Published in final edited form as:

Optom Vis Sci. 2010 April ; 87(4): 225–232. doi:10.1097/OPX.0b013e3181d408ee.

The Pathogenesis of Contact Lens-Associated Microbial Keratitis

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Abstract

Sight-threatening microbial keratitis associated with contact lens wear remains a serious concern for patients, eye-care practitioners, and the contact lens industry. Several decades of research and some major advances in lens and solution technology have not resulted in a decline in disease incidence. Here, we offer a perspective on the complex pathogenesis of microbial keratitis, the factors that have prevented a better understanding of this disease, and new approaches being used to tackle this important clinical problem.

Keywords

microbial keratitis; *Pseudomonas aeruginosa*; cornea; epithelium; innate immunity; contact lenses; hygiene; biofilms

Contact lens wear continues to be a significant risk factor for the development of acute sight-threatening corneal infections (microbial keratitis).¹⁻⁷ For more than 20 years, we and others in this field, have worked towards understanding why the corneas of contact lens wearers are more susceptible to infection.⁸⁻¹⁴

Two major stumbling blocks have hindered progress in this field. The first is the lack of basic knowledge about how the healthy ocular surface normally defends itself against infection. Naturally, it is difficult to draw conclusions about how lens wear might impact defenses that have not yet been defined. A contributor to this problem has been our sketchy

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understanding of the normal biochemistry and cell biology of the ocular surface, combined with a lack of tools for sorting out which of the many factors present at the ocular surface were critical to defense against infection. Researchers in the field have therefore resorted to making educated guesses as to what molecules, processes or cells to focus on. Sometimes associated “markers” have been examined that later turned out to have little to do with the mechanism being studied.

A second obstacle to progress in this field has been the lack of suitable animal models. Until recently, *in vivo* rodent models available to researchers studying corneal infection were limited to one that requires the cornea to be scratch-injured to expose the stroma, and another in which bacteria are injected into the stroma. These models have been of great value in discerning the inflammatory and immune responses involved in disease and its resolution once an infection is already underway^{8, 12, 15, 16}. However, these models bypass the corneal epithelium, and are not suited to studying circumstances that surround the actual initiation of contact lens-related infection, or exploring why the cornea is resistant to infection when it is healthy. To study how bacteria interact with the corneal epithelium, we and others have used corneal epithelial cells grown *in vitro*. When attempting to do such experiments *in vivo* one finds that bacteria do not interact at all with the epithelium of healthy corneas, leaving no pathology to study¹⁷. The major obstacle interfering with the development of a good contact lens infection model has been a lack of availability of contact lenses that properly fit the eyes of small animals. Use of human lenses on larger animals, (e.g. rabbits) poses similar problems, and is also prohibitively expensive for generating sufficient data for statistical analysis.

The hit and miss nature of research in this field has, in turn, made it difficult for investigators to obtain funding for basic biological research aimed at addressing pathogenesis of contact lens-related complications, further delaying progress.

While these hurdles have prevented researchers from directly addressing key questions surrounding the pathogenesis of contact lens-related keratitis for almost three decades, we have recently entered a new era of discovery that could soon lead to the eradication of contact lens-related infections. This has come about through the development of rodent contact lenses^{18, 19} (Fig. 1), and the advent of genomic sequencing of humans²⁰, of experimental animals²¹, and of the microbes that are leading causes of contact lens-associated infection²², combined with the recent development of new, highly sensitive, and readily available screening tools that can identify key molecules involved in critical biological processes (for example, those critical for defense, and those impacted during susceptibility)²³⁻²⁶. Assisting these efforts are new methods in imaging and data analysis²⁷⁻³⁰. For example, in our laboratory, we have developed novel *in vivo* and *in vitro* methods to study corneal defenses during health, that include new use of imaging technologies to enable us to “see” into living corneas so that we can observe bacteria in action while also monitoring the corneas responses. Moreover, a plethora of new relevant information is arising daily from the use of these technologies in other related fields, e.g. studies of host-pathogen interactions and medical device-related infections in other tissues^{31, 32}. Finally, because these new technologies are making research in this field more feasible (and also more interesting), leading researchers from related specialties have been

attracted into the field, which will accelerate the effort. All of these developments have ignited new optimism in the field.

This review will visit some key questions in this field, which we will attempt to answer based on published literature that include our own research on the topic over the past ~20 years. In doing so, we will also project forward by presenting hypotheses about barriers to microbes in healthy corneas and how lens wear or bacteria may compromise them to enable infection.

Would it Help if Contact Lens Wearers Were More Compliant?

Clearly, infection of the cornea requires a microbe(s). During contact lens wear microbes can enter the eye from the wearer's lid margins, their fingers upon lens insertion (or removal), or via the contact lens, from the care solutions, or the storage case. Proper use of a contact lens disinfection system (if it is effective), may reduce the probability of contamination, at least via some of these avenues. Conversely, improper use of lenses and their disinfection/cleaning systems (or lack of efficacy), could actually contribute to contamination by becoming an additional source. While improper lens care has been associated with *Acanthamoeba* spp. keratitis in the UK, poor patient compliance is not always identified as a significant risk factor for microbial keratitis in contact lens wearers^{5, 6, 33, 34}. This is not at all surprising considering that even the healthy non-lens wearing ocular surface is frequently exposed to potentially pathogenic microbes (from the environment, or from the patient themselves), yet the cornea rarely becomes infected. From this we know that contamination does not equal infection. While contamination of the ocular surface is necessary for infection to occur (obvious), it is not sufficient, and must be accompanied by changes in the way the microbes interact with the cornea, or in the way that the cornea responds to them.

Is there any value in improving user compliance? Generally speaking it is thought that good hygiene minimizes microbial contamination, and reduces the risk of infection and disease in other tissues and circumstances. Indeed, hand washing is considered one of the most effective preventive approaches against infectious disease in general. Low efficacy of certain disinfection solutions (rather than non-compliance) was implicated in the *Fusarium* spp. and *Acanthamoeba* spp. keratitis outbreaks in lens wearers.^{35, 36}

While the link between compliance and infection risk remains a grey area, improving contact lens care solutions, and education on their proper use, can only hurt if the solutions being used have undesirable side effects, e.g. they are toxic, impact immunity, or make surviving microbes more virulent or antibiotic resistant. It is important to recognize, however, that "hanging our hats" upon improving patient compliance as a magic bullet approach to preventing infections could distract researchers in the field (and contact lens/solution manufacturers) from working towards more effective ways to eradicate the problem of contact lens related-infection. It may also provide practitioners and patients with a false sense of security. Importantly, good compliance will not sterilize the environment of the contact lens wearer. Disinfectants do not kill all microbes even when properly used, and there are many other sources of potentially pathogenic microbes in our environment that

could provide an inoculum even if the lens, solution, storage case and hands are all clean. Recent research shows that the number of bacteria colonizing a single human being is staggering; with bacterial cells outnumbering our own cells 10:1. Frequently encountered microbes can include *P. aeruginosa*, *Acanthamoeba spp.*, and *Fusarium spp.* (all leading causes of contact lens-related infections), which are ubiquitous in our environment (these live in water, food and soil), and are not usually pathogenic.

An event that occurred recently in our lab provides an excellent example of how disinfection (and air-drying) can fail to protect against infection. In that study, a “control group” of three lens wearing rats (not deliberately inoculated with bacteria) all became severely infected³⁷. The source was traced back to a suction device used to insert the lenses. Six months earlier the device had been used with *P. aeruginosa* contaminated contact lenses, prior to being disinfected with 70% ethanol (considered a good disinfectant and expected to kill *P. aeruginosa*), air-dried, and then stored dry for the intervening 6 months. Incredibly, the same strain of *P. aeruginosa* (not grown in the lab since) was recovered from the device and from the rat’s eyes and lenses. In other words, a few surviving ethanol-exposed and then air-dried bacteria managed to grow in vivo on a lens and then cause severe disease. Apart from showing the potential of bacteria to foil our best efforts, this outcome also suggests that the number of bacteria needed to cause an infection can be very small, possibly beyond the limits of FDA-approved kill rates for disinfection solutions. Further, this result, combined with other data we have collected showing that the usual infection delay of ~7 days in rats can be reduced to ~2 days if lenses are transferred from infected eyes to naïve rats, suggests that the potential for microbes to cause keratitis may be dependent on the conditions they have been exposed to before entering the eye. This may also weigh into the equation when considering the impact of lens care solutions on the pathogenesis of infection.

In sum, there are logic gaps in viewing compliance as an effective way to prevent infection. Compliance will not ensure that solutions and lenses are sterile, and even if it did, there are other sources of microbes in the environment. Conversely, access of “pathogenic” microbes into the eye is not necessarily a problem since it also happens to non-lens wearers. It is likely that what happens to the eye during lens wear, or to the microbes before or after they gain access to the ocular surface, is key to the pathogenesis of contact lens-related infections and their eradication.

History shows that efforts towards improving patient compliance are generally in vain. This applies to drug therapy of life and sight threatening diseases that include hypertension, diabetes and glaucoma. Contact lens wearers are likely to have even less motivation for extra effort, particularly if they (or their friends or associates) have worn lenses for long periods of time and have not had any problems. Scare tactics such as graphic images of infections may not help, considering that horrific photos on cigarette packs have had little impact on smoking. It is of concern that efforts towards improving compliance with lens wear and existing disinfection procedures may delay progress towards understanding the fundamental causes of microbial keratitis, a path towards eliminating the problem.

Why is Extended Wear a Risk Factor?

Extended/overnight lens wear remains the most significant risk factor for infection^{1, 4, 5, 7}. Using a rat model, we have found that disease onset is delayed by approximately one week after a *P. aeruginosa* contaminated lens is placed on the eye³⁷. We have been working towards understanding this delay in disease onset in rats, which we believe may provide clues as to why extended wear is a risk factor in people, and have found that during the “incubation period” classical bacterial biofilms form on the posterior, but not anterior, lens surface³⁷. Interestingly, if we transfer a lens from an infected eye (that already harbors bacterial biofilm) to a naïve rat, the delay is reduced to approximately two days. Thus, bacteria that have already been in the eye as part of a biofilm on a lens are more adept at infecting the cornea. Subsequent experiments have confirmed that *P. aeruginosa* has the capacity to become more virulent with time if exposed to corneal epithelial cells, as demonstrated by alterations in gene expression and enhanced capacity to penetrate corneal epithelium both in vitro and in vivo (unpublished data). Thus, one reason that extended wear is a risk factor in people could be that it provides more time for bacteria to colonize the contact lens and adapt to the environment to become appropriately virulent.

Of course it is also likely that lens wear impacts the ocular surface to reduce its defense against infection. As already discussed, the healthy cornea is remarkably resistant to microbes, so infection during lens wear may require more than just microbe adaptation.

Indeed, our in vitro studies show that corneal epithelial cells grown in culture lose their normal ability to upregulate expression of an antimicrobial peptide (hBD-2) in response to bacterial factors after they have been pre-exposed to hydrogel contact lens wear, but only if that exposure is for at least 72 hours¹⁴. We have found similar results for surfactant protein-D (unpublished data), another factor involved in defense against infection at the ocular surface^{16, 17, 38, 39}. If these in vitro data showing that cultured corneal epithelial cells lose their ability to respond appropriately to bacterial challenge after lens wear translate to human lens wear in vivo, then this is also likely to contribute to the reasons why extended wear is a risk factor for infection.

Another factor important to consider in this equation is the impact of contact lens wear on tear fluid, which we have found protects corneal epithelial cells against *P. aeruginosa*⁴⁰⁻⁴². The mechanism of action of tears against microbes includes direct effects on microbes, and also upregulation of the defensive capacity of epithelial cells. Indeed, one of the reasons why corneal epithelial cells are so exquisitely resistant to bacteria in vivo, while being completely vulnerable when grown in vitro, is likely to relate to the presence versus absence of tear fluid. Tear fluid components are derived from multiple locations at and beyond the ocular surface. Thus, a contact lens has capacity to alter tear biochemistry at the corneal surface if it sits too close to the cornea (excludes tears), or if it shuts down exchange of tears (tear mixing) between the pre- and post- lens tear compartments during blinking. The consequences of changes to ocular surface biochemistry could include loss of direct and/or indirect defenses against microbes. There could be other alterations to homeostasis with detrimental effects, such as epithelial injury induced by a post-lens tear film which exaggerates normal closed eye tears^{25, 43}. In addition to impacting ocular surface

biochemistry, lack of tear exchange may also reduce the ability to remove microbes from under the lens. During extended wear, this could enable the microbes sufficient time to adapt to the in vivo ocular surface environment, as discussed above. In this respect, it is of interest that infections are more common with soft lenses that enable less tear exchange during blinking than do RGP lenses⁴⁴. Also intriguing is that reverse geometry RGP lenses used for orthokeratology (that sit closer to the cornea at their center) have been found associated with a greater risk of infection (mostly *P. aeruginosa*) than conventional RGP lenses⁴⁵⁻⁴⁷. During extended wear there is likely to be even less opportunity for tear exchange, possibly relating to the increased risk of infection with overnight wear for all lens types.

Finally, compromise to unique defenses that protect the eye during eye closure may contribute to the reasons why extended contact lens wear is a risk factor for infection versus daily wear. Indeed, it is known that the biochemistry of the closed eye ocular surface differs from that of the open eye, and these differences likely relate to defense against microbes⁴². The effect of contact lens wear on closed eye defense against microbial virulence is certainly worthy of further investigation.

Are Antimicrobial Lenses a Good Idea?

Recently, there has been interest in coating lenses (or lens cases) with antimicrobial compounds as a strategy to reduce infection risk. As discussed above, our data collected using a rat model suggest that bacterial attachment to contact lenses and subsequent biofilm formation could be critical for enabling bacteria to initiate infection. If this is also an important part of the pathogenic process during lens wear in people, blocking bacterial attachment to the posterior lens surface would be a good preventive strategy. Optimally, the strategy used would not kill microbes or inhibit their growth (provides selective pressure for resistance), and instead would target bacterial virulence, attachment, adaptation, or survival mechanisms⁴⁸. Good candidates include compounds with multiple diverse mechanisms of action to offset any resistance while offering opportunities for additive or synergistic effects. Of concern, would be agents that kill bacteria and are also used for treating infections (e.g. antibiotics), or components of our immune system (potential for serious adverse consequences).

It is clear that bacterial biofilm formation can occur in lens cases. Biofilms enable microbes within them to resist killing by antimicrobial agents, and growth in biofilm format tends to make microbes more virulent, especially if exposed to adverse conditions⁴⁹⁻⁵². In designing lens cases to resist biofilm formation, it would be important to ensure that agents used do not further enhance microbe virulence (e.g. by using agents that stress, but don't kill microbes), and that toxic factors do not eventually end up in the eye. Regular case replacement would be an alternative strategy.

Does Fluorescein Staining Predict Risk of Infection?

Fluorescein staining indicates defects/compromise to the corneal epithelium. However, our data using various in vivo models show that fluorescein staining does not necessarily correlate with susceptibility to infection. For example, using a healing model of microbial keratitis we showed that while injured corneas which had healed for either 6 or 12 hours

both stained with fluorescein, only the 6 hour healed eyes were susceptible to infection⁵³. We have also found that tissue-paper blotting of the corneal surface of mice or rats that induces extensive fluorescein staining penetrating all the way into the stroma (confirmed by confocal microscopy) does not make the cornea susceptible to *P. aeruginosa* keratitis (unpublished data). In exploring the mechanism, our data reveal that the basal lamina (the basement membrane under the epithelium) is a barrier that prevents bacteria from entering the stroma⁵⁴. Moreover, while blotting allows *P. aeruginosa* to bind to surface epithelial cells (otherwise they do not bind at all), the bound bacteria do not penetrate beyond the most superficial layer of the epithelium. We are currently investigating the role of deeper layer junction proteins⁵⁵ and epithelial-derived antimicrobial peptides⁵⁶ in preventing bacterial traversal of blotted, fluorescein-permissive epithelium. Also of relevance, we have found that blotting has no impact on the pathogenesis of contact lens induced keratitis in the rat model; the onset delay (of one week) and disease severity are similar for blotted and non-blotted eyes when they are subsequently fitted with a *P. aeruginosa*-contaminated lens³⁷. In sum, superficial blotting that enables fluorescein staining has no impact on outcome when bacteria are added to the eye with or without contact lens wear in rodent models.

Is Hypoxia a Risk Factor?

The introduction of silicone hydrogel lenses with superior oxygen transmissibility (high DK/t) has not reduced the incidence of microbial keratitis^{5, 57}, but has solved other complications known to be hypoxia-related⁵⁸. What conclusions can we draw from these studies? While the data show that low transmissibility lenses (low DK/t) are not the only lenses that cause infection, can we actually conclude that hypoxia is not required? Do we really know that the ability to transmit oxygen through a lens translates to sufficient oxygen availability at the cellular level? Or that hypoxic response pathways in cells, that can be triggered by factors other than hypoxia⁵⁹, are not activated with silicone hydrogel wear? Even if hypoxia/hypoxic responses are really not needed for infection to occur, hypoxia could still contribute to pathogenesis. Indeed, the studies of Efron and Morgan suggest that incidence of the most severe adverse events is actually reduced with silicone hydrogels as compared to hydrogels⁷. Accordingly, in vivo studies have shown that lens-induced hypoxia can negatively impact corneal epithelial cell biology in various ways. For example, low Dk rigid gas permeable lenses induced a significant reduction in epithelial cell proliferation in the central cornea of rabbits compared to high Dk rigid lenses which closely mimicked normal corneas⁶⁰. In addition, hypoxia of low Dk rigid lenses was associated with increased lipid raft formation in corneal epithelial cells and promotion of *P. aeruginosa* invasion⁶¹. A study comparing low versus high Dk hydrogel lenses in a rodent lens wearing model showed reduced incidence of *P. aeruginosa* keratitis (no infections with high Dk versus a 30 % infection rate with low Dk lenses), and significant pro-inflammatory changes in the cornea and conjunctiva involving low Dk lenses¹⁹. Other studies without lenses have also shown that hypoxia increases *P. aeruginosa* invasion of rabbit and human corneal epithelial cells, and activation of a pro-inflammatory transcription factor⁶². Thus, evidence suggests that hypoxia could impact susceptibility to infection, and that benefits of removing hypoxia with silicone hydrogel lens is masked by other lens-associated effects sufficient to allow infection. Alternatively, positive effects of removing hypoxia might be countered by

undesirable negative effects. Indeed, some effects of hypoxia could actually be protective. For example, we have found that hypoxia increases corneal epithelial cell expression of surfactant protein-D, which serves protective and immunomodulatory roles.^{17, 38, 39, 63, 64}

For all of these reasons, hypoxia remains a topic of interest for those working towards solving contact lens complications, despite the fact that contact lenses that transmit oxygen are already available.

Why *Pseudomonas aeruginosa*?

While other devastating microbes have come and gone in “outbreaks” (most recently *Acanthamoeba* spp. and *Fusarium* spp.), *P. aeruginosa* has been a staple consistent problem throughout the history of the soft lens. Indeed, prior to the introduction of soft contact lenses to the market, *P. aeruginosa* keratitis was a rare occurrence.

The capacity for *P. aeruginosa* to exploit the lens-wearing situation may relate to its very large genome and the many genes devoted to virulence, survival, and adaptation. For example, it encodes over 70 two-component “sensor-regulator” systems²² that enable it to alter gene expression to adapt to a multitude of diverse environments. Further, *P. aeruginosa* is ubiquitous in nature, and is likely to access ocular tissues often in the course of our daily lives. As a “water bug”, all lens wearers can be exposed to it whether or not they use solutions. While non-lens wearers have the benefits of blinking to regularly sweep the ocular surface, lens wear provides a means to increase contact time between microbe and the ocular surface to give those microbes with the right equipment a chance to adapt and exploit. In addition to having the right tools for sensing the environment, *P. aeruginosa* is known to encode many virulence factors with potential to enable survival at the ocular surface, including strategies for biofilm formation, for resisting killing, for communicating with one other to enhance virulence (e.g. quorum sensing), for invading epithelial cells and surviving within them, for destroying tear components, for breaking down cell-to-cell junctions and extracellular matrices, and for injecting toxins into cells^{9, 10, 13, 51, 54, 65-71}. It also possesses factors that are highly immunogenic (initiate inflammation) while being able to evade the immune responses that it initiates^{9, 15, 65, 72}. Interestingly, *P. aeruginosa* virulence factors can also confer resistance to contact lens disinfectants.⁷³

What is the Relationship between Infection and Inflammation?

Contact lens wear can predispose to microbial keratitis, but it also causes “sterile” inflammatory responses^{58, 74-76}. While there can be multiple presentations, these “sterile” events are all believed to involve inflammatory or toxic responses to immunogenic stimuli, such as microbes, or to microbial derived factors. How different are these “sterile” events from actual infections? Is it possible that they represent similar processes of varying severity? Data obtained with the rat contact lens model show that there are two different outcomes when *P. aeruginosa* contaminated lenses are placed on rat eyes. Disease onset is delayed by about one week in all eyes, but there are alternate presentations. Some eyes suffer severe disease that progresses quickly. Other eyes show milder opacity that develops slowly and does not progress substantially over time. When we examined severely infected corneas under the microscope, we found a severe inflammatory response and also large

numbers of *P. aeruginosa* throughout the cornea. While corneas showing milder opacity revealed a similar inflammatory response, we could not locate bacteria in the tissue (causative bacteria were found in large numbers only on and under the lens). At this stage, it is not clear whether the mild disease occurs because bacteria that have invaded the corneas are successfully cleared by the time we examine them, or whether inflammation occurs without bacteria entering the cornea. Conversely, we also don't yet know if the severely infected corneas become inflamed first, thereby creating the opportunity for bacteria to enter (e.g. inflammation can precede infection for *Salmonella* infection in the gut^{77, 78}). Nevertheless, these early results do suggest a relationship between infection and inflammation in etiology, even if they are not sequentially linked.

Why Not Just Advocate Daily Disposable Lenses?

Studies show that daily disposable or daily wear hydrogel lenses involve the lowest risks of microbial keratitis for soft lenses.^{5, 57} Compliant use of daily disposables eliminates solution and lens case usage, and therefore related contamination and non-compliance. Thus, it is very informative that daily disposables do not substantially/consistently reduce the infection risk compared to conventional daily wear, which involves use of solutions. Either the use of solutions and cases does not impact susceptibility, or else other factors associated with daily disposable use increase risk in other ways that cancel out the benefits. Whatever the case, these data provide further evidence that there is more to the pathogenesis of contact lens-related infection than just solution contamination and non-compliance, and that understanding lens-induced changes to normal ocular defenses against infection is warranted (Table 1, Fig. 2).

CONCLUSIONS

Continued research in the pathogenesis of microbial keratitis has significance beyond enabling us to understand contact lens infections. The study of how and why contact lens wear impacts ocular surface homeostasis advances our understanding of corneal health and disease in general. It also has the potential for development of novel methods to prevent infection of the eye and of other sites.

Of critical importance to the common goal of industry, practitioners and patients alike to eradicate contact lens infections, is that the contact lens industry has started to provide rodent contact lenses to researchers allowing them to use directly relevant models to solve this problem. We urge them companies and other industry partners to continue to develop these important tools and make them widely available to the research community.

ACKNOWLEDGMENTS

The authors wish to thank the National Eye Institute and the National Institute for Allergy and Infectious Disease for continued support of our research program (EY011221, AI079192). We would like to thank the Bill and Melinda Gates Foundation, Allergan, Inc., and Alcon Research, Inc for their generous research support. We also express our appreciation to our many colleagues in this field whose excellent work advances our understanding of microbial keratitis.

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Figure 1.
Lens-wearing murine model for investigation of microbial keratitis

Figure 2.

Schematic model of steps in the pathogenesis of *P. aeruginosa* keratitis. In order for bacteria to gain access to the corneal stroma without a full thickness epithelial defect (e.g. no scratch), the following steps are required; Step 1) bacteria survive within post-lens environment, and interact with and adhere to the corneal epithelium, Step 2) bacteria traverse the multilayered epithelium, Step 3) bacteria cross/breach basement membrane, and Step 4) for visible pathology, an inflammatory response and/or bacterial damage to the stroma are required.

Table 1

A model for the pathogenesis of contact lens-related infectious keratitis

Defenses against infection	How contact lens wear might enable bacterial infection
Physical clearance of microbes ¹⁷	<ol style="list-style-type: none"> 1. Bacterial clearance hindered by presence of lens ⁴⁴ 2. Bacteria bind to lens and form biofilm ³⁷ 3. Biofilm enables bacteria to survive and adapt to <i>in vivo</i> environment to gain virulence (unpublished data)
Protective activity of tear fluid ⁴⁰⁻⁴² a) direct activity against microbes b) upregulation of epithelial cell defenses (unpublished)	Lack of tear exchange under a lens alters corneal surface biochemistry reducing tear protective activity (hypothesis)
Epithelial cell-derived antimicrobials ^{56, 79}	Production of epithelial cell-derived antimicrobials is compromised by lens wear ¹⁴
Physical barriers that prevent microbial access; a) cell-to-cell junctions between epithelial cells ⁸⁰ b) basal lamina between epithelium and stroma ⁵⁴	<ol style="list-style-type: none"> 1. Physical barriers could be compromised by lens wear, or by bacterial virulence factors (hypothesis, ⁵⁴) 2. Persistence of bacteria on or under lens could induce inflammation that disrupts corneal physiology to enable bacterial entry (hypothesis)