Biochemistry and Biophysics Reports 23 (2020) 100786

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Meibum lipid hydrocarbon chain branching and rheology after hematopoietic stem cell transplantation

Poonam Mudgil [a], Aparna Ramasubramanian [b], Douglas Borchman [c,*]

[a] School of Medicine, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia
[b] Phoenix Children's Hospital, 1920 E Cambridge Avenue, Phoenix, AZ, 85016, USA
[c] Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, 40202, USA

ARTICLE INFO

Keywords:
Dry eye
NMR spectroscopy
Lipids
Meibum
Rheology

ABSTRACT

Purpose: Meibum from donors who have had hematological stem cell transplantations (M_{HSCT}) are susceptible to severe dry eye symptoms and exhibit very high lipid order (stiffness) compared with meibum from donors without dry eye (M_n). Since lipid order could have functional consequences, we compared the rheology and composition of M_n and M_{HSCT} to measure meibum compositional, structural and functional relationships.
Methods: The rheology and composition was measured using Langmuir trough and ^{1}H NMR spectroscopy, respectively.
Results: M_{HSCT} and M_n was studied from 16 to 43 donors, respectively, using NMR spectroscopy. M_{HSCT} contained significantly 16% more straight chain and 24% less iso-chain hydrocarbons compared with M_n. The cholesteryl ester to wax ester molar ratio, and hydrocarbon chain unsaturation were not significantly different, for M_{HSCT} compared with M_n.
Surface pressure-area isotherms of meibum from 30 donors without dry-eye were grouped into 4 pools (PC) and meibum from 32 donors with dry eye who had hematopoietic stem cell transplantation (PT) were grouped into 3 pools. Above 15 years of age the Π_{max} and $(C_s^{-1})_{max}$ increased with age for both the PC and the PT cohorts. $(C_s^{-1})_{max}$ values were higher for PT samples compared with age matched PC samples, indicating they had higher elasticity and stiffness. A more ordered lipid could contribute to the formation of a discontinuous patchy tear film lipid layer, which in turn results in deteriorated spreading, and decreased surface elasticity.
Conclusions: The composition and rheology of meibum from donors with dry eye and who have had HSCT support the idea from other studies that more ordered meibum may contribute to or be a marker of dry eye.

1. Introduction

The tear film lipid layer is a thin layer of lipid that floats on top the tear aqueous layer and is important to tear film stability [1–6]. Approximately 80% of the lipid in tears originates from the meibomian glands located in the eye lids [7–9]. The relationships between meibum lipid structural order (stiffness) measured spectroscopically, to tear film stability has been reviewed [10]. Tear film stability can be inferred from tear breakup time and blink rates measurements.

Too much lipid order may keep meibum from flowing out of the meibomian glands. Correlations between Meibum lipid order and tear film stability have been made with age between 0 and 25 years old, meibomian gland dysfunction, with donors who have had hematopoietic stem cell transplantation (HSCT) and donors with Parkinsons's disease [10]. Correlation does not necessitate cause, but the relationship between hydrocarbon chain order and tear film stability is intriguing. When tear film stability is restored with treatment, lipid order is also restored to normal levels [11] suggesting the relationship between lipid order and tear film stability may be more than coincidental. A more ordered lipid could contribute to the formation of a discontinuous patchy tear film lipid layer, which in turn results in deteriorated spreading, and decreased surface elasticity [6]. One may speculate that more ordered lipid results in attenuated capability to restore tear film lipid layer structure between blinks.

Meibum from donors who have had HSCT (M_{HSCT}) have the highest order of all of the samples measured [12–15] and the donors are very susceptible to severe dry eye symptoms [16–21]. In a recent study [15], tear lipids (TL_{HSCT}) and meibum (M_{HSCT}) from patients who had HSCT

* Corresponding author. The Kentucky Lions Eye Center, University of Louisville, 301 E. Muhammad Ali Blvd., Louisville, KY, 40202, USA.
E-mail address: borchman@louisville.edu (D. Borchman).

https://doi.org/10.1016/j.bbrep.2020.100786
Received 27 March 2020; Received in revised form 22 June 2020; Accepted 23 June 2020
Available online 20 July 2020

were pooled prospectively. Compared with M_n, M_{HSCT} had 18% fewer CH_3 moieties and TL_{HSCT} contained fewer double bonds [15]. Compared with M_{HSCT}, the phase transition temperature, cooperativity, and order were approximately 20% greater for TL_{HSCT} [15].

Since lipid order could have functional consequences, in the current study we compared the rheology and composition of M_n and M_{HSCT} using Langmuir trough and ^{1}H NMR spectroscopy, respectively to possibly measure meibum compositional, structural and functional relationships.

2. Methods

2.1. Materials

$CDCl_3$, was obtained from Sigma-Aldrich (St. Louis, MO).

2.2. Collection and processing of human meibum

In this prospective comparative study, we analyzed both eyes from all donors. Written informed consent was obtained from all donors and protocols and procedures were approved by the University of Louisville Institutional Review Board # 11.0319, August 2016. All procedures were in accordance with the Declaration of Helsinki.

Human meibum samples were collected from participants recruited from the Kentucky Lions Eye Center and the James Graham Brown Cancer Center in Louisville, Kentucky. Participants were assigned to the cohort Cn when the patient's had no history of bone marrow transplantation and their meibomian gland orifices showed no evidence of keratinization or plugging with turbid or thickened secretions and no dilated blood vessels were observed on the eyelid margin. The participants did not recall having dry eye symptoms. Participants were assigned to the cohort C_{HSCT} if they had undergone HSCT. Patients in C_{HSCT} underwent a full ophthalmic eye exam using slit lamp biomicroscopy. Tear film break up time was measured at the slit lamp after instillation of one fluorescein drop. The diagnosis of dry eye was based on the clinical examination results, including fluorescein stain uptake of the cornea or conjunctiva, irregular tear film, low tear meniscus, as well as symptoms. Symptoms that were considered positive included foreign body sensation, excessive tearing, excessive blinking, burning of eyes or blurry vision. The Schirmer's test was performed on all patients by placing a standard strip in the lower conjunctival sac without anesthesia for 5 min. Meibomian gland orifices, eyelid changes at the mucocutaneous junction and expression of meibum by gentle pressure were all evaluated for diagnosis of MGD.

Meibomian glands were gently expressed by pressing the eyelid with a fingertip with strict attention to avoid touching the eyelid margin during expression. All four eyelids were expressed, and approximately 0.5 mg of meibum lipid was collected per individual for direct spectroscopic study. The expressate was collected with a platinum spatula under a slit lamp and the meibum was immediately dissolved into 0.5 mL of deuterated chloroform in a 9-mm microvial with a Teflon® cap (Microliter Analytical Supplies Ind., Suwanee, GA). The sample in the vial was capped and frozen under argon gas until analysis. Analyses were performed within 3 weeks of collection of the sample.

2.3. NMR spectral measurements

Spectral data were acquired using a Varian VNMRS 700 MHz NMR spectrometer (Varian, Lexington, MA) equipped with a 5 mm ^{1}H {13C/^{15}N} ^{13}C enhanced PFG cold probe (Palo Alto, CA). Spectra were acquired with a minimum of 250 scans, 45° pulse width, and a relaxation delay of 1.000 s. All spectra were obtained at 25 °C.

Commercial software (GRAMS 386; Galactic Industries Corp., Salem, NH) was used for phasing, curve fitting and integrating. Hydrocarbon chain branching [22], saturation [23] and the molar ratio of cholesteryl ester to wax ester [24] were calculated from the ^{1}H NMR spectra as described previously.

Averages were compared using the Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

2.4. Meibum rheology study

Surface pressure-area profiles of meibum samples were recorded using a computer controlled Langmuir Teflon micro-trough, area 135 cm^2, volume 100 mL (Kibron MTXS; Kibron, Helsinki, Finland). The surface pressure was measured by a pressure sensor using the Wilhelmy wire probe method (dyne probe). The trough was covered with a transparent acrylic cover to avoid air currents and dust particles. An artificial tear (AT) solution [25] emulating the salt composition of human tears (NaCl 6.6 g/L; KCl 1.7 g/L; $NaHCO_3$ 1.4 g/L; $CaCl_2.2H_2O$ 0.15 g/L; $NaH_2PO_4.H_2O$ 0.1 g/L; MOPS 4.18 g/L; pH 7.4) was used as the subphase in the trough. Purified water (Milli-Q, resistance >18.2 MΩ; Millipore, Billerica, MA) was used for preparing the AT solution. The surface of the AT solution was cleaned with a vacuum aspirator to achieve a clean surface (pressure change < 0.02 mN/m upon compression and expansion of the surface area completely). The meibum sample (10 μL of 1 mg/mL) in chloroform was spread drop-wise on the surface of the AT solution using a micro-syringe (Hamilton Co, Bonaduz, Switzerland) and chloroform was allowed to evaporate for 10 min. The dynamic compression and expansion of the meibum film was done by two symmetrically moving barriers and changes in pressure with area were recorded as pressure-area profiles. The compression part of the profile gave the pressure-area isotherm. The experiments were performed at 35 °C, the physiological ocular surface temperature. Each sample was measured at least three times to ensure reproducibility.

Compressibility (C_s) of the interfacial meibum films at a given surface pressure was calculated from the pressure-area isotherms and was expressed in millinewton/meter (mN/m) [26]:

$$C_s = -1/A_\Pi \, (dA/d\Pi)$$

where A_Π is the area at a surface pressure Π. The inverse of C_s (C_s^{-1}) gave the in-plane (2 dimensional) elasticity modulus, also called as reciprocal compressibility, and was expressed in mN/m:

$$C_s^{-1} = 1/C_s = -A_\Pi \, (d\Pi/dA)$$

The inflections in the plots of C_s^{-1} as a function of surface pressure and area indicated phase transition or significant reorganization of the surface film during the compression of the film.

3. Results

3.1. Meibum composition from ^{1}H NMR

The demographics of the 16 meibum donors who underwent hematopoietic stem cell transplantation and 43 meibum donors who did not have dry eye symptoms are presented in Table 1. The C_{HSCT} contained a greater percentage of Caucasians and was an average of 13 years older compared with C_n. All of the CHSCT used for NMR spectroscopy had dry eye. Most, 47%, were diagnosed with both aqueous deficient and Meibomian gland disease, 13% were diagnosed with purely Meibomain gland disease and for 40% of the cohort, a diagnosis was not determined.

The major resonances were well resolved in the ^{1}H NMR spectra of M_{HSCT} (Fig. 1). Resonances for cholesterol/cholesterol ester, hydrocarbon chain branching and $=CCH_2$ were evident in the 1–3 ppm region (Fig. 1a, Table 2). Resonances for =CH *cis*, cholesteryl and wax esters were evident in the 4–5.5 ppm region (Fig. 1b, Table 2). M_{HSCT} contained significantly ($P < 0.05$) 16% more straight chain and 24% less iso chain hydrocarbons compared with M_n (Table 3). The average age of Cn, 23 y, was lower than that of C_{HSCT}, 47 y, however, the difference in Meibum composition between Cn and C_{HSCT} was not due to age related

Table 1
Cohort demographics of meibum samples for NMR spectroscopy.

	C_n	C_{HSCT}
Average age (y)	29 ± 16	42 ± 19
Age range (y)	13 to 80	10 to 70
Median Age (y)	23	47
Gender (% male)	59	62
Race (%)[1]	C (79)	C (88)
	B (11)	B (2)
	H (5)	H (0)
	A (5)	A (0)
Number of Donors	43	16

[1] C is Caucasian, B is Black, A is Asian, H is Hispanic. Cn is the cohort of donors who did not have dry eye or had not had a hematopoietic stem cell transplantation. C_{HSCT} is the cohort of donors who had undergone a hematopoietic stem cell transplantation.

differences because Meibum hydrocarbon chain branching did not change with age for Cn between 22 and 68 years of age, nor was it influenced by gender [22]. Four samples from C_{HSCT} with an average age of 16 (10–21 y range) showed hydrocarbon chain branching in this younger group was 19% anteiso-branched, 66% straight and 15% iso-branched, almost identical to the branching calculated for the older cohort (Table 3). The cholesteryl ester to wax ester molar ratio, and hydrocarbon chain unsaturation were not significantly different, $P > 0.05$, for M_{HSCT} compared with M_n (Table 3).

3.2. Meibum rheology

The demographics of the pooled donor samples for the rheological studies are presented in Table 4. A fraction of the pooled samples was used for an infrared spectroscopic study [15]. Surface pressure-area isotherms of pooled meibum from donors without dry-eye (PC) and pooled meibum from donors with dry eye who had HSCT (PT) are shown in Figs. 2 and 3, respectively. The code to the cohort nomenclature is P is for pooled samples, the number is the average age in years, C is for controls without HSCT, T is donors who had a HSCT. Π_{max} and $(C_s^{-1})_{max}$ values for each pool are presented in Table 4. Due to the non-linear dependence of surface pressure on C_s^{-1}, the $(C_s^{-1})_{max}$ values were

Table 2
Assignments for resonances numbered in Fig. 1.

Fig. 1 resonance #	Shift (ppm)	Resonance Assignment
1	1.00 to 0.996	Cholesterol Carbon # 19 (Fig. 1c)
2	0.906	Cholesterol Carbon #21 (Fig. 1c)
3	0.897	Cholesterol Carbon #21 (Fig. 1c)
4	0.878	Straight-chain
5	0.868	Straight-chain
6	0.858	Straight-chain
7	0.853	Iso-branched
8	0.850	Anteiso-branched
9	0.843	Iso-branched
10	0.839	Anteiso-branched
11	0.829	Anteiso-branched
12	0.821	Anteiso-branched
13	0.799	Not assigned
14	0.789	Not assigned
15	0.663	Cholesterol Carbon #18 (Fig. 1c)
16	5.36	Cholesterol Carbon #6 (Fig. 1c)
17	5.33	Hydrocarbon =CH– *cis*
18	4.6	Cholesteryl Ester Carbon #3 (Fig. 1c)
19	3.9	Wax Ester –CH_2–O–(C═O)–

Table 3
Meibum composition from ^{1}H NMR resonance intensities.

Cohort	Cn	C_{HSCT}	*P*
Anteiso Branching (%)	20 ± 1	18 ± 2	0.14
Iso Branching (%)	21 ± 1	16 ± 2	0.015*
Straight Chain (%)	59 ± 1	66 ± 2	0.0008*
Cholesteryl Ester/Wax Ester (mole/mole)	0.51 ± 0.02	0.49 ± 0.02	0.52
=CH/(Total Esters)‡	0.90 ± 0.02	0.82 ± 0.05	0.084

*statistically significant. ‡(16 + 17)/(4+(1 + 15)/3) where the numbers are the intensities of the resonances numbered in Table 2 except for 3 which is a constant. Cn is the cohort of donors who did not have dry eye or had not had a hematopoietic stem cell transplantation. C_{HSCT} is the cohort of donors who had undergone a hematopoietic stem cell transplantation.

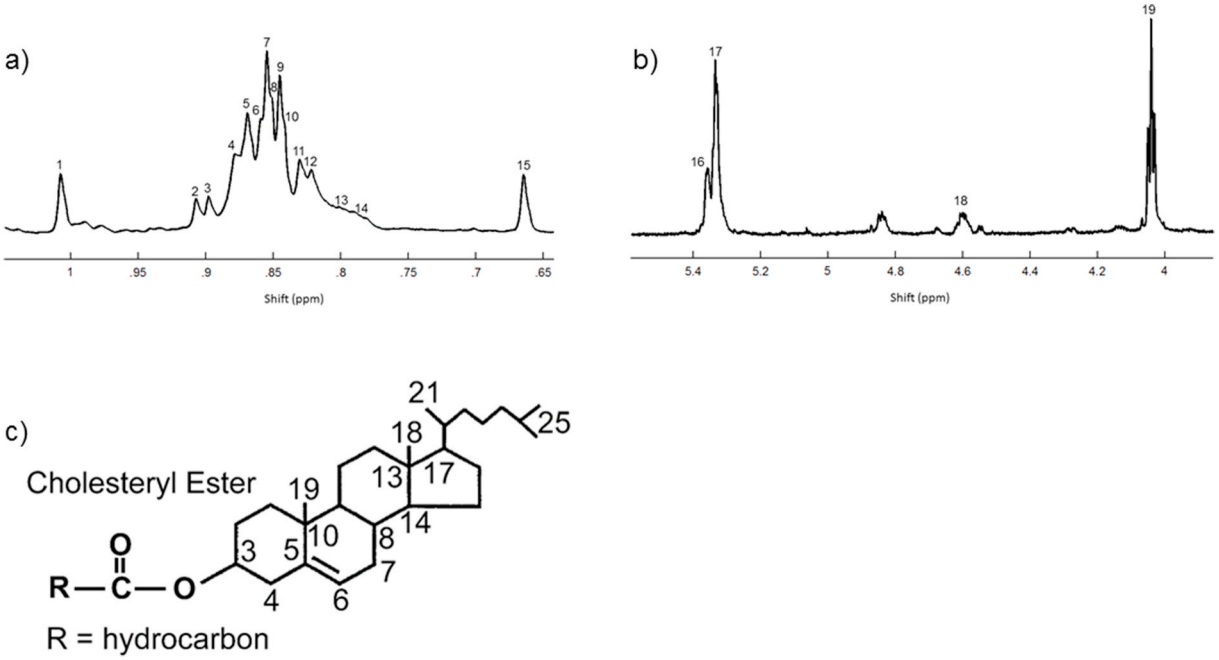

Fig. 1. Average ^{1}H NMR spectra of meibum from donors who had hematopoietic stem cell transplantation. Numbers refer to the resonance assignments in Table 2. a) The CH_3/CH_2 resonance region. b) The ester =CH region. c) Numbering of cholesteryl ester carbons.

Table 4
Demographics of pooled samples for rheology determination.

Sample	Average Age (y) ± SD	Age Range (y)	Median Age (y)	Gender (% male)	Race (%)	HSCT	# of Donors	Π_{max} (mN/m)	$(C_s^{-1})_{max}$ (mM/m)
PC1	1.6 ± 0.2	1 to 2	2	75	C (100)	No	8	21	13
PC15	15 ± 2	13 to 17	15	88	C (100)	No	8	15	13
PC27	27 ± 5	20 to 36	26	50	C(90) H(10)	No	10	24	16
PC45	45 ± 12		40	25	C(50) B(25) H(25)	No	4	28	15
PT15	15 ± 2	13 to 18	14	80	C(60) A(20) B(20)	Yes	5	25	15
PT43	43 ± 15	21 to 61	49	56	C (100)	Yes	16	32	20
PT49	49 ± 12	24 to 69	52	54	C(91) B(9)	Yes	11	28	22

*C is Caucasian, B is Black, A is Asian, H is Hispanic. HSCT is hematopoietic stem cell transplantation, Π_{max} is the maximum surface pressure, $(C_s^{-1})_{max}$ is the elasticity modulus maximum, also called reciprocal compressibility, P is for pooled samples, the number is the average age in years, C is for controls without HSCT, T is donors who had a HSCT.

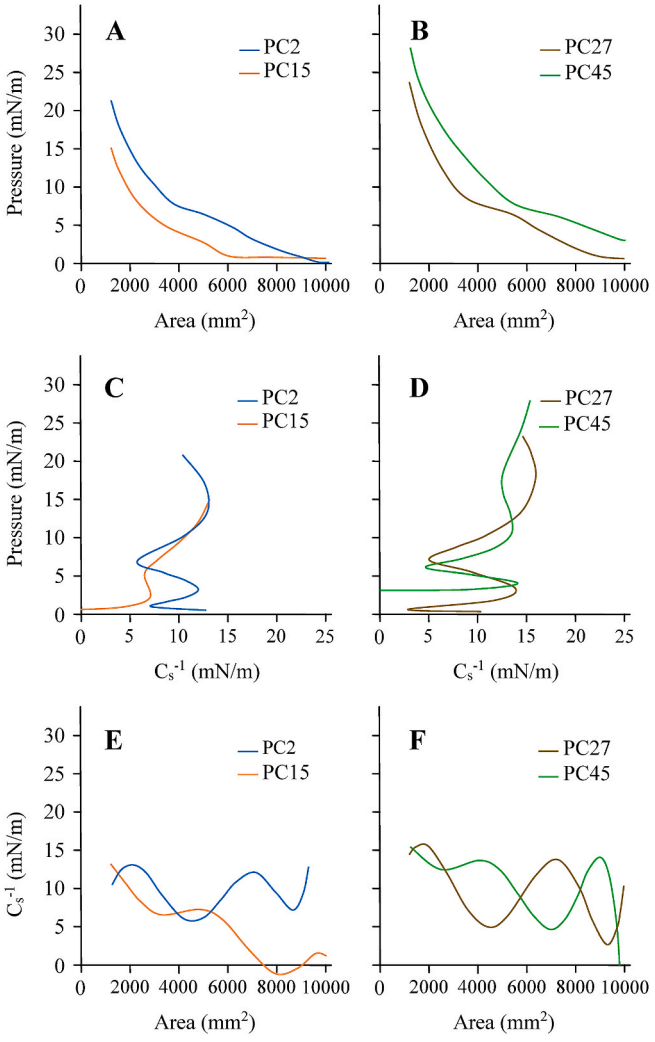

Fig. 2. Biophysical characteristics of meibum from donors without dry-eye and without hematopoietic stem cell transplantation on artificial tear solution at 35 °C. Top row: pressure-area isotherms; middle row: reciprocal compressibility modulus (C_s^{-1}) as a function of surface pressure; and bottom row: C_s^{-1} as a function of surface area.

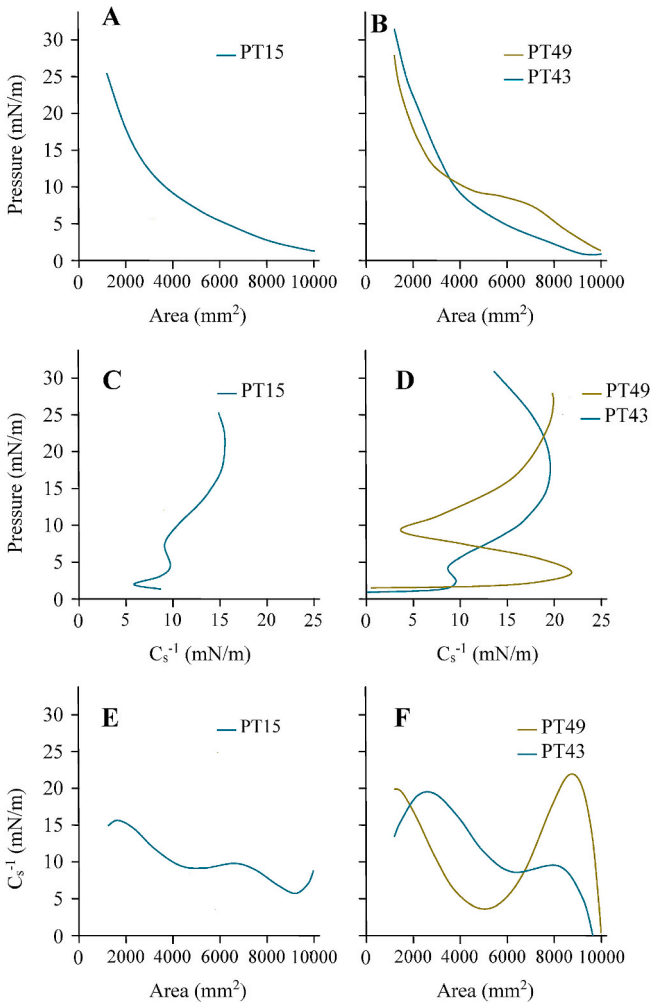

Fig. 3. Biophysical characteristics of meibum from donors with hematopoietic stem cell transplantation and dry-eye on artificial tear solution at 35 °C. Top row: pressure-area isotherms; middle row: reciprocal compressibility modulus (C_s^{-1}) as a function of surface pressure; and bottom row: C_s^{-1} as a function of surface area.

observed before Π_{max} in most samples. Note that above 15 years of age the Π_{max} (Table 4) and at all ages $(C_s^{-1})_{max}$ (Fig. 4) increased with age for the PC ($r = 0.779$ intercept = 12.9, slope = 0.0696, $P < 0.01$, $r = 0.902$

for current data only). $(C_s^{-1})_{max}$ values for PT samples (★) were significantly higher ($P < 0.01$) for above 40 years of age, and marginally higher ($P < 0.05$ for 15 years of age) compared with age matched PC samples. Higher C_s^{-1} values indicates higher elasticity and stiffness. In addition, the structural transitions in the plots of the dependence of C_s^{-1} on surface pressure/area (Figs. 2 and 3 CDEF) were longer with increasing age for the PC and PT samples, and were longer for PT samples compared with age matched PC samples.

4. Discussion

4.1. Structural-functional relationships

The correlation between meibum stiffness and tear film stability was highlighted in the Introduction and recently reviewed [10]. Too much lipid order may keep meibum from flowing out of the meibomian glands. Relevant to the structure of lipids on the tear film surface, the findings of the current rheological experiments show that above 15 years of age the Π_{max} and at all ages, $(C_s^{-1})_{max}$ increased with age for both PC and PT cohorts in accord with a previous study [27]. Higher Π_{max} and $(C_s^{-1})_{max}$ values indicate the lipids become more elastic and stiffer with age. These biophysical changes could cause the lipids to form stiffer, thicker, viscous and patchy heterogeneous films on the tear film surface. As pointed out in the Introduction, a more ordered lipid could contribute to the formation of a discontinuous patchy tear film lipid layer, which in turn results in deteriorated spreading, and decreased surface elasticity [6]. One may speculate that more ordered lipid results in attenuated capability to restore tear film lipid layer structure between blinks. In M_n lipid order increases with age between 0 and 25 years, while over 25 years lipid order slightly decreases which does not contribute to tear stability as over 25 years there is little change in tear stability [27].

Π_{max} and $(C_s^{-1})_{max}$ were higher for PT samples and therefore more ordered compared with age matched PC samples in agreement with previous infrared spectroscopic studies [11–15]. Using the same reasoning for age above, it is reasonable that the PT lipids would form stiffer, thicker, viscous and patchy heterogeneous films on the tear film surface which could lower tear film stability and impede their spreading between blinks [6]. Tear film stability, and TFLL structural order varies greatly from person to person, however, correlations and trends between the stability and structural order values could be made between large cohorts [10]. From other age related studies of meibum from normal donors and meibum from donors with dry eye from Meibomian gland dysfunction and Parkonson's disease, the current study of donors with HSCT supports the idea that a more ordered lipid could contribute to or be a marker of dry eye [10]. Thus, the presence of more ordered lipids with higher $(C_s^{-1})_{max}$ values and longer pressure-area isotherm transitions, as observed for PT lipids, are indicators of less stable lipid films at the air-tear interface [10,28].

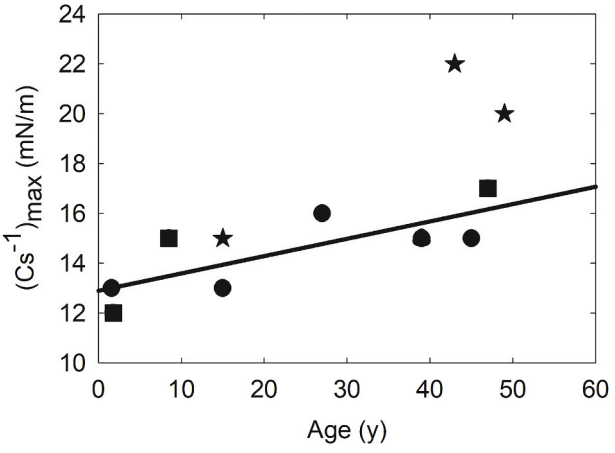

Fig. 4. Relationship between the maximum elasticity modulus, also called as reciprocal compressibility $(C_s^{-1})_{max}$ and age for human meibum from donors without dry eye and without having hematopoietic stem cell transplantation from (●) the current study, (■) from reference 27and (▲) from reference 32. (★) meibum from donors that had hematopoietic stem cell transplantation from the current study. A higher maximum elasticity modulus indicates a more elastic stiffer lipid layer.

4.2. Compositional-functional relationships

Upon comparing meibum composition with function from normal donors with age, and from donors with dry eye from Meibomian gland dysfunction and Parkonson's disease, one may note that the relationships between meibum and TFLL composition and function are less certain than the relationships between composition and structure or function discussed in the previous sections [10]. One of the major compositional findings from the current study was that M_{HSCT} contained, 16% more straight chain and 24% less iso-chain hydrocarbons compared with M_n which could contribute to the higher order reported for M_{HSCT} compared with Mn [11–15]. The difference between branching for M_{HSCT} and M_n measured using ^{1}H NMR in the current study agreed well with the findings using infrared spectroscopy [15]. Straight chain hydrocarbons are able to pack closer together and have higher van der Waal's interactions than branched chains which have bends limiting how close they can pack together [22].

In addition to hydrocarbon chain branching, hydrocarbon chain saturation is a major factor that contributes to lipid order in lens membranes [29] and human meibum [23,30–32]. Infrared spectroscopy showed that M_{HSCT} and TL_{HSCT} contained fewer double bonds compared M_n and TL_n [15]. The ^{1}H NMR results of the current study do not support this observation, however, a larger sample size may be needed for this result to be statistically significant.

For tear lipids in general, cholesteryl ester content [33,34], polar lipids and tear proteins generally enhance spreading of lipids on the surface of the aqueous layer of the tear film [35,36]. Decreased levels of certain polar lipids reported with dry eye [37,38] calls for investigations into amount of polar lipids in M_{HSCT} which might be lower and may provide further insight into the relationship between composition, structure and function of meibum from donors with HSCT. In addition, tear proteins possess surface activity and improve surface properties of meibum [39,40]. High levels of lactoferrin and albumin associated with meibum of infants [41,42], and the presence of lacritin in infants and healthy adult meibum which is downregulated in dry eye [38,42], raises the possibility that these or other proteins might be at a lower level in M_{HSCT} hindering spreading of lipids on the aqueous layer of the tear film. Therefore, an investigation into protein content in tears and meibum from donors who had HSCT will be helpful in further determining structure and function of M_{HSCT}.

5. Conclusion

The composition and rheology of meibum from donors with dry eye and who have had HSCT support the idea from other studies that more ordered meibum may contribute to or be a marker of dry eye.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Poonam Mudgil: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing. **Aparna Ramasubramanian:** Conceptualization, Resources, Writing -

review & editing. **Douglas Borchman:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Project administration, Funding acquisition, Writing - original draft.

Acknowledgement

This research was funded by National Institutes of Health EYO RO126180 (DB) and an unrestricted grant from Research to Prevent Blindness, Inc. New York, NY, USA GN151619B.

References

[1] K.B. Green-Church, I. Butovich, M. Willcox, D. Borchman, F. Paulsen, S. Barabino, et al., The international workshop on meibomian gland dysfunction: Report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease, Invest. Ophthalmol. Vis. Sci. 52 (2011) 1979–1993.
[2] A.D. Pucker, J.J. Nichols, Analysis of meibum and tear lipids, Ocul. Surf. 10 (2012) 230–250.
[3] I.A. Butovich, T.J. Millar, B.M. Ham, Understanding and analyzing meibomian lipids: A review, Curr. Eye Res. 33 (2008) 405–420.
[4] E. Knop, N. Knop, T. Millar, H. Obata, D.A. Sullivan, The international workshop on meibomian gland dysfunction: Report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland, Invest. Ophthalmol. Vis. Sci. 52 (2011) 1938–1978.
[5] J. Murube, The origin of tears: III. The lipid component in the XIX and XX centuries, Ocul. Surf. 10 (2013) 200–209.
[6] G.A. Georgiev, P. Eftimov, N. Yokoi, Structure-function relationship of tear film lipid layer: A contemporary perspective, Exp. Eye Res. 163 (2017) 17–28.
[7] D. Borchman, M.C. Yappert, S. Milliner, R. Bhola, Confirmation of squalene in human eye lid lipid by heteronuclear single quantum correlation spectroscopy, Lipids 48 (2013) 1269–1277.
[8] P. Mudgil, D. Borchman, D. Gerlach, M.C. Yappert, Sebum/meibum surface film interactions and phase transitional differences, Invest. Ophthalmol. Vis. Sci. 57 (2016) 2401–2411.
[9] I.A. Butovich, On the lipid composition of human meibum and tears: Comparative analysis of nonpolar lipids, Invest. Ophthalmol. Vis. Sci. 49 (2008) 3779–3789.
[10] D. Borchman, Lipid conformational order and the etiology of cataract and dry eye, J. Lipid Res. (2020), https://doi.org/10.1194/jlr.TR120000874 (in press).
[11] G.N. Foulks, D. Borchman, M.C. Yappert, K. Sung-Hye, J.W. McKay, Topical azithromycin therapy of meibomian gland dysfunction: Clinical response and lipid alterations, Cornea 29 (2010) 781–788.
[12] A. Ramasubramanian, R. Blackburn, H. Yeo, S.M. Sledge, Z.N. Gully, S. Singh, et al., Structural differences in meibum from donors after hematopoietic stem cell transplantations, Cornea 38 (2019) 1169–1174.
[13] A. Ramasubramanian, D. Borchman, Structural differences in meibum from teenage donors with and without dry eye induced by allogeneic hemopoetic stem cell transplantations, J. Ped. Hematol. Oncol. 42 (2) (2019) 149–151, https://doi.org/10.1097/MPH.0000000000001519.
[14] D. Borchman, The optimum temperature for the heat therapy for meibomian gland dysfunction, Ocular Surf. 17 (2019) 360–364.
[15] D. Borchman, V. Ramakrishnan, C. Henry, A. Ramasubramanian, Differences in meibum and tear lipid composition and conformation, Cornea 39 (2020) 122–128.
[16] B. Calissendorff, M. el Azazi, B. Lonnqvist, Dry eye syndrome in long-term follow-up of bone marrow transplanted patients, Bone Marrow Transplant. 4 (1989) 675–678.
[17] M.E. Flowers, P.M. Parker, L.J. Johnston, A.V. Matos, B. Storer, W.I. Bensinger, R. Storb, et al., Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: Long-term follow-up of a randomized trial, Blood 100 (2002) 415–419.
[18] A. Tichelli, T. Duell, M. Weiss, G. Socié, P. Ljungman, A. Cohen, et al., Late-onset keratoconjunctivitis sicca syndrome after bone marrow transplantation: Incidence and risk factors. European Group or Blood and Marrow Transplantation (EBMT) Working Party on Late Effects, Bone Marrow Transplant. 17 (1996) 1105–1111.
[19] Y. Ogawa, S. Okamoto, M. Wakui, R. Watanabe, M. Yamada, M. Yoshino, et al., Dry Eye after haematopoietic stem cell transplantation, Br. J. Ophthalmol. 83 (1999) 1125–1130.
[20] M.A. Lemp, C. Boudoin, J. Baum, M. Dogru, G.N. Foulks, S. Kinoshita, et al., Definition and classification of dry eye disease: Report of the definition and classification subcommittee of the international dry eye workshop (2007), Ocul. Surf. 5 (2007) 75–92.
[21] L.A. Engel, S. Wittig, F. Bock, L. Sauerbier, C. Scheid, U. Holtick, et al., Meibography and meibomian gland measurements in ocular graft-versus-host disease, Bone Marrow Transplant. 50 (2015) 961–967.
[22] D. Borchman, A. Ramasubramanian, Human meibum chain branching variability with age, gender and meibomian gland dysfunction, Ocul. Surf. 17 (2019) 327–339.
[23] S. Sledge, C. Henry, D. Borchman, M.C. Yappert, R. Bhola, A. Ramasubramanian, et al., Human meibum age, lipid-lipid interactions and lipid saturation in meibum from infants, Int. J. Mol. Sci. 18 (2017) E1862.
[24] D. Borchman, A. Ramasubramanian, G.N. Foulks, Human meibum cholesteryl and wax ester variability with age, gender and meibomian gland dysfunction, Invest. Ophthalmol. Vis. Sci. 60 (2019) 2286–2293.
[25] D. Mirejovsly, A.S. Patel, D.D. Rodriguez, T.J. Hunt, Lipid adsorption onto hydrogel contact lens materials. Advantages of Nile red over oil red O in visualization of lipids, Optom. Vis. Sci. 68 (1991) 858–864.
[26] J.T. Davies, E.K. Rideal, Interfacial Phenomena, second ed., Academic Press, New York, NY, USA, 1963, ISBN 9780323161664.
[27] P. Mudgil, D. Borchman, A. Ramasubramanian, Insights into tear film stability from babies and young adults: A study of human meibum lipid conformation and rheology, Internat. J. Mol. Sci. 19 (2018) 3502.
[28] P. Mudgil, Evaluation of use of essential fatty acids in topical ophthalmic preparations for dry eye, Ocul. Surf. 18 (2020) 74–79.
[29] D. Borchman, M.C. Yappert, Lipids and the ocular lens, J. Lipid Res. 51 (2010) 2473–2488.
[30] P. Mudgil, D. Borchman, M.C. Yappert, et al., Human meibum saturation and lipid order, Exp. Eye Res. 116 (2013) 79–85.
[31] G. Georgiev, D. Borchman, P. Eftimov, N. Yokoi, Lipid saturation and the rheology of human tear lipids, Int. J. Mol. Sci. 20 (2019) E3431.
[32] Y. Nencheva, A. Ramasubramanian, P. Eftimov, N. Yokoi, D. Borchman, G. Georgiev, Effects of lipid saturation on the surface properties of human meibum films, Int. J. Mol. Sci. 19 (2018) E2209.
[33] Z.A. Hetman, D. Borchman, Concentration dependent cholesteryl-ester and wax-ester structural relationships and meibomian gland dysfunction, Biochem. Biophys. Rep. 21 (2020) 100732.
[34] D. Borchman, A. Ramasubramanian, G.N. Foulks, Human meibum cholesteryl and wax ester variability with age, gender and meibomian gland dysfunction, Invest. Ophthalmol. Vis. Sci. 60 (2019) 2286–2293.
[35] T.J. Millar, P. Mudgil, S. Khanal, Meibomian glands and the lipid layer, in: D. A. Dartt, J. Besharse, R. Dana (Eds.), Encyclopedia of the Eye; Vol 3, Elsevier, 2010, pp. 13–20 (Chapter 48).
[36] P. Mudgil, T.J. Millar, Surfactant properties of human meibomian lipids, Invest. Ophthalmol. Vis. Sci. 52 (2011) 1661–1670.
[37] S.M. Lam, L. Tong, X. Duan, A. Petznick, M.R. Wenk, G. Shui, Extensive characterization of human tear fluid collected using different techniques unravels the presence of novel lipid amphiphiles, J. Lipid Res. 55 (2014) 289–298.
[38] M.D.P. Willcox, P. Argüeso, G.A. Georgiev, J.M. Holopainen, G.W. Laurie, T. J. Millar, E.B. Papas, J.P. Rolland, T.A. Schmidt, U. Stahl, T. Suarez, L. N. Subbaraman, O.Ö. Uçakhan, L. Jones, TFOS DEWS II tear film report, Ocul. Surf. 15 (2017) 366–403.
[39] P. Mudgil, M. Torres, T.J. Millar, Adsorption of lysozyme to phospholipid and meibomian lipid monolayers, Colloids Surf. B Biointerfaces 48 (2006) 128–137.
[40] P. Mudgil, T.J. Millar, Adsorption of apo- and holo-tear lipocalin (Tlc) to meibomian lipid films, Exp. Eye Res. 86 (2008) 622–628.
[41] M. Esmaeelpour, P.O. Watts, M.E. Boulton, J. Cai, P.J. Murphy, Tear film volume and protein analysis in full-term newborn infants, Cornea 30 (2011) 400–404.
[42] K.B. Green-Church, I. Butovich, M. Willcox, D. Borchman, F. Paulsen, S. Barabino, B.J. Glasgow, The international workshop on meibomian gland dysfunction: Report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease, Invest. Ophthalmol. Vis. Sci. 52 (2011) 1979–1993.