Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

Project

Development of an ex vivo dry eye model as an alternative to animal testing in pharmacological screenings

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Dry eye syndrome (DES) is one of the most prevalently encountered ocular diseases. It is caused by a disorder of the corneal tear film, either by an inadequate amount of the tear fluid or by a disturbance within the composition of the tear film. This disease leads to the symptoms of discomfort, stimulation of inflammatory processes on the ocular surface and, in severe cases, strong disturbance of vision. To date, most clinical and pharmacological studies targeting DES are based on *in vivo* animal models, e. g. rabbits, rats, mice, cats, monkeys and dogs. Here, mechanical removal of the main lacrimal gland, lymphocyte infiltration in the lacrimal gland, mechanical prevention from blinking and pharmacological suppression of tear segregation are used among other methods to model DES. Besides ethical concerns and subsequent legal restrictions for these animal models, the animal models suffer from highly limited reproducibility caused by inter-individual differences.

The aim of this project is to enhance a novel dry eye model system for the reduction and replacement of animal experiments during the development of new pharmaceutical products. Our new model system is based on the so called “Ex Vivo Eye Irritation Test” (EVEIT), which is a self-healing culture system using living corneas obtained from abattoir rabbit eyes. This test system is combined with high-resolution optical coherence tomography (OCT) as a monitoring tool to observe spatial and temporal structural changes on the cellular level.

Recent results indicate that DES can be simulated using enucleated rabbit eyes from abattoir refuse. The simulation model is based on increased evaporation of the simulated tear film. Alternating evaporation and lacrimation intervals induce a permanent imbalance in osmolarity of the tear film. Corneal thickness oscillations appear which result from the alternating osmolar conditions due to evaporation and lacrimation.

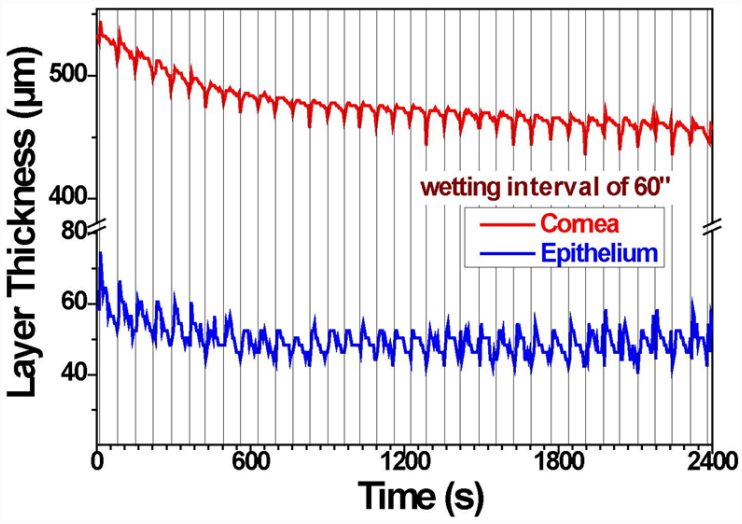

Fig. 1: Quantitative OCT analysis of the corneal layer thicknesses under simulated dry eye conditions. The layer thicknesses of the cornea and epithelium are quantitatively analysed by optical coherence tomography (OCT) using a wetting interval of 60 s. The vertical lines indicate the current moments of corneal wetting as derived from the OCT data.

The mechanical stress caused by these osmotic forces leads to a structural change in the cornea typical for DES. These structural changes can be quantified non-invasively by OCT imaging, which allows for the modeling of different severities of the dry eye syndrome and reproducible corneal damage. However, so far a healing process of dry eye induced damages has not been demonstrated in vitro, which is the most important parameter for evaluating new pharmaceutical products without animal experiments.

Within this project, our previously established damage model will be transferred to a novel corneal culture system. This allows for combining our prior simulation model with long-term *in vitro* culture techniques, resulting in a novel universal alternative test platform. The prevention and treatment of the dry eye syndrome on living tissue can be quantified and analyzed – for the first time - *in vitro*. Particularly, this crucial step will give researchers the possibility to observe healing of dry eye induced damages without animal tests. After a successful implementation of the model system, its applicability will be demonstrated exemplarily by comparing and evaluating different commercial tear-replacement solutions. It is expected that this tool will become an essential contribution for the reduction of animal experiments in pharmaceutical and scientific studies in the context of the dry eye syndrome. Thereby it is anticipated that a successful implementation will overcome the limitations of state-of-the art *in vitro* test procedures.

Institution

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ACTO is an associated research of the RWTH Aachen University. ACTO has developed the EVEIT test for non-animal testing of chemicals and cosmetics, and is currently preparing the test system for pre-validation.

Duration

01.07.2011 – 31.12.2011

Project manager

Dr. Felix Spöler

Diploma in Chemistry in 2001, PhD at the RWTH Aachen University in 2008. Now post-doc at the Institute of Semiconductor Electronics (IHT) at the RWTH Aachen University. Current focus: Novel applications of optical coherence tomography (OCT) in the field of organ culture as well as alternative methods for pharmacology and toxicology.

Prof. Dr. Norbert Schrage

Study of Medicine in Cologne and Aachen 1982-1989. Fellow of the German Research Foundation 1990-1992. Dissertation in 1990, Habilitation 1996 under Prof. Dr. M. Reim. Senior physician under Prof. Kirchhof (1995 to 2000). Acting director of the Dept. of Ophthalmology at the university Hospital Aachen 2001-2003. Now acting Director of the eye clinic Cologne-Merheim.

In 1998 he founded ACTO, since then he is acting as its chairman.

Team

Dr. Stefan Kray

Diploma degree in Computer Engineering from the University of Siegen, Germany, PhD at RWTH Aachen University. Since 2006 post-doc at the Institute of Semiconductor Electronics (IHT) at the RWTH Aachen University, Germany. He develops technical advances for OCT with a focus on ultrahigh resolution simultaneous dual-band systems and three dimensional imaging *in vitro* and *in vivo*.

Oya Kray, M.Sc.

B.Sc. in Chemistry from Istanbul Technical University, Turkey, M.Sc. in Advanced Materials from Ulm University, Germany. Now scientific assistant at the Institute of Semiconductor Electronics (IHT) at the RWTH Aachen University and at the Aachen Centre of Technology Transfer in Ophthalmology (ACTO). She investigates and develops novel organ culture techniques - including dry eye simulations in vitro - in combination with non-invasive analysis by optical coherence tomography.

Claudia Panfil, Dipl. Biol.

Trained as medical-technical assistant, diploma in Biology from the RWTH Aachen University. Now Technical Head of the laboratory work areas and workshop at the Aachen Center of Technology Transfer in Ophthalmology (ACTO). She works on design, implementation and monitoring of novel organ culture techniques.