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The ocular surface epithelial barrier and other mechanisms of mucosal protection: from allergy to infectious diseases

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Abstract

Purpose of review—Studies completed in the last decade provide new insights into the role of the epithelial glycocalyx in maintaining ocular surface barrier function. This review summarizes these findings, their relevance to allergic and infectious disease, and highlights the potential benefits of exploiting the modulation of barrier integrity for therapeutic gain.

Recent findings—The molecular components sealing the space between adjacent ocular surface epithelial cells, such as tight junctions, have been extensively characterized, and their contribution to the paracellular barrier established. A second layer of protection—the transcellular barrier—is provided by transmembrane mucins and their O-glycans on the glycocalyx. Cell surface glycans bind carbohydrate-binding proteins to promote formation of complexes that are no longer thought to be a static structure, but, instead, a dynamic system that responds to extrinsic signals and modulates pathogenic responses. While functioning as a protective mechanism to maintain homeostasis, the glycocalyx also restricts drug targeting of epithelial cells.

Summary—The traditional model of intercellular junctions protecting the ocular surface epithelia has recently been expanded to include an additional glycan shield that lines apical membranes on the ocular surface. A better understanding of this apical barrier may lead to better management of ocular surface disease.

Keywords

Epithelial barrier; galectin-3; glycocalyx; ocular surface; transmembrane mucin

Introduction

Mucosal surfaces, which comprise more than 400 m² of the total surface area in humans, are by far the largest area of contact with the external environment [1] and represent the main gateway for both allergens and pathogens to access the body. Hence, great interest and research efforts have been made to understand the molecular components that constitute the first-line of mucosal defense—from mucus secretion to the junctions between epithelial cells—and their alteration in disease. The latest evidence indicates that the glycocalyx on apical membranes of epithelial cells, which result from a complex organization of large, membrane-tethered glycoconjugates, is more robust than previously realized [2**]. Unfortunately, the many components that prevent damage to the corneal and conjunctival epithelia can be disrupted under several pathological conditions. Here, we review recent

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advances in our understanding of the ocular surface epithelial barrier, focusing on the role of the glycocalyx in ocular surface allergy and infectious disease, and its relevance to ocular surface drug delivery.

Components of the ocular surface epithelial barrier

The ocular surface is directly exposed to the outside environment, where it is especially subject to desiccation and interaction with a myriad of pathogens and allergens. The tear film plays an important janitorial role by continuously washing noxious agents out of the ocular surface. Intercellular junctions of the corneal (and to a lesser extent conjunctival) epithelium also contribute to the first line of protection against the entrance of these agents. In cornea, four types of junctions have been identified and include tight junctions (zonula occludens), desmosomes (macula adherens), adherens junctions (zonula adherens), and gap junctions. These junctional complexes exist at different depths of the stratified epithelia (Figure 1) [3].

The foremost apical layer of the epithelium contains tight junctions that appear as a series of focal connections between adjacent cells, and serve as a barrier to the diffusion of molecules through the paracellular pathway by sealing the intercellular space. These junctions are assembled by a network of integral transmembrane proteins (claudins, occludin, junctional adhesion molecule-A) and are connected to actin filaments through zonula occludens adaptor proteins such as ZO-1 [4,5]. In corneal epithelium, the resistance barrier is primarily provided by the tight junctions [6,7]. Desmosomes, which are abundant in the wing cell layers of normal corneal epithelium, adherens junctions throughout the different layers, and hemidesmosomes in the basal cell layers, provide structural integrity and anchoring function by linking the cytoskeletons of adjoining cells and maintain adhesion to the underlying substrate [3]. Gap junctions in the basal cell layers mediate intercellular communication and play an important role in cell differentiation [8]. Development of the corneal epithelial barrier function is regulated at multiple levels, starting with gene expression of transcription factors, such as Klf4, a member of the conserved family of Krüppel-like factors [9*].

While the paracellular barrier function of the intercellular junctions has been clearly defined, research performed during the last decade has been redefining the contribution of apical cell membranes on superficial cells as an additional layer of protection for the ocular surface epithelia. The stratified epithelium of the cornea and conjunctiva produces the heavily O-glycosylated transmembrane mucins MUC1, MUC4 and MUC16 [10]. MUC16, which localizes on the tips of the surface microvilli, is the largest of all of them. Indeed, with 22,152 amino acids, MUC16 is the largest human gene product after titin, a key component in the assembly and functioning of striated muscles (<http://www.uniprot.org>). It is now known that suppression of MUC16 expression in corneal epithelial cells leads to loss of surface protection, as shown by dye penetrance and adherence of *Staphylococcus aureus* [11*]. A mechanism by which transmembrane mucins provide surface protection is through association with carbohydrate-binding proteins. Interaction of galectin-3, a β -galactoside-binding lectin, with carbohydrate residues on MUC1 and MUC16, contributes to the integrity of the epithelial barrier [12**]. A model has been proposed by which galectin-3 forms multivalent complexes on the apical glycocalyx of the stratified ocular surface epithelia (Figure 1), based on findings showing that galectin-3 can polymerize through its N-terminal domain in the presence of carbohydrate ligands [13]. These complexes help organize transmembrane mucins into a physical barrier that regulates the transcellular flux of extracellular components. Both mucins and galectin-3 are among the most highly expressed glycogenes at the ocular surface epithelia, making this apical barrier a major component of the ocular surface [14].

The glycocalyx barrier in ocular allergy

The prevalence of allergic disease has dramatically increased over the last few decades. Ocular allergy, which includes distinct clinical conditions such as seasonal or perennial allergic conjunctivitis (SAC and PAC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC), represents a common disorder encountered in clinical practice [15,16]. SAC is the most common form of ocular allergy and, together with PAC, represents a mild, self-limiting disease that spares the cornea and generally does not carry any risk of long-term effects on visual function. However, both VKC and AKC constitute more severe forms that are not limited to the conjunctiva, but also affect the cornea and may cause permanent opacities that impair vision [15].

The differences in severity between the different types of allergic responses have traditionally been ascribed to different pathogenic mechanisms and immune responses against allergens. More recently, attention has focused on the dysregulation of the epithelial barrier and its contribution to allergen uptake as a primary defect in the pathogenesis of allergic reactions [17,18]. At the ocular surface, breakdown of epithelium barrier function has been associated with severe corneal damage in severe allergic eye diseases [19]. Research in a mouse model of allergic conjunctivitis indicates that the transmembrane mucin Muc4 responds in a coordinate fashion to allergen challenge, although the clinical relevance of mouse models to severe allergic conjunctivitis is uncertain [20]. More recently, clinical data on the integrity of the epithelial glycocalyx on the most severe forms of ocular allergy have been reported. These findings indicate that MUC1, 4, and 16 mRNA expression is significantly upregulated in eyes with AKC [21*,22]. It has been proposed that increased expression of transmembrane mucin may represent a defense mechanism to compensate for the loss of the goblet cell mucin MUC5AC in these patients [21,23]. Alternatively, it is possible to speculate that severe allergic eye disease may alter the glycosylation of transmembrane mucins and impair their affinity towards galectin-3, thereby decreasing the barrier function of the glycocalyx. This hypothesis, however, remains to be tested in the different forms of ocular allergy.

To date, it remains unclear what role galectin-3 of epithelial origin would have on ocular allergic response. It has emerged that carbohydrate-binding proteins recognize glycan antigens on allergens and parasitic helminths, which may contribute to the orchestration of immune responses [24,25]. Epithelial galectin-3 might also contribute to the regulation of inflammatory activities in the allergic response by binding to IgE, as previously shown in monocytes [26].

The glycocalyx barrier in infectious keratitis

A number of defense mechanisms, from individual tear components to maintaining an intact epithelium, contribute to the prevention of the sight-threatening event of infectious keratitis. In tears, proteins such as lysozyme, immunoglobulins, and lactoferrin—and likely the normal bacterial flora itself—play a crucial role in limiting the growth of pathogenic species [27]. Moreover, the gel-forming mucins act as counter receptors for pathogens, preventing their binding to corneal epithelium and potentially promoting their clearance through the lacrimal drainage system [28].

In the epithelial glycocalyx, studies using mice lacking the transmembrane mucin Muc1 have yielded controversial results on the role of this mucin in infection. Kardon et al. showed a marked propensity for development of blepharitis and conjunctivitis in those knockout mice [29]. On the other hand, Ilene Gipson's laboratory showed no ocular surface phenotype in response to deletion of Muc1, attributing the differences to housing conditions, mouse strain variation, pathogen strain variation, and environmental or epigenetic

differences [30]. More definitive evidence on the role of transmembrane mucins and their O-glycans in maintaining glycocalyx barrier function comes from studies using multilayered human corneal epithelial cell cultures. Abrogation of MUC16 expression using siRNA, or interference with mucin O-glycosylation using the chemical primer benzyl-GalNAc, has been shown to increase adherence of *Staphylococcus aureus* to these 3D cell culture systems, indicating that transmembrane mucins and their O-glycans in the glycocalyx prevent adhesion of pathogens [11*,31]. More recently, it has been shown that epidemic disease-causing species of *Streptococcus pneumoniae* secrete a protease, ZmpC, that selectively induces ectodomain shedding of MUC16, suggesting a mechanism by which bacterial enzymes remove transmembrane mucins to compromise the glycocalyx barrier and gain access to epithelial cells to cause infection [32**].

As mentioned previously, a mechanism by which transmembrane mucins provide surface protection is through association with galectin-3 at the ocular surface. It is worth to note that galectins on cell surfaces can also be exploited by viruses to allow entry and replication [33]. Indeed, recent data indicate that herpes simplex virus type 1 (HSV-1) directly binds human galectin-3, and that targeted disruption of galectin-3 impairs HSV-1 infectivity in human corneal epithelial cells, suggesting that HSV-1 uses the lectin to increase binding avidity for host cells [34*]. In this scenario, however, transmembrane mucins decrease viral infectivity, most likely by masking galectin-3 on the epithelial glycocalyx [34]. It is possible to speculate that impairment of cell surface glycosylation and enhanced galectin-3 expression during repair, following epithelial abrasion [35–37], are important molecular events that lead to increased availability of attachment sites for HSV-1, potentially triggering successful infection of the cornea. Similarly to HSV-1, *Pseudomonas aeruginosa* lipopolysaccharide also binds galectin-3, suggesting a similar role for the lectin in modulating bacterium-host interactions at the ocular surface [38].

The glycocalyx barrier in other ocular surface disease states

Several pathologies of the ocular surface are characterized by disruption of the epithelial barrier. These include dry eye, a disease affecting more than 5 million people in the US [39]. Alterations in the ultrastructural morphology of the apical epithelium, as well as changes in the content and character of transmembrane mucins and their O-glycans in the glycocalyx, have been widely reported in patients with drying diseases [40–43*]. Dry eye disease is additionally associated with loss of the enzymes responsible for adding carbohydrates to mucin protein backbones [44], and with reduction of signaling proteins involved in mucin biosynthesis, such as the Notch signaling pathway [14,45]. Alterations in transmembrane mucin biosynthesis have also been reported in pseudophakic bullous keratopathy [46], complete androgen insensitivity syndrome [47], and pterygium [48].

Although galectin-3 has gained attention in recent years as a modulatory molecule in many epithelial disorders, data on its roles in ocular surface disease and epithelial barrier dysfunction are still scarce. Based on the regional specificity and reactivity profiles, galectins have been proposed as useful markers to detect ocular disease alterations [49]. This potential use is exemplified by a study with a very limited cohort of patients with ocular inflammation who showed increased levels of galectin-3 in the tear fluid [50].

The glycocalyx barrier in drug delivery

The resistance to apical internalization of the corneal epithelium, although important to reducing the risk of developing an allergic reaction or infection, also impairs the delivery of therapeutic components into the eye. The majority of pharmacologic management of ocular disease involves the topical application of solutions to the surface of the eye in the form of

drops. However, only 1–5% of a topically applied drug permeates the cornea and reaches intraocular tissues [51]; therefore, improving permeability has been a major goal of drug-delivery research. Key reasons for such low bioavailability arise from the rapid elimination of the ophthalmic solution from the precorneal area through the nasolacrimal duct, the systemic absorption by the highly vascularized conjunctival stroma, and the multiple permeability epithelial barriers, which include the apical glycocalyx [52,53].

Several strategies have been developed to increase the residence time of drugs in the precorneal area, including the use of mucoadhesive polymers [54]. Recent methodological advances using purified transmembrane ocular mucins and recombinant galectin-3 have allowed a better understanding of the interactions between these molecules and adhesive biopolymers that may contribute to enhanced retention on apical membranes [55,56]. An alternative approach to improving drug bioavailability entails a transient increase in transepithelial penetration using penetration enhancers or promoters [57]. Other than modifying the physico-chemical properties of drugs, this approach focuses on the reversible modification of the epithelial barrier structure [58]. Administration of amphiphilic substances or chelating agents can be used to transiently modify the integrity of the corneal epithelium [59]. In addition, preservatives and surfactants such as benzalkonium chloride have been added to eye drop formulations to improve drug penetration by targeting primarily the tight junctions [60]; however, there is still concern about their toxicity and the development of toxic corneal ulcers [61,62]. It is possible to envision that transient abrogation of the ocular surface glycocalyx barrier with competitive inhibitors of galectin-3 binding could also be used as a therapeutic target for drug delivery at the ocular surface.

Conclusions

Polarized cells on mucosal surfaces have developed distinct apical and basolateral surfaces that form selective permeability barriers between biological compartments [63]. In the stratified ocular surface epithelia, apical surfaces on the most apical cell layer are directly exposed to the outside environment, while their basolateral surfaces face both adjoining and underlying cells. These two plasma membrane domains have almost completely different compositions and organizations, and serve their own functions. The apical glycocalyx contains transmembrane mucins that provide transcellular barrier function through association with the carbohydrate-binding protein galectin-3. The basolateral surfaces contain junctional complexes that promote paracellular barrier function. Alteration of glycocalyx composition and structure are associated with a number of ocular surface epithelial diseases, and may contribute to an increased risk of developing an allergic reaction or infection. Recent advances in the molecular understanding and modulation of the epithelial glycocalyx is contributing to the development of new therapeutic strategies.

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References and recommended readings

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest

**of outstanding interest.

1. Childers NK, Bruce MG, McGhee JR. Molecular mechanisms of immunoglobulin A defense. *Annu Rev Microbiol.* 1989; 43:503–536. [PubMed: 2508540]
- 2*. Kesimer M, Ehre C, Burns KA, Davis CW, Sheehan JK, Pickles RJ. Molecular organization of the mucins and glycocalyx underlying mucus transport over mucosal surfaces of the airways. *Mucosal Immunol.* 2013; 6:379–392. The authors use electron and atomic force microscopy to reveal how transmembrane mucins are finely organized in the glycocalyx to function as a selective barrier in the lung. [PubMed: 22929560]
3. Suzuki K, Saito J, Yanai R, Yamada N, Chikama T, Seki K, Nishida T. Cell-matrix and cell-cell interactions during corneal epithelial wound healing. *Prog Retin Eye Res.* 2003; 22:113–133. [PubMed: 12604055]
4. Ban Y, Dota A, Cooper LJ, Fullwood NJ, Nakamura T, Tsuzuki M, Mochida C, Kinoshita S. Tight junction-related protein expression and distribution in human corneal epithelium. *Exp Eye Res.* 2003; 76:663–669. [PubMed: 12742348]
5. Denker BM, Nigam SK. Molecular structure and assembly of the tight junction. *Am J Physiol.* 1998; 274:F1–9. [PubMed: 9458817]
6. Sugrue SP, Zieske JD. ZO1 in corneal epithelium: association to the zonula occludens and adherens junctions. *Exp Eye Res.* 1997; 64:11–20. [PubMed: 9093016]
7. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol.* 2001; 2:285–293. [PubMed: 11283726]
8. Kumar NM, Gilula NB. The gap junction communication channel. *Cell.* 1996; 84:381–388. [PubMed: 8608591]
- 9*. Swamynathan S, Kenchegowda D, Piatigorsky J. Regulation of corneal epithelial barrier function by Kruppel-like transcription factor 4. *Invest Ophthalmol Vis Sci.* 2011; 52:1762–1769. The authors provide evidence that Kruppel-like transcription factor 4 contributes to corneal epithelial barrier function by inducing expression of functionally related subsets of cell junctional proteins. [PubMed: 21051695]
10. Mantelli F, Argueso P. Functions of ocular surface mucins in health and disease. *Curr Opin Allergy Clin Immunol.* 2008; 8:477–483. [PubMed: 18769205]
- 11*. Blalock TD, Spurr-Michaud SJ, Tisdale AS, Heimer SR, Gilmore MS, Ramesh V, Gipson IK. Functions of MUC16 in corneal epithelial cells. *Invest Ophthalmol Vis Sci.* 2007; 48:4509–4518. The authors demonstrate that rose bengal dye penetrance and *Staphylococcus aureus* binding increases after MUC16 suppression in human corneal epithelial cells, suggesting that MUC16 helps provide a disadhesive protective barrier in the ocular surface glycocalyx. [PubMed: 17898272]
- 12*. Argueso P, Guzman-Aranguiz A, Mantelli F, Cao Z, Ricciuto J, Panjwani N. Association of cell surface mucins with galectin-3 contributes to the ocular surface epithelial barrier. *J Biol Chem.* 2009; 284:23037–23045. This study indicates that galectin-3, a β -galactoside-binding lectin highly expressed in human ocular surface epithelia, plays a key role in maintaining mucosal barrier function through carbohydrate-dependent interactions with transmembrane mucins. [PubMed: 19556244]
13. Ahmad N, Gabius HJ, Andre S, Kaltner H, Sabesan S, Roy R, Liu B, Macaluso F, Brewer CF. Galectin-3 precipitates as a pentamer with synthetic multivalent carbohydrates and forms heterogeneous cross-linked complexes. *J Biol Chem.* 2004; 279:10841–10847. [PubMed: 14672941]
14. Mantelli F, Schaffer L, Dana R, Head SR, Argueso P. Glycogene expression in conjunctiva of patients with dry eye: downregulation of Notch signaling. *Invest Ophthalmol Vis Sci.* 2009; 50:2666–2672. [PubMed: 19011014]
15. Hodges MG, Keane-Myers AM. Classification of ocular allergy. *Curr Opin Allergy Clin Immunol.* 2007; 7:424–428. [PubMed: 17873583]
16. Mantelli F, Lambiase A, Bonini S. Clinical trials in allergic conjunctivitis: a systematic review. *Allergy.* 2011; 66:919–924. [PubMed: 21261658]
17. Mattila P, Joenvaara S, Renkonen J, Toppila-Salmi S, Renkonen R. Allergy as an epithelial barrier disease. *Clinical and translational allergy.* 2011; 1:5. [PubMed: 22410284]

18. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, Haitchi HM, Vernon-Wilson E, Sammut D, Bedke N, Cremin C, Sones J, Djukanovic R, Howarth PH, Collins JE, Holgate ST, Monk P, Davies DE. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol*. 2011; 128:549–556. e541–512. [PubMed: 21752437]
19. Yokoi K, Yokoi N, Kinoshita S. Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis. *Br J Ophthalmol*. 1998; 82:797–800. [PubMed: 9924375]
20. Kunert KS, Keane-Myers AM, Spurr-Michaud S, Tisdale AS, Gipson IK. Alteration in goblet cell numbers and mucin gene expression in a mouse model of allergic conjunctivitis. *Invest Ophthalmol Vis Sci*. 2001; 42:2483–2489. [PubMed: 11581187]
- 21*. Dogru M, Matsumoto Y, Okada N, Igarashi A, Fukagawa K, Shimazaki J, Tsubota K, Fujishima H. Alterations of the ocular surface epithelial MUC16 and goblet cell MUC5AC in patients with atopic keratoconjunctivitis. *Allergy*. 2008; 63:1324–1334. This clinical study suggests that changes in MUC16 mRNA expression may play a pivotal role in the pathogenesis of atopic ocular surface disease. [PubMed: 18782111]
22. Dogru M, Okada N, Asano-Kato N, Igarashi A, Fukagawa K, Shimazaki J, Tsubota K, Fujishima H. Alterations of the ocular surface epithelial mucins 1, 2, 4 and the tear functions in patients with atopic keratoconjunctivitis. *Clin Exp Allergy*. 2006; 36:1556–1565. [PubMed: 17177679]
23. Dogru M, Okada N, Asano-Kato N, Tanaka M, Igarashi A, Takano Y, Fukagawa K, Shimazaki J, Tsubota K, Fujishima H. Atopic ocular surface disease: implications on tear function and ocular surface mucins. *Cornea*. 2005; 24:S18–S23. [PubMed: 16227818]
24. van den Berg TK, Honing H, Franke N, van Remoortere A, Schiphorst WE, Liu FT, Deelder AM, Cummings RD, Hokke CH, van Die I. LacdiNAc-glycans constitute a parasite pattern for galectin-3-mediated immune recognition. *J Immunol*. 2004; 173:1902–1907. [PubMed: 15265923]
25. van Die I, Cummings RD. Glycans modulate immune responses in helminth infections and allergy. *Chem Immunol Allergy*. 2006; 90:91–112. [PubMed: 16210905]
26. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol*. 2008; 8:205–217. [PubMed: 18301424]
27. McClellan KA. Mucosal defense of the outer eye. *Surv Ophthalmol*. 1997; 42:233–246. [PubMed: 9406369]
28. Fleiszig SM, Zaidi TS, Ramphal R, Pier GB. Modulation of *Pseudomonas aeruginosa* adherence to the corneal surface by mucus. *Infect Immun*. 1994; 62:1799–1804. [PubMed: 8168942]
29. Kardon R, Price RE, Julian J, Lagow E, Tseng SC, Gendler SJ, Carson DD. Bacterial conjunctivitis in Muc1 null mice. *Invest Ophthalmol Vis Sci*. 1999; 40:1328–1335. [PubMed: 10359313]
30. Danjo Y, Hazlett LD, Gipson IK. C57BL/6 mice lacking Muc1 show no ocular surface phenotype. *Invest Ophthalmol Vis Sci*. 2000; 41:4080–4084. [PubMed: 11095599]
31. Ricciuto J, Heimer SR, Gilmore MS, Argueso P. Cell surface O-glycans limit *Staphylococcus aureus* adherence to corneal epithelial cells. *Infect Immun*. 2008; 76:5215–5220. [PubMed: 18794288]
- 32*. Govindarajan B, Menon BB, Spurr-Michaud S, Rastogi K, Gilmore MS, Argueso P, Gipson IK. A metalloproteinase secreted by *Streptococcus pneumoniae* removes membrane mucin MUC16 from the epithelial glycocalyx barrier. *PLoS One*. 2012; 7:e32418. This study indicates that removal of MUC16 by bacterial enzymes leads to loss of the glycocalyx barrier function and enhanced internalization of the bacterium, thereby constituting a mechanism by which disease-causing non-opportunistic pathogens gain access to epithelial cells to cause infection. [PubMed: 22412870]
33. Vasta GR. Roles of galectins in infection. *Nat Rev Microbiol*. 2009; 7:424–438. [PubMed: 19444247]
- 34*. Woodward AM, Mauris J, Argueso P. Binding of transmembrane mucins to galectin-3 limits herpesvirus 1 infection of human corneal keratinocytes. *J Virol*. 2013; 87:5841–5847. This study indicates that herpes simplex virus type 1 can exploit galectin-3 to enhance attachment to corneal epithelial cells, but supports a protective role for transmembrane mucins under physiological conditions by their masking of galectin-3 on the epithelial glycocalyx. [PubMed: 23487460]

35. Cao Z, Said N, Amin S, Wu HK, Bruce A, Garate M, Hsu DK, Kuwabara I, Liu FT, Panjwani N. Galectins-3 and -7, but not galectin-1, play a role in re-epithelialization of wounds. *J Biol Chem.* 2002; 277:42299–42305. [PubMed: 12194966]
36. Gipson IK, Riddle CV, Kiorpes TC, Spurr SJ. Lectin binding to cell surfaces: comparisons between normal and migrating corneal epithelium. *Dev Biol.* 1983; 96:337–345. [PubMed: 6403397]
37. McLaughlin BJ, Barlar EK, Donaldson DJ. Wheat germ agglutinin and concanavalin A binding during epithelial wound healing in the cornea. *Curr Eye Res.* 1986; 5:601–609. [PubMed: 3093151]
38. Gupta SK, Masinick S, Garrett M, Hazlett LD. *Pseudomonas aeruginosa* lipopolysaccharide binds galectin-3 and other human corneal epithelial proteins. *Infect Immun.* 1997; 65:2747–2753. [PubMed: 9199445]
39. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *American journal of ophthalmology.* 2003; 136:318–326. [PubMed: 12888056]
40. Danjo Y, Watanabe H, Tisdale AS, George M, Tsumura T, Abelson MB, Gipson IK. Alteration of mucin in human conjunctival epithelia in dry eye. *Invest Ophthalmol Vis Sci.* 1998; 39:2602–2609. [PubMed: 9856770]
41. Hayashi Y, Kao WW, Kohno N, Nishihara-Hayashi M, Shiraishi A, Uno T, Yamaguchi M, Okamoto S, Maeda M, Ikeda T, Hamada H, Kondo K, Ohashi Y. Expression patterns of sialylated epitope recognized by KL-6 monoclonal antibody in ocular surface epithelium of normals and dry eye patients. *Invest Ophthalmol Vis Sci.* 2004; 45:2212–2217. [PubMed: 15223797]
42. Koufakis DI, Karabatsas CH, Sakkas LI, Alvanou A, Manthos AK, Chatzoulis DZ. Conjunctival surface changes in patients with Sjogren's syndrome: a transmission electron microscopy study. *Invest Ophthalmol Vis Sci.* 2006; 47:541–544. [PubMed: 16431948]
- 43*. Pflugfelder SC, Tseng SC, Yoshino K, Monroy D, Felix C, Reis BL. Correlation of goblet cell density and mucosal epithelial membrane mucin expression with rose bengal staining in patients with ocular irritation. *Ophthalmology.* 1997; 104:223–235. This clinical study suggests that alteration in transmembrane mucin expression may explain increased rose bengal staining in patients with ocular irritation. [PubMed: 9052626]
44. Argueso P, Tisdale A, Mandel U, Letko E, Foster CS, Gipson IK. The cell-layer- and cell-type-specific distribution of GalNAc-transferases in the ocular surface epithelia is altered during keratinization. *Invest Ophthalmol Vis Sci.* 2003; 44:86–92. [PubMed: 12506059]
45. Xiong L, Woodward AM, Argueso P. Notch signaling modulates MUC16 biosynthesis in an in vitro model of human corneal and conjunctival epithelial cell differentiation. *Invest Ophthalmol Vis Sci.* 2011; 52:5641–5646. [PubMed: 21508102]
46. Glasgow BJ, Gasymov OK, Casey RC. Exfoliative epitheliopathy of bullous keratopathy with breaches in the MUC16 Glycocalyx. *Invest Ophthalmol Vis Sci.* 2009; 50:4060–4064. [PubMed: 19420336]
47. Mantelli F, Moretti C, Micera A, Bonini S. Conjunctival mucin deficiency in complete androgen insensitivity syndrome (CAIS). *Graefes Arch Clin Exp Ophthalmol.* 2007; 245:899–902. [PubMed: 17120009]
48. Kase S, Kitaichi N, Furudate N, Yoshida K, Ohno S. Increased expression of mucinous glycoprotein KL-6 in human pterygium. *Br J Ophthalmol.* 2006; 90:1208–1209. [PubMed: 16929070]
49. Schlotzer-Schrehardt U, Andre S, Janko C, Kaltner H, Kopitz J, Gabius HJ, Herrmann M. Adhesion/growth-regulatory galectins in the human eye: localization profiles and tissue reactivities as a standard to detect disease-associated alterations. *Graefes Arch Clin Exp Ophthalmol.* 2012; 250:1169–1180. [PubMed: 22527325]
50. Hrdlickova-Cela E, Plzak J, Smetana K Jr, Melkova Z, Kaltner H, Filipec M, Liu FT, Gabius HJ. Detection of galectin-3 in tear fluid at disease states and immunohistochemical and lectin histochemical analysis in human corneal and conjunctival epithelium. *Br J Ophthalmol.* 2001; 85:1336–1340. [PubMed: 11673302]
51. Ehrhardt, C.; Kim, K-j. Drug absorption studies: in situ, in vitro and in silico models. Springer; New York: 2008.

52. Geroski DH, Edelhauser HF. Drug delivery for posterior segment eye disease. *Invest Ophthalmol Vis Sci.* 2000; 41:961–964. [PubMed: 10752928]
53. Kompella UB, Kadam RS, Lee VH. Recent advances in ophthalmic drug delivery. *Ther Deliv.* 2010; 1:435–456. [PubMed: 21399724]
54. Khutoryanskiy VV. Advances in mucoadhesion and mucoadhesive polymers. *Macromol Biosci.* 2011; 11:748–764. [PubMed: 21188688]
55. Bravo-Osuna I, Noiray M, Briand E, Woodward AM, Argueso P, Molina Martinez IT, Herrero-Vanrell R, Ponchel G. Interfacial interaction between transmembrane ocular mucins and adhesive polymers and dendrimers analyzed by surface plasmon resonance. *Pharm Res.* 2012; 29:2329–2340. [PubMed: 22565639]
56. Woodward AM, Senchyna M, Williams R, Argueso P. Characterization of the interaction between hydroxypropyl guar galactomannan and galectin-3. *Biochem Biophys Res Commun.* 2012; 424:12–17. [PubMed: 22683626]
57. Kaur IP, Smitha R. Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. *Drug Dev Ind Pharm.* 2002; 28:353–369. [PubMed: 12056529]
58. Di Colo G, Zambito Y, Zaino C. Polymeric enhancers of mucosal epithelia permeability: synthesis, transepithelial penetration-enhancing properties, mechanism of action, safety issues. *J Pharm Sci.* 2008; 97:1652–1680. [PubMed: 17828745]
59. Sasaki H, Yamamura K, Mukai T, Nishida K, Nakamura J, Nakashima M, Ichikawa M. Enhancement of ocular drug penetration. *Crit Rev Ther Drug Carrier Syst.* 1999; 16:85–146. [PubMed: 10099899]
60. Okabe K, Kimura H, Okabe J, Kato A, Shimizu H, Ueda T, Shimada S, Ogura Y. Effect of benzalkonium chloride on transscleral drug delivery. *Invest Ophthalmol Vis Sci.* 2005; 46:703–708. [PubMed: 15671302]
61. Kaur IP, Lal S, Rana C, Kakkar S, Singh H. Ocular preservatives: associated risks and newer options. *Cutan Ocul Toxicol.* 2009; 28:93–103. [PubMed: 19505226]
62. Sacchetti M, Lambiase A, Coassin M, Bonini S. Toxic corneal ulcer: a frequent and sight-threatening disease. *Eur J Ophthalmol.* 2009; 19:916–922. [PubMed: 19882588]
63. Kazmierczak BI, Mostov K, Engel JN. Interaction of bacterial pathogens with polarized epithelium. *Annu Rev Microbiol.* 2001; 55:407–435. [PubMed: 11544362]

Key points

1. Junctional complexes exist at different depths of the stratified epithelia and include tight junctions, desmosomes, adherens junctions, and gap junctions. Their role is to provide paracellular barrier function and mediate intercellular communication at the ocular surface.
2. Transmembrane mucins and galectin-3, a β -galactoside-binding lectin, are among the most highly expressed glycoproteins at the ocular surface. Their association on the apical glycocalyx of the most apical cell layer contributes to the maintenance of epithelial integrity and the transcellular barrier.
3. Glycan-dependent interactions on the epithelial glycocalyx are dynamic events that contribute to the maintenance of homeostasis, but can also promote pathological responses when dysregulated.

Figure 1.
Components of the human ocular surface epithelial barrier.