Poly-ε-Lysine or Mel4 Antimicrobial Surface Modification on a Novel Peptide Hydrogel Bandage Contact Lens

Rebecca Lace, Kyle G. Doherty, Debarun Dutta, Mark D. P. Willcox, and Rachel L. Williams*

Microbial keratitis (MK) is a serious issue in many countries and is often caused by contact lens wear. Antimicrobial peptides (AMPs) are a potentially useful tool for creating antimicrobial surfaces in light of increasing antibiotic resistance. Poly-ε-lysine (pεK) is an AMP that has been used extensively as a food preservative and Mel4 has recently been synthesized and studied as an antimicrobial coating for contact lenses. A hydrogel synthesized of pεK cross-linked with bis-carboxylic acids provides a potential lens material which has many surface free amines, that can be subsequently used to attach additional AMPs, creating an antimicrobial lens. The aim of this study is to investigate pεK hydrogels against a clinical strain of Pseudomonas aeruginosa (P. aeruginosa) for preventing or treating MK. Covalent attachment of AMPs is investigated and confirmed by fluorescently tagged peptides. Bound pεK effectively reduces the number of adherent P. aeruginosa in vitro (>3 log). In ex vivo studies positive antimicrobial activity is observed on bare pεK hydrogels and those with additionally bound pεK or Mel4; lenses allow the maintenance of the corneal epithelium. A pεK hydrogel contact lens with additional AMPs can be a therapeutic tool to reduce the incidence of MK.

Dr. R. Lace, Dr. K. G. Doherty, Prof. R. L. Williams
Department of Eye and Vision Science
Institute of Life Course and Medical Science
University of Liverpool
Liverpool L7 8TX, UK
E-mail: rhw@liverpool.ac.uk
Dr. D. Dutta,[1] Prof. M. D. P. Willcox
School of Optometry and Vision Science
University of New South Wales
Sydney, New South Wales 2052, Australia

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/admi.2020001232.

© 2020 The Authors. Published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Present address: Department of Optometry, School of Life and Health, Aston University, Birmingham, B4 7ET, UK

DOI: 10.1002/admi.2020001232

Poly-ε-lysine (PeK) is a naturally occurring AMP that is non-toxic and used as both an emulsifier and food preservative. Melimine is a cationic AMP which has previously been shown to produce a family of hydrogels synthesized from pεK peptide, cross-linked with bis-carboxylic acids to produce tailorable, transparent hydrogels that are nontoxic to corneal cells in vitro. They have demonstrated the antimicrobial properties of these hydrogels against S. aureus and they can be cast into contact lens molds.[13,15] Free amine groups are present on the hydrogel surface allowing the covalent binding of additional AMPs. In 20 studies,[1b] The traditional treatment method, in over 90% of cases,[14] for MK is a broad spectrum of fortified antibiotic eye drops. However, prolonged use of antibiotics is toxic to the cornea and can have a detrimental effect on corneal re-epithelialization.[1b] There is also a lack of efficacy with the use of antibiotic eye drops with only approximately 5% being absorbed.[15] In addition, antimicrobial resistance has been identified by the World Health Organisation as a growing threat.[1a,6] In some cases, bandage contact lenses are applied to aid wound healing and prevent further microbial insult during healing.[7] Therefore, there is potential in exploring a synthetic antimicrobial contact lens which could prevent bacterial adhesion when the epithelium is compromised, such as during corneal collagen cross-linking, or be used as a tool in the treatment for MK.

Several antimicrobial peptides (AMPs) have been found in the ocular surface, such as defensins and LL-37.[8] The typical, although not exclusive, general mode of action of cationic peptides is disruption of the microbial membrane.[8,9] The cationic peptides interact with the anionic components of microbial membranes, causing disruption and permeabilization, leading to cell death.[8,10] The Willcox group at University of New South Wales (UNSW) is experienced in AMP modification of contact lenses. The group has reported primarily on the AMP melimine and its shorter derivative, Mel4.[11] Melimine is a cationic AMP which has previously been shown to have a broad spectrum of antimicrobial activity however superficial corneal staining was reported in a human trial.[11a,b,d,e,f,h] Mel4 is a smaller derivative of melimine, which had less corneal staining compared to melimine lenses.[11d] Contact lenses modified with Mel4 at a concentration of 3.1 mg cm⁻³ have been reported to be active against many bacterial strains with no toxicity or ocular irritation in an animal study.[11c,f,g]
In this study, we aim to combine our expertise and investigate the antimicrobial activity of two promising AMPs, pεK and Mel4, covalently bound to a hydrogel contact lens, against *P. aeruginosa* for the prevention and treatment of MK.

pεK was cross-linked 60% with octanediol-acid using N-hydroxysulfo succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), to a polymer density of either 0.1 or 0.13 g cm⁻³ (named 60%0.1 and 60%0.13, respectively, hereafter) as previously described, see Table S1 (Supporting Information).[7] The physical properties of the hydrogels including their contact angle, water content, tensile properties, and transparency were investigated. Both hydrogels were highly hydrophilic and had an average contact angle of 15.1° ± 4° and 16.3° ± 5.6 for 60%0.1 and 60%0.13, respectively; hydrogels were not significantly different (*p = 0.775*) (Figure 1a), meaning water spread easily across the surface. High wettability of contact lenses has been linked to better comfort.[14] The hydrophilic nature of the pεK hydrogels is likely due to the presence of many polar amine groups on the materials surface, as well as the high water content of hydrogels. Both pεK hydrogels had a high water content similar to commercially available contact lenses,[15] 71.8% ± 1.5 and 68.5% ± 5.8 for 60%0.1 and 60%0.13, respectively (*p = 0.38; Figure 1b). Water in the hydrogel can facilitate oxygen permeability and transport; which may help prevent corneal edema.[14a] The elastic moduli of both hydrogels was similar; 60%0.1 was stiffer at 402.4 kPa ± 72.1 compared to 256.8 kPa ± 45.9 for 60%0.1 (*p = 0.078*) (Figure 1c). The ultimate tensile strength (UTS) was significantly different (*p = 0.021*); 60%0.1 had a UTS of 45.9 kPa whereas 60%0.13 was higher at 72.1 kPa (Figure 1c). However, 60%0.1 was easier to handle and less prone to snapping when pinched. These values are within the range of soft hydrogels, especially the Acuvue lens product family.[15] Previous work on these hydrogels indicated there was a slight increase in stiffness, but an insignificant change in UTS following covalent attachment of additional pεK.[7] Both hydrogels had excellent transparency, allowing passage of light across all the tested wavelengths of the visible spectrum; 560 nm was chosen as a representative wavelength. The % light transmission was 94.4% ± 0.7 and 93.1% ± 0.2 for 60%0.1 and 60%0.13, respectively (Figure 1d). Light transmission for other wavelengths can be found in Figure S1 (Supporting Information). These were comparable to a control commercial contact lens (Acuvue 2) at 98.2% ± 0.8, however both hydrogels were significantly different to the control (*p < 0.001*). These physical properties of the hydrogels demonstrate their suitability and potential to be used as a therapeutic contact lens.

To demonstrate whether additional peptides successfully bound to the hydrogels, pεK and Mel4 were fluorescently tagged with Alexa Fluor 594 NHS ester (named +pεK 594 and +Mel4 394), prior to covalently binding them to pεK hydrogels. Untagged peptides (+pεK and +Mel4) served as controls. Covalent binding of untagged peptides caused no change in fluorescence compared to bare gel (60%0.1). Hydrogels with fluorescently tagged peptides had approximately 12-fold increase in fluorescent signals, despite copious washes with water; confirming the peptides were covalently bound to the hydrogels (Figure 1e). Due to the strong fluorescent signal, the widely reported carbodiimide chemistry methods, and the observed antimicrobial activity, we did not repeat this experiment for statistical analysis.

Lenses were incubated with 1 × 10⁶ CFU *P. aeruginosa* overnight and the number which had bound to the lenses were determined. The polymer density in the hydrogel had an insignificant effect on bacterial adhesion, irrespective of subsequent peptide modification. The bare pεK hydrogels had a small log reduction of 0.6 (60%0.1) and 0.7 (60%0.13) compared to inoculum (Figure 2). PεK is innately antimicrobial due to its cationic nature but nine or more free lysine residues are required for optimal antimicrobial activity.[16] This is likely the reason why bare pεK hydrogels are not inherently antimicrobial, due to their cross-linked nature they do not have many molecular chains long enough to disrupt bacterial membranes.

These hydrogels, however, have an abundance of free amine groups which can be further used to covalently bind cell adhesive motifs,[13a] ionically trap antimicrobial drugs, or covalently bind AMPs.[7,13b] The UoL group has previously demonstrated that we can impart antimicrobial activity by binding additional pεK peptides to the surface of the hydrogels using a similar method to the hydrogel synthesis but in the absence of a cross-linking diacid. This method was effective at producing an ~2 log reduction in attached *Staphylococcus aureus* compared to the bare gel. A similar reduction in planktonic *S. aureus* was observed when hydrogels were impregnated with penicillin G.[7] Here, we demonstrate that when additional pεK was bound to both hydrogels (60%0.1 + pεK and 60%0.13 + pεK) there was a 3 log reduction in CFU for *P. aeruginosa* compared to the inoculum (Figure 2). These were significantly different to bare lenses (*p < 0.05*).

The addition of Mel4 resulted in a 0.2 log reduction compared to inoculum on 60%0.1 + Mel4 hydrogels (*p = 0.0085 and 0.011 compared to 60%0.1 + pεK, and 60%0.13 + pεK, respectively), and less on the 60%0.13 + Mel4 (*p = 0.0066 and 0.0085 compared to 60%0.1 + pεK, and 60%0.13 + pεK, respectively). Addition of Mel4, using the same binding method to that used for pεK, on the commercial polyhydroxyethyl methacrylate (pHEMA) contact lenses (Acuvue 2, Etaflexon A) resulted in a 0.5 log reduction in CFU. Previous work by the Willcox group has demonstrated a >2 log reduction against *P. aeruginosa* when Mel4 bound was bound to silicone hydrogel lenses[13c] and a 1.3 log reduction when bound to pHEMA.[13d] In these studies a different method of binding the Mel4 was used which would influence the way in which the AMP was attached to the surface. When pεK was bound to commercial pHEMA lenses a lesser effect (1.2 log reduction) was observed compared to the 60%0.1 + pεK and 60%0.13 + pεK hydrogel equivalents, this was not significantly different compared to any lenses (*p > 0.05*, Figure 2).

There are two main considerations which may explain why we observe differences in this and earlier studies. The first consideration is the difference in peptide binding methods due to the different surface chemistry of pεK hydrogels and pHEMA. The pHEMA or acrylic acid-modified silicone lenses contain surface carboxyl groups whereas the pεK hydrogels contain surface amine groups. The EDCI/NHS reacts with the carboxyl groups and promotes the peptide bond formation with the amine functional groups.[17] The amine rich pεK hydrogel surface encourages the carboxyl of the peptide C-terminus to bind to the surface, whereas any amine along the pεK peptide may bind to the “activated” carboxyl groups of the pHEMA,
Figure 1. Physical properties of 60%0.1 and 60%0.13 pK hydrogels demonstrated: a) Hydrogels were very hydrophilic (15.1° – 16.3°). b) Hydrogels had a high water content (63.7 – 67.8%). c) The pK hydrogels stiffness ranged between 256.8 and 402.4 kPa and ultimate tensile strength (UTS) ranged between 45.9 and 72.1, which was significantly different to each other, p = 0.021. d) Hydrogels were extremely transparent (93.1–94.4%), however, both hydrogels were significantly different to the control (98.2%), p < 0.001. 560 nm shown as representative wavelength. e) Hydrogels bound with fluorescently tagged peptides had a high relative fluorescence signal (12-fold increase) even after extensive washing, confirming covalent binding. Error bars represent ±1 standard deviation. *p < 0.05, ***p < 0.001.
thereby shortening the AMP chain length. This could influence the 
antimicrobial activity of the bound AMP, resulting in the 
significant reduction in antimicrobial activity observed when 
peptide was bound to pHEMA lenses compared to pK hydrogels 
(Figure 2). However, previous work by UNSW has shown that 
the activity of melamine is reduced when bound to a surface 
by its C-terminus compared to when bound by its N-terminus 
or at an approximate central position. These may explain the 
reduction of antimicrobial activity of Mel4 compared to 
previous work. This work may indicate that aminated surfaces are 
beneficial for modification by some AMPs, but not others and 
so the optimal binding method should be considered for each 
AMP.

The second consideration is the difference in length of the 
peptides; pK used in this study contains approximately 25–35 
residues, whereas the Mel4 contains only 17 residues, which 
may explain the differences in antimicrobial activity between 
peptide and Mel4 observed in this study. Liu et al. also showed that 
increasing the length of AMPs improved their antimicrobial 
activity by synthesizing repeated units of Arg(R) and Trp(W), 
where n = 1–5, whereas the longer peptide melamine (29 resi- 
dues) has greater antimicrobial activity compared to the shorter 
Mel4. Researchers from UNSW “activated” the surface 
carbonyl groups on the acrylics prior to adding AMPs, whereas 
within this and previous studies from Ulm researchers, AMPs and 
EDCI/NHS are added simultaneously. Adding AMP and EDCI/NHS simultaneously could cause some AMPs to 
bind to each other, effectively shortening the chain of free 
amino acids, creating an interconnected net-like structure, 
which may reduce the antimicrobial effect although the mecha-
nisms are likely to be more complicated for AMPs which have 
a variety of amino acids compared to the simple pK. For 
example work by Juba et al. demonstrated differences in efficacy 
and mechanism between the AMP peptide NA-CATH and its 
truncated isomers a- and b-ATRA-1A; NA-CATH had a greater 
antimicrobial activity. These different binding methods could 
also explain the reduced efficacy of Mel4 when bound to 
pHEMA in this work compared to previous publications. Mel4 
was added at the same concentration as the previously reported 
work. The binding method reported in this work is expected to 
behave similarly.

For the ex vivo study only 60%0.1 pK hydrogels were used 
as they had favorable handling properties with no difference 
in antibacterial properties between the two formulations. We 
adapted an ex vivo culture model and damaged the cornea by 
ethanol treatment and mechanical scraping then pipetted the 
bacteria onto the cornea. This successfully removed the super-
ficial stratified epithelium, observed as densely stained 
areas (Figure 3a), similar to that reported by Crespo-Morales 
et al. Corneas incubated overnight without inoculation with 
P. aeruginosa had begun to regrow a squamous epithelial 
layer (Figure 3b–g). Removal of the superficial layers allowed 
P. aeruginosa to infect the corneas where no hydrogels were 
present, which resulted in disintegration of the epithelium. 
During overnight incubation the epithelium became compro-
mised and dissociated from the stroma (Figure 3h) and a high 
density of Gram-stained bacteria was observed on the exposed 
stroma (Figure 3h).

The H&E and Gram staining demonstrated that if a hydrogel 
(60%0.1, 60%0.1 + pK or 60%0.1 + Mel4) was placed on the 
cornea after inoculation the epithelium beneath the hydrogels 
was preserved and remained largely intact (Figure 3d–f), 
compared to infection control samples with no hydrogel (Figure 3c). 
Although some bacteria were observed on the apical surface 
of the epithelium, there were no bacteria observed within the 
epithelium or the stroma beneath these hydrogels suggesting 
that direct contact of the AMPs killed the bacteria before they 
were able to invade the stromal tissue, similar to a recently 
published study. These lenses may be useful as a bandage 
contact lens following corneal collagen cross-linking to prevent 
microbial keratitis, as current bandage lenses have been 
identified as a possible risk factor. Many bacteria were observed 
on the apical surface beneath bare 60%0.1 hydrogels (Figure 3i), 
whereas only a few bacteria were observed beneath +pK 
(Figure 3k) or +Mel4 hydrogels (Figure 3m). Gram stained 
bacteria were present at the outer edges of the corneas which 
were not beneath the hydrogels and the epithelium appeared similar 
to the infection control (Figure 3j,l,n). This suggests that the 
AMPs were covalently bound to the hydrogels as antimicrobial 
action was only observed when in contact with the infected 
area. A dual action lens could be developed with pK bound to 
hydrogel which was soaked in an antimicrobial agent that could 
elute over time to add further functionality to these lenses.

We have demonstrated that a pK-based hydrogel can impart 
antimicrobial activity in vitro and in a porcine ex vivo model 
against a clinical isolate of P. aeruginosa. A >3.0 log reduction 
can be achieved when additional pK is bound to the surface
Figure 3. a–f) H&E and g–n) gram staining of formalin-fixed paraffin-embedded porcine corneas. a) Nonwounded no infection control shows the intact porcine cornea; b) wounded no infection control demonstrates removal of apical stratified squamous layers by wounding; c) infection control demonstrates destruction of the cornea after 18 h incubation with *P. aeruginosa*; d) 60%0.1 hydrogel demonstrates preservation of the epithelium despite *P. aeruginosa*; e) shows epithelial preservation by 60%0.1 + pεK hydrogel; f) similarly demonstrates epithelial preservation by 60%0.1 + Mel4 hydrogel; g) no infection control demonstrating stratified epithelium and one layer of regrowth of the apical squamous epithelium; h) demonstrates complete removal of the epithelium and bacterial penetration into the stroma tissue 18 h after inoculation with *P. aeruginosa* 6294; i) demonstrates the area beneath 60%0.1 hydrogel, showing many bacteria near the detached epithelial cells; j) shows the edge of the same in an area not underneath the hydrogel; k) demonstrates an intact epithelium beneath a 60%0.1 + pεK hydrogel with very few bacteria present; l) represents an area of the same cornea not beneath the hydrogel; m) also demonstrates perseveration of the corneal epithelium beneath a 60%0.1 + Mel4 hydrogel with only a few bacteria visible on the apical surface; n) demonstrates a compromised epithelium in an area not beneath the 60%0.1 + Mel4 hydrogel. Black arrows indicate *P. aeruginosa*. Scale bar = 10 µm
of the hydrogel. Mel4 does not effectively impart antimicrobial activity when bound to the peK surface or the pHEMA surface using this binding technique. Ex vivo analysis demonstrated that hydrogels preserved the epithelium after 18 h of incubation with *P. aeruginosa* 6294 and demonstrated positive antimicrobial activity when hydrogels were bound with additional AMPs (+peK and +Mel4) in the area underneath the gels suggesting that direct contact with the bound AMPs is necessary to kill the bacteria.

**Experimental Section**

See supporting information. The data used in publication can be found at [https://doi.org/10.17638/dataset.liverpool.ac.uk/1149](https://doi.org/10.17638/dataset.liverpool.ac.uk/1149). The porcine corneas for the ex vivo model were obtained as a waste product from the meat industry, and did not require ethical approval.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

R.L. and K.G.D. contributed equally to this work and they are joint first authors. This work was funded by EPSRC grant no: EP/M002209/1. The authors would like to thank SpheriTech Ltd for providing poly-ε-lysine.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords**

antimicrobial peptides, contact lens, microbial keratitis, *Pseudomonas*

---

[1] a) C. Ezisi, C. Ogbonnaya, O. Okoye, E. Ezeanosike, H. Ginger-Eke, O. Arinze, *Niger. J. Ophthalmol*. 2018, 26, 13; b) L. Ung, P. J. M. Bispo, S. S. Shanbhag, M. S. Gilmore, J. Chodosh, *Surg. Ophthalmol.* 2019, 64, 253.

[2] A. Shah, A. Sachdev, D. Coggon, P. Hossain, *Br. J. Ophthalmol.* 2011, 95, 762.

[3] J. Averyard, R. C. Deller, R. Lace, R. L. Williams, S. B. Kaye, K. N. Kolegraff, J. M. Curran, R. A. D’Sa, *ACS Appl. Mater. Interfaces* 2019, 11, 37491.

[4] a) P. Garg, S. Sharma, G. N. Rao, *Ophthalmology* 1999, 106, 1319; b) S. Tuft, M. Burton, *Royal Collage of Ophthalmologists Focus* 2013, 5, https://www.rcophth.ac.uk/wp-content/uploads/2014/08/Focus-Autumn-2013.pdf.

[5] R. L. Williams, H. J. Levis, R. Lace, K. G. Doherty, S. M. Kennedy, V. R. Kearns, in *Encyclopedia of Biomedical Engineering* (Ed: R. Narayan), Elsevier, Oxford 2019, pp. 289–300.

[6] a) World Health Organisation, *Global Action Plan on Antimicrobial Resistance 2015*, https://www.who.int/antimicrobial-resistance/publications/global-action-plan/en/; b) M. Cabrera-Aguas, P. Khoo, C. R. George, M. M. Lahra, S. L. Watson, *Clin. Exp. Ophthalmol.* 2020, 48, 183.

[7] A. G. Gallagher, J. A. Alorabi, D. A. Wellings, R. Lace, M. J. Horsburgh, R. L. Williams, *Adv. Healthcare Mater.* 2016, 5, 2013.

[8] A. M. McDermott, *Ocul. Surf.* 2004, 2, 229.

[9] a) J. D. F. Hale, R. E. W. Hancock, *Expert Rev. Anti-Infect. Ther.* 2007, 5, 951; b) T. Berry, D. Dutta, R. Chen, A. Leong, H. Wang, W. A. Donald, M. Parviz, B. Cornell, M. Willcox, N. Kumar, C. G. Cranfeld, *Langmuir* 2018, 34, 11586; c) M. Yasir, D. Dutta, M. D. P. Willcox, *Sci. Rep.* 2019, 9.

[10] a) Y. Shai, J. Pept. Sci. 2002, 66, 236; b) B. Bechinger, S.-U. Gor, *J. Dent. Res.* 2017, 96, 254.

[11] a) M. D. P. Willcox, E. B. H. Hume, Y. Aliwarga, N. Kumar, N. Cole, *J. Appl. Microbiol.* 2008, 105, 1817; b) R. Rasul, N. Cole, D. Balasubramanian, R. Chen, N. Kumar, M. D. P. Willcox, *Int. J. Antimicrob. Agents* 2010, 35, 566; c) D. Dutta, T. Zhao, K. B. Cheah, L. Holmlund, M. D. P. Willcox, *Contact Lens Anterior Eye* 2017, 40, 175; d) D. Dutta, A. K. Vijay, N. Kumar, M. D. P. Willcox, *Invest. Ophthalmol. Visual Sci.* 2016, 57, 5616; e) D. Dutta, J. Ozkan, M. Willcox, *Optom. Vision Sci.* 2014, 91, 570; f) D. Dutta, N. Kumar, M. D. P. Willcox, *Biofouling* 2016, 32, 429; g) D. Dutta, B. Kamphuis, B. Ozcelik, H. Thissen, R. PinARBasi, N. Kumar, M. D. P. Willcox, *Optom. Vis. Sci.* 2018, 95, 937; h) D. Dutta, N. Cole, N. Kumar, M. D. P. Willcox, *Invest. Ophthalmol. Visual Sci.* 2013, 54, 175.

[12] M. Hyldgaard, T. Mygind, B. S. Vad, M. Stenvang, D. E. Otzen, *J. Pept. Sci.* 2006, 12, 7758.

[13] a) S. Kennedy, R. Lace, C. Carisorders, A. G. Gallagher, D. A. Wellings, R. L. Williams, H. J. Levis, J. *Matr. Sci.: Mater. Med.* 2019, 30, 102; b) A. G. Gallagher, K. McLean, R. M. K. Stewart, D. A. Wellings, H. E. Allison, R. L. Williams, *Invest. Ophthalmol. Visual Sci.* 2017, 58, 6499.

[14] a) C. S. A. Musgrave, F. Fang, *Materials* 2019, 12, 261; b) M. Guillon, C. Maissa, *Contact Lens Anterior Eye* 2007, 30, 5.

[15] a) S. Shima, H. Matsuoka, T. Iwamoto, *J. Antibiot.* 1984, 37, 1449; b) T. Yoshida, T. Nagasawa, *Appl. Microbiol. Biotechnol.* 2003, 62, 21.

[16] a) Z. Grabarek, J. Gergely, *Anal. Biochem.* 1990, 185, 131; b) G. L. Grobe III, P. L. Valint Jr., D. M. Ammon Jr., *Anal. Biochem.* 1990, 185, 131; c) M. Yasir, D. Dutta, J. Ozkan, M. Willcox, *Invest. Ophthalmol. Visual Sci.* 2018, 95, 937; h) D. Dutta, N. Cole, N. Kumar, M. D. P. Willcox, *Biofouling* 2016, 32, 429; g) D. Dutta, B. Kamphuis, B. Ozcelik, H. Thissen, R. PinARBasi, N. Kumar, M. D. P. Willcox, *Optom. Vis. Sci.* 2018, 95, 937; h) D. Dutta, N. Cole, N. Kumar, M. D. P. Willcox, *Invest. Ophthalmol. Visual Sci.* 2013, 54, 175.

[17] M. L. Juba, D. K. Porter, E. H. Williams, C. A. Rodriguez, S. M. Barksdale, B. M. Bishop, *Biochim. Biophys. Acta, Biomembr.* 2007, 175, 11586; c) M. Yasir, D. Dutta, J. Ozkan, M. Willcox, *Optom. Vision Sci.* 2014, 91, 570; f) D. Dutta, N. Kumar, M. D. P. Willcox, *Biofouling* 2016, 32, 429; g) D. Dutta, B. Kamphuis, B. Ozcelik, H. Thissen, R. PinARBasi, N. Kumar, M. D. P. Willcox, *Optom. Vis. Sci.* 2018, 95, 937; h) D. Dutta, N. Cole, N. Kumar, M. D. P. Willcox, *Invest. Ophthalmol. Visual Sci.* 2013, 54, 175.