Multipurpose Care Solution–Induced Corneal Surface Disruption and *Pseudomonas aeruginosa* Internalization in the Rabbit Corneal Epithelium

Leila C. Posch, Meifang Zhu, and Danielle M. Robertson

Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, Texas, United States

Correspondence: Danielle M. Robertson, Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX 75390-9057, USA; Danielle.robertson@utsouthwestern.edu.

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PURPOSE. To evaluate the effects of a chemically preserved multipurpose contact lens care solution (MPS) on the corneal epithelial surface and *Pseudomonas aeruginosa* (PA) internalization in the rabbit corneal epithelium.

METHODS. Rabbits were fit in one eye with a silicone hydrogel lens (balafilcon A) soaked overnight in a borate-buffered MPS (BioTrue). The contralateral eye was fit with a lens removed directly from the blister pack containing borate-buffered saline (control). Lenses were worn for 2 hours. Upon lens removal, corneas were challenged ex vivo with invasive PA strain 6487 and assessed for PA internalization. Ultrastructural changes were assessed using scanning electron (SEM) and transmission electron microscopy (TEM).

RESULTS. Scanning electron microscopy showed frank loss of surface epithelium in MPS-exposed eyes, while control eyes exhibited occasional loss of surface membranes but retention of intact junctional borders. Transmission electron microscopy data supported and extended SEM findings, demonstrating the presence of epithelial edema in MPS-treated eyes. There was a 12-fold increase in PA uptake into the corneal epithelium following wear of the MPS-treated lens compared to control ($P = 0.008$).

CONCLUSIONS. These data demonstrate that corneal exposure to MPS during lens wear damages the surface epithelium and are consistent with our previous clinical data showing an increase in bacterial binding to exfoliated epithelial cells following MPS use with resultant increased risk for lens-mediated infection. These findings also demonstrate that the PA invasion assay may provide a highly sensitive quantitative metric for assessing the physiological impact of lens-solution biocompatibility on the corneal epithelium.

Keywords: contact lens, corneal epithelium, microbial keratitis, *Pseudomonas aeruginosa*, multipurpose solution

Microbial keratitis (MK) is a serious, sight-threatening multifactorial disease. Numerous risk factors for MK have been identified and include contact lens use, ocular trauma, preexisting ocular disease, and history of ocular surgery.^{1–3} Of these, MK is most frequently associated with contact lens wear.^{1–3} While contact lenses are relatively safe for the majority of wearers, contact lens wear has been shown to alter the normal biology of the corneal epithelium and provides a vector for the introduction of foreign pathogens to the eye. Likewise, contact lens storage cases are frequently colonized with dense microbial biofilms.^{4–6} The inappropriate use of contact lens cleaning solutions further stimulates microbial growth in lens cases and on contact lens surfaces. Multiple epidemiological studies have independently confirmed that the pathogenic gram-negative bacterium *Pseudomonas aeruginosa* (PA) is responsible for greater than half of all cases of lens-related MK.^{1,7–11} These studies have further provided evidence supporting a role for lens-induced hypoxia during overnight wear as a key risk factor.^{7,8} While some gains have been realized with the widespread use of silicone hydrogel lenses, which have been shown to eliminate hypoxia-mediated corneal surface damage, the overall incidence rates of contact lens-

related MK have remained essentially unchanged over the past three decades.^{12–15}

Chemically preserved multipurpose solutions (MPS) are widely used by the majority of contact lens wearers to routinely disinfect their lenses on a nightly basis.^{16,17} While MPS are required to achieve a certain level of bactericidal efficiency in isolated testing conditions prior to gaining Food and Drug Administration (FDA) approval, studies have shown that many commercially available MPS are ineffective in removing bacterial biofilms formed on lens surfaces.¹⁸ Moreover, the antimicrobial properties of the various MPS can vary greatly with different lens-solution combinations and when used with particular lens cases, and have prompted the FDA to initiate new “real-world” testing.¹⁹ In addition to disinfection efficacy, recent attention has focused increasingly on the direct effects of chemically preserved multipurpose cleaning solutions (MPS) on the corneal epithelium and their role in solution-induced corneal staining and the onset of corneal inflammatory events.^{20,21} In concert with this, our clinical data evaluating bacterial binding to exfoliated epithelial cells after wear of different contact lens polymers, with and without MPS use, have suggested that MPS may contribute to surface epithelial damage and lens-related risk for infec-

tion.²² While this finding has been confirmed by recent unpublished epidemiological data showing an increased risk of MK with MPS when compared to peroxide (OR [odds ratio]: 41, 95% CI [confidence interval] = 0.22–0.75, $P < 0.004$; Stapleton F, written communication, 2009), the exact role that MPS may play in the pathogenesis of infection is unclear.²³

It is well established that MPS are absorbed by the lens during overnight soaking and later released during lens wear, resulting in direct exposure of the chemical preservatives to the surface epithelium.²⁴ In vitro studies have demonstrated varying levels of corneal epithelial cell toxicity following incubation of corneal epithelial monolayers in MPS and using an established lens–corneal onlay model.^{25–28} Similarly, MPS use has been shown in both animal and cell culture studies to decrease the expression and release of certain ocular mucins, which function to impede bacterial adherence.^{17,29,30} In addition to the above, available data also suggest that MPS may contribute to the breakdown of the cornea's protective barrier through direct damage to corneal epithelial tight junctions.^{16,31} These laboratory-based findings are confounded by the controversial clinical observation of fluorescein staining of the human cornea following wear of various lens–MPS combinations and the role of lens-solution biocompatibility.^{29,32}

Corneal fluorescein staining has been reported to be maximal after 2 to 4 hours of contact lens wear, suggesting that this may represent the time point at which the cornea is most vulnerable to solution-induced effects.³³ The purpose of this study was to investigate the impact of MPS on the rabbit corneal surface in vivo after 2 hours of lens wear and the corresponding effects of lens-released MPS on the ability of PA to invade the corneal epithelium. Importantly, we provide evidence that wear of a specific lens–solution combination is associated with surface epithelial damage that corresponds to an increase in bacterial uptake into the corneal epithelium. These findings suggest that the use of intracellular invasion assays may provide a valid, physiological metric for assessing lens-solution biocompatibility during lens wear.

MATERIALS AND METHODS

Contact Lenses and Test Solutions

Prior to fitting in the rabbit, new sterile balafilcon A contact lenses (PureVision; Bausch & Lomb, Rochester, NY, USA) were soaked overnight in a borate-buffered MPS containing 0.00013% polyaminopropyl biguanide and 0.0001% polyquaternium (BioTrue; Bausch & Lomb). For control eyes, lenses were removed directly from the blister pack (BP) solution containing sterile borate-buffered saline, immediately prior to insertion. All lenses had a power of -0.50 and a base curve of 8.6 mm. All lens manipulations were performed in a cell culture hood using sterile forceps.

Bacteria

Pseudomonas aeruginosa strain PA-6487, an invasive corneal isolate stably conjugated to green fluorescent protein containing a carbenicillin selection cassette, was used (gift of Suzanne M. Fleiszig, University of California, Berkeley, CA, USA). Bacterial stocks were maintained in tryptic soy broth containing glycerol at -80°C . Prior to use, bacteria were grown on tryptic soy agar supplemented with 300 $\mu\text{g}/\text{mL}$ carbenicillin. From this master plate, a single clone was selected and grown overnight on Mueller Hinton agar (Sigma-Aldrich Corp., St. Louis, MO, USA) slants at 37°C . Bacteria were resuspended in phosphate-buffered saline (PBS), and the bacterial concentration was adjusted to 1×10^9 CFU/mL

(optical density of 0.411–0.418) using a SmartSpec 3000 Spectrophotometer (Bio-Rad, Hercules, CA, USA) at 650 nm. The bacterial suspension was then diluted with antibiotic-free minimum essential medium (MEM; Mediatech, Inc., Manassas, VA, USA) to 1×10^8 CFU/mL.

Animals and Contact Lens Fitting

Ten New Zealand White rabbits (body weight 2.5–3.5 kg; Charles River Laboratories, Wilmington, MA, USA) were used in the study. All rabbits were treated humanely according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Prior to lens fitting, animals were anesthetized with an intramuscular injection of 30 mg/kg ketamine HCl (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA, USA) and 1.5 mg/kg xylazine (Anased, Shenandoah, IA, USA) to facilitate ease of lens insertion. Both eyes of each rabbit were fit with lenses as described above. To standardize test conditions and prevent lenses from drying, 10 μL 0.9% balanced NaCl solution at pH 7.2 was instilled into each eye at 5-minute intervals followed by manual blinking. After 2 hours, the contact lenses were removed and the rabbits were euthanized with an intravenous injection of 120 mg/kg pentobarbital sodium (Euthasol; Virbac Animal Health, Fort Worth, TX, USA). Both eyes were enucleated immediately and processed as indicated in the following sections.

Gentamicin Invasion Assay

Enucleated whole globes were placed corneal surface up in sterile plastic well plates, and a 1-cm-deep plastic tube that encompassed the entire corneal surface including the limbus was placed on the eye with a water-tight seal. All reagents and bacteria suspensions were placed in the resulting reservoir to ensure equal exposure of all parts of the cornea. The corneas were washed with MEM three times for 5 minutes each with gentle agitation to remove any remaining MPS, followed by the addition of PA (0.5 mL, 1×10^8 CFU/mL) suspended in MEM. The eyes were incubated in the PA-MEM solution for 30 minutes at 37°C . After another three washes with MEM, the eyes were incubated in MEM (0.5 mL) for an additional 30 minutes at 37°C to allow internalization of the bacteria. Bacteria internalization was quantified with a gentamicin invasion assay. Briefly, the corneas were exposed to 200 $\mu\text{g}/\text{mL}$ gentamicin in MEM, a cell-impermeable antibiotic, for 2 hours at 37°C to kill extracellular bacteria. The eyes were then washed three times with MEM, and the corneal epithelial cells were collected by scraping the entire cornea with a Lance number 11 sterile surgical blade (Cincinnati Surgical, Cincinnati, OH, USA). The collected cells were incubated for 15 minutes at room temperature in MEM containing 5 mg/mL saponin. To determine the number of viable bacteria, the cell lysate was serially diluted and plated on Mueller-Hinton agar (BD Biosciences, San Jose, CA, USA) in triplicate, and CFU were determined.

Scanning Electron Microscopy (SEM)

After enucleation as described above, eyeballs were placed in sterile well plates and fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer pH 7.4 for 15 minutes. The corneas were then removed from the rest of the eye using a sterile surgical blade, placed in fresh glutaraldehyde, and allowed to sit overnight at 4°C . Corneas were processed in the Electron Microscopy Core at University of Texas Southwestern Medical Center according to the following protocol. Briefly, tissue was washed in 0.1 M cacodylate pH 7.4 and then subjected to secondary fixation using 1% osmium. After rinsing with water, corneal tissue underwent an ethanol dehydration series followed by drying with hexamethyldisilazane (Sigma-Aldrich

FIGURE 1. Scanning electron microscopy images of the corneal surface after 2 hours of lens wear. (A–D) Blister pack control. (A) Region showing intact surface epithelium following lens wear; (B) magnified view of the epithelial surface. (C) Region showing epithelial disruption after wear of the control lens; (D) magnified view showing loss of the surface membrane with retention of nuclei (*arrow*) and well-defined cell borders. (E–H) MPS-treated lens. (E) Region of smooth surface epithelium with only occasional surface peeling (*arrow*); (F) magnified view of surface epithelium showing surface epithelial edge lift, or peeling (*arrow*). (G) Area with epithelial damage resulting from wear of the MPS-treated lens; (H) magnified view of the region of surface damage with irregular borders and loss of epithelial nuclei (*arrow*), as shown in (G). Images in (A, C, E, G) taken at $\times 200$ magnification. Scale bar: 100 μm . Images in (B, D, F, H) taken at $\times 1000$ magnification. Scale bar: 20 μm . Images representative of two different corneas per lens group.

Corp.). Tissue was air dried, mounted on aluminum stubs, and sputter-coated with gold-palladium in a Cressington 108 auto sputter coater (Cressington Scientific, Watford, UK). Images were acquired in a Philips XL 30 ESEM (FEI, Hillsboro, OR, USA) operating in high vacuum mode with an accelerating voltage of 15.0 kV.

Transmission Electron Microscopy (TEM)

For TEM, primary fixation was performed as above. After washing in 0.1 cacodylate buffer pH 7.4, corneal tissue was fixed in 1% osmium containing 0.8% $\text{K}_3\text{Fe}(\text{CN})_6$. Tissue was rinsed with water and prestained with 4% uranyl acetate in

50% ethanol. Following an ethanol dehydration series, tissue was incubated in propylene oxide for 10 minutes and then infiltrated using a graded series of propylene oxide and resin. After embedding in 100% resin, samples were allowed to polymerize overnight. Tissue sections were cut using a Reichert-Jung Ultra-cut ultramicrotome (Leica Microsystems, Heidelberg, Germany) and imaged on the FEI Tecnai G2 Spirit Biotwin (FEI) at 120 kV.

Toluidine Blue Staining

Resin-embedded tissue was sectioned (semithin thickness of 0.25 and 0.6 μm) using a Reichert-Jung Ultra-cut ultramicro-

FIGURE 2. Toluidine blue-stained semithin sections. (A, C) Representative images showing an intact epithelial surface after wear of the control lens. (B, D) Representative images of the damaged corneal surface following wear of the MPS-treated lens. Note the loss of surface epithelium (B, arrows) and an edematous cornea (D). Scale bar: 50 μ m. Images representative of three different corneas per lens group.

tome. Following heat fixation, tissue was stained with 1% toluidine blue in 2% sodium borate. After washing with water, samples were air dried. Tissue sections were viewed using a Zeiss Axioscop equipped with an Axiocam HRC color digital camera (Carl Zeiss Microscopy, Jena, Germany).

Statistical Analysis

Data were represented as mean $\pm$ standard error. *Pseudomonas aeruginosa* internalization data were analyzed using a Student's *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

In all experiments, one eye of each rabbit was fit with a lens soaked overnight in MPS and the other eye was fit with a control lens from the BP. Scanning electron microscopy imaging revealed obvious regions of epithelial disruption for both lens conditions (Fig. 1). However, the pattern of disruption was distinctly different for MPS compared to the control lens. After wear of the control lenses from the BP, the corneal surface revealed patches of damaged epithelial cells that appeared to have lost their surface membrane but retained their crisp, linear

FIGURE 3. Transmission electron microscopy images of the surface corneal epithelium. (A) Representative image of an undisturbed corneal epithelium after 2 hours of wear of the control lens. (B) In regions of epithelial cell damage, TEM confirmed loss of the outer cell membrane with partial retention of cytoplasmic contents. Intact cell junctions were evident (arrows). (C) Region of surface epithelial damage following wear of the MPS-treated lens. In addition to loss of surface epithelial cells (asterisk), peeling of residual surface cells is seen (dotted arrows), exposing deeper cell layers. Scale bar: 2 μ m. Images representative of three different corneas per lens group.

FIGURE 4. Representative high-magnification TEM images of the surface epithelium and intercellular space. (A) Corneal surface following wear of the control lens without obvious edema. (B) After wear of the MPS-treated lens, intercellular edema was seen between surface cells, indicating breakdown of the tight barrier. (C) Visible surface damage and cellular desquamation resulting from toxicity and junction loss. Scale bar: 0.5 μm . (D–F) Higher-magnification images corresponding to (A–C). (D, E) Difference in the intercellular junctions in the surface epithelium. In (F), note the rounded, dying cell preparing to desquamate but still anchored to the epithelial surface by desmosomal contacts (arrow). Scale bar: 1 μm . Images representative of three different corneas per lens group.

boundaries (Figs. 1A–D). Both residual cytoplasm and nuclei were present in all damaged cells. In contrast, SEM of the corneal surface following wear of lenses soaked in MPS showed ragged and torn patches with fragmented cell borders where entire cells had been peeled away and lost (Figs. 1E–H). In these corneas, small depressions were left behind from absent nuclei.

To examine a high-resolution view of the full-thickness corneal epithelium, semithin sections were stained with toluidine blue (Fig. 2). The corneal epithelium from control lenses was intact and smooth without visibly apparent edema (Figs. 2A, 2C). In contrast to this, corneas from MPS-treated lenses demonstrated a frank loss of surface epithelial cells (Fig. 2B) and significant epithelial disorganization indicating the presence of epithelial edema arising from loss of barrier function (Fig. 2D).

To further examine the impact of MPS-treated lens wear on the epithelium, TEM was used to assess ultrastructure. Similar to the SEM data, control eyes revealed areas with no damage to the cornea (Fig. 3A) interspersed with patches of torn or damaged cells (Fig. 3B). Torn or split epithelial cells had residual cytoplasm and intact junctional borders. Corneas exposed to MPS-treated lenses, however, showed areas of complete cell loss and peeling or lifting of exposed surface cells (Fig. 3C). Higher-magnification imaging confirmed differences in intercellular spacing between the test and control lens, indicating the presence of edema in the MPS test group (Figs. 4A, 4B, 4D, 4E). In addition to corneal epithelial swelling, occasional rounded cells were seen on the damaged epithelial surface. These rounded cells represent nonviable cells in the process of desquamating, as they were still held intact to the corneal

surface by desmosomal contacts (Figs. 4C, 4F). A montage of the corneal epithelium after wear of each lens showed that the degree of epithelial damage extended beyond the surface epithelium, reaching as deep as three or four cell layers (Fig. 5). In contrast, control eyes had normal, intact intercellular junctions throughout the full-thickness epithelium.

To investigate the physiological consequence of lens-induced surface epithelial damage on PA internalization, a gentamicin invasion assay was performed. Importantly, challenge with PA in the MPS-treated eye resulted in a 12-fold increase in PA internalization in corneal epithelial cells compared to the control ($P = 0.008$, Fig. 6).

DISCUSSION

This study showed that, in the rabbit model, exposure of the surface corneal epithelium to chemically preserved MPS during lens wear is associated with disruption of the corneal epithelial surface. This is supported by ultrastructural assessment of the epithelial surface showing evidence of surface epithelial cell loss and pronounced intercellular edema. Importantly, these changes were also associated with a significant increase in the amount of internalized PA after wear of an MPS-soaked lens. The finding that corneal epithelial cells internalized greater PA following exposure to MPS is not altogether unsurprising. Previous clinical studies have shown that bacterial binding to exfoliated corneal epithelial cells is increased following repeated instillation of MPS.³⁴ Similarly, wear of silicone

FIGURE 5. Transmission electron microscopy montage of corneal epithelium from apical surface to basal. **(A)** Cornea wearing the control lens; **(B)** cornea wearing the MPS-treated lens. Junctional disruption was not restricted to the surface epithelium but extended basally three or four cell layers (arrows). Scale bars: 2 μ m. Images representative of three different corneas per lens group.

hydrogel lenses with concomitant MPS use also promoted PA attachment to exfoliated corneal epithelial cells when compared to peroxide-based care systems.^{22,35}

The mechanism for the increase in PA attachment and internalization is unclear. Work by Imayasu et al.³⁶ using SV40 in vitro cell cultures has confirmed an increased rate of PA attachment to corneal epithelial cells when treated with borate-buffered care solutions. Consistent with this work, the presence of borate in lens packaging solutions and care systems has been proposed as a direct mediator of epithelial damage.^{25,37} Using markers for apoptotic cell death, Gorbett and colleagues³⁷ demonstrated a relationship between the use of borate buffers in lens packaging solutions and corneal epithelial cell viability. While boric acid was a component of the MPS formation used in this study, the inclusion of a borate buffer in the packaging solution used as a control indicates that the presence of borate alone does not account for the increased bacterial internalization that we found after MPS exposure.

A second potential mechanism that may explain the increased adherence and internalization of PA associated with MPS use is the detrimental effects on membrane-associated mucin. Corneal epithelial cell mucins are a vital component of the innate immune system as mucins bind bacteria and prevent interactions between bacteria and surface epithelia. The exact effects of lens wear and MPS on mucin expression and release, however, is controversial. Gordon et al.³⁰ used a telomerase-immortalized corneal limbal epithelial cell line to investigate the effects of commercially available MPS on MUC16. The findings from their study showed that increased release of MUC16 into cell culture media was associated with increased PA internalization in cultured cells and that this effect was dependent on the MPS tested. Imayasu and colleagues^{17,24} also investigated the effects of MPS on mucin production in an SV40 epithelial cell line and in the rat cornea in vivo. From their results, they concluded that use of certain MPS reduced MUC1 and MUC16 expression in the corneal epithelium, with increased release of MUC16 into culture media. While the

FIGURE 6. Bacterial invasion assay. Wear of the MPS-treated lens increased bacterial uptake into the corneal epithelium 12-fold compared to the control lens (Ctrl, $P = 0.008$, $n = 5$ rabbits, t -test).

effect of MPS on mucin expression was not tested in the current study, future studies to evaluate the relationship between mucin expression and PA internalization in the lens-wearing rabbit model are required.

In the present study, we found varying degrees of epithelial surface damage following lens wear in both the MPS-treated group and the control. Interestingly, the pattern and location of damage were distinct between groups. In control corneas, there was evidence of widespread frictional damage likely arising from lens dryness during wear. This took the form of tearing or splitting of the cells and loss of the exposed phospholipid membrane but retention of intracellular constituents and well-defined borders between neighboring epithelial cells. Indeed, the rabbit has a reduced blink rate compared to the human eye, as well as differences in tear composition including osmolarity, divalent cations, and protein profiles that may contribute to the increased dryness and superficial damage observed in this model.^{19,35,38} While clinically the surface epithelial damage reported with the control lens in this study would manifest as a corneal staining response following the instillation of fluorescein, our findings suggest that this is not indicative of a breached barrier, which is thought to be required for PA adhesion and invasion into deeper corneal epithelial cells.

In contrast, this pattern of superficial damage was not present with the MPS-treated lens. We hypothesize that this effect was a result of the combination of hyaluronan and poloxamine in the test MPS. Instead, widespread regions showing surface epithelial peeling were evident with discrete regions of localized tissue damage. Unlike the control eye, which showed the retention of distinct polygonal epithelial cell borders, MPS-exposed corneas showed areas of focal cell loss without defined epithelial cell borders and associated intercellular edema. The presence of edema and disorganization in intercellular spacing is consistent with loss of the corneal epithelium's tight barrier function. In agreement with these findings, a small number of recent studies have focused on the effects of MPS on corneal tight junction and barrier function properties. Imayasu et al.³¹ demonstrated that cultured human corneal epithelial cells treated with MPS containing poloxamine and boric acid showed discontinuous and disrupted ZO-1 staining at cell-cell borders using laser

confocal microscopy versus the normal continuous linear staining pattern seen in control non-MPS-exposed cells.³² Similarly, Chuang et al.¹⁶ demonstrated changes in ZO-1 staining as well as disturbed patterns of occludin staining when corneal epithelial cell monolayers were exposed to various MPS, with the poloxamine and boric acid-buffered solution having the greatest impact. In addition to the in vitro findings, Paugh and colleagues³⁹ used fluorescein penetration in human subjects as a measure of barrier function decline. In their work, they showed that barrier disruption was associated with the use of MPS containing the preservative polyhexamethylene biguanide. When combined with the ultrastructural findings in this study, these data collectively indicate that certain MPS have the potential to negatively impact the natural barrier function of the cornea. To establish the exact role of different MPS constituents including preservatives, buffers, and surfactants in mediating corneal epithelial surface damage, further work is needed.

A significant amount of research has been invested in assessing the effects of lens-solution interactions on fluorescein staining of the cornea. It has been argued that solution-induced corneal staining or preservative-associated transient hyperfluorescence may indicate a range of processes including fluorescein uptake and metabolism by viable cells, acute surface toxicity, and charged interactions between fluorescein and the biocide present underneath the lens.^{21,27,28} While we chose to use the peak 2-hour time point that has been reported in the staining literature, we did not evaluate staining as an outcome measure in this study.³ We do expect that the pattern and extent of surface epithelial damage seen in the lens-wearing rabbit eye, both with and without MPS, would present clinically as fluorescein staining and that changes in epithelial surface permeability that facilitate either inter- or intracellular pooling may be a contributing factor. However, it is well accepted clinically that the solution reaction giving rise to the appearance of fluorescein staining is visible with white light prior to fluorescein instillation, indicating some level of epithelial disruption. Given the significant differences between differentiated surface epithelial cells compared to monolayer cultures, in vivo modeling is essential in the assessment of lens-solution biocompatibility. Based upon our findings, we propose that the use of bacterial invasion assays may provide a sensitive physiological in vivo metric for assessing the interactive effects of lenses and solutions on the biology of the corneal surface, independent of the causative mechanism underlying staining.

In summary, the findings from the present study indicate that some MPS have the capacity to disrupt the innate surface epithelial barrier to invading organisms. The potential loss of the tight barrier, as seen in this study, and associated defense mechanisms such as mucins would set the stage for increased bacterial adherence and internalization in the corneal epithelium. The extent to which this phenotype applies to other MPS and MPS-lens combinations is of high clinical interest and requires additional investigation. However, based on our current findings, we hypothesize that the resultant increased level of corneal epithelial-associated bioburden represents a significant risk factor for lens-related infection. In agreement with this hypothesis, epidemiological data confirm that MPS use is associated with a 2.5-fold increased risk of infection when compared to peroxide.²³ Given the highly multifactorial nature of contact lens-related MK, the optimization of lens-solution biocompatibility alone will not be sufficient to reduce lens-related complications. Further development involving increased compliance measures and

enhanced tear exchange under the lens to remove accumulated debris is necessary.

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