Contact Lens and Anterior Eye 42 (2019) 652–657

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

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

The effect of Blephadex™ Eyelid Wipes on *Demodex* mites, ocular microbiota, bacterial lipase and comfort: a pilot study

Katherine Wong[a,*], Judith Flanagan[b], Isabelle Jalbert[a], Jacqueline Tan[a]

[a] School of Optometry and Vision Science, UNSW Sydney (The University of New South Wales), Australia
[b] Brien Holden Vision Institute, Sydney, Australia

ARTICLE INFO

Keywords:
Demodex
Microbiota
Comfort
Eyelid
Lid hygiene

ABSTRACT

Purpose: To investigate the effect of Blephadex™ Eyelid Wipes on *Demodex* mites, ocular microbiota, bacterial lipase, tear film characteristics and ocular comfort after one month of daily use.
Methods: Twenty subjects were randomly assigned to use the Blephadex™ Eyelid Wipes on either eye once daily for 30 days whilst the contralateral eye was left untreated in this observer-masked, within-subject study. *Demodex* count, eyelid bacterial colony count, Tearscope Plus non-invasive tear break up time (NITBUT), Lipiview® tear film lipid layer thickness and phenol red thread test tear volume were measured at baseline and 30 days. Bacterial lipase was quantified from single bacterial colonies using a glycerol monolaurate assay. Ocular comfort was assessed at both visits using the Ocular Surface Disease Index (OSDI) questionnaire and visual analogue scales (VAS) to capture monocular symptoms of itching, dryness and overall discomfort.
Results: Six males and 14 females, median age 63.5 (range 48–76) completed the study. A statistically significant reduction in *Demodex* count was observed in treated eyes only (median ± IQR: treated eyes 2 ± 3 vs. 0 ± 2, ANOVA $p = 0.04$). Bacterial colony count, lipase production, NITBUT, lipid layer thickness and tear volume remained unchanged ($p > 0.05$). Overall comfort improved over time in treated eyes only (15 ± 32 vs. 10 ± 16, $p = 0.05$). Dryness symptoms significantly reduced in both treated and untreated eyes (23 ± 42 vs. 12 ± 21 and 23 ± 41 vs. 10 ± 15, $p = 0.02$). The OSDI and ocular itch scores remained unchanged ($p > 0.05$).
Conclusion: In this pilot study, no changes were observed in ocular microbiota, tear film characteristics or bacterial lipase in eyes treated with Blephadex™ Eyelid Wipes after one month of daily use in this normal healthy population. Although a statistically significant reduction in *Demodex* count was observed in treated eyes, overall numbers of *Demodex* were low. A parallel group, placebo-controlled, randomised clinical trial in a population with active blepharitis is warranted to further elucidate these preliminary findings.

1. Introduction

Demodex mites are commonly found on the periocular skin and eyelashes, with increasing rates observed with age. [1,2] However, these microscopic ectoparasites found on the skin of healthy asymptomatic humans have also been shown to have pathogenic potential [3,4]. *Demodex* have been associated with anterior and posterior blepharitis, disorders of the eyelashes, keratoconjunctivitis and contact lens wear. [1,5] Furthermore, ocular discomfort has been also been associated with higher *Demodex* counts. [1,6] *Demodex* mites are known to feed on sebum and therefore tend to propagate around hair follicles and sebaceous glands. [1] Bacteria, such as *Staphylococci* spp. which are known to play a significant role in blepharitis, have been found on the surface of the *Demodex* mite body. [1,7] These bacteria produce lipase that has the potential to alter meibomian secretions that are essential for providing a smooth optical surface for the eye and are thought to be crucial to impeding evaporation of the tear aqueous [8–10]. It has been postulated that lipases are also necessary for *Demodex* to digest the sebum it feeds on. [1,11] Free fatty acids that result from the action of lipases on meibum are toxic to the ocular surface [10,12]. Increased amounts of lipases and degraded lipids have been observed in the tear film of symptomatic contact lens wearers and associated with higher dry eye history and symptom scores [13].

Agents that have been investigated as a potential treatment for ocular *Demodex* include pilocarpine gel, metronidazole gel and mercury oxide ointment. [14,15] However more recent studies which are summarised in the TFOS DEWS II Management and Therapy Report, have found topical products containing tea-tree oil or oral ivermectin to be

* Corresponding author at: School of Optometry and Vision Science, University of New South Wales, Gate 14 Barker St, UNSW, Sydney, NSW 2052, Australia.
E-mail address: katherine.y.wong@unsw.edu.au (K. Wong).

https://doi.org/10.1016/j.clae.2019.06.001
Received 20 February 2019; Received in revised form 24 May 2019; Accepted 11 June 2019

more appropriate [16]. Tea tree oil, which also has antimicrobial and antifungal properties, has been demonstrated to be effective for eliminating ocular *Demodex* and improving subjective ocular symptoms in both *in vitro* and *in vivo* prospective, randomised studies. [17–21] However, ocular irritation has been reported with use of tea tree oil if not diluted appropriately [19,22]. Commercial eyelid wipes offer a mode of treatment which can be applied at-home and have been found to be favoured by patients for convenience and ease of use. [23] One previous study demonstrated that eyelid hygiene using commercial eyelid wipes in conjunction with eyelid massage is associated with improved ocular comfort in people with blepharitis and meibomian gland dysfunction [24]. Another more recent study found that a commercial eyelid wipe containing 0.5% 1,2-Octanediol used daily for 60 days was able to reduce *Demodex* numbers and symptoms. [25]

Blephadex™ Eyelid Wipes (Lunovus, LLC, Morris, Alabama USA, http://optimed.com.au/images/stories/Products/dryeye/BlephadexPatientBrochure.pdf) are a commercially available product marketed as a treatment option for blepharitis and *Demodex* mites. The Blephadex™ Eyelid Wipes contain tea tree oil and also coconut oil which has been shown to have antimicrobial and anti-inflammatory activity. [26,27] Additionally, the main constituent of coconut oil is lauric acid, a derivative of glycerol monolaurate which has been demonstrated to inhibit bacterial lipase production [28–30].

No studies to date have been conducted to evaluate the effect of the Blephadex™ Eyelid Wipes on *Demodex* numbers, ocular comfort and bacterial lipase production. Therefore, the purpose of this pilot study was to investigate the feasibility of daily eyelid cleaning with the Blephadex™ Eyelid Wipes and evaluate the effect of such treatment on *Demodex* numbers, *S. aureus* and *S. epidermidis* counts, and lipase production. Secondary aims of the study were to assess tear film characteristics and subjective ocular comfort. The hypotheses of the current study were that a reduction in *Demodex* count, bacterial burden and lipase production would be seen after 30 days use of Blephadex™ Eyelid Wipes. A concomitant reduction in ocular symptoms would also be expected.

2. Materials and methods

2.1. Materials

Blephadex™ Eyelid Wipes are classed as a cosmetic application and are covered under the National Industrial Chemicals and Assessment Scheme (NICNAS) number 7845. The key ingredients are listed in Table 1, however the concentrations of each ingredient have not been specified by the manufacturer.

2.2. Participants

Twenty participants aged 45 years or above were enrolled in this pilot, prospective, randomised, controlled single-centre, observer-masked, within subject design study. The study protocol was approved by the University of New South Wales Human Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki. All participants were given a written information form and indicated consent to participate by signing the informed consent record.

This study was registered on the Australian New Zealand Clinical Trials Registry (ACTRN12618001368224).

Table 1
Key ingredients and their proposed functions in the Blephadex™ Eyelid Wipe.

Ingredient	Function(s)
Glycerin	Humectant [31]
Aloe Barbadensis (Aloe Vera) Leaf Juice	Anti-bacterial, wound-healing and anti-inflammatory [32]
Cocos Nucifera (Coconut) oil	Antipyretic [26], anti-inflammatory [26], antimicrobial [27] and analgesic [26]
Melaleuca Alternifolia (Tea Tree) Leaf oil	Anti-bacterial [17], anti-fungal [18] and acaricidal [19]
Sodium Laureth Sulfate	Surfactant [33]
Cocamidopropyl Betaine	Surfactant [34]
Lauryl Glucoside	Surfactant [33]

2.3. Inclusion and exclusion criteria

Participation in this study was restricted to subjects aged 45 years and above, with the rationale that *Demodex* infestation is more common in an older population. [2]

All subjects were assessed for suitability for inclusion in the study by conducting a general history and symptomatology assessment, visual acuity and slit lamp examination of the anterior eye and adnexa. Patients with any active anterior segment disease, with the exception of blepharitis, were excluded from participating in the study. Any participants who had started ocular medications or systemic medications that might affect tear film or ocular microbiota (including corticosteroids, antibiotics, immunosuppressants, fish oil, flaxseed oil and other omega-3 supplements) less than 3 months prior to the baseline visit or if change in dosage was anticipated during the study were also excluded. A history of ocular surgery in the last 6 months, use of contact lenses within the last 6 months, pregnancy or lactation, or known allergy to tea tree oil, coconut oil or fluorescein dye also excluded participants. Heavy makeup users (defined as regular use of eye makeup including mascara, eyeliner, eye shadow and eye creams) were also excluded due to the likelihood of interocular cross contamination, and to eliminate the possibility of participants using additional eye makeup removers during the study. Use of facial makeup and moisturiser did not exclude participants, as these are usually applied to the face with caution to avoid the eye area. Therefore, the risk of cross contamination between the eyes was minimal.

History of epilepsy or migraines exacerbated by flashing lights also excluded participants due to the strobe-like nature of the LipiView interferometer. Participants were also required to have similar vision in each eye determined by having monocular best corrected visual acuity of each eye within 2 lines of each other on a logMAR chart at 6 m distance, to ensure an unbiased perception of ocular comfort, as previous evidence suggests that reduced visual quality increases subjective ocular discomfort symptoms. [35]

2.4. Methods

Upon completion of the baseline visit, eligible participants were randomly assigned to use the Blephadex™ Eyelid Wipes once daily on either the right or the left eye for 30 days using the web based GraphPad software (https://www.graphpad.com/), to generate the randomisation sequence. A sealed envelope system was employed to notify the participant which eye was allocated to the treatment, and the designated eye was also labelled on the inside of the box of lid wipes by an unmasked investigator. The contralateral eye was to receive no treatment and serve as the control. Whilst investigators were masked to the treatment allocation for the duration of the study, it was impossible to mask participants. All participants underwent the following procedures at both the baseline and 30 day follow-up visit, in the order as listed.

2.4.1. Subjective ocular comfort

Subjective ocular comfort was assessed using the Ocular Surface Disease Index (OSDI) to elicit binocular symptoms of ocular dryness. [36] Additionally, a visual analogue scale was administered to capture monocular symptoms of itching, dryness and overall discomfort, which are symptoms which have been specifically demonstrated as related to the presence of *Demodex* [20,21].

2.4.2. Ocular surface assessment

Observation of tear film characteristics included measurement of the non-invasive tear break up time (NITBUT) using the Tearscope Plus (Keeler Ltd, Windsor, UK) in conjunction with a slit lamp biomicroscope. An average of three readings was taken for each eye. Average lipid layer thickness was measured using the LipiView ocular surface interferometer (TearScience, Morrisville, N.C.). Tear volume was measured using the phenol red thread tear test (Tianjin Jingming New Technological Development Co. Ltd, Tianjin, China). The phenol red thread test has been shown to have strong agreement with the Schirmer test, and was used in the current study as a measure of tear volume. [37]

Slit lamp biomicroscopic examination involved assessment of the lid margin, cornea and conjunctiva using white light. Corneal and conjunctival staining were also observed and graded according to the Oxford scheme, subsequent to instillation of fluorescein and lissamine green dyes, respectively.

2.4.3. Demodex count

Demodex count was performed using procedures as previously reported in the literature. [19] In brief, four lashes were epilated from each eye (one lash from each half of the superior and inferior eyelids) and placed on a glass slide. Lashes with cylindrical dandruff were preferentially selected, as the results of a previous study demonstrated a higher prevalence of *Demodex* in these lashes. [38] A coverslip was placed over the lashes and fluorescein was applied to the edge of the coverslip to enhance the appearance of *Demodex* [39]. *Demodex* were observed and counted under a light microscope at 40X magnification after 20 min, to allow dissolution of cylindrical dandruff and *Demodex* mites to migrate away from the lash. Images were captured using the Olympus DP80 digital microscope camera.

2.4.4. Eyelid aerobic bacteria analysis

A swab of the eyelid was taken by rolling a moistened cotton tip on the inferior lid margin of each eye.

Isolation and identification of bacteria was conducted essentially as reported by Wu et al. [40] with the following modifications: swabs were infused in a phosphate-buffered saline solution and agitated for 60 s using a vortex mixer; 400 µL of the solution was plated out on soya nutrient agar, then cultured aerobically at 37 °C for 24 h. Colony morphology and number of colony forming units (CFU) was recorded, and each type of morphology was speciated by means of Gram staining, catalase and coagulase testing.

2.4.5. Bacterial lipase assay

After bacterial colony count was completed, two colonies from each plate were isolated for inoculation in individual tubes of 5 mL of trypticase soy broth (TSB) and incubated for 24 h at 37 °C with continuous agitation. A stock was then made comprising of 500 µL broth and 500 µL of 50% glycerol solution and stored at −80 °C. Following all sample collection, frozen stocks were cultured in trypticase soy broth overnight at 37 °C with continuous agitation. From each of the bacterial colony cultures, 50 µL microlitres of the cultured supernatant was then aliquoted into 6 mm diameter wells on 1.5% agarose gel plates containing glycerol monolaurate (500 µg/mL). Plates were incubated for 18 h at 37 °C. Geh Lipase in the supernatant is known to hydrolyse glycerol monolaurate within the agarose plates, which is observed as a zone of clearance. [41] Lipase production was quantified by measuring the diameter of the clearance zone and subtracting the diameter of the well (Fig. 1). An average lipase clearance zone was calculated from the two zones observed from the two bacterial colony cultures.

2.4.6. Statistical analysis

The Statistical Package for Social Sciences (SPSS 23.0 for Windows, Chicago, IL, USA) was used to analyse the data. A two-way repeated measures analysis of variance (ANOVA) was conducted to compare the treated and untreated eyes, and the change in variables between visits. In the presence of a statistically significant interaction, it was appropriate to use paired t-tests to compare change between visits in the treated and untreated groups. All analyses were two-tailed and $p < 0.05$ was considered statistically significant. Normality was assessed by observation of Q-Q plots and data were log transformed for variables with a skewed distribution. Spearman's correlation test was performed to determine whether any associations exist between *Demodex*, bacterial lipase, bacterial colony count and lipid layer thickness NITBUT, tear volume or ocular symptoms.

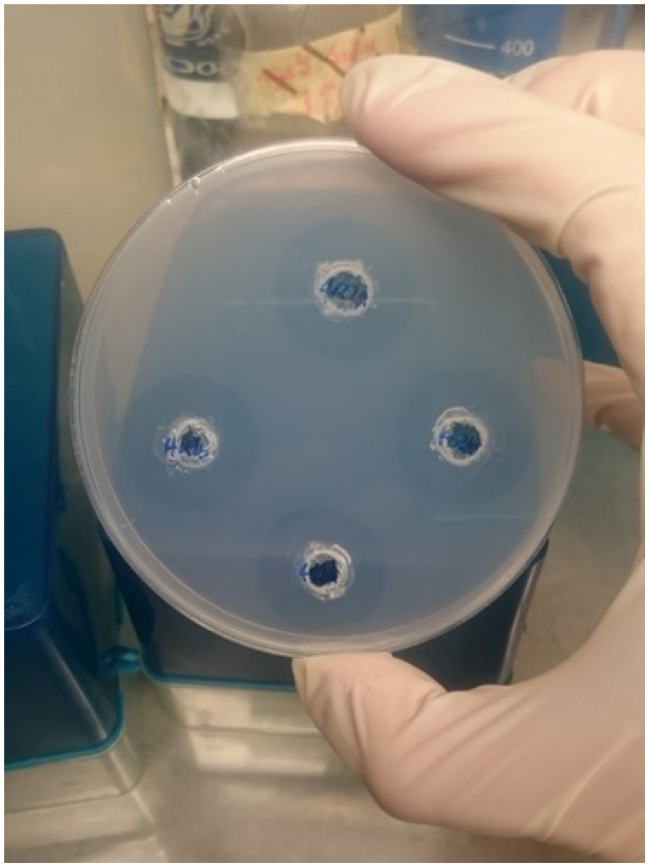

Fig. 1. Clearance zones indicating degradation of glycerol monolaurate.

3. Results

A total of 20 participants (6 males and 14 females) with median age of 63.5 (range 48–76) completed the study over the period of November 2016 to April 2017. Participant compliance was verified by checking that the number of unused wipes returned was consistent with the number of days in the trial. 95% of participants used the expected number of wipes and 5% of participants were more than 10% non-compliant. No adverse events were reported throughout the study and the product was well tolerated by participants with only one reporting slight discomfort upon using the wipe initially. This participant continued to use the wipe and did not experience ongoing discomfort.

Overall, *Demodex* counts were low in this healthy population (Table 2) but nevertheless, a statistically significant reduction in the *Demodex* count was observed between baseline and the 30 day visit for the treated eyes only (median ± IQR: 2 ± 3 vs 0 ± 2, ANOVA $p = 0.04$, respectively). This represents a 50% reduction in *Demodex* burden after treatment (median ± IQR: 2 ± 3 vs 0 ± 2, paired *t*-test $p < 0.01$) compared to an 11% reduction in *Demodex* in the untreated eyes between visits (median ± IQR: 3 ± 5 vs 2 ± 4, paired *t*-test $p = 0.45$).

Fig. 2 shows *Demodex* mites embedded in a cylindrical dandruff at

Table 2
Effect of daily use of Blephadex™ Eyelid Wipes on treated versus untreated eyes after 30 days.

VARIABLE	TREATED		UNTREATED		ANOVA p value
	Baseline Median (IQR)	Day 30 Median (IQR)	Baseline Median (IQR)	Day 30 Median (IQR)	
Demodex (number)	2 (3)	0 (2)	3 (5)	2 (4)	0.04*
Colony Count (number)	60 (149)	70 (120)	118 (198)	83 (100)	0.29
Lipase clearance zone (mm)	16 (8)	15 (4)	15 (6)	15 (2)	0.25
NITBUT Average (sec)	5.4 (1.6)[a]	4.9 (1.0)[a]	4.9 (1.3)[a]	4.9 (1.7)[a]	0.40
Lipiview Mean (nm)	70.4 (25.5)[a]	68.5 (24.8)[a]	71.4 (22.3)[a]	68.3 (23.7)[a]	0.44
Tear Volume (mm)	10 (8)	11 (9)	14 (8)	11 (10)	0.06
OSDI (0-100)	9 (15)	9 (14)	9 (15)	9 (14)	0.15
Itching (0-100 VAS)[b]	10 (25)	5 (21)	10 (24)	6 (17)	0.31
Overall discomfort (0-100 VAS)[b]	15 (32)	10 (16)	14 (32)	10 (21)	0.05*
Dryness (0-100 VAS)[b]	23 (42)	12 (21)	23 (41)	10 (15)	0.02*

P value refers to difference between visits.
[a] Mean (SD) displayed for normally distributed variables.
[b] VAS = visual analogue scale; 0 = No symptoms; 100 = maximum symptoms.
* Asterix indicates statistically significant difference.

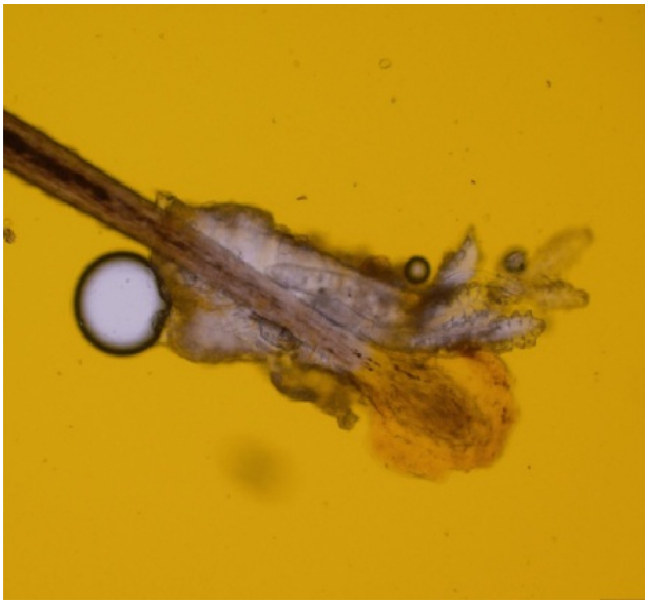

Fig. 2. Multiple *Demodex* mites embedded in a cylindrical dandruff.

the base of a lash collected at the baseline visit. Out of the 20 participants, six participants had no *Demodex* in either eye at both the baseline and 30 day visit. Of the 14 participants with *Demodex* present at the baseline visit, a larger percentage of eyes in the treated group had *Demodex* count decrease to zero (50% of eyes in the treated group compared to 29% in the untreated group). However, whilst nine participants had reduced *Demodex* count in the treated eye, nine participants also had reduced *Demodex* count in the untreated eye.

No significant difference was found between treated and untreated eyes for bacteria colony count or lipase production. Only gram-positive bacteria were isolated from the lid cultures with *Staphyloccocus epidermidis* being cultured from all 80 lid swabs and in addition, Sta*phylococcus aureus* being cultured from 3 lid swabs. NITBUT, lipid layer thickness and tear volume did not change over time in the treated or untreated eyes (Table 2, ANOVA $p > 0.05$).

Comfort ratings improved for treated eyes only (VAS median ± IQR: 15 ± 32 vs. 10 ± 16, ANOVA $p = 0.05$). A statistically significant reduction in dryness symptoms was demonstrated in both treated and untreated eyes (ANOVA $p = 0.02$). However, there were no significant changes in the OSDI or ocular itch scores over time (ANOVA $p > 0.05$). Interestingly, when participants were stratified into dry eye and normal subgroups using a criteria of OSDI < 13 being normal [42], the median *Demodex* count was numerically higher in the dry eye subgroup compared to the normal group (median 8 vs. 2) at baseline. However, the number of participants in each subgroup ($n = 9$ in dry eye group, $n = 11$ in normal subgroup) was too small to perform any meaningful statistical analysis or draw any conclusions. Correlations analysis showed that Demodex count had a significant positive correlation with OSDI ($r = 0.31$, $p = 0.01$) and itching measured by the VAS ($r = 0.28$, $p = 0.01$).

4. Discussion

This pilot study was the first to investigate the effect of the commercially available product Blephadex™ Eyelid Wipes which contain both tea tree oil and coconut oil, on *Demodex* numbers, ocular comfort and bacterial lipase production. Post-hoc power calculation based on a sample size of 20 determined that a numerical reduction in *Demodex* of 3.2 (0.5 ± 0.75 log units) could be detected with a power of 80% and at a 5% level of significance. Previous research has suggested that symptoms of ocular irritation were correlated with a greater than nine mites *Demodex* count on average [43], whereas in the current study, the mean number of *Demodex* mites at baseline was equivalent to six mites per participant on average. Given that the number of *Demodex* mites observed in the study cohort was low, the reduction in *Demodex* count whilst statistically significant, may not be clinically significant. This supports the need for further investigation amongst a population with confirmed *Demodex* blepharitis, rather than a normal healthy population.

Although no change in *Staphylococcus* spp. counts or lipase production were observed in this study, there was a statistically significant reduction in *Demodex* numbers in the treated eyes only. Gao et al. [19] was the first to suggest the use of tea tree oil for *Demodex* treatment, based on its known acaricidal properties and ability to kill head lice. Concentrations of tea tree oil varying from 5% to 50% have been reported to reduce *Demodex* counts. [19,21] This study demonstrated a statistically significant reduction in *Demodex* count after daily use of Blephadex™ Eyelid Wipes for 30 days. However, this was not accompanied by a significant change in bacterial colony count. This suggests that bacteria and *Demodex* mites may operate somewhat independently in their pathogenic effect. Previous studies have reported that bacterial burden is similar in people with *Demodex* and without. [6] The concentration of tea tree oil and coconut oil contained in the Blephadex™ Eyelid Wipes has not been specified by the manufacturer and may in fact be below the minimum inhibitory concentration for *Staphylococcus* spp. The absence of change in the bacterial colony count may also

suggest that treatment period of longer than 30 days may be required to produce a significant reduction. No change in the NITBUT, tear film lipid layer thickness or tear volume were observed in this study. This corroborates the findings of Gao et al. [22] where lipid layer thickness was not statistically different after treatment with tea tree oil.

Although no statistically significant change in lipase production was observed between the baseline and 30 day visit, bacterial lipase production was measured from two isolated colonies *in vitro*. However, the bacterial colonies cultured may not be clonal and therefore sampling single colonies would not represent the lipase production potential of all bacterial colonies present. Measuring lipase directly from tears may reflect lipase production *in vivo* more accurately, since any reduction in lipase production observed might be more a reflection of the bacterial community dynamics as a whole.

Demodex infestation was shown to have significant correlation with ocular discomfort and itching which was consistent with findings in previous studies. [20,21] However, no significant change was observed in the OSDI or ocular itch scores (ANOVA $p > 0.05$) with treatment, despite the reduction in *Demodex* numbers observed in the current study. This may be attributed to the fact that this study was conducted on a normal healthy population, where ocular symptoms and numbers of *Demodex* were low at the baseline visit compared to studies of subjects with blepharitis.

A statistically significant improvement in comfort rating using a visual analogue scale was observed between baseline and final visit for treated eyes only, although subjects were not masked to the treatment. It is interesting to note that a statistically significant reduction in ocular dryness symptoms was seen in both treated and untreated eyes between the baseline and 30 day visit. This may relate to the perception of comfort in one eye having a carry-over effect to the contralateral eye. Standard deviations of the visual analogue scale scores were also high, indicating a high level of variability. Whilst visual analogue scales are a simple and commonly used method for assessing intensity of pain in clinical practice, such scales have received criticism for low levels of repeatability. [44] Numerical rating scales have been shown to have better compliance [44] and may be incorporated in future studies to measure symptoms. In accordance with the recommendations of the recently published TFOS DEWS II Diagnostic Methodology Report, the DEQ-5 questionnaire should also be considered in future studies as a valid assessment that is both short and discriminatory [45,46].

Weaknesses of a contralateral study design include relying on the participant to comply with using the eyelid wipe on the allocated eye only. It is not possible to ensure complete compliance with at-home treatments. Another limitation of using the contralateral eye as a control is that it was not possible for the participant to be masked to the treatment. Therefore, changes in symptoms may be biased. It would be valuable in future studies, to implement a double masked parallel group design comparing the use of Blephadex™ Eyelid Wipes in one group with a placebo eyelid wipe containing no active ingredients in a second group. This would further elucidate if the effect on *Demodex* numbers is mechanical or due to the active ingredients.

The population in this study reflected a normal healthy population of 45 years and above. Being the first study to evaluate the product Blephadex™ Eyelid Wipes, a normal population was selected to evaluate the feasibility of the product as an at-home treatment. However, it would be pertinent to preselect participants with symptomatic blepharitis for future studies. It may also be worthwhile to study treatment over a longer period of time to ascertain if the length of treatment would also affect symptoms, bacterial colony counts and lipase production.

5. Conclusions

In this pilot study, no changes were observed in ocular microbiota, tear film characteristics or bacterial lipase in eyes tested with Blephadex™ Eyelid Wipes after one month of daily use in this normal healthy population. Although a statistically significant reduction in *Demodex* count was observed in eyes treated with Blephadex™ Eyelid Wipes, the overall numbers of *Demodex* were low. A parallel group, placebo controlled, randomised clinical trial in a population with active blepharitis is warranted to further elucidate these preliminary findings.

Declarations of interest

None.

Acknowledgements

The authors thank Dr Ajay Vijay and Dr Jaya Sowjanya Siddyreddy for their advice on conducting lipase assay. We also thank Dr Thomas Naduvilath for his valuable time and assistance in the statistical data analysis. OptiMed Pty Ltd supplied Blephadex™ Eyelid Wipes at no cost. However, OptiMed Pty Ltd had no other role in the study and none of the investigators are receiving benefits or have any financial interest with OptiMed Pty Ltd. No conditions were imposed upon the use, ownership of results or material by OptiMed Pty Ltd or any other party.

References

[1] J. Liu, H. Sheha, S.C. Tseng, Pathogenic role of Demodex mites in blepharitis, Curr Opin Allergy Clin Immunol 10 (5) (2010) 505.
[2] C.F. Post, E. Juhlin, Demodex folliculorum and blepharitis, Arch Dermatol 88 (3) (1963) 298–302.
[3] N. Lacey, S.N. Raghallaigh, F.C. Powell, Demodex mites–commensals, parasites or mutualistic organisms, Dermatology 222 (2) (2011) 128–130.
[4] G. Geerling, J. Tauber, C. Baudouin, E. Goto, Y. Matsumoto, T. O'Brien, et al., The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction, Invest Ophthalmol Vis Sci 52 (4) (2011) 2050–2064.
[5] I. Jalbert, S. Rejab, Increased numbers of Demodex in contact lens wearers, Optom Vis Sci 92 (6) (2015) 671–678.
[6] S.H. Lee, Y.S. Chun, J.H. Kim, E.S. Kim, J.C. Kim, The relationship between Demodex and ocular discomfort, Invest Ophthalmol Vis Sci 51 (6) (2010) 2906–2911.
[7] L.R. Groden, B. Murphy, J. Rodnite, G.I. Genvert, Lid flora in blepharitis, Cornea 10 (1) (1991) 50–53.
[8] G.N. Foulks, The correlation between the tear film lipid layer and dry eye disease, Surv Ophthalmol 52 (4) (2007) 369–374.
[9] J.P. Craig, A. Tomlinson, Importance of the lipid layer in human tear film stability and evaporation, Optom Vis Sci 74 (1) (1997) 8–13.
[10] J.M. Dougherty, J.P. McCulley, Bacterial lipases and chronic blepharitis, Invest Ophthalmol Vis Sci 27 (4) (1986) 486–491.
[11] F. Jimenez-Acosta, L. Planas, N. Penneys, Demodex mites contain immunoreactive lipase, Arch Dermatol 125 (10) (1989) 1436–1437.
[12] J.M. Dougherty, J.P. McCulley, R.E. Silvany, D.R. Meyer, The role of tetracycline in chronic blepharitis. Inhibition of lipase production in staphylococci, Invest Ophthalmol Vis Sci 32 (11) (1991) 2970–2975.
[13] M. Glasson, F. Stapleton, M. Willcox, Lipid, lipase and lipocalin differences between tolerant and intolerant contact lens wearers, Curr Eye Res 25 (4) (2002) 227–235.
[14] G. Fulk, C. Clifford, A case report of demodicosis, J Am Optom Assoc 61 (8) (1990) 637–639.
[15] G.W. Fulk, B. Murphy, M.D. Robins, Pilocarpine gel for the treatment of demodicosis–a case series, Optom Vis Sci 73 (12) (1996) 742–745.
[16] L. Jones, L.E. Downie, D. Korb, J.M. Benitez-del-Castillo, R. Dana, S.X. Deng, et al., TFOS DEWS II management and therapy report, Ocul Surf 15 (3) (2017) 575–628.
[17] A. Raman, U. Weir, S. Bloomfield, Antimicrobial effects of tea-tree oil and its major components on Staphylococcus aureus, Staph. Epidermidis and Propionibacterium acnes, Lett Appl Microbiol 21 (4) (1995) 242–245.
[18] K. Hammer, C. Carson, T. Riley, Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil, J Appl Microbiol 95 (4) (2003) 853–860.
[19] Y. Gao, M. Di Pascuale, W. Li, A. Baradaran-Rafii, A. Elizondo, C. Kuo, et al., In vitro and in vivo killing of ocular Demodex by tea tree oil, Br J Ophthalmol 89 (11) (2005) 1468–1473.
[20] H. Koo, T.H. Kim, K.W. Kim, S.W. Wee, Y.S. Chun, J.C. Kim, Ocular surface discomfort and Demodex: effect of tea tree oil eyelid scrub in Demodex blepharitis, J Korean Med Sci 27 (12) (2012) 1574–1579.
[21] Y.-Y. Gao, Xu D-l, R. Wang, S.C. Tseng, Treatment of ocular itching associated with ocular demodicosis by 5% tea tree oil ointment, Cornea 31 (1) (2012) 14–17.
[22] Y.-Y. Gao, M.A. Di Pascuale, A. Elizondo, S.C. Tseng, Clinical treatment of ocular demodecosis by lid scrub with tea tree oil, Cornea 26 (2) (2007) 136–143.
[23] J.E. Key, A comparative study of eyelid cleaning regimens in chronic blepharitis, CLAO J 22 (3) (1996) 209–212.
[24] M. Guillon, C. Maissa, S. Wong, Symptomatic relief associated with eyelid hygiene in anterior blepharitis and MGD, Eye Contact Lens 38 (5) (2012) 306–312.
[25] O. Murphy, V. O'Dwyer, A. Lloyd-McKernan, The efficacy of tea tree face wash, 1, 2-

Octanediol and microblepharoexfoliation in treating Demodex folliculorum blepharitis, Cont Lens Anterior Eye 41 (1) (2018) 77–82.
[26] S. Intahphuak, P. Khonsung, A. Panthong, Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil, Pharm Biol 48 (2) (2010) 151–157.
[27] V.M. Verallo-Rowell, K.M. Dillague, B.S. Syah-Tjundawan, Novel antibacterial and emollient effects of coconut and virgin olive oils in adult atopic dermatitis, Dermatitis 19 (6) (2008) 308–315.
[28] A. Marina, Y.C. Man, S. Nazimah, I. Amin, Chemical properties of virgin coconut oil, J Am Oil Chem Soc 86 (4) (2009) 301–307.
[29] J.L. Flanagan, N. Khandekar, H. Zhu, K. Watanabe, M. Markoulli, J.T. Flanagan, et al., Glycerol monolaurate inhibits lipase production by clinical ocular isolates without affecting bacterial cell viability, Invest Ophthalmol Vis Sci 57 (2) (2016) 544–550.
[30] P. Schlievert, J.R. Deringer, M.H. Kim, S.J. Projan, R. Novick, Effect of glycerol monolaurate on bacterial growth and toxin production, Antimicrob Agents Chemother 36 (3) (1992) 626–631.
[31] M. Lodén, W. Wessman, The influence of a cream containing 20% glycerin and its vehicle on skin barrier properties, Int J Cosmet Sci 23 (2) (2001) 115–119.
[32] R.M. Shelton, Aloe vera: its chemical and therapeutic properties, Int J Dermatol 30 (10) (1991) 679–683.
[33] Y. Takagi, M. Shimizu, Y. Morokuma, M. Miyaki, A. Kiba, K. Matsuo, et al., A new formula for a mild body cleanser: sodium laureth sulphate supplemented with sodium laureth carboxylate and lauryl glucoside, Int J Cosmet Sci 36 (4) (2014) 305–311.
[34] S.E. Jacob, S. Amini, Cocamidopropyl betaine, Dermatitis 19 (3) (2008) 157–160.
[35] E. Papas, E. Chan, L. Sarian, J. Tan, Does the Quality of Vision Affect the Perception of Ocular Discomfort? Invest Ophthalmol Vis Sci 44 (13) (2003) 3694 E-Abstract.
[36] R.M. Schiffman, M.D. Christianson, G. Jacobsen, J.D. Hirsch, B.L. Reis, Reliability and validity of the ocular surface disease index, Arch Ophthalmol 118 (5) (2000) 615–621.
[37] S. Vashisht, S. Singh, Evaluation of phenol red thread test versus Schirmer test in dry eyes: a comparative study, Int J Appl Basic Med Res 1 (1) (2011) 40.
[38] Y.-Y. Gao, M.A. Di Pascuale, W. Li, Liu DT-S, A. Baradaran-Rafii, A. Elizondo, et al., High prevalence of Demodex in eyelashes with cylindrical dandruff, Invest Ophthalmol Vis Sci 46 (9) (2005) 3089–3094.
[39] A. Kheirkhah, G. Blanco, V. Casas, S.C. Tseng, Fluorescein dye improves microscopic evaluation and counting of Demodex in blepharitis with cylindrical dandruff, Cornea 26 (6) (2007) 697–700.
[40] Y.T. Wu, H. Zhu, N.Y. Harmis, S.Y. Iskandar, M. Willcox, F. Stapleton, Profile and frequency of microbial contamination of contact lens cases, Optom Vis Sci 87 (3) (2010) E152–E158.
[41] A. Ruzin, R.P. Novick, Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in Staphylococcus aureus, J Bacteriol 182 (9) (2000) 2668–2671.
[42] K.L. Miller, J.G. Walt, D.R. Mink, S. Satram-Hoang, S.E. Wilson, H.D. Perry, et al., Minimal clinically important difference for the ocular surface disease index, Arch Ophthalmol 128 (1) (2010) 94–101.
[43] A.B. de Venecia, Lim Bon Siong R. Demodex sp. Infestation in anterior blepharitis, meibomian-gland dysfunction, and mixed blepharitis, Phillipp J Ophthalmol. 36 (1) (2011) 15–22.
[44] M.J. Hjermstad, P.M. Fayers, D.F. Haugen, A. Caraceni, G.W. Hanks, J.H. Loge, et al., Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review, J Pain Symptom Manage 41 (6) (2011) 1073–1093.
[45] J.S. Wolffsohn, R. Arita, R. Chalmers, A. Djalilian, M. Dogru, K. Dumbleton, et al., TFOS DEWS II diagnostic methodology report, Ocul Surf 15 (3) (2017) 539–574.
[46] R.L. Chalmers, C.G. Begley, B. Caffery, Validation of the 5-Item Dry Eye Questionnaire (DEQ-5): discrimination across self-assessed severity and aqueous tear deficient dry eye diagnoses, Cont Lens Anterior Eye 33 (2) (2010) 55–60.