Spectroscopic Characterization of Human Meibum

Borchman, Foulks, Yappert
Composition → Structure → Function
Meibomian Gland
Meibomian Gland
Lid Margin
Composition ➔ Structure ➔ Function
MAJOR REVIEW

The Correlation Between the Tear Film Lipid Layer and Dry Eye Disease

Gary N. Foulks, MD FACS

Available online at www.sciencedirect.com


Review

Functional aspects of the tear film lipid layer

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Tear Film Lipid Layer Function

- Decrease the rate of evaporation
- Reduce the free energy at the tear film surface and increase aqueous layer thickness
- Maintain Viscosity and surface tension
Evaporation Rate (mg/min/cm^2) for Lactoglobulin (mg/mL) with and without wax treatments. From Borchman et al., Eye & Contact Lens. In Press, 2009.
Composition

Structure

Function
Composition

There are over 30,000 molecular species of lipids in human meibum (Nicolaides and Santos, 1985)
TABLE 1 Comparison of the major lipid composition of normal human meibomian lipids

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Composition

Alkanes compose 0 % to 36 % of the meibum
Composition

Esters compose between 20-80% of the meibum

Wax Ester
13-32%
Composition

Cholesterol esters make up 7-50% of the meibum
Is the large variation in lipid classes due to experimental variation, or variation from person to person, time, sex, age or Meibomian Gland dysfunction?
Small variation from sample to sample and with age

62% rise in cholesterol esters
Fourier Transform
Infrared and Raman Spectroscopies
Meibum Lipid Composition is Different from Tear Film Lipid Composition
Raman Spectra of Human Meibum and Standards
Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS)
Tear lipids minus waxes
Meibum lipids minus waxes
Tear lipids
Meibum lipids
Lipids were measured with a sensitivity of 9 pmoles.

In spectra where the wax was made to be invisible, tear fluid lipid composition was much more complex than for meibum lipid. The opposite was true for spectra where the wax was made visible.
Composition

Structure

Function
What is Known about Tear Lipid Molecular Structure/ conformation?
Nothing
Tear Lipid Hydrocarbon Chain Conformation Using FTIR

- $\text{CH}_2$ asymmetric
- $\text{CH}_3$ asymmetric
- $\text{CH}_3$ asymmetric
- $\text{CH}_3$ moiety Fermi resonant
- $=\text{CH} \text{ cis}$
- CH$_3$ symmetric
- CH$_2$ symmetric
Ordered (Rigid)

all trans
Disordered (Fluid)
\[ \nu_{\text{sym}} = P_1 + \left( \frac{P_2}{(1+P_3/T)^P_4} \right) \]
As the temperature increased from 25 to 40°C, hydrocarbon chains became disordered (20% to 62%). These structural changes may contribute to the therapeutic spreading of meibum with heat.
Crystal Field Splitting

A)

B)

Wavenumber (cm⁻¹)

Wavenumber (cm⁻¹)
Does Meibum Lipid Composition/Molecular-Structure Change With Age or Meibomian Gland Disfunction?
Meibum from Normal Donors

CH$_2$ Symmetric Stretching Frequency (cm$^{-1}$)

Temperature (°C)

- 3 years old
- 84 years old
Meibum from Normal Donors

Hydrocarbon Chain Order (% trans)

Age (y)

Graph showing the relationship between hydrocarbon chain order and age with data points and trend line.
From Raman Spectra of Human Meibum

Conjugated C=C bonds from carotinoids
cicatricial disease
Lipid Order at 34°C (% trans rotomers)

Phase Transition Temperature (°C)

Normal

Dry Eye

Lipid Order at 34°C (% trans rotomers)

Normal

Dry Eye

Phase Transition Temperature (°C)
Conclusions

- Meibum lipid composition changes with age and dry-eye symptoms. These compositional changes are enough to change lipid hydrocarbon conformation and lipid-lipid interactions.
- Wax, a major component of meibum, binds to proteins found in tears. Wax bound to proteins in solution does not influence the rate of evaporation in vitro. However, lipid on the surface strongly lowers the rate of evaporation.
- This work also highlights the power of spectroscopic methods to characterize tear film lipid composition-structure-function relationships and lipid-protein interactions that will be applied in future studies in relation to age, sex and dry eye symptoms.
The End