Perspiratio Insensibilis of the Cornea

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Introduction

How does the cornea lose its water? This fundamental question on the homeostasis of the cornea is investigated in this project. The corneal water content is about 70-80% (Kampa et al. 2002) [1]. This specific hydration is crucial for the optical transparency of the corneal stroma (Ehlers et al. 1966) [2]. Furthermore, there is a need of the correct composition of collagens and proteoglycans. Under physiological conditions of closed epithelium with tight junctions water mainly enters the corneal stroma via the endothelium, very little amounts of additional water derive from tears and from the scleral base. As soon as the pressure in the eye increases or any layer of the cornea become dysfunctional, the corneal water starts to grow up (Ehlers et al. 1967) [3]. The former research found that semi-permeable layers and the active transport of water are essential for maintaining corneal transparency (Kuerten et al. 2015) [4]. Corneal opacities with intact active transport of the endothelium are typically caused by endothelia injuries which result in an increased water inflow into the corneal stroma. The same mechanism combined with intra-epithelial edema can be seen in acute glaucoma when the intraocular pressure is >= 70 mmHg and water is physically pressed into the corneal stroma (Ehlers et al. 2004) [5]. This becomes obvious when the intraocular pressure exceeds 70 mmHg (Ytteborg et al. 1965) [6]. Another way of corneal hydration imbalance is, finally, the opening of the scleral entry port and submersion in MEM for corneal grafts in the European corneal culture system, which leads to

Abstract

Introduction: The deswelling of the cornea via the natural pathways of perspiratio insensibilis and endothelial pump has been known for a long time. However, reliable data on the amount of perspiratio insensibilis, especially in edematous corneas, have been lacking until now.

Material and Methods: In the Ex Vivo Eye Irritation Test (EV-EIT), corneas can be observed purely physically under defined and stable biochemical conditions. The thickness and temperature of the corneas is measured by infrared thermography and Optical Coherence Tomography (OCT).

Three corneas in stable MEM EVEIT culture and three corneas with induced and reversible edema in an EVEIT osmolar deficiency culture were placed in at 20°C and 60% humidity while tracking thickness and temperature for 30 minutes.

Results: Over a period of 30 minutes, the temperature of both, healthy and edematous EVEIT corneas decreased from an initial 28°C to 21°C. The corneal thickness decreased by 51 ±12 µm in the healthy corneas and by 131 ±16 µm in the edematous corneas.

Conclusion: The considerable thickness differences between healthy and swollen corneas might give a physical and physiological explanation of some observable clinical effects in Fuchs’s endothelial dystrophy. The clinically observed effect of clearing of the cornea during the waking period. Furthermore, some phenomena of Dry Eye Syndrome (DES) can be explained by this difference of increased water release from the eye.

Keywords: Cornea; Dry Eye Syndrome; Perspiratio Insensibilis; Fuchs’s endothelial dystrophy.
considerable swelling of the corneas with the need of deswell-
ing (Lindstrom et al. 1990) [7] before grafting either with dex-
tran (Reim et al. 2008) [8] or as in the American system, where
chondroitin sulphate is added

Our objective in this was to find out, to which end the grafting,
performed to complete this clinical quality control. The exact
conditions have been published previously (Frentz et al. 2008)
with GOD-PAP and LOD-PAP; Greiner Diagnostic GmbH, Bahlin-
gen, Germany) in the medium passed through the chamber to
with cobalt blue light to demonstrate a fully intact epithelium.
The surface was stained with sodium fluorescein and illuminated
to evaporation from skin, mucous membranes, alveolar surfaces,
and other surfaces. In this article we try to quantify the loss of
water over the cornea measured by the loss of thickness [11].

An infrared camera (Seek-Thermal (Seek Thermal, Inc. 6300
Hollister Ave Santa Barbara, CA 93117.), which is controlled via
an i-Phone12 mini (Apple, Apple, One Apple Park Way, Cuper-
tino, CA 95014) was used for the thermography. For the touch
free measurement of corneal thickness, we used an OCT from
Thorlabs. Device: Ganymed Spectral Reader, Thorlabs HL GmbH,
Münchner Weg 1, Bergkirchen, Germany).

Healthy EVEIT Cornea in Standard Culture

We took 3 rabbit corneas directly from the animal slaughter
and cultured them within 4 hours. The eyes were pre-cultured in
serum-free culture with Minimal Essential Medium (MEM) in
the EVEIT chamber system and treated for 24 h in the incubator
under EVEIT culture conditions. Subsequently, the thickness of
the corneas was measured by OCT. For vitality and quality con-
trol a glucose lactate measurement in the culture medium was
performed. This must contain minimum criteria of more than 2
mmol glucose/l and more than 1.5 mmol lactate/l (measured
with GOD-PAP and LOD-PAP; Greiner Diagnostic GmbH, Bahlin-
gen, Germany) in the medium passed through the chamber to
work with the corneas. Furthermore, the corneal surface was stained with sodium fluorescein and illuminated with cobalt blue light to demonstrate a fully intact epithelium. Complete transparency of the cornea was measured by OCT measurements set-up enables a temperature /

Corneal Edema in the EVEIT Cornea by Osmolar Deficient
Culture Medium

In another experiment, we cultured 3 additional EVEIT rab-
bit corneas that were vital and fully epithelialized as described
above. After 24 hours in the regular medium, we changed the
MEM with a hypoosmolare swelling medium (MEM + 0.3%
saline solution 1:4). According to the experiments by Panfil et
al., these corneas (Dutescu et al. 2015) [13] showed uniform
increase in corneal thickness under culture conditions in the
incubator at 32°C temperature and 99% humidity.

Sequence of Measurements

All corneas were removed from the incubator at 99% humid-
ity and 32°C and immediately covered with metal lids to main-
tain temperature and vapor pressure stable for a short time.
When all measuring equipment was organized after less than
2 minutes the covers were removed, and the thickness of the
corneas was measured by OCT. During OCT measurements
the temperature of the apex cornea was measured at 20°C room
temperature and 60% humidity by infrared thermography cam-
ara.

Both sets of measurement were performed in intervals of
6 minutes. This measurement set-up enables a temperature /
corneal thickness diagram (Figure 5).

Results

For healthy corneas under normal culture medium, there is a
maximum change in thickness of 51.1 µm (Figure 3). Figure 4
shows a considerable swelling of the corneal stroma to thick-
nesses around 760 µm (±58 µm) in EVEIT corneas in the defi-
ciency medium culture. While the corneal temperature drops
from 28°C to 21°C as in healthy corneas, the corneal thickness
changes for a total of -138 µm (±16) over 30 minutes at 20°C
room temperature. There seems to be a considerable differ-
ence in evaporation for healthy and swollen corneas. Com-
puting the volume change we see -0.00578 ml in healthy and
-0.015 ml in swollen rabbit corneas with a mean diameter of 12
mm. The difference is considerable and cannot be explained by
temperature and vapor pressure which were similar. Under the
same environmental conditions (temperature and humidity),
the swollen corneas lose more than 2.5 times of the volume
compared to healthy corneas in the same culture and condition.
This shows that a swollen cornea is significantly more affected
by evaporation than a non-swollen cornea.
Figure 2: Ex vivo Eye Irritation Test (EVEIT) Chamber with rabbit cornea, left directly after opening the metal lid. Temperature of the chamber 30°C of the apex cornea 28°C, right 15 minutes later with clear cooling of the apex cornea to 23°C, the chamber itself is still 29°C (red ring).

Figure 3: Healthy EVEIT cornea taken in culture (without swelling). The cornea initially stands for 30 min at 20°C room temperature and 60% humidity. The corneal thickness decreases continuously. With a mean initial thickness of all corneas (n=3) of 558 µm (standard deviation (Std) ±22 µm), the mean decrease over 30 min was 51.1 µm (Std±12 µm). At t>30 min the ambient temperature was lowered to 9 °C and 40% humidity. This then leads to a significant increase in corneal thickness if the surface continues to lack wetting.

Figure 4: EVEIT corneas in the deficiency medium culture show a clear swelling of the corneal stroma. The average thickness at the beginning of the experiment was 760 µm (±58 µm). The change in corneal thickness was -138 µm (±16 µm) over 30 minutes at 20°C room temperature and 60% humidity.

Figure 5: The thickness changes of the healthy cornea are approximately linear in relation to the temperature change, whereas the thickness change of the swollen corneas shows hyperbolic dynamics. Using 3rd degree polynomials, the corneal thicknesses can be mapped against temperature with a good correlation (dashed lines).

Discussion
In the following we want to distinguish between swollen and healthy corneas in terms of perspiratio insensibilis under culture conditions of the EVEIT. Furthermore, a clinical example is made based on physical principles.

Volume Loss
As described above, we observe a difference in the amount of water evaporating from healthy and swollen corneas. One possible explanation for the high evaporation rate in swollen corneas is a less stable epithelium with increased permeability to water. This is suggested by the work of Kinoshita et al. 1995 [14] who found a 4-fold higher fluorescein permeability of the epithelium in patients with corneal stromal edema and an almost 100-fold higher permeability of the epithelium in patients with epithelial edema. Another possible explanation of this difference is the reduced colloidal binding of excess water in the anterior stroma resulting in a facilitated and higher transfer of water towards the epithelium. At the same time, increased permeability of the epithelium results in a water shift with high water loss in the anterior stroma of the cornea (Baudouin et al. 2013) [15].

All these differences in deswelling readily explain the typical improvement in vision and decrease in corneal edema in Fuchs’ dystrophy of the cornea over the day. Furthermore, this explains the phenomena of anterior stromal damage in Dry Eye Syndrome (DES) (Messmer et al. 2015) [16]. When looking at the change in corneal thickness versus temperature change, the graph shown in Figure 5 is obtained for healthy versus edematous corneas. There is a significant difference between the two curves, with a swollen cornea losing twice the thickness of a non-swollen cornea at a temperature change of 6°C and almost three times the thickness of a non-swollen cornea at 7°C. This also indicates a significantly higher change in corneal thickness. This also indicates a higher susceptibility of the edematous corneas to evaporation. Furthermore, the temperature drops are steeper due to the increased evaporation. This can be impressively demonstrated in this system of a uniform culture chamber, under stable ambient conditions with defined humidity. With the standard enthalpy of water, this volume loss can be converted into an amount of energy. To evaporate a volume of 0.015 ml requires an energy of 0.015 ml * 0.832 *10^-3 mol at a standard enthalpy (25°C, isobaric) of 43.99 kJ / mol H₂O and at a molecular weight of water of 18.01528 g/mol. Accordingly, an energy of 36.6 joules is needed to evaporate this amount of water. In the healthy cornea, this is only 14.11 joules. Conversely, this observation explains the stronger cooling of the edematous cornea.
Transferred to the patient with DES or Fuchs’s endothelial dystrophy, this results in an earlier trigger for blinking (Schrage et al. 1997) [17] due to the faster cooling of the cornea. This leads to the typical premature eyelid closure in DES (Ding et al. 2021 and Bron 2021) [18,19]. The increased energy required for vaporization of water also explains the typical overheating and reddening of the eyes in DES, as this more than double amount of energy cannot be provided by aqueous humour activity alone. A combination of premature eyelid closure and hyperemia brings in enough blood to maintain the temperature of the cornea.

Clinical Observations

Overall, these results explain the slow increase in swelling of the cornea in the absence of perspiratio insensibilis when the eyelids are closed, e.g., during night sleep, which decreases again during the waking phase over the course of the day. By this there is improved corneal clarity and thus better vision in patients with Fuchs’ dystrophy after a distinct time with eye open. This mechanism only works if the endothelium and the perspiratio insensibilis remove more water from the cornea during the day than flows into the corneal stroma during the night.

With this basic work on corneal physiology, we can physically explain some effects being observed in DES. Another example of perspiratio insensibilis is visible within the clearing of corneal transplants during a chaud keratoplasty with swollen grafts from the European system grafts, which is frequently observed. Furthermore, the effects of excessive water loss from the superficial corneal tissue in dry eye can also be explained. Allevaporation of water leads to a thinning remnant of the salts staying in place with a hyperosmolar load of the epithelia (Cui et al. 2014) [20]. But by an increased salt concentration in the superficial stroma and tears results. This could explain the tear film osmolality of up to 519 mOsmol/kg in DES (Gilbard et al. 1979) [21]. The amount of water evaporated in this test alone (approx. 10% of the total volume) is sufficient to increase the osmolality of 320 mOsmol/kg by 10% to approx. 350-360 mOsmol/kg. This in turn causes stress on the epithelial barrier and its tight junctions and thus changes the epithelial integrity of the. This stress results in permeability leaks of epithelium and might be one trigger of the inflammatory mechanisms of DES.

The marketing departments of various manufacturers of artificial tears propagate hypo-, iso- or hyper-osmolar or osmo- buffers as tear substitutes, depending on the product. Because of our results, a sequence from hyperosmolar to iso- and then to hypo-osmolar seems to be optimal for patients with a DES, and exactly the opposite with corneal edema. This approach probably protects the epithelial barrier of the corneal epithelium and thus supports the improvement of surface permeability as a driving factor of what is happening to the cornea. Notes on the osmolar compositions of the eye drops can be found e.g., in Dutescu et al. (2015) [13].

Finally, our experiments with corneal cooling to 9°C (Figure 3 t>30 minutes) and an increase in corneal thickness confirm the old clinical work of Redbrake et al (1997) [22] and Champman-Smith (1977) [23].

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Statements on Ethics Issues

Approval by an ethics committee is not required for the experiments on eyes of animals for slaughter in this case.

Declaration of Pecuniary Interest

The authors have no relevant financial or non-financial interests to disclose.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed mainly by Thomas Schrage with the help of Claudia Panfiland Marc Urbach. The manuscript was written by Thomas Schrage and all authors contributed to the manuscript. All authors read and approved the final manuscript.

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