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Changes to the Ocular Biota with Time in Extended- and Daily-Wear Disposable Contact Lens Use

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Gram-negative bacteria may play a role in the etiology of certain soft contact lens (SCL)-related diseases. Contact lens (CL) wear may modify the normal ocular biota, providing a more favorable environment for potential pathogens. This study reports temporal changes in ocular biota in daily-wear (DW) and extended-wear (EW) disposable SCL use in experienced and neophyte wearers. Lid margin and bulbar conjunctival biota were sampled prior to CL fitting in 26 previous DW SCL users, 18 previous EW SCL users, and 26 neophytes. Wearers were fitted with an etafilcon A CL in one eye and a polymacon CL in the fellow eye. Lenses were worn on a daily basis by the 26 previous DW SCL wearers and on an EW basis by the remaining 44 subjects. The ocular biota was further sampled after 1, 3, 6, 9, and 12 months of wear. The ocular biota consisted of coagulase-negative staphylococci, *Corynebacterium* spp., *Micrococcus* spp., and *Propionibacterium* spp. Potential pathogens were rarely isolated at baseline. No significant trend of increasing ocular colonization was shown for extended CL wear. Lid and conjunctival colonization increased with DW SCL use ($P < 0.001$), although this increase occurred for nonpathogenic species only. Fewer potential pathogens were isolated from DW SCL than from EW SCL users ($P < 0.05$). The lid margin consistently showed greater colonization than the conjunctiva and may be a source of potential pathogens during CL wear. Hydrogel CL wear appears to modify the ocular biota. An increased number of commensal organisms were present in DW SCL use. EW SCL use altered the spectrum of organisms isolated. These alterations may suppress the normal ocular defense mechanisms and may be relevant in the pathogenesis of CL-related disease.

Corneal infection is a rare but potentially severe complication of cosmetic contact lens wear, which is attributable in 70% of cases to *Pseudomonas aeruginosa* (9, 23). Less commonly, other gram-negative organisms and rarely gram-positive organisms have been implicated (9). Additionally, other less severe, but more common, acute adverse responses in hydrogel contact lens wear may be associated with certain gram-negative organisms (2). The source of pathogenic organisms in lens wearers is not always clear, although lens care material (15), manual (10), and ocular contamination may be implicated.

Several studies have documented conjunctival biota in non-lens wearers. In the absence of contact lens wear, the ocular surface is sparsely colonized with small numbers of coagulase-negative staphylococci, *Corynebacterium* spp., and *Propionibacterium* spp. (7, 16, 18, 25, 29). Controversy exists, however, as to the effect of hydrogel contact lens wear on the ocular biota. Increased conjunctival biota has been reported in hydrogel lens wearers (5, 14, 17), although the spectrum of organisms was not found to differ from pre-lens-wear biota. This increase in conjunctival biota may be secondary to quantitative changes in lid margin biota (14). In one study, the conjunctival biota could be related to the contaminants of the contact lens storage case (18), although no such association was confirmed in a more recent study (7). An alteration in the spectrum of organisms was found to occur in a mixed group of lens wearers (11), where increased numbers of both negative cultures and gram-negative organisms were found. Other studies, however, have reported no differences in conjunctival biota between lens wearers and non-lens wearers (20, 28). Despite finding no significant overall differences between wearers and non-lens-wearing controls, Fleiszig and Efron (7) found an increased rate of positive cultures in former lens wearers associated with certain modes of lens wear and disinfection systems. Fewer pathogens were recoverable among aphakic extended wear (EW) lens users than from a group of preoperative cataract patients (24). Comparisons between these studies are not always possible because of differences in sampling techniques, subject sampling, and methodologies and variations in geographical locations, contact lens care systems, and modes of lens wear. Additionally, the majority of studies have sampled the ocular biota on a single occasion only.

Alteration of the normal ocular biota during contact lens wear may suppress the ocular defense mechanisms and enable colonization by pathogenic organisms. Characterization of any temporal changes in the ocular biota with different modalities of lens wear will enhance our understanding of the pathogenesis of certain acute adverse responses.

The aims of this study were to evaluate long-term temporal changes in ocular biota with lens wear and to compare the effects of daily wear (DW) and EW of hydrogel lenses used on a disposable basis in both experienced and neophyte populations. The effects of different lens types on ocular biota were evaluated. To establish possible sources for pathogenic organisms, biota was sampled from different ocular sites.

MATERIALS AND METHODS

Subjects. Seventy subjects participated in the study, which was conducted at the Cornea and Contact Lens Research Unit, School of Optometry, University of New South Wales, Sydney, Australia. Twenty-six subjects used soft contact lenses

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TABLE 1. Subject details

Lens group	*n*	Age (yr [mean ± SD])	Females/ males	Lens replacement schedule	Previous wear experience (mo [mean ± SD])
Experienced DW	26	31 ± 8	14/12	Fortnightly	62 ± 41
Experienced EW	18	33 ± 6	8/10	Weekly	57 ± 39
Neophyte EW	26	25 ± 6	13/13	Weekly	None

on a DW basis and 44 on an EW basis. Of the DW group, all subjects had previously worn DW soft contact lenses, whereas the EW soft contact lens group consisted of 18 previous EW soft contact lens users and 26 subjects with no previous lens wear experience (neophytes). (To show a difference in ocular biota of one classification level or more, given a power of 90% and a significance level of 0.05, the minimum sample size required was estimated to be 12 subjects per group.) Patient details are summarized in Table 1. All subjects were free of ocular and systemic disease, had no previous ocular surgery, and required visual correction for low refractive errors only. Informed consent was obtained, and subjects underwent an ocular examination, including a detailed history and slit lamp biomicroscope examination, prior to lens fitting.

Lenses. The base materials of the lenses used were etafilcon A (Acuvue; Vistakon, Johnson & Johnson, Jacksonville, Fla.), a 58% water content ionic hydrogel material, and polymacon (SeeQuence 2; Bausch & Lomb, Rochester, N.Y.), a 38% water content nonionic hydrogel material. A different lens type was worn in each eye, and lenses were allocated randomly. EW lenses were worn on a 6-continuous-nights-per-week schedule, with lenses replaced weekly. DW users replaced lenses fortnightly, and the daily care regimen consisted of a rub, rinse, and disinfection procedure with Bausch & Lomb ReNu multipurpose solution with Allergan Lens Plus (Allergan, Irvine, Calif.) spray saline for rinsing.

Procedure. In experienced EW lens users, ocular sites were sampled by use of either cotton or calcium alginate swabs moistened with sterile saline. For other groups of wearers, calcium alginate swabs only were used. This method has been shown to enable good recovery of organisms from ocular sites (4). Samples were taken from the upper bulbar conjunctiva, avoiding contact with the lids, lashes, and tarsal conjunctiva. A second swab was passed along the lower lid margin, avoiding contact with the bulbar conjunctiva and lashes.

The choice of sample site was based on a previous study (19), which demonstrated that eye closure resulted in a large increase in biota at all ocular sites, but the greatest increase was shown for the upper bulbar conjunctiva. It was postulated that this site may also show the largest increase with contact lens wear. For comparison purposes, the lower lid was also sampled since this has been shown to have the greatest yield of all ocular sites.

Swabs were immediately placed into 3 ml of Calgon Ringer's solution and vortexed for 30 s. After removal of the swab, 0.4-ml aliquots were used to inoculate three chocolate plates and one Sabouraud agar plate. The Sabouraud plate and one chocolate plate were incubated aerobically at 35°C. One each of the remaining chocolate plates was incubated at 35°C under conditions of increased carbon dioxide and anaerobically, respectively. All plates were read initially after 48 h, and anaerobic plates were reincubated for up to 5 days. Colonies were enumerated and identified by use of Gram stain, standard biochemical methods (3), and API strips (BioMerieux, Marcy l'Etoile, France) for gram-negative isolates.

Baseline biota was sampled prior to lens fitting, and subsequent sampling was performed after 1, 3, 6, 9, and 12 months of wear.

Data analysis. For statistical analysis, culture results were classified by the levels and potential pathogenicity of the organisms isolated, as follows: 0, no growth; 1, <10 CFU of normal ocular biota; 2, ≥10 CFU of normal ocular biota; 3, ≥1 CFU of any gram-negative organism, *Staphylococcus aureus*, *Streptococcus* spp., or fungi (normal ocular biota consisted of coagulase-negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Propionibacterium* spp., and *Corynebacterium* spp.). The mean level of colonization based on these classifications was calculated.

Multiple analyses of variance were performed to evaluate changes with time for each group of wearers, and the Mantal-Haenszel test of trend was used to assess linear associations. Within each analysis of variance, the effects of the duration of previous hydrogel lens wear history and swab type on biota were also evaluated. Individual comparisons were made with a chi-square test or Wilcoxon sign rank test where appropriate.

RESULTS

Spectrum of ocular biota. Tables 2 and 3 summarize the isolation rates of different organisms from conjunctival and lid sites at baseline. Tables 4 and 5 summarize the frequency of isolation for all other sampling times.

Temporal changes in ocular biota. (i) Conjunctival biota. Changes in conjunctival biota with time were evaluated for each mode of wear. For comparison purposes, trends were evaluated for up to 12 months of lens wear for each group of wearers. When controlling for the duration of previous lens wear experience, increased conjunctival biota with wearing time was found for DW lens users ($P = 0.001$) (Fig. 1). No trend of increasing colonization with lens wear was found for the neophyte EW lens users (Fig. 2). For experienced EW lens users, when controlling for the effects of duration of previous lens wear experience and swab type, conjunctival biota was found to reduce with time ($P < 0.05$) (Fig. 3).

TABLE 2. Conjunctival biota—frequency of isolation of organisms at baseline[a]

Organism	Frequency of isolation (%) in lens users[b]		
	Experienced DW	Experienced EW	Neophyte EW
Coagulase-negative staphylococci[c]	21	36	40
Corynebacterium spp.	4	0	0
S. aureus	0	8	0
Unidentified organism	0	3	0
No growth	75	53	60

[a] Where more than one organism was recovered, all organisms were recorded per sampling occasion.
[b] Total sampling occasions for experienced DW, experienced EW, and neophyte EW lens users were 52, 36, and 52, respectively.
[c] Coagulase-negative staphylococci consisted predominantly of *S. epidermidis*. Other species identified included *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.*

A significantly greater proportion of potential pathogens were recovered from the conjunctiva of EW lens users (23 of 404 [6%]) than of DW lens users (6 of 282 [2%]; $P = 0.04$). Potentially pathogenic organisms were present at a single sampling time in only 23 of 29 cases and on consecutive occasions in 6 cases.

No significant differences in conjunctival biota between experienced and neophyte EW lens users were found for any sampling times. At all sampling times and for all wearers, there were no significant differences in conjunctival biota between etafilcon A- and polymacon-lens-wearing eyes.

(ii) Lid biota. At all sampling times, a higher proportion of positive cultures were recovered from the lid margin (440 of 722) than from the conjunctiva (246 of 722; $P = 0.00001$).

TABLE 3. Lid biota—frequency of isolation of organisms at baseline[a]

Organism	Frequency of isolation (%) in lens users[b]		
	Experienced DW	Experienced EW	Neophyte EW
Coagulase-negative staphylococci[c]	52	28	52
Corynebacterium spp.	0	5.5	6
S. aureus	0	5.5	0
Bacillus spp.	0	3	0
Micrococcus spp.	0	3	0
Unidentified organism	4	0	0
No growth	44	55	42

[a] Where more than one organism was recovered, all organisms were recorded per sampling occasion.
[b] Total sampling occasions for experienced DW, experienced EW, and neophyte EW lens users were 52, 36, and 52, respectively.
[c] Coagulase-negative staphylococci consisted predominantly of *S. epidermidis*. Other species identified included *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.*

TABLE 4. Conjunctival biota—frequency of isolation of organisms during wear[a]

Organism	Frequency of isolation (%) in lens users[b]		
	Experienced DW	Experienced EW	Neophyte EW
Coagulase-negative staphylococci[c]	31	30	31
Corynebacterium spp.	5	9	9
Staphylococcus aureus	0.5	2.5	2
Streptococcus spp.	0.5	1	4
Propionibacterium spp.	1	0.5	3
Micrococcus spp.	0.5	0	0
Bacillus spp.	1	0.5	0
Gram-negative rods[d]	2	1	4
Fungus	0.4	0.5	0.5
Unidentified organism	0	0	0.5
No growth	60	69	65

[a] Where more than one organism was recovered, all organisms were recorded per sampling occasion.
[b] Total sampling occasions for experienced DW, experienced EW, and neophyte EW lens users were 230, 198, and 178, respectively.
[c] Coagulase-negative staphylococci consisted predominantly of *S. epidermidis*. Other species identified included *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.*
[d] Gram-negative rods isolated were *Achromobacter* spp., *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Flavimonas* spp., *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp., and *Xanthomonas* spp.

Changes in lid biota with time were evaluated for each mode of wear. When controlling for the duration of previous lens wear experience, lid biota was found to increase significantly with time for DW lens users ($P = 0.00006$) (Fig. 1). No significant trend of increasing colonization with time was found for neophyte EW lens users ($P = 0.07$) (Fig. 2). For experienced EW lens users, when controlling for the effects of the duration of previous lens wear and swab type, lid biota was found to reduce with time ($P < 0.05$) (Fig. 3).

EW lens users were found to have a greater proportion of negative cultures (193 of 440 [44%]) at all sampling times compared with DW lens users (91 of 284 [32%]; $P = 0.002$). Increased lid biota was found in neophyte compared with experienced EW lens users ($P = 0.001$).

For all ocular samples, fungi were isolated in 5 of 564 (0.9%) instances from DW lens users, in 3 of 468 (0.6%) instances from experienced EW users, and in 7 of 408 (1.5%) instances from neophytes. Differences between wear modes were not significant, and similar rates of isolation were encountered for both ocular sites and lens types. No differences between etafilcon A- and polymacon-lens-wearing eyes were statistically significant at any sampling time.

TABLE 5. Lid biota—frequency of isolation of organisms during wear[a]

Organism	Frequency of isolation (%) in lens users[b]		
	Experienced DW	Experienced EW	Neophyte EW
Coagulase-negative staphylococci[c]	65	47	58
Corynebacterium spp.	16	11	17
Staphylococcus aureus	4	6	2
Streptococcus spp.	0.5	1	2.5
Propionibacterium spp.	4	3	7
Micrococcus spp.	1	1.5	1
Bacillus spp.	0.5	0.5	0.5
Gram-negative rods[d]	2.5	1.5	2
Fungus	1	1	2
Unidentified organism	2	1.5	1
No growth	30	47	36

[a] Where more than one organism was recovered, all organisms were recorded per sampling occasion.
[b] Total sampling occasions for experienced DW, experienced EW, and neophyte EW lens users were 230, 198, and 178, respectively.
[c] Coagulase-negative staphylococci consisted predominantly of *S. epidermidis*. Other species identified included *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus.*
[d] Gram-negative rods isolated were *Achromobacter* spp., *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Flavimonas* spp., *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp., and *Xanthomonas* spp.

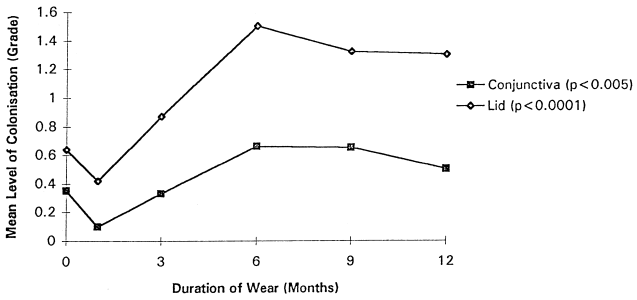

FIG. 1. Mean levels of conjunctival and lid biota plotted against duration of lens wear in experienced DW lens users ($n = 26$). P values indicate whether a linear trend of increasing colonization with time was present, when controlling for the duration of previous hydrogel lens wear experience.

DISCUSSION

This study reports temporal changes in ocular biota with different lens wear modalities and lens types in a population of experienced contact lens wearers and neophytes. This study has aimed to ascertain whether a predictable increase in ocular colonization occurs with lens wear and whether ocular biota is a potential source for pathogenic organisms, which have been implicated in the etiology of certain adverse responses to lens wear.

Data from the neophyte group ($n = 26$) show a resident ocular biota of small numbers of *Staphylococcus epidermidis* and *Corynebacterium* spp., which concurs with previous studies (16, 18, 20, 25). In 50% of the samples, the ocular surface was negative. Other organisms were present as transient colonizers of the ocular surface. With overnight use of lenses, no significant increase in conjunctival colonization was found for either experienced or neophyte wearers. Recent studies have demonstrated that eye closure causes an increase in numbers of

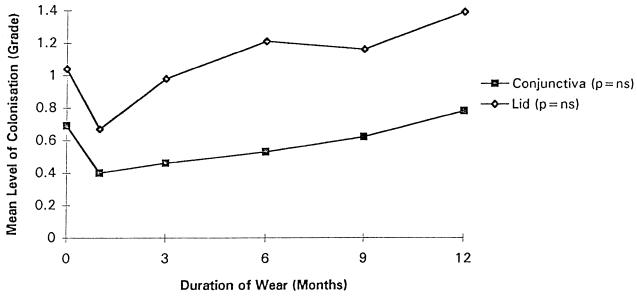

FIG. 2. Mean levels of conjunctival and lid biota plotted against duration of lens wear in neophyte EW lens users ($n = 26$). P values indicate whether a linear trend of increasing colonization with time was present. ns, not significant.

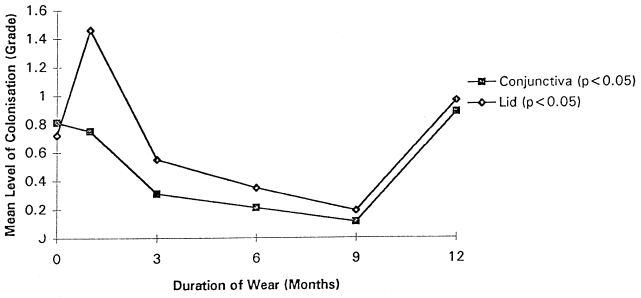

FIG. 3. Mean levels of conjunctival and lid biota plotted against duration of lens wear in experienced EW lens users ($n = 18$). P values indicate whether a linear trend of decreasing colonization with time was present, when controlling for the duration of previous hydrogel lens wear experience and swab type.

normal biota recoverable from the ocular surface (19), although the spectrum of organisms is unchanged. In the present study, the ocular surface was sampled at routine aftercare visits (i.e., sampling times), some hours after eye opening. This suggests that the increased numbers of resident biota are cleared from the ocular surface after eye opening, even in the presence of a contact lens, during uncomplicated lens wear.

EW of gas-permeable hard lenses has been shown to cause increased conjunctival colonization by pathogenic organisms (8). This trend was not found in the current study with overnight use of hydrogel lenses. However, pathogenic organisms were present more frequently in association with overnight use of lenses than with daily use. These organisms appeared to be present as transient colonizers of the ocular surface rather than as part of the resident population, during uncomplicated lens wear. The presence of such organisms plus the known existence of a nocturnal subclinical inflammatory environment (22) may predispose the eye to acute inflammatory or infective disorders during overnight wear. Where pathogens are present, adherence of organisms to the contact lens itself may occur, with subsequent lens colonization, and the normal mechanism for clearing microorganisms on eye opening may be impeded. Adherence and colonization of lenses by pathogenic organisms has been reported in association with lens-related keratitis (27), and high numbers of pathogenic organisms have been recovered from lenses during episodes of acute adverse responses (2). One possibility is that pathogenic organisms adhere more strongly to lenses and are not removed as easily as normal biota are. Alternatively, disruption of the normal ocular biota may suppress the production of antimicrobial substances produced by resident organisms which normally inhibit colonization by pathogenic organisms.

Resident mucosal biota are likely to mediate normal defense mechanisms. *Propionibacterium* spp. have been implicated in nonspecific systemic and local immune defense mechanisms in nonocular sites (21). In the nasal mucosa, *S. epidermidis* and *Corynebacterium* spp. have been shown to retard colonization by *S. aureus* (6). Studies of ocular biota in the absence of lens wear have suggested a synergistic relationship between *S. epidermidis* and *Corynebacterium* spp. (1, 20); these interactions may be significant in maintaining normal biota and ocular defense mechanisms. Rauschl and Rogers (20) postulated that *S. epidermidis* may produce necessary growth factors for *Corynebacterium* spp. and that lens wear may alter this relationship, such that increased colonization by *S. epidermidis* is associated with a simultaneous reduction in numbers of *Corynebacterium* spp. In the current study, these possible effects were evaluated. Among experienced wearers, there was an increase in coagulase-negative staphylococci on the lids after 12 months of wear, but there was no significant concurrent change in the numbers of *Corynebacterium* spp. recovered.

Increased lid colonization with time was not apparent for either group of overnight lens users, although a significant increase occurred in association with DW of lenses. This change represented increased numbers of normal ocular biota rather than increasing colonization by potential pathogens. A concurrent increase in conjunctival biota was apparent for these wearers. Additionally, overnight lens users were found to have a higher frequency of negative lid cultures compared with DW lens users. These findings may reflect manual inoculation through repeated lens handling in DW as opposed to EW. Lid margin and conjunctival biota also demonstrated a greater number of species recoverable during wear compared with the number of species at baseline. This finding has been reported previously in DW lens users (14). These authors speculated that conjunctival biota increased possibly secondarily to increased lid colonization. The absence of gram-negative contamination of the lids in DW lens users would suggest that carryover from lens storage case contaminants is an unlikely source for increased lid contamination, since gram-negative organisms are common contaminants of storage cases for hydrogel lenses (13).

The present study evaluated changes in ocular biota in EW lens users who used a disposable lens system, thus eliminating the variable effects of both disinfecting solutions and contaminants from the lens storage case. Significant changes in ocular biota with overnight use are thus likely to represent the true effects of lens wear alone. No significant trend of increasing ocular surface colonization was apparent in neophyte EW lens users, whereas experienced users showed reducing ocular surface colonization with time. This reduction appears to be attributable to increasing numbers of negative ocular surface cultures with increased duration of wear. In addition, overnight use caused an increased frequency of recovery of potential pathogens, although the overall frequency was low (6%). Similar alterations in biota have been reported to persist after ceasing lens wear (7). It is not clear, however, whether this effect is limited to wearers discontinuing lens wear because of adverse responses, which may have been associated with alterations of the ocular biota.

Other differences in ocular biota between experienced and neophyte lens wearers were not found to be significant, although neophytes were found to have significantly higher levels of lid colonization. This may be an effect of increased lid manipulation in neophyte wearers.

No individual wearers within the study were found to have consistently culture-positive or culture-negative samples, and multiple isolates were cultured only sporadically. It is therefore unlikely that individual data distorted the overall changes in biota with time. Ocular contamination by *P. aeruginosa*, which is known to have pathologic significance in contact lens wearers, was found to be rare and sporadic in this group of asymptomatic wearers. There was no prolonged ocular carriage of this organism, and there was no apparent trend towards increased or reduced ocular presence of this organism in association with lens wear.

Although seasonal variations may influence the ocular biota, individuals were sampled over a 3-year period, minimizing these effects. Additionally, such variations have not been reported in previous studies (20).

Previous studies have demonstrated a greater recovery of organisms from ocular sites when calcium alginate swabs rather than cotton swabs were used (4). Within the experienced EW lens-wearing group, samples were taken with either

a calcium alginate or cotton swab. However, with multiple analyses of variance, no significant effect of swab type was shown.

Other than the ocular biota, alternative sources of organisms may be present in individuals experiencing adverse responses. This has been demonstrated in therapeutic lens wearers, where the ocular biota appears to be unaltered (12) despite these individuals having a higher risk of infective keratitis. Possible alternative sources include manual contamination of lenses or exogenous sources. In one study where lenses were manually contaminated, no organisms could be recovered after wear (10). In addition, in a study of wearers with culture-proven corneal infections, the causative organism could not be isolated from a variety of skin and other body sites (26). However, it is conceivable that lid and conjunctival manipulation during lens insertion and removal increases ocular biota in DW lens users. Contact lens storage cases have been confirmed as a source of causative organisms in microbial keratitis (15). Alternatively, organisms may be derived from environmental sources.

This study has demonstrated alterations in the ocular biota with contact lens wear. Overnight use of lenses resulted in a greater frequency of isolation of pathogenic organisms from the conjunctiva, and DW lens users showed increasing conjunctival and lid colonization with time. EW lens users with previous lens-wearing experience showed a reduction in colonization with time, which was due to the increased frequency of negative cultures over time. No significant trend with time was observed in neophyte wearers. No differences were found with different types of lenses. These specific changes imply that long-term overnight use of contact lenses may interfere with the normal clearing of pathogenic organisms from the eye following sleep. Disruption to the normal ocular flora and inhibition of clearing of pathogenic organisms from the eye may be contributing factors which modulate increased ocular susceptibility during contact lens wear.

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