# SUMMARY (ENGLISH)

The following dissertation is a far-reaching work with the purpose to find a new macro-type biomarker that could be objectively and non-invasively measured as an indicator of DED, specifically at its early stage. Attempting to achieve this goal, it proposes new metrics of tear dynamics quantification. By tracking visit-to-visit longitudinal trends of these measures over a period of one year, it explores their role in supporting DED diagnosis in subjects reported with incipient symptomatology.

The hypothesis behind developing the new macro-type biomarkers of DED is that the *loss of homeostasis of the tear film*, which describes the core pathophysiological mechanism of DED, may not only be expressed as disturbed tear film morphology, but also by a lack of equilibrium between hydro-dynamic processes occurring in the tear fluid or tear menisci. These phenomena are in a state of equilibrium regulated by the lacrimal functional unit (LFU). A disturbance of this subtle balance may ultimately lead to DED. Based on the abovementioned observation, the tear clearance rate (TCR) and tear turnover rate (TTR) were chosen as the potential macro-type biomarkers of DED. The multifactorial nature of the disease can be expressed by TCR or TTR, as they consider all the hydrodynamic phenomena occurring in the tear fluid and were shown to perform well in DED differential diagnosis.

Several ocular measures that could be interpreted as potential quantifiers of TCR or TTR, have been developed. Further, several existing ocular measures have been identified as potential DED biomarkers. These measures and quantifiers can be non-invasively assessed and objectively analysed and thus are appropriate to fulfil DED definition and, what is most important, can be assessed in a clinical setting. This includes tear osmolarity, meniscometry,

tear film stability assessment with non-invasive, objective technique and TCR estimation with optical coherence tomography (OCT).

This dissertation is divided into two major parts: ***Experimental part*** and **the *longitudinal study of biomarkers' trends*.** The experimental chapter (***Chapter II***), in a form of three separate experiments, proposes new methodologies for tear dynamic quantification. The second part (***Chapter III***) describes the one-year-long longitudinal study, which was arranged to follow the above-mentioned visit-to-visit biomarkers' trends.

It is of interest to develop simpler and, by extension, more clinically applicable methodologies for TCR quantification. Techniques described in ***Chapter II*** can be used to follow, analyse and quantify different aspects of tear fluid dynamics. Temporal measures of TCR and TTR were reported in several studies as markers of the integrity of the LFU and tear exchange on the ocular surface. These measures have the potential to become the new macro-type biomarkers of DED. Considering the probable applicability of TCR measurements in supporting DED diagnosis, this dissertation proposes such new solutions. It describes two alternative methodologies for TCR assessment - one that utilizes the newly-developed device for corneo-scleral topography to follow tear fluorescence intensity decay after fluorescein instillation (***Experiment 1***) and the latter, which allows estimation of an early-phase TCR (***Experiment 3***) with the use of spectral-domain OCT-based dynamic meniscometry algorithm proposed in the ***Experiment 2***.

To observe temporal, longitudinal changes in ocular physiology (***Chapter III),*** subjects were fitted with modern, daily disposable Silicon Hydrogel (SiHy, Delefilcon A) or Hydrogel (Hy, Omafilcon A) soft contact lenses. Free supply of lenses aided attendance outcomes and ensuing a systematic schedule, which made the study design more robust and has minimized the number

of drop-outs. The clinical part of the study lasted for 12 months. The hypothesis driving this longitudinal study was that contact lens wear will somehow impact ocular physiology over the period of 12 months, so the biomarkers' trends can be observed. The longitudinal study protocol consisted of:

- qualifying visit (Baseline visit);
- contact lens fitting visit (following day - 'Day 2' visit);
- contact lens fit control at two weeks after refitting;
- follow-up visits at three months, six months and 12 months post-refitting;
- post-study assessment after three days (Control visit).

Laboratory temperature (°C) and humidity (%RH) were monitored. The most appropriate ocular measures to fulfil the definition of DED and its sub-classifications were chosen from the ones that could be estimated in a clinical setting. Thus, the protocol of measurement included chronologically:

- Ocular Surface Disease Index (OSDI) evaluation and calculation;
- 5-Item Dry Eye Disease Questionnaire (DEQ-5);
- tear meniscus height (TMH) measurements with Oculus Keratograph 5M (K5M);
- tear osmolarity measurements acquired from the inferior tear meniscus with TearLab Osmolarity System;
- non-invasive tear film break-up times (NIKBUT) assessment with K5M;
- slit lamp anterior eye examination.

Subsequently, ocular measures proposed in the experimental part were used as additional DED biomarkers, including:

- dynamic meniscometry (tear meniscus height, depth and area estimation) assessed with OCT;
- TCR assessment based on dynamic tear meniscus morphology

and was followed by:

- ocular surface and lid wiper staining with lissamine green and fluorescein;
- infrared meibography recording with K5M.

Measurements were performed with objective, automated and clinically applicable methods, using infrared radiation and non-invasive alternatives to traditional measures, whenever possible.

The methods for assessing tear dynamics were proven to be not time-consuming, easy to perform and clinically applicable. Fluorescein profilometry (***Experiment 1***) can be used to follow subtle, dynamic changes occurring in the tear film on the whole exposed corneo-scleral surface and the measurements are not limited by corneal permeability to fluorescein. The method was also reported repeatable. A custom-written software allowing dynamic meniscometry (***Experiment 2***) was proposed to enhance the precision of tear meniscus morphology measurements with OCT, by minimizing the effect of tear meniscus nonconfluence after each blink on the reported estimations. OCT can be used as a rapid, qualitative and quantitative method of determining tear meniscus parameters and TCR. With this new algorithm, tear meniscus parameters can be calculated more precisely. It was also observed that the dynamic meniscometry method may provide clinicians with different information than the meniscometry performed based on static, single OCT B-scan.

OCT-based measurements of early-phase TCR (***Experiment 3***) are non-invasive, relatively rapid and simpler to perform than the traditionally used tear exchange tests. OCT allows more in-depth visualization of tear menisci and TCR observation.

Fifty-five subjects participated for the whole duration of the longitudinal study. The group mean age was (mean ± standard deviation) 26 ± 4 y/o and was ranging from 20 to 37 y/o. Based on the contact lens fitting procedure, 38 subjects (25 females and 3 males) were fitted with Silicone-Hydrogel (SiHy) and 17 subjects (11 females and 6 males) were fitted with Hydrogel (Hy) daily disposable soft contact lenses.

Since there were no statistically significant differences noted between right and left eye of each subjects and between SiHy and Hy-fitted group or the group of males and females in any of the assessed ocular measures, the group of subjects was unified into one cohort and temporal changes of biomarkers' trends were analysed for the whole study group. Non-parametric two-way ANOVA showed statistically significant temporal trends in OSDI and DEQ-5 in subjects with incipient symptomatology and temporal changes in tear osmolarity, non-invasive objective measures of tear film break-up time (M-NIKBUT and F-NIKBUT), tear meniscus height assessed with dynamic meniscometry, TCR and in staining with vital dyes, lid wiper epitheliopathy (LWE) scores, corneal thickness and quantification of Meibomian gland drop-out.

Statistically significant difference between Baseline and Control visit was not noted in some of these measures, suggesting that the temporal changes induced by contact lenses could be short-term. No statistically significant differences were noted in bulbar and limbal redness scores, which may suggest that changes observed in the study are not of inflammatory nature.

Over the time-course of the study, a gradual decrease of tear clearance and tear osmolarity was observed. Tear meniscus area significantly negatively linearly correlated with tear osmolarity. Additionally, dynamic meniscometry method was proven to be sensitive enough to reveal potential changes in ocular surface shape.

This dissertation proposes new metrics of tear dynamics quantification and explores the pathophysiological role of several ocular biomarkers of tear homeostasis. By tracking visit-to-visit trends of several potential biomarkers over the period of one year, it shows that tear osmolarity may be used to track slight changes in tear film physiology. Changes in tear osmolarity corresponded with the beneficial effect of contact lens wear on the tear film both in habitual, symptomatic contact lens wearers and in subjects initially reported as asymptomatic. All changes in tear osmolarity over a period of one year were statistically significant. Additionally, meniscometry based on dynamic changes of tear meniscus height seemed to respond to very subtle changes in tear meniscus parameters over the time-course of the study. These changes were additionally expressed as changes in OCT-based TCR.

Summarizing, this dissertation proposes new methods of tear clearance and turnover assessment and shows that tear osmolarity, TCR and dynamic meniscometry measures could be used as potential biomarkers for supporting DED diagnosis, that are sensitive enough to follow the progression of subtle ocular changes in time and response to effective therapy.