The handle http://hdl.handle.net/1887/65534 holds various files of this Leiden University dissertation.

**Author:** Raus, P.P.M.
**Title:** Innovative strategies to clinically characterize the human tear proteome: from fundamental exploration to ophthalmological relevance
**Issue Date:** 2018-09-04
Summary

“Innovative strategies to clinically characterize the human tear proteome; from fundamental exploration to clinical ophthalmological relevance”

Ophthalmologists are seriously concerned about the chronic and frequently progressive course in many patients of a problematic and possibly vision-threatening chronic condition known as Dry Eye Disease (DED) [DEWS, 2017, Chapter 2]. This multifactorial ophthalmologic disorder is known to affect a large number of people. Prevalence of DED is reported to vary from 7% in the United States to 33% in Taiwan and Japan. A grade 1 or mild DED condition may even be observed in 100% of patients over 60 years of age [Shah et al., 2015].

DED may have many causes, and, as such, occurs in various forms and degrees of severity [Murube et al., 2005 & Chapter 2]. Currently, in a majority of cases no clear diagnosis can be made. As a consequence, many patients are not effectively treated and their condition does not improve or even worsens.

In recent years, several lines of clinical as well as molecular and cell biological research have yielded relatively limited novel insights into cause and progression of DED [Olligris et al., 2014]. Clinical research has largely focused on the classification of various disease grades. Extensive pathophysiological studies including the cellular and molecular alterations in DED pathogenesis are rare [Wei et al., 2014].

One of the issues, which we feel to be hampering progress in the field of DED, is the apparent difficulty for clinicians and cell and molecular biology research scientists to find, let alone understand, each other.

This thesis wants to contribute to the noble goal of bridging the gap between clinical practice in the field of DED and present day (as well as future) bio-analytical research science. In particular, we elected to focus on the analytical discipline of proteomics, which complements other “omics” approaches, including genomics, transcriptomics and metabolomics. As such we attempt to help establish an integrated approach of clinical and bio-analytical protein research, to further our understanding of DED causes and progression.

Chapter 1 introduces the field of DED, by describing some fundamental knowledge of the disease, its diagnosis and standard treatments. We focus on the status praesens concerning clinical knowledge of DED, including several recent developments in diagnostics and treatment. Besides the assessment of a current practicing ophthalmologist, also the vision of an analytical molecular biologist as well as the view of a contemporary patient is given.

Chapter 2 includes a review on radiofrequency surgery and elaborates on various aspects of our earlier personal eye surgical practice, which were instrumental (sometimes literally, as in Chapter 2.4) in our later development of innovative DED treatments.

Chapter 3 continues by describing the various innovative treatments which we introduced in our personal practice treating DED patients. This includes the so-called ‘Chedly punctal Occluder’ [Chapter 3.1], and the, in our eyes, most important example in this respect, i.e. the
first transplantation in Belgium (one of the first in Europe) of labial salivary glands to the conjunctival side of the eyelids [Chapter 3.2].

Initial mass-spectrometric analyses and (proteomics) comparison of tears and saliva from healthy individuals revealed the different protein compositions of tears and saliva, confirming a high degree of similarity between both ‘secretomes’ [Chapter 4], as previously suggested by Murube et al. [1998].

As such, Chapter 4 of this thesis constitutes the first effective pillar of our bridge between clinical and molecular (protein) biological understanding of DED.

Throughout our thesis work we tried to keep track of the numerous analytical and technological developments in the field of proteomics, and we got more and more convinced of the huge potential modern mass spectrometry (MS) approaches in the analysis of tears have for continued research leading to improved diagnostics for DED and to novel insights into DED pathogenesis.

As such, Chapter 5 reports on our first use of an orbitrap equipped MS system. This work was the first published record that proteomics analysis of human tears can be successfully and reproducibly performed on material from a single (DED) patient.

Chapter 6 summarizes a set of current ongoing research, in which we set off to explore the potential which the recently made available top-down proteomics tools (both hardware as well as software) are bringing to the field. The preliminary results which we have obtained so far are overwhelming. These include a number of novel developments and improvements of top-down mass spectrometric analysis of the tear proteome for future use in the clinical practice. It describes innovative approaches for the full identification of tear proteins with all the post-translational modifications present on them, i.e. the actual tear ‘proteoforms’.

As such we are confident to conclude (Chapter 7) that (top-down) MS will live up to its promise of being a very valuable analytical tool to further our understanding of DED pathophysiology, enabling the discovery of innovative diagnostics as well as therapeutics for DED. At the end of this thesis, we give our personal view on future research directions and novel clinical developments (including MS applications) in DED.

We prophesize that the top-down proteomics study of tear proteins (‘tear proteoforms’) will represent an innovative approach to help evaluate the general health and disease state of, not only, DED patients, but of patients suffering from a wide variety of metabolic, age-related, and other diseases which characterize our modern society.

References:
