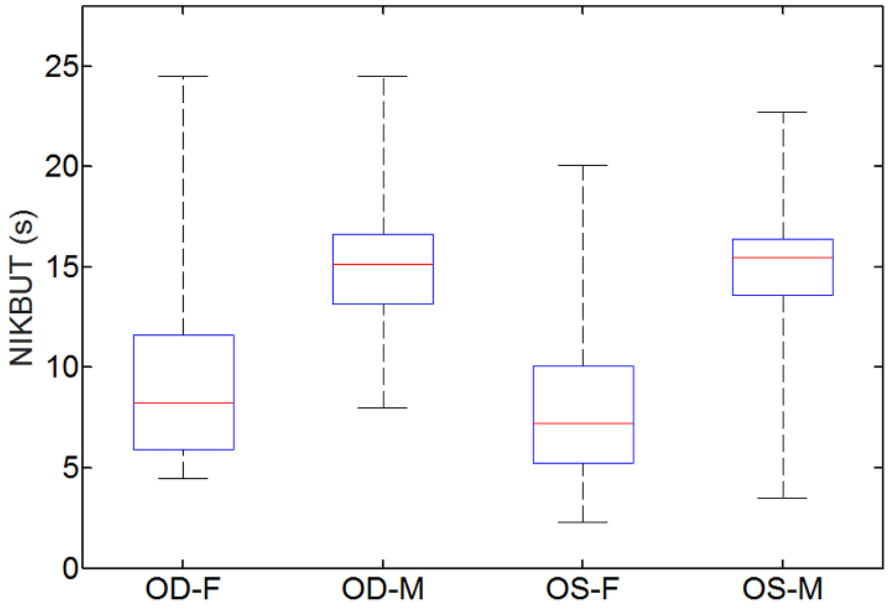

Figure 32. FNIKBUT (F) and MNIKBUT (M) before fitting contact lens (Baseline). OD and OS describe the right eye and left eye, respectively

As stated previously, the NIKBUT estimates were not used for selecting the most suitable CL for the subjects. On their own, if only tear film quality was considered, FNIKBUT would select the SiHy CL in 54% of subjects (25 out of 46) while MNIKBUT would indicate that lens for 52% of subjects (24 out of 46). Further, the NIKBUT based decision was contrasted against the decision made earlier that was based on VA, contact lens fit and non-related to tear film clinical indications. The comparison between the qualitative clinical assessment and the decisions made based on NIKBUT is shown in Table 7. A statistically significant difference was observed between the qualitative analysis-based decision and that based on the mean NIKBUT (P = 0.049). The results have also shown that, for the majority of cases, the decision based on the first NIKBUT corresponds to that based on the mean NIKBUT and the difference between them was not statistically significant. Correlation analysis showed that the NIKBUT based decision is not correlated to the clinical one (R^2 =0.002, P = 0.754 and R^2 =0.030, P = 0.252 for the first and the mean NIKBUT, respectively).

Table 7. The results of comparison between the qualitative decision and that made based on NIKBUT for the first and mean values.

		Counts	Percentage (%)	p-value
Qualitative vs FNIKBUT	Matched:	18	39.1	0.061
	Didn't match:	28	60.9	
Qualitative vs MNIKBUT	Matched:	16	34.8	0.049*
	Didn't match:	30	65.2	
FNIKBUT vs MNIKBUT	Matched:	40	86.9	0.671
	Didn't match:	6	13.1	

*asterisk denotes statistical significance (two-sided Wilcoxon sign rank test)

IV. DISCUSSION

Recent studies and patents revision have revealed an evolution in soft contact lens technology and its materials (Nicolson and Vogt, 2001; Calo and Khutoryanskiy, 2015). This evolution is the result of better understanding of the physiological characteristics of tear film and cornea, which has led to alleviate some of the issues (such as discomfort) that patients have experienced to other types of contact lenses. Their soft surface permits them to be flexible and being able to modify their shape to different eyes. Daily disposable lenses are the leading modality recommended by eye care professionals. The greater convenience including flexibility in prescribing, excluding lens cleaning, and storing enhance the lens wearer compliance. Needless to say, as with any lens type, there may also be some disadvantages and modern daily disposable contact lenses cost noticeably higher than the other types of lenses.

The study considered the impact of modern daily disposable soft contact lenses on ocular physiology over the course of twelve months. To the best of my knowledge this is the first longitudinal study to consider whether taking additional measurements, to the standard clinical procedure, provide useful information during CL fit to avoid contact lens discomfort, which has been defined as a condition related to lens wear and the goal of CL fit to keep ocular surface minimally affected. The project consisted of different stages, which helped to shape this thesis. One stage was to summarise the part of the data leading to a publication. The scientific work is discussing the importance of contact lens impact on ocular health and vision emphasising the fundamental coexistence between anterior eye and contact lens fit, concluding the advantage of tear film surface quality measurement to follow the extent of lens induced changes.

In the following, I provide a detailed discussion on the results of the longitudinal study. At the Baseline visit, none of the parameters measured showed statistically significant differences between right and left eyes (Table 5). This facilitated application of two different contact lenses (SiHy and Hy) on Day 2. The mean Baseline tear osmolarity, mean corneal and conjunctival staining score, FBUT and NIKBUT were comparable with the values reported for healthy individuals (Szczesna-Iskander, 2016, Korb et al., 2001, Wolffsohn et al., 2009, Wolffsohn et al., 2017). Baseline median OSDI score was slightly higher than values reported for healthy population (i.e., median value of 11.5). However, the group of subjects partially consisting of habitual contact lens wearers may have contributed to this difference, as they are more likely to report dry eye symptoms (Nichols, 2005).

Regarding self-reported comfort, two drop in the OSDI and CLDEQ-8 scores were observed for both SiHy-fitted and Hy-fitted groups: one at the two-week visit and one at the final control visit. After initial drop at two-weeks, the values returned to the baseline level at the six-month visit and remained stable at the 12-month visit. This drop in the OSDI and CLDEQ-8 scores could be attributed to the difference between current and previous lens wear modalities as well as the difference between the lens materials (Jones, 2002). The positive effect of lens refitting has been previously observed across different materials and modalities in other studies (Riley et al., 2006, Young et al., 2007, Fahmy et al. 2011).

The frequency of self-reported dry eye symptoms among the contact lens wearers has been reported to be high (Wolffsohn et al., 2017). However, in this study the recorded OSDI and CLDEQ-8 twelve-month scores (except for one subject) were not higher than those registered at baseline (see Table 2). This indicates that the wear of the daily disposable contact lenses used in this study did not have an effect on the comfort of the participants

during the course of the study. It is worth noting that elimination of all ocular dryness symptoms due to contact lens wear would be a vast improvement. However, it would be unreasonable to expect all signs and symptoms to be relieved if some proportion of them may have been present without lenses at the Baseline visit.

It has been suggested that contact lens wear alters the normal tear film structure and affects its rate of evaporation potentially leading to adverse ocular surface health effect (Nichols, 2011, Chalmers, 2015). However, daily disposable lenses used in this study did not show adverse wear effects. It has also been suggested that contact lens wear influences tear osmolarity, particularly in changing environmental conditions. In one study (Kojima, 2011), both SiHy and Hy lenses were considered showing increased osmolarity for subjects wearing Hy lens when placed for 20 minutes under a controlled adverse environment but not for those wearing SiHy. Generally, it has been suggested that contact lens wear increases tear osmolarity (Nichols et al., 2006). For example, Miller et al. (2004) showed increased levels of tear osmolarity in both SiHy and Hy contact lenses in comparison to non-contact-lens wearers while Best et al. (2013) found no change in tear osmolarity between baseline and a 6-month visit for SiHy contact lenses. Contrarily to those previous works, this study showed a statistically significant decrease in tear osmolarity for both SiHy-fitted and Hy-fitted group. In the SiHy group there was a steady decrease in tear osmolarity but in the Hy-fitted group there was a sudden decrease and then the osmolarity remained the same at the further visits. The results of decreased osmolarity further indicate, as suggested by the evidence from the OSDI and CLDEQ-8 scores, that the modern daily disposable contact lenses may not necessarily lead to typically known adverse effects of contact lens wear, such as ocular discomfort or inflammation of the ocular surface (Nichols et al., 2011, Craig et al., 2013).

It could be assumed that the decrease in tear osmolarity is associated with seasonal changes in temperature and humidity as the study was performed within a year in a country with four seasons. However, Khanal and Millar (2012) found no correlation between tear osmolarity measurements with the temperature or humidity. Hence, those environmental factors could not substantially affect the tear osmolarity results. Another reason may be measuring osmolarity shortly after contact lens removal or it may be due to corneal desensitisation after prolonged CL wear – since the osmolarity values go even smaller during follow up visits. However, it may be indorsed by modern daily disposable materials and healthier contact lens wearing habits.

The NIKBUT parameters have been primarily designed for assessing pre-corneal tear film surface quality. The prospect of using pre-lens NIKBUT measurement has been suggested earlier (Best et al., 2013) and eventuated in a recent study (Mousavi et al., 2018). Here, it was assumed that NIKBUT parameters, which essentially measure tear film surface levelling/de-levelling properties, quantify tear film surface quality on either eye or a contact lens. The NIKBUT results showed that the estimates of the pre-lens tear film surface quality acquired on Day 2 visit were not statistically significantly different to those recorded at the 3-month, 6-month and 12-month visits. Hence, for modern daily disposable lenses, measurement of pre-lens tear film surface quality after a four-hour wear may provide sufficient information on the suitability of contact lens material for an individual subject (Szczesna-Iskander et al., 2012, Szczesna-Iskander et al., 2014). Of importance is the result of MNIKBUT for the Hy-fitted group were found no statistically significant differences between any of the visits, indicating that there was no clinically important difference between the baseline pre-corneal tear film surface quality and that acquired from the lens surface. Pearson's correlation was used to show the correlation between two different

methods of tear film quality assessment. Throughout the time-course of the study low or non-significant correlations were shown between these two methods, except for 6-month visit. However, as noted earlier, this correlation was artificially inflated due to multi-modality of the data. While TFSQ-NIBUT measure is characterised by statistically significant steady decline, the FNIKBUT value dropped at Day 2 and stayed constant until 12-month visit. The repeatability (Szczesna-Iskander et al., 2010) and objective nature of the noninvasive tear film measurement techniques contributes to the detection of significant differences associated with contact lens type (Szczesna-Iskander et al, 2012). However, in this study, no statistically significant difference was noted between lens types in the 12-month time-course. This may suggest that two lenses achieved similar performance, regardless of their different material properties and in vitro wetting characteristics.

TFSQ-NIBUT and FNIKBUT represent different tear film characteristics. FNIKUT is related to the image intensity of reflected Placido disc rings, while the TFSQ-NIBUT is the assessment of the dynamics of the tear film, derived from textural image information, and it may correlate with dewetting, rather than with evaporation (Szczesna-Iskander and Iskander, 2014).

A stable tear film has a levelling phase that lasts until the next blink, unless becomes unstable right after the blink, which the tear film is classed as thin.

The FBUT measurements followed a similar trend of no change in the Hy-fitted group as the NIKBUT and conjunctival staining. For the SiHy-fitted group statistically significant differences were found but the post-hoc analysis did not confirm that result. There was a decreasing trend in the median value of FBUT for across the term of the study

for the SiHy-fitted group but not for the Hy-fitted group, suggesting that in the case of FBUT Hy lenses might have less effect on tear film stability.

The results of corneal staining showed improvement of ocular surface health at the final control visit while those of conjunctival staining showed no significant differences between the Baseline and the final Control Visit. Using Efron Grading Scales for Contact Lens Complications, the grade of conjunctival staining was mild and within the norm of typically recorded values for severity (Efron, 2012). The grading was marked no higher than mild (median grading value of trace).

This study has some limitations. Firstly, the type of lens was not masked from the observer and could result in some potential bias. However, an important premise of this research study was to equip the subject for the following 12-month with the better of the two lenses, using both subjective assessment of the contact lens fit as well as the objective measures of ocular surface physiology. One could argue that non-randomising the lenses in the group in order to achieve similar number of SiHy and Hy- fitted lenses could have also created a potential bias. However, for the subject to be able to wear newly-fitted contact lenses safely for a period of 12 months it was necessary to fit them with the most comfortable and properly fitted contact lens. Randomising lens type could have resulted in drop-outs and potential health risk. Therefore, the duration of the study justifies this approach. Secondly, the compliance of subject is an important factor that should be considered. Providing free contact lenses could bias the results of OSDI and CLDEQ-8 questionnaires as inadvertently the Hawthorne effect might have been introduced (Foulks et al., 2013). However, the remaining objective measures of ocular physiology should be unaffected by this possibility. Also, the free supply of lenses aided attendance outcomes, following a systematic schedule, which made the study design more robust. Another

limitation of this study is a lack of control over temperature and relative humidity in the laboratory environment. Environmental changes related to seasons could not be avoided unless a strictly controlled environmental chamber is used. Conductivity-based tear osmolarity measure is temperature dependent (Stalh et al., 2012). Nevertheless, for each subject, the difference between the maximum and minimum temperatures across the time-course of the study were only 1.3 [°C] and 4.7 [°C], respectively. Differences in tear film osmolarity do not seem to correspond with changes in the laboratory temperature. Additionally, subjects were allowed to have an environmental adjusting period if they arrived to the laboratory directly from the outdoors. The season of the year could affect the subjects' subjective comfort assessment and one could expect a drop in questionnaires scores in the middle of the project – 6-month visits – taking place between April and June, when the heating season is finished. However, the drop in the comfort score was recorded only in Hy-fitted group for the CLDEQ-8 and it was not statistically significant.

Modern daily disposable soft contact lenses minimally affect ocular physiology, if they are properly fitted and used. The decrease in tear osmolarity may be attributed to healthier contact lens wearing habits, moderate wearing schedule, appropriate contact lens fit and control. Subjects were becoming more responsible knowing that they will be regularly checked by the eye care professional and a lack of adherence could get them excluded from participation. The majority of the subjects, before participating in the study, were using monthly and 2-weekly contact lenses and had limited knowledge about contact lens wear effect on the ocular health. A supposition can be made that a refit to daily disposable lenses, moderate wearing schedule and the simplified contact lens hygiene could have all contributed to this decrease in the assessed parameters. This highlights the importance of the role of the eye care professional not only as a contact lens prescriber but also as a

continuing educator. Also, although measuring tear film surface quality is not used routinely in practice, this study showed, that this measurement certainly adds an extra information, which may be valuable to assess long-term contact lens performance and add this examination as another routine test in practice.

V. SUMMARY

This project is part of the European Dry Eye Network (EDEN). The aims of EDEN consist of developing modelling tools to improve dry eye disease diagnosis and therapeutic alternatives. Since dry eye disease has been also associated with contact lens wear, this project has been assigned to examine ocular health during contact lens wear. Each project coordinates research and training collaboration among the network. Researchers who participated in the network have been trained in state-of-the-art methods necessary to studying dry eye disease through training courses, research and scientific exchange within and beyond the network. In spite of the three years' time limit including training courses and secondment, the longitudinal format was chosen due to the limited studies following such a design as it could be costly and time consuming. Another great advantage of this project was that the finance received was not restricted by any commercial sponsors and as a result any potential bias was avoided. Therefore, this project was a result of careful discussions, literature reviews and meetings over a period of a year to provide a solid pillar for the study design.

Clinical measurements such as visual acuity is used to provide information about the refractive error and its correction. This information then is used by spectacle and contact lens wearers to provide them with vision correction. However, it is essential to consider different approaches such as a detailed questionnaire to indicate the impact of those measurements on each individual as a whole and their quality of life (Pesudovs et al., 2006), as for some patients the contact lens corrective modality might be of paramount importance. Soft contact lenses, especially daily disposable CLs are becoming more common form of vision correction and have certain advantages in comparison with the spectacles. For example, the prismatic effects of ophthalmic lenses are not encountered by contact lens

wearers (Efron, 2017). Contact lens wearers mostly rely on their lenses for all day, every day for a variety of reasons. High prescriptions in spectacles can not only degrade the quality of vision through spherical aberration, improper frame adjustment or inaccurate pupillary distance measurements but also degrade a patient's quality of life in regards to how they are perceived by their peers with spectacles and also how a patient may subconsciously perceive himself.

In vivo measurements of ocular surface characteristics are not performed routinely in practice but they are important for a successful contact lens wear. Although there are crude traditional measurements such as fluorescein tear film break up time, which has been done routinely in practice, there is no non-invasive method being used to assess tear film physiology routinely. In addition, the invasive techniques used during that exam and fitting may subconsciously provide a patient with a reason to avoid the routine anterior eye health check, which could potentially exacerbate any issues they may have. With many professions today involving long hours if not a full workday in front of a computer screen with minimal blink rates, contact lens discomfort abounds. Eyes that could tolerate a full day of computer use in contact lenses or simply every day contact lens wear may require more monitoring. Non-invasive techniques to determine ocular surface health can be used to make ongoing ocular health examinations easier, faster and more convenient not only for the patient, but for the eye care professional as well. Potentially, this results in less chair time overall for the professional leaving more time for other patients during the course of the day. Non- invasive techniques could spell faster exams for the examiner and happier contact lens patients on the basis of helping to provide that ounce of prevention more often in a contact lens wearer's life, hopefully long before that pound of cure is ever needed. This study provides a better understanding of the relation between tear film and contact lens

wear and insight on tear film surface quality during long-term contact lens use. Although there is no ideal contact lens material that would fit all eyes, as hypothesised, this study demonstrates that modern daily disposable soft contact lenses minimally impact tear film and anterior eye surface physiology regardless of their material, if well fitted and assuming that a firm controlled regime is implemented by an advising optometrist.

Despite making every possible effort to set up a diligent protocol, some limitations existed and there are some aspects of the study that could have been done differently to establish a more solid protocol. For instance, masking the examiner during the contact lens fit to avoid bias. It stands to reason that there is still room for further research on comparing lenses with different geometrical characteristics.

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VII. APPENDICES

Appendix 1. Baseline visit evaluation sheet

Contact lenses to be removed and eye drops not used at least 3 days prior to evaluation

Subject code ☐☐☐☐ **Date:** ☐☐ / ☐☐ / ☐☐ **TIME:** ☐☐

At what time did the subject wake up? ☐☐ : ☐☐

Room Temperature: ☐☐.☐ **[°C/F]** **Relative Humidity:** ☐☐.☐ **[% RH]**

Number of blinks ☐☐ **/30s; Incomplete blinking noted? Yes / No;**

WHICH EYE (OD if Si-Hy was worn, OS if Hy was worn): ☐☐☐☐

DED Questionnaires: DEQ-5 score ☐☐ **OSDI** ☐ **Score** ☐ / ☐ / ☐

Answers ☐☐

TMH with OCULUS: ☐.☐☐ **[mm]**

TFSQ-NIBUT: NBC OD ☐ **OS** ☐**, SBC, OD** ☐ **OS** ☐**, OD** ☐**, OS** ☐

Tear Film Osmolarity: ☐☐☐, ☐☐☐, ☐☐☐

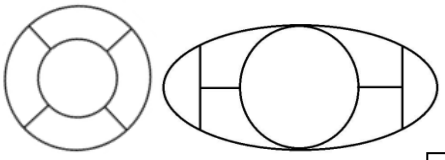
M-NIKBUT 1) ☐☐ **2)** ☐☐ **3)** ☐☐

F-NIKBUT 1) ☐☐ **2)** ☐☐ **3)** ☐☐

SLIT LAMP EXAMINATION OD ☐ **(Use the slit lamp protocol)**
Fluorescein Tear Film Break-up Time
OD: 1) 2) 3)
OS: 1) 2) 3)

Fluo imaging OD: ☐ **OS:** ☐

Ocular Staining (slit lamp protocol)



Conjunctival staining with LG ☐

Lid wiper staining: Upper ☐ **Lower** ☐

Meibomian gland imaging ☐ **Upper lid**, ☐ **Lower lid**

Appendix 2. Contact lens fitting evaluation sheet

Newly-fitted contact lens should be worn at least for 4 hours prior to afternoon visit

Subject code ☐ **Date: / / / Time**
Room Temperature [°C] Relative Humidity [%Rh]

Instruction about CL'S usage and hygiene signed, explained and understood ☐
Contact Lens Fit (morning visit) - Px will be fit with Si-Hy in OD and hydrogel in OS

FITTED LENSES ☐ OD ☐ OS

AFTER 4 HOURS of CLS wear

Time:
Choose the CL based on the fit, subjective rating comfort, slit lamp, TFSQ-NIBUT assessment and vision.

	OD	OS
Fit:		
Centration:		
Horizontal Lag:		
On Blink		
PU Test		
Vision		

Better fit ☐ **Better comfort** ☐ **Better fit** ☐ **Better comfort** ☐

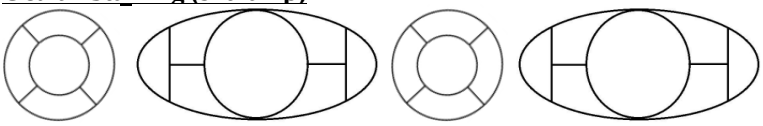
CONTACT LENS ASSESSMENT – VIDEO ☐ **OCULUS**
Assess and capture the subjective CL fit of the chosen lens
Primary gaze 3s. Temporally 3s, Nasally 3s
Blink in up gaze x3 waiting 3s after each; Push-up to mid cornea with lower lid and pull lid down as release x 3 waiting 5s after each.

TFSQ-NIBUT: NBC OD ☐ **OS** ☐**, SBC, OD** ☐ **OS** ☐**, OD** ☐**, OS** ☐

NIKBUT Oculus

M-NIKBUT	**OD:**	**1)**	**2)**	**3)**
	OS:	**1)**	**2)**	**3)**
F-NIKBUT	**OD:**	**1)**	**2)**	**3)**
	OS:	**1)**	**2)**	**3)**

Ocular Staining (slit lamp)



OD FBUT: .....[s] ….....[s] ….....[s] **OS:** FBUT:.....[s]….....[s].....[s]

Appendix 3. Evaluation sheet - 2-week follow-up

Subject code **Date: / /** TIME

At what time did the patient woke up? ☐☐:☐☐

Room Temperature ☐ **°C** **Relative Humidity** ☐ **%RH**

DED Questionnaire:

OSDI ☐ **Score** ☐/☐/☐, **DEQ-5** ☐ **Score** ☐☐

SLIT LAMP CONTACT LENS SURFACE QUALITY ASSESSMENT

Observations: Clean/ Debris Yes / No /Oily / Protein deposit

Other artefacts:

AFTER LENS REMOVAL:

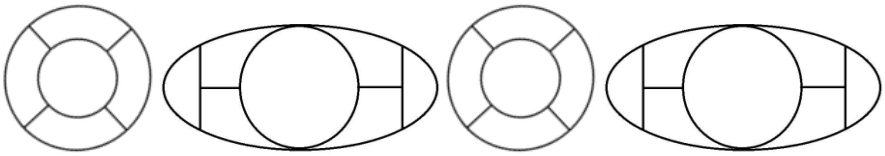
TEAR MENISCUS HEIGHT Oculus OD ☐ **OS** ☐

TFSQ-NIBUT: NBC OD ☐ **OS** ☐**, SBC, OD** ☐ **OS** ☐**, OD** ☐**, OS** ☐

***Instil fluorescein* - Ocular health examination (slit lamp)**

Ocular Staining (slit lamp)

OD, FBUT: .....[s] ….....[s] ….....[s] **OS,** FBUT: .....[s] ….....[s] ….....[s]



Observations:

Appendix 4. Dry eye symptoms questionnaires

Have you experienced any of the following *during the last week*:

	All of the time	**Most of the time**	**Half of the time**	**Some of the time**	**None of the time**	
1. Eyes that are sensitive to light?	4	3	2	1	0	**N/A**
2. Eye that feel gritty?	4	3	2	1	0	**N/A**
3. Painful or sore eyes?	4	3	2	1	0	**N/A**
4. Blurred vision?	4	3	2	1	0	**N/A**
5. Poor vision?	4	3	2	1	0	**N/A**

Have problems with your eyes limited you in performing any of the following *during the last week*:

6. Reading?	4	3	2	1	0	**N/A**
7. Driving at night?	4	3	2	1	0	**N/A**
8. Working with a computer or a bank machine (ATM)	4	3	2	1	0	**N/A**
9. Watching TV?	4	3	2	1	0	**N/A**

Have your eyes felt uncomfortable in any of the following situations *during the last week*:

10. Windy conditions?	4	3	2	1	0	**N/A**
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	**N/A**
12. Areas that are air conditioned?	4	3	2	1	0	**N/A**

1 Questions about EYE DISCOMFORT:

a. During a typical day in the past month, **how often** did your eyes feel discomfort?	**0** **Never**	**1** **Rarely**	**2** **Sometimes**	**3** **Frequently**	**4** **Constantly**

b. When your eyes felt discomfort, **how intense was this feeling of discomfort** at the end of the day, within two hours of going to bed	**Never have it** **0**	**Not at All Intense** **1**	**2**	**3**	**4**	**Very Intense** **5**	

2 Questions about EYE DRYNESS:					
a. During a typical day in the past month, **how often** did your eyes feel dry?	**0 Never**	**1 Rarely**	**2 Sometimes**	**3 Frequently**	**4 Constantly**
b. When your eyes felt discomfort, **how intense was this feeling of dryness** at the end of the day, within two hours of going to bed	**Never have it**	**Not at All Intense**			**Very Intense**
	0	**1**	**2**	**3**	**4** **5**
3 Questions about WATERY EYES:					
During a typical day in the past month, **how often** did your eyes feel dry?	**0 Never**	**1 Rarely**	**2 Sometimes**	**3 Frequently**	**4 Constantly**
4 Questions about IRRITATED EYES:					
During a typical day in the past month, **how often** did your eyes feel irritated?	**0 Never**	**1 Rarely**	**2 Sometimes**	**3 Frequently**	**4 Constantly**

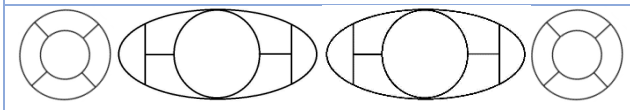
Have you had a previous clinical diagnosis of dry eye? Yes ☐ No ☐

Appendix 5. Medical history chart

Date (First visit):		Time:	
Occupation:		Px ID:	
Surname:			
Forename:			
Date of Birth:			
Telephone number:			
Refracting Correction: OD	VA	OS	VA
Distant Vision:			
Near Vision:			
General Health:			
Ocular Health:			
Medication:			
Allergies:			
Last Eye Examination:			
Last Medical Examination:			
Family Ocular History:			
Family Medical History:			
Driver:			
Visual Display Unit (TV, computer…) (how many hours per day):			
Hobbies:			
Smoker:			
Current Contact Lens history: OD: How often:		OS: How long:	
Comments			

Appendix 6. Slit lamp examination protocol

ID: **……** ***Date:*****……**

	OCULUS DEXTER	**OCULUS SINISTER**
TEAR FILM ***qualitative assessment***	Good / polluted / excessive lipid / watery / reflex tearing / artefacts / no artefacts / foam / other:	Good / polluted / excessive lipid / watery / reflex tearing / artefacts / no artefacts / foam / other:
TEAR MENISCI	Even / uneven / reflex tearing / low volume/ other:	Even / uneven / reflex tearing / low volume/ other:
BLINKING	Complete / forced / tic / lid inversion / other: …… INCOMPELTE BLINKING:	Complete / forced / tic / lid inversion / other: …… INCOMPELTE BLINKING:
LIDS AND LASHES	LIDS: no abnormalities / scaling / mucus / hyperaemia / redness / puss / oedema / frothy tear film / entropion / ectropion / pigmented lesions / thickening / other: EYELASHES: no abnormalities / ingrown / multiple / deposits / discharge / other:	LIDS: no abnormalities / scaling / mucus / hyperaemia / redness / puss / oedema / frothy tear film / entropion / ectropion / pigmented lesions / thickening / other: EYELASHES: no abnormalities / ingrown / multiple / deposits / discharge / other:
LID MARGIN *(normal: up to 6 glands obstructed with clear discharge)*	Lid margin: glands unobstructed / pus / oedema / frothy tear film / even / uneven / Meibomian glands obstruction / discharge / notched lid margin / other: MGD: 0 / 1 / 2 / 3 / 4	Lid margin: glands unobstructed / pus / oedema / frothy tear film / even / uneven / Meibomian glands obstruction / discharge / notched lid margin / other: MGD: 0 / 1 / 2 / 3 / 4
CORNEA AND LIMBUS	Clear / transparent / limbal vascularization / micro cists / vacuole / scars / oedema/ other: ……..…… LIMBAL REDNESS: 0/1/2/3/4 LIMBAL VASCULARIZATION: 0/1/2/3/4	Clear / transparent / limbal vascularization / micro cists / ulcers / vacuole / scars / oedema/ other: ……... LIMBAL REDNESS: 0/1/2/3/4 LIMBAL VASCULARIZATION: 0/1/2/3/4
FBUT	.....[s] ….....[s] ….....[s]	.....[s] ….....[s] ….....[s]
FLOURESCEIN STAINING SCORE		
DED DIAGNOSIS	At least two out of the following: ☐ OSDI ≥ 25 ☐ Conjunctival staining score ≥ 2 ☐ Corneal staining score ≥ 2 ☐ FBUT ≤ 7 s	**NOTES:**

Appendix 7. Instructions on contact lens care and wear

CONTACT LENS ADVICE

DO:

- Always wash your hand thoroughly before inserting, removing or handling your lenses and ensure hands are dry
- Remove lenses in the event of persistent irritation and contact us

DON'T:

- Sleep in your contact lenses,
- Lick your lenses or put them in your mouth,
- Use tissues or handkerchiefs to rub your lenses,
- Wear your lenses longer than advised,
- Wear your lenses if you think you may have an eye injury, infection or the lens might be damaged,
- Share your lenses with anyone else,
- Swim in your contact lenses,
- Wear for long plane journeys -they may dry out or you may want to sleep.
-

NEVER USE TAP WATER TO CLEAN YOUR LENSES!

Make-up advice:

- Apply make-up on after inserting contact lenses,
- Do not use mascara that flakes,
- When using hair spray, close your eyes to prevent it getting onto your lenses, or spray before inserting contact lenses,
- Do not share make-up tools with anyone and ensure they are not expired, as this could result in an infection.

With my signature I declare that I understand the abovementioned instructions and I am obliged to wear my newly-fitted contact lenses 5 days per week and not exceed 12 hours of daily contact lens wear

DATE:

Patients signature: ……..

(one signed copy for the subjects and one copy for the ESR)

Appendix 8. 3-month, 6-month and 12-month visit

Subject code Date: Time: Patient woke up at: and put the lenses on at:

Room Temperature °C/F Relative Humidity [%Rh]

OSDI ☐ **Answers:** ☐☐ **DEQ-5** ☐ **Score** ☐☐

WITH the contact lens on

NIKBUT Oculus

M-NIKBUT	**OD:**	**1)**	**2)**	**3)**
	OS:	**1)**	**2)**	**3)**
F-NIKBUT	**OD:**	**1)**	**2)**	**3)**
	OS:	**1)**	**2)**	**3)**

LENS REMOVAL

TMH with OCULUS: OD mm OS mm

TFSQ-NIBUT: NBC OD ☐ **OS** ☐**, SBC OD** ☐ **OS** ☐**, OD** ☐**, OS** ☐

Tear Film Osmolarity

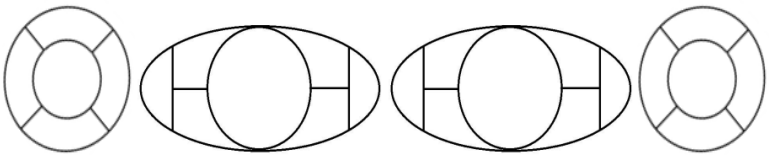
OD	**1)**	**2)**	**3)**
OS	**1)**	**2)**	**3)**

Ocular Redness OD: Bulbar (Efron Scale): Limbal (Efron Scale):
Temporal Nasal Temporal Nasal

Ocular Redness OD: Bulbar (Efron Scale): Limbal (Efron Scale):
Temporal Nasal Temporal Nasal

Ocular Staining (slit lamp)

OD + Fluo Image, FBUT: .....[s] ….....[s] ….....[s]



OS + Fluo Image, FBUT: .....[s] ….....[s] ….....[s]

Conjunctival staining with LG (primary gaze) ☐

Lid wiper staining: Upper Marx's line ☐ **Lower Marx's line** ☐

Meibomian gland imaging ☐ **Upper lid**, ☐ **Lower lid**

Appendix 9. Control visit evaluation sheet

Contact lenses to be removed and eye drops not used at least 3 days prior to visit

Subject code Date: TIME: At what time did the subject wake up?

Room Temperature: [°C/F] Relative Humidity: [% RH]

Incomplete blinking noted? Yes / No;

WHICH EYE (OD if Si-Hy was worn, OS if Hy was worn): ☐

DED Questionnaires: DEQ-5 Score ☐☐ OSDI Score ☐☐

TMH with OCULUS: ☐.☐☐ [mm]

TFSQ-NIBUT: NBC OD☐ OS☐, SBC, OD☐ OS☐, OD☐, OS ☐

Tear Film Osmolarity: ☐☐☐, ☐☐☐, ☐☐☐

M-NIKBUT	**1)**	**2)**	**3)**
F-NIKBUT	**1)**	**2)**	**3)**

Ocular Redness: Bulbar (Efron Scale): Limbal (Efron Scale):

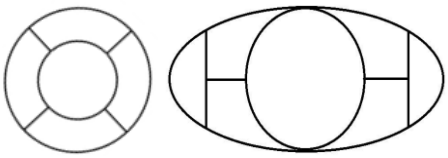
Temporal	Nasal	Temporal	Nasal

SLIT LAMP EXAMINATION OD ☐ (Use the slit lamp protocol)

Fluorescein Tear Film Break-up Time 1) 2) 3)

Ocular Staining (slit lamp protocol)

Fluo Image, FBUT: .....[s] ….....[s] ….....[s]



Conjunctival staining with LG (primary gaze) ☐

Lid wiper staining: Upper Marx's line ☐ Lower Marx's line ☐

Meibomian gland imaging: Upper lid, ☐ Lower lid ☐

VIII. LIST OF FIGURES

IX. LIST OF TABLES

X. SCIENTIFIC CONTRIBUTIONS RELATED TO THE THESIS

The scientific achievements resulting from this thesis were published in either scientific journals or international conference proceedings. All scientific contributions were peer-reviewed and are indicated in the following two subsections.

Journal Articles

[J1] **Mousavi M**, Garaszczuk IK, Szczesna-Iskander DH, Iskander RD. Ocular physiology during the wear of modern daily disposable soft contact lens. PLOS ONE, 2018 [In second round review].

[J2] **Mousavi M**, Jesus DA, Garaszczuk IK, Szczesna-Iskander DH, Iskander RD. The utility of measuring tear film break-up time for prescribing contact lenses, Contact Lens and Anterior Eye, 2017; 1:105-9.

[J3] Llorens-Quintana C, **Mousavi M**, Szczesna-Iskander DH, Iskander DR. Non-invasive pre-lens tear film assessment with high-speed videokeratoscopy. Contact Lens and Anterior Eye, 2017; 1:18-22.

[J4] Garaszczuk IK, **Mousavi M**, Cerviño Exposito A, Bartuzel MM, Montes-Mico R, Iskander DR. Tear Clearance Rate evaluation with optical coherence tomography. Contact Lens and Anterior Eye, 2017; 1:54-9.

Conference Proceedings

[C1] **Mousavi M**, Garaszczuk IK, Szczesna-Iskander DH, Iskander RD. Changes in tear film physiology during soft contact lens wear. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2018, Honolulu, United States.

[C2] Garaszczuk IK, **Mousavi M**, Montés-Micó R, Cerviño EA, Iskander DR. Changes in the lower tear meniscus morphology during contact lens wear. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2018, Honolulu, United States.

[C3] **Mousavi M**, Jesus DA, Garaszczuk IK, Szczesna-Iskander DH, Iskander DR. The Utility of Measuring Tear Film Break-up Time for Prescribing Contact Lenses. The 2018 Marie Curie Alumni Association (MCAA) Conference and General Assembly, 2017, KU Leuven, Belgium.

[C4] Jesus DA, Llorens-Quintana C, **Mousavi M**, Iskander DR. A new perspective about the corneal structure based on Optical Coherence Tomography speckle. 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 2016, Florida, United States.

[C5] **Mousavi M**, Garaszczuk IK, Jesus DA, Szczesna-Iskander DH, Iskander DR. The impact of daily disposable soft contact lens wear on tear film surface quality over a three-month period. Acta Ophthalmologica, Publication date 2017/9/1.

[C6] Garaszczuk IK, **Mousavi M**, Cerviño EA, Szczesna-Iskander DH, Iskander DR, Jesus DA. Scleral radius estimation based on anterior eye surface. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2017, Baltimore, Maryland, United States.

[C7] Jesus DA, Garaszczuk IK, **Mousavi M**, Iskander DR. Influence of IOP fluctuation on corneal micro-structure. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2017, Baltimore, Maryland, United States. Research in Vision and Ophthalmology (ARVO) 2017, Baltimore, Maryland, United States.

[C8] Llorens-Quintana C, **Mousavi M**, Szczesna-Iskander DH, Iskander DR. Assessing the morphology of high-speed videokeratoscopy recordings for the evaluation of tear film surface quality in contact lens wear. The Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2016, Seattle, United States.

Oral Presentations

[O1] Relative performance of well-fitted hydrogel and silicone hydrogel contact lenses, British Contact Lens Association (BCLA) Clinical Conference 2017, 9-11 June 2017, ACC Liverpool, UK.