Article title: Microwave Decontamination of Eyelid Warming Devices for the Treatment of Meibomian Gland Dysfunction

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Abstract

Purpose: The role of bacteria in meibomian gland dysfunction is unclear, yet contamination of compresses used as treatment may exacerbate this condition. This study therefore determined the effect of heating on bacteria on two forms of compress.

Methods: Cotton flannels and MGDRx EyeBags (eyebags) were inoculated by adding experimental inoculum (*S. aureus*, *S. pyogenes*, *P. aeruginosa*; one species for each set of 3 eyebags and flannels). One of each were then randomised in to 3 groups: no heating (control); therapeutic (47.4±0.7°C); or sanitisation (68±1.1°C). After treatment, bacteria cell numbers were calculated. The experiment was repeated in triplicate.

Results: There was a statistically significant difference between each treatment with the eyebag for *S. aureus* (control=7.15±0.11logC/ml, therapeutic heating=5.24±0.59 logC/ml, sanitisation heating=3.48±1.43logC/ml; p<0.001) and *S. pyogenes* (7.36±0.13, 5.73±0.26, 4.75±0.54; p<0.001). *P. aeruginosa* also showed a significant reduction (p<0.001) from control (6.39±0.34) to therapeutic (0.33±0.26) and sanitisation (0.33±0.21), but the latter were similar (p=1.000). For the flannels, there was significant difference between each treatment for *S. aureus* (6.89±0.46, 3.96±1.76, 0.42±0.90; p<0.001). For *S. pyogenes,* there was a significant reduction (p<0.001) from control (7.51±0.10) to therapeutic (5.91±0.62) and sanitisation (5.18±0.8), but the latter were similar (p=0.07). For *P. aeruginosa*, there was a significant difference (p<0.001) from control (7.15±0.36) to sanitisation (5.83±0.44); but not to therapeutic (6.84±0.31) temperatures (p=0.07).

Conclusions: Therapeutic heating produces a significant reduction in bacteria on the eyebags, but only sanitisation heating appears effective for flannels. However, patients should be advised to heat the eyebag to sanitisation temperatures on initial use.

INTRODUCTION

Treatment of meibomian gland dysfunction (MGD) is typically focussed on eyelid hygiene and eyelid warming therapy to melt abnormal meibum, clear the obstructive material and therefore unblock the meibomian gland to help restore normal function.[1] Effective eyelid warming devices reported in the scientific literature include the use of moist air goggles, combined heat and pulsatile pressure devices, and eye-masks that are heated by light-emitting-diodes or controlled chemical reactions [1, 2, 3]. In clinical practice, therapy frequently involves the recommendation of patient applied traditional warm compresses that can be performed with either a moistened flannel, or an eye-mask, such as the dry seed-filled MGDRx EyeBag, that can be heated within the home using a microwave oven [4, 5, 6]. However, these patient applied procedures nearly always involve the re-use of the same device, and little information, if any at all, is provided with respect to their maintenance, storage, and decontamination.

Indeed, the role of bacteria in the pathophysiology of MGD remains unclear [7]. Previous histopathologic studies by Gutgusell *et al*. (1982) showed that inflammatory cell infiltration was absent or minimal in MGD patients, suggesting that inflammation secondary to bacterial infection did not play an important role [8]. However, in studies on the bacteriology of healthy controls and patients with chronic blepharitis, who often present with concomitant MGD*, Staphylococcal aureus*, *Corynebacterium spp.*, and *Propionibacterum acnes* was frequently isolated from the eyelid margin and expressed meibum, suggesting bacteria normally reside inside or within the vicinity of the meibomian gland [9, 10]. Moreover, studies have shown that lipases and esterases from commensal bacteria are able to modify or degrade the meibum lipids and increase levels of free fatty acids, rather than cause direct infection and damage [7, 10, 11]. Although these lipid changes may affect the composition of meibum and increase viscosity as described above, free fatty acids have been suggested to also cause irritation, inflammation, and stimulate keratinisation of the eyelid margin epithelium in patients with chronic blepharitis [10, 12]. In addition, bacterial growth may even be encouraged by the increased availability of cholesterol due to bacterial esterase [12, 13]. Therefore, stasis and obstruction of the meibomian gland may lead to increased levels of pre-existing bacteria that can exacerbate MGD, rather than a primary infective cause [7].

Although heating of the compress device material at regular intervals may decontaminate it before application, temperatures obtained at therapeutic levels of heating are not sufficient to do so effectively. Temperatures that melt the abnormal meibum effectively while remaining safe (no thermal injury, cataract formation, corneal deformation, or ocular surface damage) and comfortable typically range between 40-45°C [2, 4, 5, 6]; whereas heat sterilisation of medical instruments require a minimum of 121°C to 134°C (ISO17665-1:2006). Thus, eyelid warming devices that make contact with the eyelids and surrounding tissue that are not sufficiently heated to decontaminate them may inadvertently prolong and or reinforce MGD. While there are no published reports showing infection caused by the use of such treatments, the presence of significant eyelid contamination is associated with an increased risk of ocular discomfort and infection in contact lens wearers [14]. However, there appears to be no reports in the scientific literature relating to the decontamination of eyelid warming devices or how this may be achieved. Therefore, this study aimed to determine the effectiveness of microwave decontamination of an eyebag and traditional compress (flannel) on bacterial isolates representative of those naturally existing on the eyelid margin.

MATERIALS AND METHODS

The study was designed as a randomised, controlled, examiner masked in-vitro experiment..

Experimental Protocol

Separate *Staphylococcus aureus* (NCTC10788; National Collection of Type Cultures, Public Health England), *Streptococcus pyogenes* (NCTC8198), and *Pseudomonas aeruginosa* (NCTC10332) experimental inoculum were prepared by placing a few colonies of the bacteria in 10mL of nutrient broth and incubating at 37°C for 24 hours. A haemocytometer (Neubaur, Germany) was used to count the number of cells (C/ml) in each overnight culture, before diluting in nutrient broth to obtain a bacterial count of $2-6 \times 10^7$ per ml for each species.

A set of 9 of each new and unused eyebags and flannels (unfolded 30cmX30cm 100% cotton flannels; soaked in sterile water for 10 seconds and then excess water wrung until no further water was liberated) were microwaved (centre of the carousel tray) for 60 seconds at 800W and left to cool to 25°C), before being inoculated by adding ten evenly spaced 0.1mL drops of the experimental inoculum (one species for each set of 3 eyebags and flannel compresses) and left for five minutes to allow the inoculum to be absorbed. One of each inoculated eyebag and flannel in each set was randomised into one of the following:

Control: did *not* receive any heat treatment. This group not only served as a control but also allowed the sensitivity of the bacterial recovery method to be determined.

Therapeutic: heated for 30 seconds in an 800W microwave oven to an immediate surface temperature of 47.4±0.7°C (ThermoTracer 7102MX, NEC, Japan).

Sanitisation: heated for 60 seconds in the same 800W microwave oven to produce an immediate surface temperature of 68±1.1°C

After treatment, all eyebags and flannels were placed into separate sterile stomacher bags and left to cool for 70 minutes. Nutrient broth (200mL) was then added to each bag and kneaded for 2 minutes using a stomacher machine (Stomacher 400 Circulator, Steward, West Sussex, UK). A stomacher is a laboratory homogeniser, where a series of paddles manipulate the contents of the bag creating a washing effect to drive more microorganisms from the sample into the surrounding diluent, thereby providing better recovery for analysis. A 0.1mL sample of this solution was then taken and 1:10 dilution series was created in 0.85% sodium chloride for each bag. A 0.1mL sample of each dilution was spread evenly on separate nutrient agar (*S. aureus; P. aeruginosa*) or blood agar (*S. pyogenes*) plates (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours before counting and calculating the number of cells. This experimental protocol was repeated in triplicate using new and unused eyebags and flannels each time.

Due to the destructive nature of the testing, an additional 9 eyebags and flannels that were neither inoculated nor heat-treated underwent microbiological analysis to determine the pre-existing bacterial load. Here, the eyebags and flannels were placed into separate stomacher bags and 200mL of nutrient broth was added before kneading for 2 minutes using the stomacher machine to release any organisms. The solution from each bag was then inoculated on to a range of selective culture media (nutrient agar CM0003, violet red bile

glucose agar CM1082, manitol salt agar PO0151, and *Pseudomonas* agar base CM0559 with selective supplement SR103). Any cultures of characteristic morphology were observed using microscopy and staining to arrive at a presumptive identification. Further investigation with biochemical profiling tests (API20E and API20NE, BioMerieux UK Ltd., Hampshire, UK) was performed to confirm the bacterial species. Another 9 un-inoculated eyebags and flannel compresses had the sanitisation protocol applied and then also underwent the same analysis to determine if sanitisation temperatures affected the levels of bacteria previously identified.

In all cases sterile disposable gloves were worn at all times while handling the eyebags and compresses.

Statistical Analysis

As the data were normally distributed (Kolmogorov-Smirnov test p>0.05), one-way ANOVA was performed to determine if there were any significant differences between the levels of each bacterium in the controls and after heat and sanitisation treatment. Tukey's post-hoc test was performed to determine which group differed from the others for each bacterium if appropriate. Unpaired Student's *t*-test was performed to determine if there were any significant differences in the change in bacteria levels between the eyebags and flannel compresses, and before and after treatments. Statistical significance was taken as p<0.05.

RESULTS

Effect of Heating on Pre-existing Bacteria

Microbiological analysis of the 9 un-inoculated and un-heated eyebags revealed pre-existing bacteria including the species *Bacillus spp.* (mean 1.63±0.98 logC/ml) *E. sakazakki* (4.13±1.56 logC/ml) and *S. aureus* (4.40±0.42 logC/ml). Heating of the other 9 un-inoculated eyebags for 60 seconds at 800W (sanitisation protocol) demonstrated a statistically significant reduction in bacterial load for *Bacillus spp.* (0.76±1.40 logC/ml; p=0.006), *E. sakazakki* (2.56±1.17 logC/ml; p=0.001) and *S. aureus* (2.47±1.10; p<0.001).

Pre-existing bacteria on the 9 un-inoculated and un-heated flannels included *Bacillus licheniformis* (mean 3.64±2.82 logC/ml), *P. aeruginosa* (2.56±2.80 logC/ml), and *Pseudomonas luteloa* (5.46±0.17 logC/ml). Sanitisation heating of the other 9 un-inoculated flannels demonstrated no statistically significant difference in the bacterial load for *Bacillus licheniformis* (2.28±2.50 logC/ml; p=0.39) and *P. aeruginosa* (1.65±2.55 logC/ml; p=0.57), except for *Pseudomonas luteloa* (1.48±2.29 logC/ml, p=0.002).

Effect of Heating on Known Levels of Bacteria

The mean initial culture bacterial counts before application to the experimental eyebags and flannels were 7.48±0.21 logC/ml for *S. aureus*, 7.86±0.25 logC/ml for *S. pyogenes*, and 7.54±0.19 logC/ml for *P. aeruginosa*; thus simulating a very high level of inoculation. Using non-treated (control) eyebags, the sensitivity of bacterial recovery was 95.6% for *S. aureus*, 93.7% for *S. pyogenes*, and 84.6% for *P. aeruginosa* (Figure 1). For the flannel compresses, the sensitivity of bacterial recovery was 92.0% for *S. aureus*, 95.6% for *S. pyogenes*, and 94.7% for *P,aeruginosa* (Figure 2).
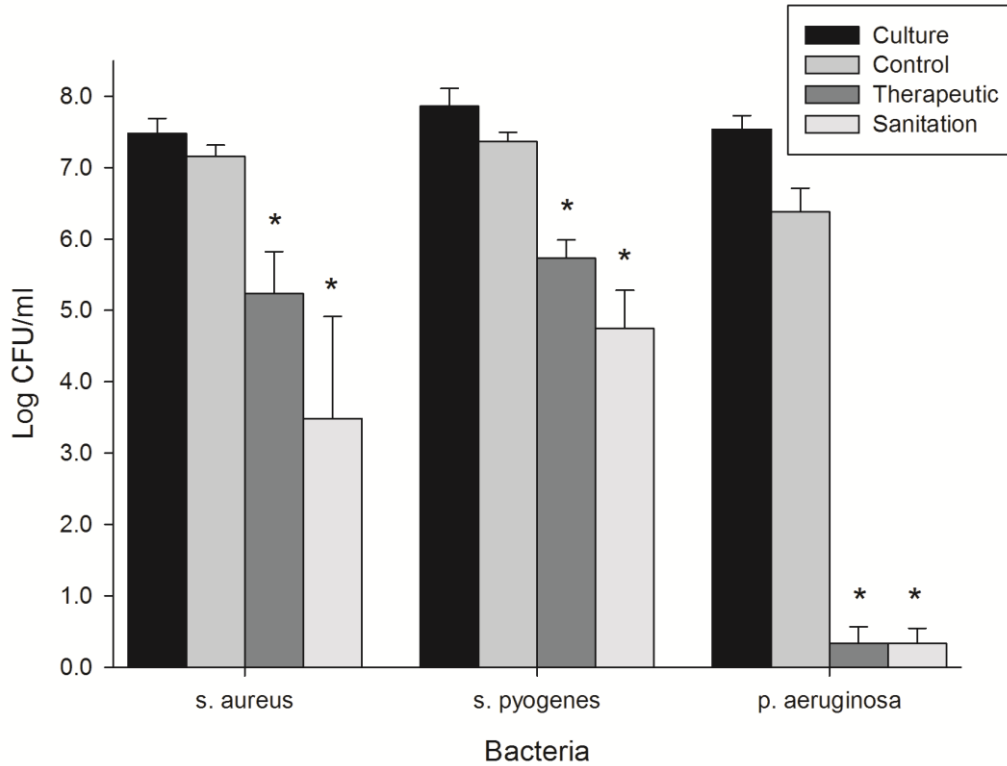
Figure 1: Bacterial cell counts (log CFU/ml) in control, therapeutic and sanitisation treatments for each bacterium (S. aureus, S. pyogenes, and P. aeruginosa) using the eyebags. Error bars represent ±1 standard deviation. Asterisks represent a statistically significant difference compared to the respective control cell count.
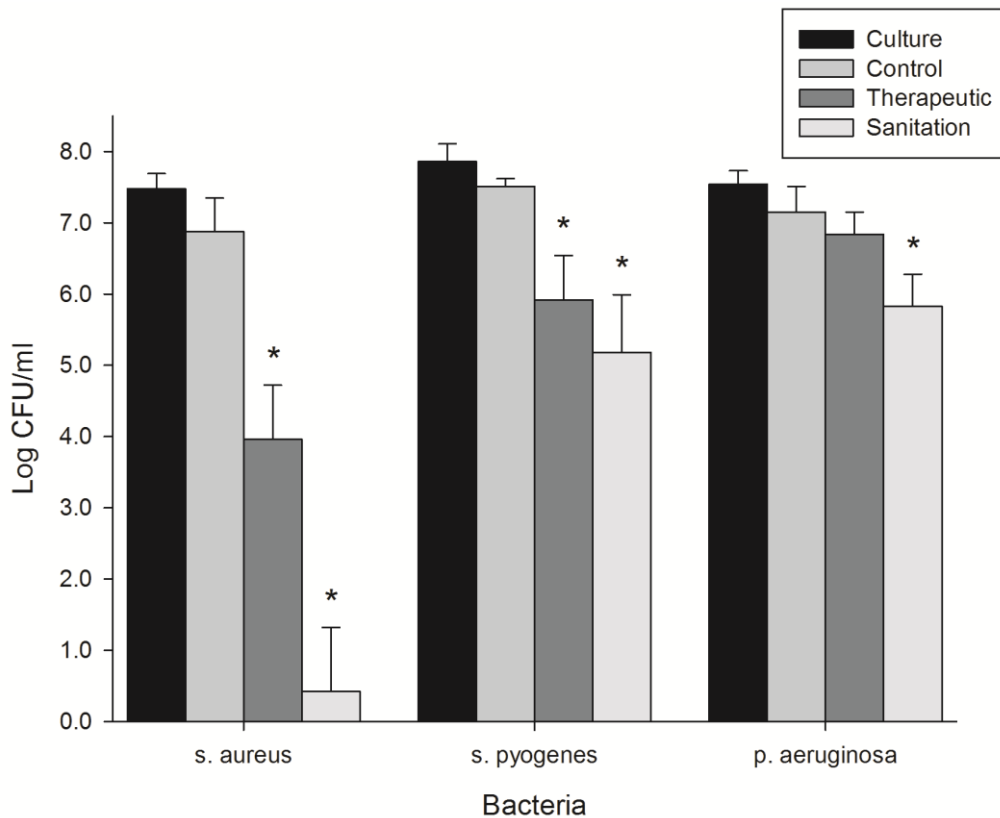
Figure 2: bacterial cell counts (log CFU/ml) in control, therapeutic and sanitisation treatments for each bacterium (S. aureus, S. pyogenes, and P. aeruginosa) using the flannel compresses. Error bars represent ±1 standard deviation. Asterisks represent statistically significant difference ($p < 0.05$) compared to the respective control cell count.

S. aureus

For the eyebags (Figure 1) and flannels (Figure 2), there was a statistically significant difference between controls, therapeutic and sanitisation treatment for *S. aureus* (F=37.85, $p < 0.001$; F=67.94, $p < 0.001$). There was a statistically significant reduction in bacteria compared to control levels between the eyebag and flannels for sanitisation heating (mean reduction 3.67±1.47 logC/ml versus 6.47±0.97 logC/ml; $p < 0.001$) but not therapeutic heating (1.92±0.50 logC/ml versus 2.93±0.87 logC/ml; p=0.13).

S. pyogenes

For the eyebags, there was a statistically significant difference between controls, therapeutic and sanitisation treatment for *S. pyogenes* (F=127.60, $p < 0.001$; Figure 1), but for the flannels (F=42.01, $p < 0.001$; Figure 2) there was no difference between therapeutic and sanitisation treatment (p=0.070). There was no statistically significant difference in the change in bacterial levels compared to control levels between the eyebag and flannels for therapeutic (mean reduction 1.63±0.28 logC/ml versus 1.59±0.66 logC/ml; p=0.88) and sanitisation heating (2.62±0.59 logC/ml versus 2.33±0.87 logC/ml; p=0.43).

P. aeruginosa

For the eyebags, there was a statistically significant difference between controls, therapeutic and sanitisation treatment for *P. aeruginosa* (F=155.72, p<0.001; Figure 1), but not between therapeutic and sanitation treatment (p=1.000; Figure 2). For the flannels, there was a significant difference between the sanitisation treatment and both the controls and therapeutic treatment (F=67.94, p<0.001), but the control colonisation was similar to that after therapeutic treatment (p=0.070). There was a statistically significant difference in the change in cell count compared to control levels between the eyebag and compress for therapeutic (mean reduction 6.04±0.95 logC/ml versus 0.31±0.51 logC/ml; p<0.001) and sanitisation heating (6.04±0.95 logC/ml versus 1.32±0.45 logC/ml; p<0.001).

DISCUSSION

Staphylococcus and Streptococcus species (both Gram-positive) are considered part of the normal flora that exist along the eyelid margin and meibomian glands, but over colonisation may initiate or exacerbate MGD [9, 13, 15]. Thus *S. aureus*, one of the most common species isolated from the eyelid margin and meibum, and *S. pyogenes*; which is also frequently detected, was included in the present study. Pseudomonas species was also selected not only as a representative Gram-negative bacterium, but due to its high resistance to antibiotics, ubiquitous nature, and potentially devastating effect on vision if infection arises [16, 17]. The eyebags and flannel compresses were inoculated with very high bacterial loads, beyond that which normally reside on ocular and surrounding tissue [9], in order to elicit any decontaminating effect in an extreme scenario.

Pre-existing bacteria detected on the 9 un-inoculated eyebags and flannel compresses included several Gram-negative species, but at levels far less than that used to inoculate the eyebag (mean 3.39±0.44 logC/ml). Pre-existing levels of *S. aureus* (4.40±0.42 logC/ml) were also detected on the eyebags. These bacteria are typically associated with seeds/grains and manual handling so were not unexpected owing to the composition of the eyebag. After heat-treating the un-inoculated eyebags, the levels of pre-existing species identified were significantly lower, confirming that the experimental eyebags were successfully decontaminated prior to inoculation. However, it is likely that any residual levels of S. aureus (if initially present) that survived this prior heat treatment may have contributed to the inoculum. Of concern is that pre-existing levels of *P. aeruginosa* were detected on the flannels - although levels reduced with sanitisation heating, this was not statistically significant.

Therapeutic heating of the eyebag, as would be prescribed by a practitioner to treat MGD produces a statistically significant reduction in cell count relative to the control group for all three species, particularly for *P. aeruginosa* (mean reduction 6.05±0.95 logC/ml). Although contact lens wear may be contraindicated in moderate to severe MGD cases, this result is somewhat reassuring in that the bacterial load of this species, which is the most frequently isolated organism in contact lens related microbial keratitis [16], is minimised following normal treatment if contact lenses are worn. However, only sanitisation heating significantly reduced levels of *P.aeruginosa* with the flannel compresses, but far less than for the eyebags – combined with the lack of significant reduction in pre-existing load, this suggests that *P. aeruginosa* is not effectively minimised during traditionally recommended wet flannel warm compress.

Although *S. aureus* and *S. pyogenes* levels decreased less (*S. aureus*: by 1.92±0.51 logC/ml; *S. pyogenes*: by 1.63±0.29 logC/ml) with the eyebags, this still represents a reduction of approximately 2 orders of magnitude. However, sanitisation temperatures produced a greater decontamination effect for both *S. aureus* (mean reduction 3.67±1.47 logC/ml) and *S. pyogenes* (2.62±0.59 logC/ml) compared to therapeutic heating, but the effect on *P. aeruginosa* was very similar (6.06±0.95 logC/ml). It should be noted that the effect of both therapeutic and sanitisation heating on *S. aureus* may be under-estimated, owing to its possible presence prior to eyebag inoculation. It may be that *S. aureus* and *S. pyogenes* survived better than *P.aeruginosa* as the inoculum containing them penetrated the eyebag deeper, and were insulated by the surrounding flaxseed. Moreover, the presence of flaxseed may have acted as a growth medium and thus encouraged proliferation. Indeed,

although *S.aureus* was reduced to a greater extent with therapeutic heating and to negligible levels with sanitisation heating with the flannel compress, the reduction in *S pyogenes*, was similar; the levels of *P. aeruginosa* was also significantly higher compared to the eyebag suggesting the material and dimensions of eyelid warming compress affect the ability of microwave energy to kill a particular bacterium.

Under normal user conditions, the initial bacterial load of the eyebag following removal from the packaging is considerably less than the inoculum used in this study thus, based on the linear (log) decontamination rate expected for bacteria [18, 19], therapeutic heating is likely to reduce the bacterial load, at least for *P. aeruginosa* and other (pre-existing) Gram negative bacteria on the eyebag to negligible levels. Conversely, sanitisation heating may be needed to decontaminate all (including Gram-positive) bacteria from the eyebag, as confirmed by the experiment on the 6 un-inoculated eyebags. Given that disinfection techniques are considered effective with a 3 log reduction in bacterial cell count, heating for 60 seconds with an 800W microwave compares well to this figure and can be performed easily within the home environment. Since the temperature achieved is a function of wattage and time of exposure and that patients may have different power microwave ovens in the home, the following has been calculated for comparison: therapeutic: 55s at 450W, 40s at 600W, 25s at 1000W, 20s at 1250W; sanitisation: 106s at 450W, 80 at 600W, 48s at 1000W, 38s at 1250W.

However, given that the sanitisation temperatures were beyond that used for therapeutic purposes, the eyebag becomes very hot to the touch and thus patients may risk burn injury. Smouldering was not observed when heating the eyebags to such temperatures. In a pilot study of 5 new and 5 unused eyebags heated to sanitation temperatures, allowed to cool and then heated to therapeutic levels after which the cooling profile was monitored using the laser thermometer at 5 minute intervals for 1 hour, the cooling profiles were comparable (F=0.11, p=0.98) to those of 5 eyebags heated to therapeutic temperatures without prior sanitisation.

CONCLUSIONS

In conclusion, the decontamination of the eyebag with heat is more effective than the traditionally recommended wet flannel compress. It is recommended that the eyebag and flannel compresses (wetted with sterile water) should undergo sanitisation temperatures and allowed to cool in the microwave before heating to therapeutic temperature on first use. Thereafter, therapeutic heating as per normal application is likely to eliminate or minimise the bacterial load of the eyebag to clinically insignificant levels. Patients should also be advised to ensure they wash their hands and remove any make-up before each use to minimise contamination and spoilage of the compress; and in-between use, it should be stored in a sealed container. The effect of heat treatment on melting the blocked meibum and providing symptomatic relief [5, 6] would appear to outweigh any effect of bacteria being introduced to the closed eye and surrounding tissue.

Competing Interests & Funding

References

1. G. Geerling, J. Tauber, C. Baudouin C, E. Goto, Y. Matsumoto, T. O'Brien, M. Rolando, K. Tsubota, K. K. Nichols, The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction, Invest. Ophthal. Vis. Sci. 52 (2011) 2050-2064.

2. S. S. Lane, H.B. DuBiner, R. J. Epstein, J. V. Greiner, D. R. Hardten, E. J. Holland, M. A. Lemp, J. E. McDonald II, D. I. Silbert, C. A. Blackie, R. Bedi, A new system, the LipiFlow, for the treatment of meibomian gland dysfunction, Cornea 31 (2012) 396-404.

3. C. Purslow, Evaluation of the ocular tolerance of a novel eyelid-warming device used for meibomian gland dysfunction, Cont. Lens. Ant. Eye. 36 (2013) 226-231.

4. C. A. Blackie, J. D. Solomon, J. V. Greiner, M. Holmes, D. R. Korb, Inner eyelid surface temperature as a function of warm compress methodology, Optom. Vis. Sci. 85 (2009) 675-683.

5. P. S. Bilkhu, S. A. Naroo, J. S. Wolffsohn. Randomised masked clinical trial of the MGDRx eyebag for the treatment of meibomian gland dysfunction-related evaporative dry eye, Br. J. Ophthalmol. 98 (2014) 1707-1711

6. P. S. Bilkhu, S. A. Naroo, J. S. Wolffsohn. Effect of a Commercially Available Warm Compress on Eyelid Temperature and Tear Film in Healthy Eyes, Optom. Vis. Sci. 91 (2014) 163-170.

7. 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.

8. V. J. Gutgesell,, G. A. Stern, C. I. Hood. Histopathology of meibomian gland dysfunction, Am. J. Ophthalmol. 94 (1982) 383-387.

9. J. M. Dougherty, J. P. McCulley, Comparative bacteriology of chronic blepharitis, Br. J. Ophthalmol. 68 (1984) 524-528.

10. J. M. Dougherty, J. P. McCulley, Analysis of the free fatty acid component of meibomian secretions in chronic blepharitis, Invest. Ophthalmol. Vis. Sci. 27 (1986) 52-56.

11. W. E. Shine, J. P. McCulley, Meibomian gland triglyceride fatty acid differences in chronic blepharitis patients, Cornea 15 (1996) 340-346.

12. W. E. Shine, J. P. McCulley, Role of wax ester fatty alcohols in chronic blepharitis, Invest. Ophthalmol. Vis. Sci. 34 (1993) 3515-3521.

13. W. E. Shine, R. Silvany, J. P. McCulley, Relation of cholesterol-stimulated Staphylococcus aureus growth to chronic blepharitis, Invest. Ophthal. Vis. Sci. 34 (1993) 2291-2296.

14. L. B. Szczotka-Flynn, S. Bajaksouzian, M. R. Jacobs, A. Rimm, Risk factors for contact lens bacterial contamination during continuous wear, Optom. Vis. Sci. 86 (2009) 1216-1226.

15. J. P. McCulley, W. E. Shine, Changing concepts in the diagnosis and management of blepharitis, Cornea 19 (2000) 650-658.

16. M. D. Willcox, Pseudomonas aeruginosa infection and inflammation during contact lens wear: a review, Optom. Vis. Sci. 84 (2007) 273-278.

17. M. D. Willcox, Review of resistance of ocular isolates of Pseudomonas aeruginosa and staphylococci from keratitis to ciprofloxacin, gentamicin and cephalosporins, Clin. Exp. Optom. 94 (2011) 161-168.

18. A, Crabbe, P. Thompson, Testing of a dual-mode microwave care regimen for hydrogel lenses, Optom. Vis. Sci. 81 (2004) 471-477.

19. M. Sharma, J. Eastridge, C. Mudd, Effective household disinfection methods of kitchen sponges, Food Control 20 (2009) 310-313.