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Perspective

The Need for Improved Therapeutic Approaches to Protect the Cornea Against Chemotoxic Injuries

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Cornea, a highly specialized transparent tissue, is the major refractive element of the eye. The cornea is highly susceptible to chemotoxic injury through topical exposure to vapors, microparticles, and aqueous drops, as well as through systemically absorbed chemicals that access the cornea via tear film, aqueous humor, and limbal vasculature. Corneal injury activates a carefully orchestrated series of repair processes capable of resolving minor lesions over time, but it often fails to overcome the menace of moderate, severe, and chronic injuries and secondary pathophysiologies that permanently impair vision. The most serious complications of chemical injuries—persistent corneal edema, neovascularization, scarring/haze, limbal stem cell deficiency, and corneal melting—often manifest over months to years, suggesting that a better understanding of endogenous regenerative mechanisms of corneal repair can lead to the development of improved treatments that may attenuate or prevent corneal defects and protect vision.

Overview

Corneal exposure to toxic chemicals can occur through accidental exposure to industrial and household chemicals,[1,2] as well as by deliberate misuse of highly toxic chemicals.[3,4] Although minor chemical trauma to corneal tissues can readily heal, more severe injuries may result in chronic pathophysiologies to the cornea that can permanently impair vision. In recognition of a shared requirement for improved treatments of corneal chemical trauma, the National Institutes of Health (NIH) and the U.S. Army Medical Research Institute of Chemical Defense jointly convened a trans-agency scientific meeting in February 2020 under the auspices the ocular Countermeasures Against Chemical Threats (CounterACT) research program. This meeting assembled subject matter experts from civilian, commercial, and military research communities; regulatory experts; and program managers from various federal funding agencies to discuss the state-of-the-art research aimed at understanding and treating corneal chemical trauma. Here, we provide additional context to this meeting by (1) describing aspects of corneal anatomy and function that render the cornea highly susceptible to chemical injury, and (2) identifying key research gaps that should be prioritized in the near future. Our collective goal is to synergize new research collaborations focused on developing treatments that reduce the severity of chemical lesions and protect vision.

Unique Aspects of Corneal Structure and Function Increase Corneal Susceptibility to Chemical Toxicity

The refractive power and transparency of the cornea depend on its precise curvature and highly organized microanatomy. Optical transparency emerges from a trilaminar structure comprised of a stratified corneal epithelium, collagen-rich stroma, and

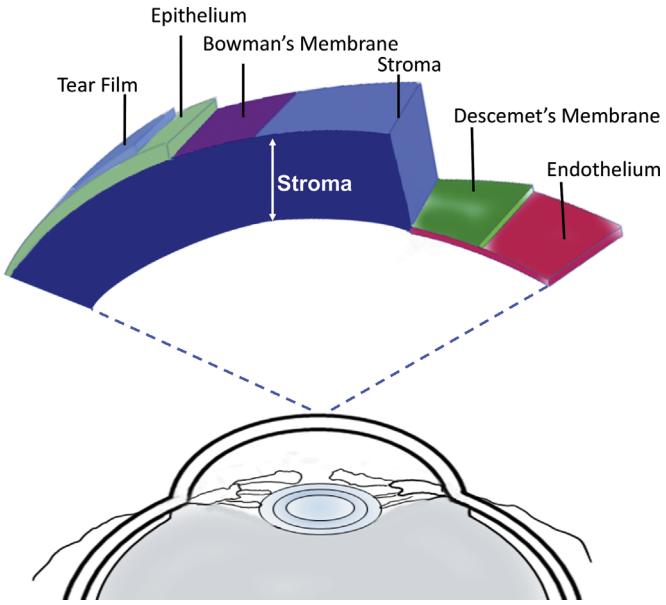

Figure. Schematic illustrating layers of the human cornea.

metabolically active but mitotically inactive endothelial cell monolayer (Fig.). The epithelium is bathed by a complex, multilayered tear film that ensures a smooth corneal surface, and the endothelium is bathed by nutrient-rich aqueous humor. The corneal epithelium and endothelium maintain stromal hydration in a narrow range required for proper refraction,[5,6] and the stroma employs a combination of microanatomical regularity, avascularity, and sparse cellular content to ensure transparency. Corneal structure is critically dependent on the dynamic maintenance of corneal hydrostasis to ensure proper hydration. Given the critical functions of the corneal epithelium and endothelium in maintaining the stroma in a deturgescent state, chemotoxic injuries to these tissues that exceed regenerative capacities can have severe acute and long-term effects on visual acuity.

The Corneal Epithelium

The corneal epithelium is a squamous, stratified epithelium that maintains homeostasis through a constant process of cell replacement.[7] Structurally, the corneal epithelium is comprised of three to four outer cell layers of flattened squamous cells, one to three layers of mid-epithelial cells, and a single layer of columnar basal cells, which are anchored to the anterior stroma through matrix adhesion molecules and hemidesmosome attachments.[8] Tight junctions between the outermost epithelial cells form a passive barrier to tear film.[9] Chemotoxic disruption of the epithelial barrier has two major effects: (1) tear film enters the stroma, causing corneal edema; and (2) matrix-active enzymes and signaling molecules from tear film and damaged epithelial cells activate diverse wound repair pathways.[10,11] The corneal epithelium is highly regenerative and, following even a large scarification, can re-establish an impermeable epithelial barrier within 7 days and a fully stratified epithelium with anchoring corneal adhesions within 1 to 2 months.[12,13] The high degree of regenerative capacity of the epithelium is mediated by on-demand stem cells located in the corneal limbus, which produce transient amplifying cells that undergo centripetal and superficial migrations to maintain epithelial homeostasis and restore damaged epithelia.[7] Similar to other stem cell niches, the limbus is highly vascularized and exceptionally sensitive to inflammatory stress.[14] Limbotoxic stress can deplete limbal stem cell reserves, predisposing the cornea to the delayed manifestation of limbal stem cell deficiency. Although stem-cell-based treatment strategies can be effective in repairing limbal stem cell loss,[15] treatment of limbal stem cell deficiency in the context of chronic corneal edema can be clinically challenging.[16,17]

The Corneal Stroma

The stroma constitutes 90% of the cornea and accounts for the transparent nature and refractive power of the cornea. The stroma contains 200 to 500 lamellae, which are organized by the glycosaminoglycan chains of proteoglycans into tightly packed, regularly spaced collagen fibrils.[18] This quasi-uniform organization of lamellae is optimized to balance transparency and light transmission with mechanical toughness. Lamellae in the anterior third of the stroma are extensively interwoven, thus increasing their resistance to deformation and swelling; however, the posterior stroma contains planar arrays of lamellae, which are more susceptible to swelling but concomitantly have decreased light-scattering properties.[19,20] The proper organization of lamellae, and therefore visual acuity, requires the stroma to be maintained in a dehydrated state.[21,22] The stroma is composed of proteoglycan molecules that produce a strong stromal swelling pressure, and, if unconstrained, fluid uptake causes the stroma to swell to several times its normal thickness.[19,23] The cornea uses both passive (epithelium) and active (endothelium) processes to counteract this stromal swelling pressure.

Under normal conditions, human stroma is sparsely populated with quiescent keratocytes at 2.1×10^4 cells/mm.[24] Keratocytes normally reside between lamellae, where they maintain the slow turnover of

stromal collagen and proteoglycans.[25] Following a chemical injury, keratocytes positioned beneath the lesion can rapidly undergo necrosis in response to acute chemical toxicity or, alternatively apoptosis in response to interleukin-1 (IL-1) signaling from tear fluid or damaged corneal epithelium.[26] Within 24 hours, keratocytes at the lesion periphery are activated by tumor necrosis factor-α to proliferate and migrate into the damaged region, where they undertake remodeling of minor lesions. It has also been proposed that multiple myofibroblast populations accumulate in the damaged cornea, originating from different locations and having distinct functions.[27] Upon completion of wound healing, upregulation of IL-1 by epithelial and stromal cells triggers apoptosis of activated keratocytes.[28] Naïve keratocytes then re-infiltrate the repaired lesion and gradually repair the stromal matrix.[29]

Following a more severe injury, activated keratocytes transition to myofibroblasts in response to diverse pro-inflammatory mediators, including transforming growth factor-β (TGF-β).[30] Myofibroblasts proliferate within the lesion (or edematous region), depositing aberrant matrix protein without the organizing proteoglycans and disrupting the stromal microarchitecture.[31] If TGF-β continues to be expressed, such as in response to chronic edema or inflammation, then a pathological fibrosis develops in which myofibroblasts hyperproliferate and secrete excessive amounts of aberrant matrix, causing hypertrophic scarring and persistent corneal opacity.[31–33] The precise conditions that determine whether corneal lesions undergo wound healing or fibrosis remain unclear but are likely to result from incomplete resolution of corneal edema in combination with sustained pro-inflammatory conditions.

The role of corneal nerves in wound healing remains largely obscure.[34] The cornea is the most densely innervated tissue in the body, consisting of 50 to 450 sensory neurons that originate from the ophthalmic division of the trigeminal nerve (cranial nerve V), enter the cornea from the limbus, and form a rich plexus subjacent to Bowman's layer. Nerve fibers then penetrate the Bowman's layer into the epithelium and extensively ramify, producing long bundles of nerve processes that extend into the central corneal epithelium.[35] In addition to controlling the blink reflex and modulating tear film production, corneal nerves release numerous trophic substances that reciprocally influence corneal epithelial homeostasis and keratocyte properties, including substance P, nerve growth factor, brain-derived neurotrophic factor, and neurotrophin.[36] Nerve fibers are highly sensitive to chemical injury, and failure of nerve fibers to regenerate from a chemotoxic lesion can result in neurotrophic keratopathy, which can cause recurrent epithelial defects that eventually progress to corneal ulceration in addition to destructively interfering with keratocyte wound healing responses.[37]

The Corneal Endothelium

The corneal endothelium is a thin monolayer of hexagonal, epithelial-like cells lining the posterior cornea. Corneal endothelial cells (CECs) dynamically regulate corneal hydration through a pump–leak function, in which nutrient-rich aqueous humor leaks through the semipermeable endothelial monolayer into the stroma, and excess fluid is actively transported back out of the stroma via Na^+/K^+-ATPase osmotic pumps.[38,39] CECs require high levels of metabolic activity to sustain osmotic pump activity,[40] rendering them particularly susceptible to chemicals that interfere with mitochondrial respiration or glycolytic metabolic pathways.[41] In contrast to the highly regenerative epithelium, human CECs have limited proliferative capacity in vivo.[42,43] Instead, CECs respond to endothelial lesions by migrating, thinning, and spreading to re-establish adhesions with proximal cells.[44] Despite a steady age-related loss of endothelial cells ($\sim$0.6% per year), corneal transparency is maintained as long as the cell density exceeds 1000 cells/mm^2.[45] Adult human corneas have an average CEC density of $\sim$2500 cells/mm^2, suggesting that the endothelium has a modest capacity to repair lesions that is diminished over time.[46] Chemical exposures that cause a significant degree of CEC toxicity result in acute edema. If the corneal endothelium heals, then edema will resolve; however, the cornea will be predisposed to the future emergence of endotheliitis when the cumulative effects of acute CEC loss and age-related loss exceed the threshold for decompensation. Clinical endotheliitis emerges when the endothelium can no longer compensate for the stromal swelling pressure. The resulting stromal edema disrupts clarity and visual acuity and elicits secondary keratopathies such as neovascularization, bullous keratopathy, and limbal toxicity, which further compromise corneal function.[47] Currently, endotheliitis can only be treated by corneal transplant surgeries.[48,49] Although there is preliminary evidence that rho-kinase (ROCK) inhibitors may potentiate corneal endothelial wound healing,[50] the precise therapeutic mechanisms and clinical efficacy remain to be convincingly demonstrated. Notably, ROCK inhibitors have been reported to facilitate cell-based regenerative strategies for corneal endothelium, in which CECs are expanded in vitro, without losing their endothelial phenotype, and then injected into the

anterior chamber as a cell suspension or implanted as a sheet onto the Desçemet's membrane.[51]

In addition to contributing to corneal edema, severe endothelial lesions can cause an endothelial-to-mesenchymal transition, involving a change in morphology to a spindle shape, expression of smooth muscle actin and vimentin, loss of zonula occludens-1, increased migratory and proliferative capacity, and secretion of fibrotic matrix leading to formation of a retrocorneal fibrous membrane (RCFM).[21,52–54] RCFM formation is observed in clinical conditions associated with profound damage to the corneal endothelium and is thought to represent an end-stage disease process that is only treatable by corneal transplant.[55–57]

The Effects of Chemical Toxicants on Corneal Function

Corneal chemical toxicants can be roughly divided into two general classes: chemicals that cause toxicity by denaturation and/or chemical hydrolysis, such as acids and bases; and chemicals that cause toxicity by formation of covalent adducts on biological macromolecules. The effects of hydrolytic chemical toxicants on the cornea are generally well studied and include exposure to strong alkali agents (e.g., NH_3, NaOH), which saponify cell membranes and hydrolyze stromal matrix, and strong acids (e.g., HCl, H_2SO_4), which cause coagulation of cellular and matrix protein that, paradoxically, limits corneal penetration (with the exception of the efficient penetrant HF). Toxic signs of injury usually rapidly emerge following corneal exposure to these highly reactive chemicals. In contrast, covalent adducts can result in cellular toxicity via damage to metabolic pathways or cytological processes. These molecular toxicities may cause cell death within minutes or over days. This variability in toxic manifestation is due to the different modes of toxic mechanisms, including (but not limited to) genotoxicity in response to DNA alkylation (e.g., mitomycin C, sulfur mustard); disruption of energy metabolism due to adduct formation on thiol groups (e.g., lewisite and other arsenic-containing compounds); and disruption of cellular metabolism through oxidative stress (e.g., adduct formation by hydrazine and methyl ethyl ketone peroxide-derived free radicals).[58]

Corneal recovery from a toxic chemical injury requires the proper orchestration of a complex sequence of events in various corneal subcompartments, including rapid regeneration of epithelium, resolution of stromal edema, remodeling of lamellae, prevention of neovascularization, restoration of keratocyte function, and maintenance of healthy endothelium. In many cases, the toxic injury occurs rapidly; thus, treatment strategies are focused on reducing secondary injuries and promoting regeneration. Metabolic toxins that cause cytotoxicity without directly affecting corneal matrix may confound wound healing processes, leading to inefficient or aberrant injury resolution; for example, cornea injuries may appear to heal but subsequently transition to a chronic keratopathy after an asymptomatic period. In other cases, wound healing may transition to hyper cellularization and fibrosis, resulting in the progressive disruption of corneal structure. Secondary inflammatory responses can compound the acute toxicity by damaging corneal tissues unaffected during the original chemotoxic exposure. Chronic stromal edema can evoke irreversible pathologies that decrement visual acuity, such as neovascularization. The sheet-like migration of basal epithelial cells onto denuded basement membrane requires the deliberate degradation of existing basement membrane molecules and deposition of provisional matrix.[59] Thus, chemotoxic damage to the basement membrane may delay or impair re-epithelialization. Finally, injuries that stress the regenerative capacity of corneal cell populations, such as by creating limbotoxic conditions, causing excess CEC loss, or preventing keratocyte repopulation, may effectively "age" the cornea, predisposing it to degeneration and failure.

Future Directions for Treatment of Corneal Chemotoxicity

Currently, numerous limitations impede our ability to treat the broad spectrum of potential corneal chemical exposures. There is a general lack of understanding of specific mechanisms of the action of many corneal toxicants and how those toxic mechanisms influence both acute injury and recovery (or lack thereof). Signaling processes that promote pathological healing responses, such as neovascularization and scar formation, are largely uncharacterized and thus represent a fertile target for topical pharmacotherapies to mitigate chronic sequelae. Although preliminary evidence indicates that sensory neurons and limbal niche cells affect the corneal wound healing response, the specific contributions of these and other less-understood cell populations to corneal repair remain unclear. Discovering mechanisms to direct TGF-β activity from profibrotic to antifibrotic would have enormous value in influencing the wound healing

process. Finally, improved treatment strategies that compensate for limited regenerative capacity are critically important for reducing the need for corneal transplant surgeries, including approaches based on stem cell replacement, cell transplantation, bioengineered tissue replacements, supplementation with extrinsic growth factors, and enhanced wound repair environments.

In this special edition, we highlight several ideas emerging from the 2020 trans-agency meeting. These papers represent funding opportunities in ocular chemotoxicity research, novel approaches to understanding mechanisms of corneal toxicity, wound healing and tissue repair, and promising therapeutic approaches for developing medical countermeasures. The article from Araj et al.[60] describes objectives of the NIH ocular CounterACT research program and highlights the need for and importance of attracting established and young eye researchers, postdoctoral students, and graduate students to the field. Deng et al.[61] discuss an emerging therapeutic approach involving extracellular vesicles derived from mesenchymal stem cells, in particular, stromal stem cells, for corneal function and vision restoration. Gouveia and Connon[62] present an approach to biomechanical modulation therapy that promotes the regeneration of corneal/ocular tissues via restoration of the limbal stem cell niche. McDaniel et al.[63] illustrate the potential of an ocular wound chamber for treating corneal surface injuries and infections, and Tripathi et al.[64] highlight the importance of developing novel, multimodal, non-steroidal topical eye drops capable of utilizing concomitant mechanisms of action in preventing and treating corneal damages caused by toxic chemicals such as mustard gas in vivo. Although these papers represent an exciting cross-section of the corneal chemotoxicity wound healing community, a vast amount of information about corneal physiology and regeneration remains elusive, offering both established and early career scientists the rare opportunity to study this remarkable tissue.

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