Review Article

Invisible Shield: Review of the Corneal Epithelium as a Barrier to UV Radiation, Pathogens, and Other Environmental Stimuli

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Abstract

The ocular surface is comprised of the cornea and conjunctiva, which are structures that not only protect the eye but also enable vision. The corneal epithelium is the most superficial layer of the cornea, and therefore first line of defense against external assaults. Damage to this highly specialized structure could lead to vision loss, making it an important structure to investigate and understand. Here, we conducted a search of the current literature on the mechanisms the corneal epithelium has adapted against three frequent insults: UV-radiation, pathogens, and environmental assaults. This review systematically examines the corneal epithelium's response to each assault in order to maintain its role as an invisible shield. The goal of this review is to provide insight into some of the critical functions the corneal epithelium performs that may be valuable to current regenerative studies.

Keywords: Apoptosis; Barrier; Cornea; Epithelium; Inflammation; Neurosensory; Ocular Surface; Pathogen; Sensory; UV-radiation

INTRODUCTION

The cornea is a highly specialized tissue that, along with the conjunctiva, comprises the ocular surface (OS).[1] It is a transparent structure composed of five layers. The most superficial is the corneal epithelium, followed by Bowman layer, stroma, Descemet membrane, and endothelium. The transparency and curvature of the cornea permit and refract light onto the retina.[1] Like other exposed surfaces, the cornea also shields the vulnerable intraocular structures from external insults resulting in repetitive injuries.[2] Unlike other surfaces, however, when the cornea repairs itself, it needs to restore optical clarity in order to maintain vision. If an injury is significant and deep enough, corneal scarring ensues.[3] Currently, it has been estimated that vision loss from corneal scarring is second only to cataracts worldwide.[4] It is commonly accepted that early treatment of superficial epithelial wounds prevents the development of deeper, visually debilitating wounds.[5] Therefore, optimal corneal epithelial wound healing is essential to preserve vision. Wound healing involves the coordinated regulation of cellular proliferation, migration, and differentiation.[6] While the renewal and

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wound healing of the corneal epithelium have been studied,[7] there remains a need to precisely define the sequence of biological events required for restoring the corneal epithelium. This review covers the corneal epithelium's role as a protective barrier from external insults, specifically: (1) UV radiation, (2) pathogens, and (3) external stimuli. Collectively, these roles allow the cornea to not only maintain vision but also protect the intraocular structures. Recent relevant discoveries within each area will be covered with the goal of promoting further investigation.

ULTRAVIOLET RADIATION

The two surfaces that are exposed to chronic UV radiation are skin and OS.[8] Extensive research has been dedicated to the effects of UV radiation on cutaneous keratinocytes, but fewer studies have been performed using corneal epithelial cells.[9] Clinically, acute UV exposure can cause photokeratitis, which is analogous to a sunburn, producing haze, edema, and opacification from damage to the corneal epithelium, stroma, and endothelium.[10] Chronic UV exposure can lead to climatic droplet keratopathy, endothelial dysfunction, and tumors.[10-14] However, the risk of developing a malignancy of the OS from sun-exposure is extremely low, around 0.3 per million in the United States. Moreover, the majority of cases do not arise from or involve the corneal epithelium but develop in the conjunctiva.[11,15] Compared to cutaneous non-melanoma skin cancer, which is estimated to be over 14,000 per million in the United States, the corneal epithelium is significantly less susceptible to UV-induced cancer.[8,16] Although the corneal epithelium shares many similarities with skin, there is a clear difference in risk for UV-induced neoplasia between corneal epithelial cells and keratinocytes.[8] These observations suggest that corneal epithelium is resistant to UV-induced carcinogenesis. Identifying the mechanisms that make corneal epithelium resistant to UV-induced carcinogenesis could illuminate potential therapeutic approaches for cancers.[8]

A recent study by Mallet et al revealed that corneal epithelium is primed for efficient repair of UV induced DNA mutations.[8] UV light is partitioned into three categories: UVA (320-400 nm), UVB (280-320 nm), and UVC (100-280 nm).[17,18] Nearly all UVC and the lower range of UVB (280-290 nm) are blocked by the ozone layer and atmosphere, and are not as relevant as UVA and UVB radiation.[18] The role of UVB, in particular, has been well validated in sun-related cancers.[19,20] The OS receives around 59% to 77% of the UV light directed to the head.[21] The tear film absorbs wavelengths up to 300 nm,[22] while the cornea absorbs wavelengths under 290 nm and most wavelengths above 300 nm, covering much of the UVB spectrum.[18,23-26] The corneal stroma, on the other hand, is moderately permeable.[18] Thus, the corneal epithelium absorbs much of the UV radiation, protecting underlying structures from the harmful effects of UV rays.[8,18,23,25]

While UVA radiation causes DNA damage through oxidative stress, UVB radiation is directly absorbed by DNA, resulting in production of cyclobutane pyrimidine dimers (CPD) and pyrimidine (6–4) pyrimidone photoproducts (6-4PP).[27-30] CPDs are the most frequent and pro-mutagenic signature UVB-induced DNA product, causing C→T and CC→TT substitutions at dipyrimidine sites.[8,28-31] Mallet et al previously demonstrated the presence of CPDs in corneas at similar levels to skin.[8,18] In general, it is thought cells have three main mechanisms to mitigate damage caused by UV radiation: (1) resistance to UV-induced DNA damage, (2) repair of damage, and (3) apoptosis.[32,33] The corneal epithelium lacks features skin possesses to resist UV damage, such as a thicker epithelial layer, stratum corneum, and melanocytes, which all help to mitigate the effects of UV radiation.[34,35] Mallet et al show instead that corneal epithelium appears to repair signature UV-induced mutations more efficiently.[8] Using cultured human corneal epithelial cells, they found that CPD mutations decreased by half within the first 12 hours after UV exposure, which is 4× faster than epidermal keratinocytes.[8] Mallet et al deduced that the faster repair rate was from prolonged stabilization of proteins that recognize CPDs, specifically, DDB2, XPC, and p53, which led to higher intracellular levels of them.[8]

Intriguingly, in the same study, Mallet et al did not find increased levels of UV-induced apoptosis in corneal epithelial cells but the contrary.[8] Several previous groups have shown corneal epithelial cells exhibit UV-induced apoptosis.[8,36,37] Podskochy et al, for example, demonstrated that the lower range of UVB radiation causes apoptosis in corneal epithelial cells and superficial keratocytes of rabbits, while the higher range induced apoptosis in all corneal layers.[36] However, Mallet et al found lower levels of apoptosis in corneal epithelial cells compared to keratinocytes for the same amount of UV radiation.[8] They believe the lower UV-induced apoptosis sensitivity in corneal epithelial cells could be related to the more efficient repair of UV-induced DNA mutations. Additionally, other studies have shown that UV radiation induces the production of matrix metalloproteinases (MMP) by corneal cells.[38,39] This is the primary mechanism of sterile keratolysis, which may cooperate with UV induced apoptosis.[40] The need to eliminate highly damaged cells by apoptosis would therefore be reduced when coordinated with MMP secretion.[8]

PATHOGENS

Corneal inflammation from external pathogens, particularly infectious agents, is a major cause of

preventable blindness in the world.[5] In the United States, it incurs about $175 million dollars in direct health care costs.[41] It is considered a clinical emergency since it frequently leads to ulceration that requires immediate intervention to prevent irreversible scarring.[42] Accurate diagnosis of the pathogen, however, can be difficult; and often entails ancillary testing to identify the cause.[42] Given this potential threat, the cornea has adopted protective mechanisms that include physical, chemical, and signaling methods.

A major physical barrier of the corneal epithelium stems from the intercellular junctions between the cells.[3,43] This is corroborated by the clinical observation that microbial invasion often requires disruption of the epithelial barrier except for a few exceptions like contact lens related infections.[43] Four types of junctions have been identified in the corneal epithelium, which include tight junctions (zonula occludens), desmosomes (macula adherens), adherens junctions (zonula adherens), and gap junctions.[3] Many of the junctions contribute to the structure of the tissue and adherence to extracellular matrices, but the tight junctions in the apical epithelial layer create a resistance barrier to external pathogens.[3,44] This barrier is further enforced by the production of heavily O-glycosylated transmembrane mucins, MUC1, MUC4, and MUC16, by corneal epithelial cells.[45,46] It has been shown that the mucin layer prevents pathogens from binding to the corneal epithelium, and promotes clearance from the OS in the tear film.[43,47] Suppression of MUC16 expression, for example, leads to increased adherence of *Staphylococcus aureus*,[48,49] while epidemic-causing strain of *Streptococcus pneumonia* produces a zinc metalloprotinase that targets MUC16 in order to gain access to corneal epithelial cells.[50] Furthermore, MUC1 knockout mice have been found to be more susceptible to blepharitis and bacterial conjunctivitis.[51]

Transmembrane mucins have also been shown to decrease viral infectivity, most likely by masking galectin-3 on the epithelial glycocalyx.[52] Galectins are utilized by viruses to invade host cells and replicate.[53] Recent data have demonstrated that herpes simplex virus type 1 (HSV-1), one of the most common viral pathogens of the cornea, directly binds to human galectin-3. Disruption of galectin-3 impairs HSV-1 infectivity in human corneal epithelial cells, suggesting viruses use galectin-3 to invade the cells.[52] *Pseudomonas aeruginosa*, a common contact lens related pathogen, also binds galectin-3, indicating a similar role for galectin in bacterial-host interactions at the OS.[54]

If the corneal epithelium is breached, cell surface glycosylation becomes impaired and galectin-3 expression increases. These are two of many molecular events that render the cornea vulnerable to infections.[55-57] Corneal epithelial cells respond by producing antimicrobial peptides, such as β-defensins.[58] *In-vivo* studies showed that β-defensins 2 and 3 have an important role in protection against *Pseudomonas aeruginosa*.[59,60] A recent study by Tam and Fleiszig also identified a novel class of antimicrobial peptides constitutively produced by corneal epithelial cells.[61] These keratin-derived antimicrobial peptides, or KDAMPs, appear to be distinct from β-defensins and have broad anti-microbial activity.[62] Additionally, corneal epithelial cells possess phagocytic properties, and have been shown to phagocytize foreign particulates, including live and dead bacteria.[63–66]

The corneal epithelium also assists in modulating the immune response. Normally, the corneal epithelium and stroma contain heterogeneous populations of macrophages and dendritic cells, such as Langerhans cells.[67–71] Epithelial exposure to live or killed bacteria have been shown to activate signaling pathways in resident myeloid and corneal epithelial cells, leading to secretion of pro-inflammatory and chemotactic cytokines in the cornea. The Toll-Like Receptor (TLR) family is a class of single membrane-spanning receptors that recognize conserved motifs derived from microbes.[62] When the corneal epithelium is violated, resident immune cells in the cornea respond to TLR2, TLR3, TLR5, and TLR9 ligands and secrete chemokines that help recruit bone marrow derived cells.[72–76] Human corneal epithelial cells likely contribute to this response through TLR receptors as well.[77–79] In response to TLR2, TLR3, and TLR5 ligands, for example, corneal epithelial cells release β-defensins and cathelicidin.[73,80,81] Membrane nanotubes are thought to facilitate cell-cell communication in the cornea and modulate these responses.[68]

SENSORY

Similar to other epithelial surfaces, the cornea serves as a sensory device, which, in addition to many functions, helps protect the OS and intraocular structures from potential damage.[82,83] Physical stimuli, for example, generates the blink reflex. However, it is diminished or abolished in certain pathologies such as herpetic keratitis.[84,85] As part of the peripheral nervous system (PNS), the corneal nerves originate from the ophthalmic branch of the trigeminal ganglion; travel in the suprachoroidal space; then enter the corneal stroma near the limbus.[85] Because of their clinical relevance and expanding appreciation of their involvement in wound healing, functions of these nerves are a growing interest.[86–89]

Sixty to eighty myelinated trunks enter the corneal stroma, where they eventually lose their myelin sheathes in order to preserve optical clarity. Anteriorly, the nerves form the subepithelial plexus, which is a dense network of nerves located between Bowman layer and anterior stroma. The nerves then pierce through the corneal epithelial basement membrane, forming the subbasal

plexus. Subbasal corneal nerves remain within the corneal epithelial basal cells where β4 integrin is expressed.[90] The nerves continue as free naked nerve endings towards the superficial epithelial layers, where they are referred to as intraepithelial corneal nerves (ICNs).[85,91–94] ICNs terminate at the suprabasal and wing cell layers, where β4 integrin is no longer expressed.[86]

In general, PNS nerves are accompanied by neural crest-derived Schwann cells (SCs).[95] SCs are the primary glial cells of the PNS. Depending on their subtype, their functions can include myelin production, phagocytosing axonal debris during development and wound healing, and coordinating cytokine signaling and inflammatory responses.[86] When the corneal nerves leave the stroma, however, SCs are no longer with them in the epithelium.[87] In fact, ICNs travel for several millimeters within the epithelium without SC support. The only PNS nerves that are not associated with SCs are sensory free nerve endings, such as in skin and hair follicles. Nevertheless, in skin and hair follicles, free nerve endings are typically shorter than 100 μm long. In the cornea, they extend for millimeters.[92,93] Imaging studies have shown that the corneal nerves, instead, become surrounded by the plasma membranes of epithelial basal cells.[85,93]

Because of their close anatomical relationship, it has been proposed that corneal epithelial cells function as glial cells.[86] The corneal epithelium may have evolved to take over these functions in order to maintain transparency.[86] One supporting arm of this theory is that corneal epithelial cells express SC specific proteins, such as GAP-43 and p75NTR.[86,96,97] Through a combination of different animal models and *in-vitro* studies, many SC proteins have been uncovered in corneal epithelial cells.[86,98–100] Han et al also show precedence for this theory by demonstrating that epidermal cells are the primary phagocytes for clearing degenerating dendrites during dendrite pruning and injury of sensory neurons in *Drosophila*.[101] Given their capability for phagocytizing pathogens and other debris, it plausible that corneal epithelial cells could assist in ICN phagocytosis.[86] Stepp et al show that corneal epithelial cells phagocytize distal axon fragments within hours of ICN crush wounds *in vitro*.[7] In addition, six hours after crush wounds, degenerating ICN fragments are found to co-localize with LAMP1, a lysosomal protein, in corneal epithelial cells.[7] The molecular and cellular responses of corneal epithelial cells to axonal injury appear to be similar to those of SCs during Wallerian Degeneration, which is the major nerve injury response that involve SCs.[7,97,102]

In return, ICNs may provide corneal epithelium with nutrients and raw materials that it can utilize rapidly.[86] Since the cornea is an avascular tissue, corneal epithelial cells contain large stores of glycogen as their primary energy source.[103] Phagocytosis of axonal debris would eliminate damaged lipids and proteins that corneal epithelial cells could amass and use.[86] In addition, SCs also store glycogen, which they release to support axonal function.[104,105] When corneal epithelium is wounded, SCs that persist in the subepithelial plexus could provide the corneal epithelial cells with a source of energy during wound repair. In support of this, studies have shown that glycogen could be visualized in the corneal subbasal nerves.[94,105]

SUMMARY

Diseases of the OS are one of the leading causes of blindness worldwide, second only to cataracts.[4] According to the World Health Organization estimates, 4.9 million people suffer from bilateral blindness, while 23 million people suffer from unilateral vision loss due to OS disease.[106] Unilateral and bilateral vision loss have been both associated with decreased quality of life and activities of daily living; high disability adjusted life years; and depression.[106-109]

Vision requires an intact, functional OS. To refract light, the cornea must maintain its transparency and curvature. As an exposed surface, however, the cornea also serves as first line defense against external insults.[4,106] The corneal epithelium endures steady damages, and therefore must activate wound healing pathways to repair itself. These pathways are then deactivated once the epithelium has been reconstituted. When wounds penetrate deeper, violating the epithelial basement membrane and Bowman layer, scarring occurs.[3] Currently, surgical interventions are the only treatment options available for visually debilitating corneal opacification. Such treatments, in of themselves, can be associated with poor outcomes and complications, as well as being inaccessible universally.[5] It is generally acknowledged that small, corneal epithelial abrasions or non-healing corneal epithelial defects often precede the development of visually debilitating corneal ulcers.[4,5] If a therapeutic intervention could be developed to repair these initial defects, this strategy would provide the most benefit to patients in cases of preventable corneal scarring.[4,5] Therefore, much focus has been on regenerating the corneal epithelium. However, re-establishing the corneal epithelium is beyond just replacing the morphology of the epithelial surface. Hopefully, this review provided insight into some of the other critical functions the corneal epithelium serves that also need to be addressed in regenerative studies.

Conflicts of Interest
There are no conflicts of interest

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