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Coordinated modulation of corneal scarring by the epithelial basement membrane and Descemet's basement membrane

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Abstract

Purpose: To provide an overview of the importance of the coordinated role of the epithelial basement membrane (EBM) and Descemet's basement membrane (DBM) in modulating scarring (fibrosis) in the cornea after injuries, infections, surgeries and diseases of the cornea.

Methods: Literature review.

Results: Despite their molecular and ultrastructural differences, the EBM and DBM act in coordinated fashion to modulate the entry of transforming growth factor beta (TGF β), and other growth factors, from the epithelium/tear film and aqueous humor, respectively, into the corneal stroma where persistent levels of these modulators trigger the development and persistence of myofibroblasts that produced disordered, opaque extracellular matrix not usually present in the corneal stroma. The development of these myofibroblasts and the extracellular matrix they produce is often detrimental to visual function of the cornea after PRK, LASIK buttonhole flaps, persistent epithelial defects, microbial keratitis, DSEAK, DMEK, while being beneficial in other situations such as the scarred edge of LASIK flaps and donor-recipient interface in penetrating keratoplasty (PKP). Efforts to modulate the repair or replacement of the EBM and DBM, and thereby the development or disappearance of myofibroblasts should be a major emphasis of treatments provided by refractive and corneal surgeries, infections trauma or diseases of the cornea.

Conclusions: The EBM and DBM are critical modulators of the localization of pro-fibrotic growth factors, such as TGF β , that modulate the development and persistence of myofibroblasts that produce corneal scars (stromal fibrosis). Therapeutic efforts to regenerate or repair EBM and/or DBM, and interfere with the development of myofibroblasts or facilitate their disappearance, are often the key to clinical outcomes.

The critical role of the EBM and DBM in modulating corneal myofibroblast development and scarring

The central role of the epithelial basement membrane (EBM)¹⁻⁴ and Descemet's basement membrane (DBM)^{4,5} in modulating scarring (stromal fibrosis) that may develop after surgeries, injuries, infections and diseases of the cornea has been illustrated by numerous

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studies published over the past decade. Although the ultrastructure of EBM and DBM with the transmission electron microscope is very different (Fig. 1),⁶ and many of their functions are distinctive, they share and coordinate in the critical function of limiting the entry of profibrotic growth factors, such as TGF β , from the epithelium, tear film and aqueous humor into the corneal stroma.^{7,8,1,4,5}

In normal corneal *stroma*, the level of TGF β , and other pro-fibrotic growth factors, are necessarily low—otherwise normally quiescent keratocytes would transition into corneal fibroblasts and begin to develop into scar-producing alpha-smooth muscle actin (SMA)-positive myofibroblasts.^{7,8,9,10} TGF β is normally produced by corneal epithelial and endothelial cells,^{11,12} although the amounts of TGF β produced by these cells likely increases after injury. TGF β is also normally present in the tears^{13,14} and aqueous humor.^{15,16} What keeps this TGF β from entering the corneal stroma from the epithelium and tears or endothelium and aqueous humor in normal uninjured corneas? It has become clear the *normal intact* EBM and DBM are the critical structures that limit TGF β entry into the stroma.¹⁻⁵ Conversely, any type of injury to the EBM and/or DBM—surgical, infectious, traumatic or via disease—allows TGF β to pass into the stroma and bind to receptors on keratocytes—initiating their development into corneal fibroblasts, and eventually—if TGF β levels do not decrease in a timely manner—into myofibroblasts. Also, injury to either the epithelium or endothelium triggers the release of interleukin-1 alpha from these cells that binds to receptors on keratocytes and stimulates these cells to produce chemokines that attract bone marrow-derived cells into the stroma from the limbal blood vessels.¹⁷ Among these bone marrow-derived cells that enter the cornea after injury are *fibrocytes*—that also develop into myofibroblasts when exposed to sufficient levels of TGF β .^{18,19} Thus, myofibroblasts develop in the cornea from at least two completely different cell types, keratocytes and fibrocytes. These different myofibroblasts have many similar functions but may make unique poorly characterized contributions to fibrosis in the cornea based on proteomic studies currently in progress (P. Saikai, S. E. Wilson and J. Crabb, unpublished data, 2019). Some studies also suggest that under certain circumstances corneal myofibroblasts can also develop from Schwann cells.²⁰

How do EBM and DBM bind TGF β and other growth factors that promote the development of myofibroblasts? Both corneal basement membranes are composed of multiple components including laminins, perlecan, nidogens, and collagens, including collagen type IV.¹²⁻²³ Among these EBM and DBM components, several bind specifically to TGF β and other profibrotic growth factors, include perlecan (binds TGF β 1, TGF β 2, PDGF AA, and PDGF BB), collagen IV (binds TGF β 1 and TGF β 2), and nidogens (bind PDGF AA and PDGF BB).²⁴⁻³⁰ Perlecan also creates a high negative charge in both EBM and DBM because it contains three heparan sulfate side chains.^{24,25} Therefore, perlecan also provides a non-specific barrier to some growth factors—such as TGF β .

These observations regarding TGF β being a critical modulator after either EBM or DBM injury suggests that TGF β inhibitors could be useful adjuvants to decrease or block fibrosis after injuries to these basement membranes from trauma, infection or surgery.

An extremely important concept regarding the basement membranes in the cornea is that they are produced and regenerated after injury through the *coordinated* action of both the corneal epithelial cells and keratocytes in the case of the EBM and corneal endothelial cells and keratocytes in the case of the DBM. Thus, while corneal epithelial cells produce laminins, perlecan, nidogen-1, nidogen-2 and collagen type IV, so do the keratocytes,³¹⁻³⁴ and human keratocytes upregulate the production of some components like perlecan and nidogen-2 after corneal epithelial injury.³¹ Laminins produced by the epithelium initiate the regeneration of damaged EBM after a corneal epithelial-EBM-stromal injury such as PRK and, therefore, epithelium must be present to initiate the EBM repair process. It is our working hypothesis (Fig. 2) that once the self-polymerizing laminin layer is established beneath the healing epithelium, the deeper layers of the EBM must, at least in part, be provided by keratocytes that cooperate directly with the overlying epithelial cells in regenerating the mature EBM that has lamina lucida and lamina densa when examined with transmission electron microscopy. Corneal fibroblasts and myofibroblasts can also produce some of these EBM components,³²⁻³⁴ but studies with organotypic cultures of corneal epithelial cells with the different types of corneal stromal cells suggest that corneal fibroblasts and myofibroblasts cannot coordinate with the overlying epithelial cells to properly localize the EBM components to produce mature EBM (Unpublished data, P. Saikia and S.E. Wilson, 2019). There is evidence that the corneal endothelial cells and posterior keratocytes similarly cooperate in the production of Descemet's basement membrane,⁵ but more work is needed for confirmation.

What factors lead to injury and defective regeneration of the EBM or DBM after corneal injury? Studies have thus far identified three major contributors. First, the epithelial cells or endothelial cells must heal over the injury. These cells produce the laminin that forms the nascent EBM or DBM, and without them it cannot begin to regenerate. Corneal endothelial cells, even in rabbits, show much less capacity to heal over a site of posterior corneal injury to begin DBM regeneration.^{4,5} In the case of anterior corneal injuries, even very severe ones caused by a pseudomonas aeruginosa corneal ulcer,⁴ the epithelium shows amazing facility to eventually heal and regenerate the EBM. However, anterior scarring (fibrosis) can still occur despite the epithelium healing normally, as in photorefractive keratectomy in both humans³⁵ and rabbits.³⁶ Surface irregularity has been shown to be one factor that directly relates to defective EBM regeneration, myofibroblast generation and anterior stromal scarring after treatments such as PRK.³⁷ This surface irregularity presumably interferes directly with the formation of a continuous laminin nascent EBM by the epithelium. Also, since keratocytes participate in EBM regeneration, severe injuries that result in the loss of large numbers of keratocytes, such as high level corrections with PRK³⁶ or severe corneal ulcers,⁴ increase the odds that a cornea will heal with scarring fibrosis rather than transparency. There may be other factors discovered in the future that favor corneal scar formation vs. transparency after injury, surgery, infection or disease—including unknown genetic factors that contribute to bilateral haze formation in some patients with minor EBM injuries or low PRK that rarely results in “late haze.”

Every injury that involved the EBM or the DBM initiates the process towards scar formation.¹⁹ Thus, even a simple corneal abrasion that removes a patch of epithelium and EBM, results in TGF β entry into the stroma and transition of keratocytes into corneal

fibroblasts that could continue development into myofibroblasts that produce scarring (Fig. 3). Fortunately, after these types of simple injuries, including most low correction PRK procedures in humans or rabbits, the epithelium heals promptly, the anterior stroma is repopulated with normal keratocytes and the EBM is regenerated.³ This mature EBM takes eight to ten days to regenerate in rabbits, and once it forms, TGF β from the epithelium is blocked from entering the stroma and any corneal fibroblasts or fibrocytes that began to develop into myofibroblasts die by apoptosis since they are deprived of their requisite source of TGF β .³ The more severe the injury—whether it occurs by trauma, toxic exposure, surgery, infection or disease—the more likely the EBM will not regenerate normally, TGF β will continue to penetrate into the stroma, myofibroblasts will develop and scarring fibrosis will be generated. Thus, every injury to the EBM initiates a race of sorts between processes that would restore the BM and lead to apoptosis of myofibroblast progenitors vs. processes that would lead to defective EBM regeneration and development of mature myofibroblasts that form a cellular barrier between the epithelium and interacting keratocytes. The clinician often has an early opportunity to facilitate the transparency pathway by encouraging epithelial healing, smoothing the stromal surface, and inhibiting myofibroblast development (mitomycin C). Posterior fibrosis after injuries, surgeries or infections of the cornea are different because the endothelium often has a difficult time healing over bare stroma, even in rabbits where the endothelial cells have much higher proliferative potential. Thus, in a rabbit model of severe *Pseudomonas aeruginosa* ulcers of the cornea,⁴ the EBM was regenerated by two months after sterilization of the ulcer (Fig. 4)—resulting in apoptosis of myofibroblasts and restoration of transparency in the anterior cornea—but the DBM showed no tendency towards regeneration and the posterior stromal myofibroblasts and scarring fibrosis persisted. Even when the DBM-endothelial complex is removed surgically over the posterior 8mm of the cornea in the rabbit (Fig. 5), no tendency for the DBM to regenerate was noted by one month after the surgery.⁵ Coverage of the bare stroma could occur with much longer follow-up, but by that time dense fibrosis would likely be established with high levels of dense extracellular matrix. Thus, injuries or infections that produce extensive DBM injury or removal that begin to develop posterior scarring fibrosis, should likely be treated in a timely manner with surgically replacement of the DBM-endothelial complex, or penetrating keratoplasty may become the only therapeutic option.

Some anterior corneal scars resolve completely over a period of six months to several years. For example, in human corneas that develop severe anterior stromal scarring fibrosis after PRK, the cornea is often transparent for two to three months after surgery, and then rapidly develops dense anterior stromal fibrosis (late haze). The “haze” will often begin to clear in transparent areas called “lacunae” at about a year after surgery and over time these can enlarge and coalesce to restore complete transparency. In rabbits, this process proceeds more rapidly with the late haze beginning to develop by two weeks after –9D PRK, peaking at one month after surgery and clearing completely by two months after PRK.³ These clear lacunae that develop within the fibrosis are probably areas where keratocytes have managed to penetrate the layer of fibrosis produced by myofibroblasts and coordinate with the epithelium to regenerate EBM—causing underlying myofibroblasts to undergo apoptosis so that the keratocytes can remove the disordered extracellular matrix the myofibroblasts produced, and restore normal collagen lamellae and transparency.³ If a cornea that has had

mitomycin treatment after PRK subsequently develops scarring fibrosis (late haze) it is referred to as “breakthrough haze.” Interestingly, breakthrough haze shows much less tendency to resolve over time spontaneously. Presumably, this is attributable to a change in keratocyte phenotype or density produced by the mitomycin C that retards regeneration of the EBM for a prolonged period of time.

Now that the basic principles of the role of the EBM and DBM in modulating stromal scarring fibrosis have been highlighted, it will be helpful to clinicians to apply this knowledge to the development of corneal scarring after some of the disorders and surgeries in which it often occurs.

Scarring in photorefractive keratectomy and phototherapeutic keratectomy

Fortunately, anterior stromal fibrosis (“late haze”) after PRK (Fig. 3A) is not nearly the clinical problem it was when PRK was initially introduced in the 1990s. At that time, it would be noted in approximately 5% of corneas with corrections over six diopters of myopia or in the midperiphery of hyperopic corrections. This is because of the efficacy of mitomycin C treatment after PRK in blocking the proliferation of myofibroblast precursor cells in the cornea stroma and thereby preventing the development of large numbers of myofibroblasts that produce the haze.³⁸ Unfortunately, it is still occasionally seen after PRK when mitomycin C treatment is omitted or as “breakthrough haze” that occurs despite mitomycin C treatment. These cases may have central islands or peninsulas on corneal topography that indicates there was corneal surface irregularity that mechanically impeded EBM regeneration. If no mitomycin C was used, the haze typically resolves spontaneously over one to two years and observation is recommended. If mitomycin C was used and breakthrough haze occurred, then, unfortunately, the haze usually persists much longer and may show no tendency to spontaneously resolve. Some of these patients may have underlying unknown genetic factors, possibly involving basement membrane components or their assembly. If after a year of observation, no tendency for spontaneous resolution is noted (clear lacunae within the haze) then other measures may be needed. Usually, PTK with mitomycin C is the first treatment attempted, and it is critical that a masking smoothing step be included to smooth the stromal surface and facilitate regeneration of the normal EBM.³⁹ This is because surface irregularity may have been a factor in the original development of the breakthrough haze and would facilitate its recurrence. It is important to note that it is often not necessary to remove all the anterior stromal haze to improve visual function. Each 50 pulses of PTK removes approximately 12 microns of tissue and induces approximately 1 diopter of hyperopic shift in refraction. Thus, if 300 pulses are applied to remove all the haze, the eye will end up with +5 to +6 diopters of hyperopic change. That could be beneficial if the eye originally had a -8 diopter correction that recurred with the late haze, but it would not be thought of as a success by the patient if the eye was originally a -2 diopters of myopia and ends up +4 diopters of hyperopia. If the PTK with mitomycin C and masking smoothing is not effective, or severe haze recurs, then other measures such as lamellar keratoplasty or penetrating keratoplasty may be needed to restore vision.

LASIK flap edge and buttonhole flaps

Stromal fibrosis is always noted at the LASIK flap edge where the EBM was transected by either a femtosecond laser or microkeratome bed (Fig. 6A), although the density of this fibrosis varies in the eyes of different patients. This fibrosis is helpful to prevent late dislocations of the flap due to minor trauma that can result in late diffuse lamellar keratitis or epithelial ingrowth. Unfortunately, this flap edge fibrosis can also make the flap difficult to lift for LASIK enhancement more than 6 to 12 months after the original surgery.

LASIK buttonhole flaps produced by microkeratomes will usually lead to surrounding stromal fibrosis (haze) that typically begins to be visible at the slit lamp at approximately two weeks after the complication (Fig. 6B). This fibrosis tends to intensify over the months after the LASIK surgery and is usually spotty in distribution. These buttonholes also commonly develop epithelial ingrowth. Buttonhole flaps tend to be very thin in the periphery (usually less than approximately 50 μm) with central thicknesses dropping to zero μm at the buttonhole. This can be verified with OCT. Since spotty haze and epithelial ingrowth result in irregular excimer laser ablations, my approach has been early removal of the flap by transepithelial PRK with mitomycin C that provides 90% of the original intended correction to compensate for greater refractive correction obtained with this approach.^{40,41} The change made in this original strategy is to perform the follow-up PRK approximately 10 to 14 days after the complicated microkeratome cut when best spectacle-corrected vision has returned to nearly normal—which allows time for the epithelium to smooth over the surface irregularity of the buttonhole that would otherwise be imprinted on the stroma if the excimer laser surgery were performed earlier—but before significant fibrosis or epithelial ingrowth can develop to further complicate the situation. If a buttonhole flap occurs in a hyperopic eye, a two-staged procedure is usually the best approach since hyperopic PRK ablations remove stromal tissue in the midperiphery. First, the central flap and buttonhole is removed with transepithelial PTK with mitomycin C (the amount of ablation needed can be approximated with OCT, using the approximation that each pulse of excimer laser will remove 0.25 micron of corneal tissue). Then, approximately six months later, a secondary PRK with mitomycin C is performed to correct the residual refractive error. My approach is to not correct more than 4.5 to 5 diopters of hyperopia in these eyes because of the risk of impaired vision quality with higher hyperopic corrections.

Persistent epithelial defects of the cornea

It has been appreciated by ophthalmologists for many decades that scarring will begin to appear in a cornea with a persistent epithelial defect (PED) if it is not healed within two to four weeks. This is the case whether the PED occurs spontaneously or is associated with other disorders such as a neurotrophic cornea, ocular surgery, exposure, diabetes mellitus or infection. Therefore, clinicians typically strive to heal such defects in a timely manner. A recent study in rabbits with spontaneous persistent epithelial defects demonstrated that this scarring is fibrosis mediated by myofibroblasts that is associated with defective regeneration of the EBM.² The EBM cannot begin to regenerate in the absence of epithelial closure because only the epithelium can provide the laminin 511 and/or 521 that are the only self-polymerizing laminins that initiate regeneration of the nascent EBM (unpublished data, P

Saikia and SE Wilson, 2019) before other components can be contributed by the epithelium and keratocytes.^{2,3,31-34} Thus, in the absence of epithelium, there can be no EBM to block tear film TGF β from continuously penetrating into the stroma and driving the development of myofibroblasts from keratocyte and/or fibrocyte precursor cells.² Therefore, every effort should be made by ophthalmologists to heal persistent epithelial defects, regardless of the initiating disorder, within two to four weeks of the disorder appearing—because this is the time it takes in humans for mature alpha-smooth muscle actin (SMA)+ myofibroblasts that produce disordered extracellular matrix in corneal scars to begin to develop.³⁶ Katzman and Jeng⁴² have detailed the measures that can be used to facilitate healing of persistent epithelial defects—including lubrication, addressing medicamentosa, surgical repair of eyelid defects, bandage contact lenses, punctal occlusion, epithelial debridement to freshen the epithelial edges, tarsorrhaphy, autologous serum drops and amniotic membranes.

EBM regeneration after therapeutic debridement or PTK for recurrent erosions

In eyes with recurrent corneal erosions due to anterior basement membrane dystrophy or after corneal trauma, it is often necessary to perform therapeutic debridement of the epithelium and redundant epithelial basement membrane or to perform PTK. Studies in rabbits have shown that the EBM fully regenerates at 8 to 10 days after treatments like PRK that remove epithelium and EBM.^{3,23} No studies have examined the time required to regenerate anchoring fibrils that also fix the epithelium to the underlying stroma. Therefore, after corneal therapeutic debridement or PTK,³⁹ my clinical practice is to maintain a bandage contact lens with prophylactic antibiotic for three to four weeks after the procedure to allow more than adequate time for EBM and anchoring fibril regeneration. Following removal of the bandage contact lens, lubricants—including bedtime ointments—should be continued for several more months (Wilson SE, personal observation).

Microbial corneal ulcers

Treatments for microbial corneal ulcers—bacterial, fungal, viral, acanthamoeba, etc.—should not only include appropriate antimicrobials, but also measures to facilitate closure of the epithelium, so the EBM can regenerate and corneal scarring (fibrosis) can be minimized. Some of the scarring caused by microbial ulcers may be attributable to damage to the corneal stromal lamellae produced by the microorganisms or the associated stromal inflammation. However, a significant amount of the scarring is likely to be due to the development of myofibroblasts, as was demonstrated in a study of *Pseudomonas aeruginosa* ulcers in rabbits.⁴

Measures discussed previously for persistent epithelial defects may also be clinically useful for microbial corneal ulcers that are started at the time of beginning antimicrobials or at least within a few days of initiating treatment. In addition, the judicious use of topical corticosteroids after an infection is under good control may decrease inflammation and likely facilitate EBM regeneration.

Corneal crosslinking and scarring

Transient haze that occurs in the cornea after normal epithelium-off or epithelium-on riboflavin UV-crosslinking is not myofibroblast-mediated corneal scarring (fibrosis).⁴³ Rather it is likely the generation of corneal fibroblasts (also referred to as activated keratocytes), that are transient and either die by apoptosis or revert to keratocyte phenotype, as well as fibrocytes and their progeny that migrate into the cornea from the limbal blood vessels after the initial corneal crosslinking injury. But these progenitor cells normally do not complete their development into mature SMA+ myofibroblasts because the epithelium heals, the basement membrane is regenerated, and the supply of TGF β needed to drive full development of myofibroblasts is cut off—resulting in apoptosis of these immature SMA–myofibroblasts. However, in some cases the epithelium does not heal in a timely manner after riboflavin-UV crosslinking and then persistent scarring caused by mature SMA+ myofibroblasts may occur due to the non-healing epithelial defect—as was discussed in an earlier section. In crosslinking cases where the epithelium doesn't heal within 7 to 10 days, the measures described for non-healing epithelial defects should be begun to try to prevent stromal scarring (fibrosis).

Corneal transplantation

In penetrating keratoplasty (PKP) or deep anterior lamellar keratoplasty (DALK) surgeries it is helpful to have uniform scarring around the periphery of the transplanted tissue at the donor-recipient interface. This scarring (fibrosis) holds the donor tissue in place and is produced by mature SMA+ myofibroblasts that develop in the first month or two after the transplant surgery. If, unfortunately, the scarring is not uniform around the periphery of the transplanted tissue, variable levels of astigmatism typically develops. At present, there is no way to control this scarring, except sutures should be removed in a timely manner (usually 6 to 12 months) and early suture removal is indicated in any quadrants where blood vessels cross the donor-recipient interface, since these vessels typically indicate that adequate scarring has occurred at that site.

Endothelial replacement surgeries such as Descemet's stripping automated endothelial keratoplasty (DSAEK) or Descemet Membrane Endothelial Keratoplasty (DMEK) are a special case. First of all, when corneas decompensate in Fuchs' corneal dystrophy, pseudophakic bullous keratopathy or other diseases where bullous keratopathy occurs, the endothelial transplant should be performed in a timely manner because eventual damage to Descemet's membrane due to edema and inflammation is likely to occur over time and result in stromal scarring that occurs because of SMA+ myofibroblast development in the posterior cornea, as we demonstrated in a recent study in rabbits.⁵ This scarring occurs because once Descemet's membrane function is compromised, TGF β from the aqueous humor can penetrate into the stroma at sufficient levels to drive the development of mature myofibroblasts from keratocyte precursors. In advanced cases of Fuchs' dystrophy or bullous keratopathy, the EBM can also be destroyed, resulting in myofibroblast-mediated subepithelial fibrosis.⁴⁴ Transplantation of a donor Descemet's membrane-endothelium with either DSAEK or DMEK restores normal Descemet's basement membrane and usually halts this pathological process.

Secondly, when DSAEK or DMEK is performed, every effort should be made to cover all the stromal surface that is exposed by the Descemetorhexis of diseased recipient tissue because leaving gaps between recipient and donor Descemet's membranes is likely to lead to localized fibrosis in the stroma anterior to those gaps (Wilson SE, personal observation, 2019). This may not affect visual function or the health of the graft in many cases if it remains in the periphery of the transplanted tissue. But there is a possibility that such fibrosis could progress over time in some cases and result in fibrosis overlying the pupil or even destroy endothelial cells in the transplant. Thus, it is advisable to strive to leave no gaps of exposed posterior stromal surface. An area of future research would be to determine whether it might be advisable to prepare the donor tissue with a slightly larger diameter than the Descemetorhexis performed in the donor so no bare stromal gap remains.

Finally, in endothelial transplantation cases where the donor tissue gets displaced or dislocated, the tissue should be repositioned or replaced in a timely manner because the processes that can lead to SMA+ myofibroblast-mediated posterior fibrosis⁵ likely begin at the moment the donor tissue is no longer in position (Wilson SE, personal observation, 2019). These processes that could lead to posterior stromal fibrosis would likely be halted by repositioning or replacement of the Descemet's membrane because the entry of TGF β from the aqueous humor to the stroma would be cut off.

Descemetorhexis without endothelial transplantation

Descemetorhexis without endothelial transplantation has been investigated as a potential treatment for Fuchs' dystrophy corneas that decompensate, and encouraging results have been reported with this approach because viable endothelial cells remain in the periphery of these diseased corneas and appear to migrate and, possibly, proliferate.⁴⁵⁻⁴⁷ However, posterior stromal fibrosis has been noted in some human corneas after Descemetorhexis without endothelial transplant,^{48,49} similar to what we have noted in rabbits that have Descemetorhexis without endothelial transplantation.⁵ Rabbit corneas have more tendency to develop fibrosis than human corneas.⁵ However, in cases where posterior stromal scarring (fibrosis) is noted after Descemetorhexis without endothelial transplantation, the mechanism is likely related to the removal of Descemet's membrane over the central cornea and the ensuing pathophysiology mediated by aqueous humor TGF β noted in rabbit corneas.⁵ Importantly, ROCK inhibitors could be useful therapeutically in these cases to promote endothelial regeneration and, possibly, regeneration of a Descemet's basement membrane-like layer.⁵⁰

Corneal endotheliitis

Many corneas that develop corneal endotheliitis caused by herpes simplex, cytomegalovirus, or autoimmune processes develop posterior corneal scarring (fibrosis).⁵¹ It is likely that Descemet's basement membrane injury has occurred in endotheliitis cases that develop posterior scarring. Penetrating keratoplasty has long been a mainstay of treatment for eyes with significant vision loss due to endotheliitis. Once the infectious and/or inflammatory disorder was under control, several groups reported good outcomes with endothelial replacement surgery in treating corneal edema and/or scarring that impedes vision.^{52,53} This

approach may be optimal since damage to Descemet's basement membrane could be associated with the late posterior scarring in these cases.⁵

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Fig. 1. Ultrastructure of the epithelial basement membrane and Descemet's basement membrane. **A.** The rabbit corneal EBM at 32,000X magnification shows the lamina lucida (arrowheads) and lamina densa (arrows). E is the epithelium and S is the stroma. **B.** The rabbit corneal DBM at 9000X magnification in a 12 week old rabbit is much thicker than the EBM. The anterior banded layer is indicated by the white arrowheads. The remainder of DBM is the posterior non-banded layer. e is the endothelium and S is the stroma. The black arrowhead is a keratocyte.

Fig. 2.

Schematic illustration showing injury to the corneal epithelial basement membrane (EBM) and defective regeneration leading to myofibroblast development and fibrosis, followed by keratocyte contributions to regeneration of the EBM and removal of fibrotic extracellular matrix produced by myofibroblasts. **A)** The normal unwounded cornea has intact epithelial basement membrane (EBM) comprised of laminin 332, nidogen-1, nidogen-2, perlecan, and other components not depicted—such as collagen type IV. The underlying stroma has keratocytes that maintain the highly-organized stromal collagen lamellae and cornea transparency. Epithelium-derived transforming growth factor beta (TGFβ) and platelet-derived growth factor beta (PDGF) are blocked from penetration into the underlying stroma by binding to normal EBM components such as perlecan and collagen type IV. **B)** After severe epithelial-stromal injuries, such as infections, trauma and surgeries—such as high correction photorefractive keratectomy—the epithelium and EBM are disrupted and activated TGFβ and PDGF penetrate into the underlying stroma at sufficient concentrations to drive the development of mature myofibroblasts from keratocyte-derived and bone marrow-derived (fibrocyte) precursor cells. Myofibroblasts are also opaque (relative to keratocytes) and secrete large amounts of disordered collagen type I, collagen type III and other matrix materials that disrupt the organization of the normal stromal lamellae to produce corneal scarring (fibrosis). **C)** Over months to years following the initial injury, keratocytes penetrate the anterior stromal myofibroblast layer and facilitate EBM regeneration via the production of laminins, nidogens, and perlecan in coordination with overlying epithelial cells that also produce these and other EBM components. The working hypothesis is that once the nascent laminin-332 layer is produced by the epithelium, more posterior EBM components must come from keratocytes to fully regenerate the normal EBM. This causes a decrease in TGFβ and PDGF penetration into the stroma from the epithelium and triggers myofibroblast apoptosis. This EBM regeneration process begins in a random spotty distribution within the

stromal opacity to produce clear areas within the scarring called “lacunae,” that can enlarge and coalesce over weeks to months to fully restore stromal transparency. **D.** In many corneas, depending on the type and severity of the initial injury, all the myofibroblasts disappear and keratocytes fully-repopulate the stroma and reabsorb the remaining disorganized extracellular matrix materials secreted by the myofibroblasts to completely restore the normal morphology of the collagen lamellae and stromal transparency. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2019. All Rights Reserved.

Fig. 3.

Corneal fibrosis in PRK, **A.** Slit lamp photograph of severe anterior fibrosis (arrows), clinically referred to as late haze, in a human cornea at 3 months after high correction PRK without mitomycin C. Mag 25X. **B.** A rabbit cornea with alpha-smooth muscle actin+ myofibroblasts (arrows) in the sub-epithelial stroma at one month after high correction -9D PRK. Blue is DAPI staining of all nuclei. e is epithelium. Mag. 400X. **C.** Transmission electron microscopy of a rabbit cornea with severe fibrosis (late haze) at one month after -9D PRK showing there is no detectible EBM lamina lucida or lamina lucida (arrowheads) where the normal EBM should be noted. Arrows indicate layered myofibroblasts in the subepithelial stroma that are noted in **B.** e is epithelium. Mag 13,000X

Fig. 4.

Pseudomonas aeruginosa keratitis in rabbit treated after 24 hours with antibiotics at one month after infection (SL). Note dense corneal scarring (fibrosis) and vascularization. Mag 25X. Immunohistochemistry (IHC) for myofibroblast marker alpha-smooth muscle actin (200X Mag.) and transmission electron microscopy (TEM) (23,000X mag.) of control unwounded corneas (A, B), and corneas at one month (C, D), two months (E, F) and three months (G, H) after *Pseudomonas aeruginosa* keratitis. In a normal cornea (A), there are no α -SMA+ myofibroblasts detected in the stroma and the underlying stroma (s) is populated with keratocytes with nuclei stained with DAPI. Normal EBM lamina lucida and lamina densa (arrows in B) are present beneath the epithelium (e). At one month after keratitis (C), the anterior stroma is filled with SMA+ myofibroblasts (arrows). In this cornea, antibiotics halted the infection before the corneal endothelium was destroyed and the most posterior stroma is populated by normal SMA-negative keratocytes (*). TEM at one month after keratitis (D) found no detectible EBM beneath the epithelium (e) in all sections imaged. At one month after keratitis, the anterior stroma (s) is filled with large myofibroblasts with rough endoplasmic reticulum (arrows). These are the SMA+ cells in IHC in panel C. At two months after *Pseudomonas* keratitis (E), there are only a few SMA+ cells present in the anterior half of the stroma. Those SMA+ cells that persist in the anterior stroma (arrows) are likely pericytes associated with the invading blood vessels. Note that no corneal endothelial cells (SMA-) are present on the posterior surface of the cornea since in this cornea the *Pseudomonas* infection destroyed the Descemet's membrane and endothelium. In the TEM of this cornea at two months after keratitis (F), EBM lamina lucida and lamina densa (arrows) have regenerated beneath the epithelium (e). The myofibroblasts that were present in the anterior stroma (s) at one month after infection (D) are no longer seen at two months after infection (F). In IHC at three months after *Pseudomonas* keratitis (G), almost all SMA + myofibroblasts have disappeared in the anterior 80% to 90% of the stroma. The only SMA + cells in the mid-stroma are associated with penetrating blood vessels (arrows), indicating they are pericytes. In the posterior 10% of the stroma SMA+ myofibroblasts (arrowheads) persist and no SMA- corneal endothelium is noted. In the TEM of this cornea at three months after keratitis (H), normal lamina lucida and lamina densa (arrows) are noted beneath the epithelium (e) and no cells with large amounts of rough endoplasmic reticulum were present in the stroma (s). IHC and TEM of corneas at 4 months after *Pseudomonas*

keratitis were similar to those at 3 months (not shown). Reprinted with permission from Marino, et al. Exp. Eye Res. 2017; 161:101-105.

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Fig. 5.

Posterior corneal fibrosis after removal of Descemet's membrane-endothelial complex. **A.** A slit lamp photo of the same cornea four weeks after 8 mm diameter Descemet's-endothelial removal shows persistent stromal edema and posterior stroma fibrosis (scarring). Mag 25X. **B.** One month after excision of the Descemet's basement membrane-endothelial complex without transplant over the central 8mm of the cornea in rabbit corneas. A zone of fibrosis (f) occupied the posterior 30 to 40% of the corneal stroma and was filled with alpha-smooth muscle actin (SMA)+ (red) myofibroblasts in all corneas that had the Descemetorhexis. The anterior stroma (k) in this cornea was occupied by normal keratocytes that are here stained for the keratocyte marker keratocan (green). Between the posterior fibrotic zone and anterior keratocyte-populated stroma there was a band (arrows) of keratocan- SMA- stromal cells that likely included corneal fibroblasts, fibrocytes, and possibly other infiltrating cells, which occupied 3% to 5% of the stromal volume in each of the individual corneas in this group. In a few localized spots in each section, there were small areas where there were no keratocan- SMA- stromal cells and, thus, there was direct intermingling of keratocan+ keratocytes with SMA+ myofibroblasts (within boxes). Notice there were no endothelial cells detected where the posterior surface of the cornea can be seen in one small area (arrowheads). E is epithelium. Mag. 200X **C.** Another rabbit cornea one month after 8mm Descemetorhexis without transplant. The posterior stroma is filled with SMA+ (red) myofibroblasts—many of which are also vimentin+ (SMA+ vimentin+ = yellow). Some keratocytes (arrowheads), especially in the subepithelial stroma, are also vimentin+ (green) but SMA-. Cells that stain only green in the posterior stroma are vimentin+ precursors to myofibroblasts undergoing differentiation and which at this time are SMA-. Blue is DAPI stain of all nuclei. e is epithelium. Mag. 150X. Reprinted by permission from Mederois, et al. Invest Ophth Vis Sci. 2019;60:1010-1020.

Fig. 6. Fibrosis in LASIK. A. Normal corneal fibrosis at the flap edge that is normal after LASIK. In this case, there is a double line with the internal line indicated by arrows and the outer line indicated by arrowheads. This fibrosis is mediated by myofibroblasts. Mag. 25X. B. A flap complication with a microkeratome resulted in patches of subepithelial fibrosis (arrows) surrounding the linear cut through the epithelium and into the stroma. This fibrosis is also mediated by the development of myofibroblasts. Mat. 25X.