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Original Research

Light transmission/absorption characteristics of the meibomian gland

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ABSTRACT

Purpose: While meibography has proven useful in identifying structural changes in the meibomian gland (MG), little is known regarding the MG spectral transmission and absorption characteristics. The purpose of this study was to measure the transmission/absorption spectra of the MG compared to other eyelid tissues.

Methods: Human and rabbit eyelids were fixed in paraformaldehyde, serial sectioned (50 μm) using a cryotome and imaged by brightfield and reflectance microscopy. Eyelid regions (MG, muscle, tarsus and dermis) were then illuminated by a 100 μm spot using a infrared enhanced white light source. Transmission spectra over a 550–950 nm range were then measured using a spectrometer and differences compared using two-way analysis of variance.

Results: Brightfield microscopy of both human and rabbit eyelid tissue showed a marked decrease in light transmission for MG acini compared to other eyelid tissues. In rabbit, the dermis showed 5× and the muscle showed 2× more light transmission compared to MG ($P < .001$ and $P < .001$, respectively). For human, the muscle showed 14× and the tarsus showed 84× more light transmission compared to MG ($P < .01$ and $P < .001$, respectively). No specific spectral region of light absorption could be detected in either rabbit or human MG. Loss of light transmission in MG was localized to acini containing small lipid droplets, averaging 2.7 ± 0.8 μm in diameter.

Conclusions: The data suggest that light transmission is dramatically reduced in the acini due to light scattering by small lipid droplets, suggesting that Meibography detects active lipid synthesis in differentiating meibocytes.

1. Introduction

Dry eye syndrome is a common eye disorder comprising both an aqueous deficient and evaporative dry eye disease [1]. One of the major causes of evaporative dry eye syndrome is meibomian gland dysfunction (MGD) [1–3]. Meibomian glands are modified sebaceous glands that are embedded in the tarsal plate, underlying the orbicularis muscle of both the upper and lower eyelids [4]. The meibomian gland excretes oil, or meibum, onto ocular surface, which forms part of the lipid layer of the tear film. Thinning of the lipid layer caused by meibomian gland dysfunction (MGD) leads to excessive evaporation of water from the ocular surface leading to increased tear osmolarity, ocular surface damage and evaporative dry eye syndrome [5,6].

Detection of MGD has relied principally on meibography, which is an imaging technique used to detect meibomian gland dropout [7,8]. Traditional meibography uses transilluminated light applied to the skin side of the eyelid with imaging of the glands taken from the conjunctival side using an infrared detector or film [9,10]. Using transillumination meibography, meibomian glands appear as dark, grape like clusters of acini that extend the length of the tarsal plate. More recently a noncontact form of meibography has been developed that uses an infrared light source focused on the conjunctiva [11]. In noncontact meibography, acini appear as bright clusters within the tarsal plate.

Why acini of meibomian glands show distinct differences in their transillumination and reflected light appearance is not known, but likely involves the spectral absorption/scattering characteristics of the gland compared to other eyelid structures. While the spectral absorption characteristics of the eyelid muscle has been previously studied [12], to our knowledge the spectral absorption/scattering characteristics of the meibomian gland have not been previously reported. As suggested by Pult and Nichols in 2012, knowing the spectral absorption characteristics of the meibomian gland may provide better insight into the assessment of gland structure and function [13]. To address this

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issue, we have measured the spectral absorption profile for the meibomian gland and other eyelid structures including eyelid muscle and tarsal plate. We report that the meibomian gland shows a greatly reduced transmission of light that increases with increased wavelength into the infrared. This pattern appears to be caused by increased light scattering from small lipid droplets within differentiating meibomian gland acinar cells that is likely due to the difference in refractive indices between cell cytoplasm and meibum lipid (1.35 vs 1.48) [14,15]. This increased light scattering and not absorption likely explains the dark appearance on transillumination and the white appearance on reflection of the meibomian gland acini.

2. Methods

2.1. Tissue preparation

Human eyelid tissue was obtained from the upper eyelid of a 64 year-old female with sebaceous cell carcinoma of left upper eyelid and was fixed in 4% paraformaldehyde (PFA). Tissue was obtained from Seoul St. Mary's Hospital as approved by the Institutional Review Board of Seoul St. Mary's Hospital (IRB #KC14TISI0671). PFA fixed rabbit eyelid tissue was obtained from a one-year old New Zealand pigmented rabbit that was humanely sacrificed under an IACUC approved protocol from the University of California, Irvine. Tissues were embedded in O.C.T. Compound (Tissue-Tek, Torrance, CA), snap frozen in liquid nitrogen, and cryo-sectioned using a Leica CM1850 cryostat (Leica Biosystems, Buffalo Grove, IL). Tissue sections (50 µm thick) were collected and placed in phosphate-buffered saline (PBS), pH 7.2 and stored at 4 °C.

2.2. Brightfield microscopy

Tissue sections were mounted onto glass slides (Micro Slides, Corning, NY) in PBS and covered by a thin glass coverslip. Brightfield microscopy was then performed using a Leica DMI6000 B (Leica Microsystems, Wetzlar, Germany) with an automated stage. The eyelid section was then digitally imaged using Metamorph (Molecular Devices, San Jose, CA) and a montage of images over the entire eyelid section were collected and stitched using the Scan Slide subroutine of the imaging application program.

2.3. Reflectance microscopy

Reflectance microscopy of tissue sections was taken with Stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy GmbH, Jena, Germany). Tissue sections were illuminated at a 45° angle respective to the tissue section and images collected using a Nikon D200 digital camera connected to the microscope using a C-mount.

2.4. Measurement of spectral transmission of eyelid structures

Spectral transmission characteristics of eyelid structures including the meibomian gland acini, muscle, dermis, and tarsus were measured using a spectrometer (CCS200/M, Thorlabs, Newton, NJ) and an infrared-enhanced, broadband white light source (OSL2 and OSL2BIR, Thorlabs, Newton, NJ). Sections were placed on the glass slide with a cover slip, and mounted on a BH2 Olympus microscope (Olympus Optical, Tokyo, Japan). Regions of interest were first identified using a 10 × 0.1 NA, DPlan 4 objective (Olympus, Feasterville, PA). The eyelid structures of interest were then illuminated using the system shown in Fig. 1A that was attached to the BH2 Olympus microscope. Briefly, white light was delivered by a optic fiber cable (M93L01, Thorlabs, Newton, NJ) focused through a 100 µm-diameter pinhole (P100S, Thorlabs, Newton, NJ) and then collimated light directed to specific regions of the eyelid tissue using a mirror (Fig. 1B). Light passing through the tissue was then collected using a reflective collimator

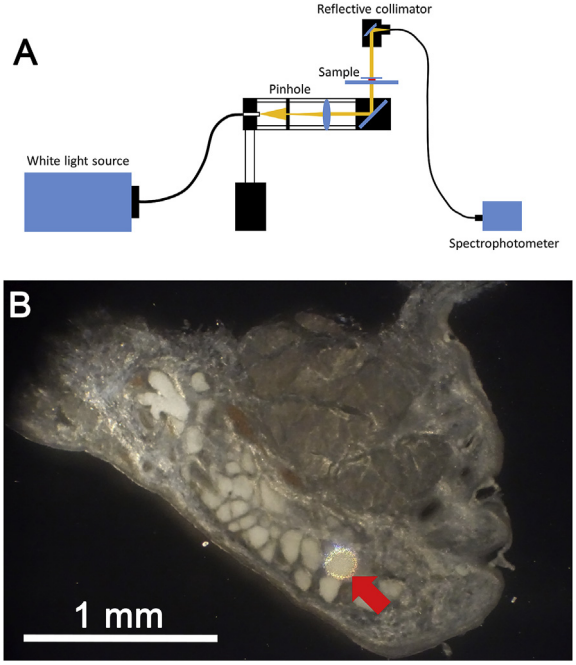

Fig. 1. Measurement of eyelid tissue optical transmission. A. Light from a broad spectrum, white light source was focused on a 100 µm diameter pinhole, and light collimated and focused onto the eyelid tissue through a reflecting mirror. Light passing through the eyelid tissue was then reflected to fiber optic cable connected to a spectrometer. B. Reflected light image of rabbit eyelid tissue section showing a 100 µm illuminated spot (Red arrow) that covered the area of a single acini.

(RC04SMA-P0, Thorlabs, Newton, NJ), which was rotated into place by the objective turret holding the 10× objective. Light passing through the reflective collimator was then directed to the sensor of the spectrometer. Because the transmitted light through the acinus was very weak, the measurements were done in a dark room.

The transmittance through the meibomian gland acini, muscle, and dermis were measured for rabbit eyelid and the transmittance through meibomian gland acini, muscle and tarsus were measured for human eyelid. In the rabbit eyelid, ten, thick (50 µm) tissue sections covering multiple meibomian glands, were used. In each section, one point within the acinus, muscle, and dermis was chosen for measuring the transmittance spectrum. In human eyelid, five, thick (50 µm) tissue sections covering 3 meibomian glands were used. In each section, one point within acinus, muscle, and tarsus was chosen for transmittance measurements. First, the light spectrum through a control region of the glass slide, PBS and cover slip was measured (A). Then the light spectrum through glass slide, PBS, tissue section, and cover slip was measured (B). Then transmittance was defined as the ratio (100% *B/A) within the transmittance spectrum from 550-950 nm. For 10 tissue sections of rabbit eyelid, mean transmittance was calculated for each region (acinus, muscle, dermis) by averaging the percent transmittance over the spectral range of 550 nm–950 nm and reported as $\%T_{550\text{-}950}$, and the average and standard deviation calculated for all 10 samples. Differences between regions were determined using Student-Newman-Keuls, multiple comparison, two-way ANOVA. For human tissue, the same analysis was performed on 5 sections and region measurements were made of acinus, muscle and tarsus.

2.5. Lipidtox staining

To measure lipid droplet size, tissue sections were stained with LipidTox (Thermo Fisher Scientific, Eugene, OR), a fluorescent neutral lipid stain. The human eyelid was cryosectioned (10 µm thickness), stained with LipidTox and imaged using a Leica DMI6000 B.