

Feb 10, 2025

Long-term alterations of collagen reconstruction and basement membrane regeneration after corneal full-thickness penetrating injury in rabbits

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvr9weovmk/v1>

Jingjing Chen¹, Yuqing Luo¹, Luting Xie¹, Na Meng¹, Sumei Li¹, Shifang Xiao¹, Xia Li¹

¹the First Affiliated Hospital of Guangxi Medical University

Jingjing Chen: The first author

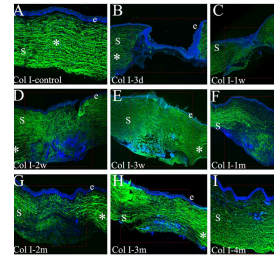
Yuqing Luo: Co-first author

Xia Li: Corresponding author



Xia Li

the First Affiliated Hospital of Guangxi Medical University



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvr9weovmk/v1>

Protocol Citation: Jingjing Chen, Yuqing Luo, Luting Xie, Na Meng, Sumei Li, Shifang Xiao, Xia Li 2025. Long-term alterations of collagen reconstruction and basement membrane regeneration after corneal full-thickness penetrating injury in rabbits.

protocols.io <https://dx.doi.org/10.17504/protocols.io.6qpvr9weovmk/v1>

Manuscript citation:

1. Connon CJ, Meek KM. Organization of corneal collagen fibrils during the healing of trephined wounds in rabbits. *Wound Repair and Regeneration*. 2003;11(1):71-78. doi: 10.1046/j.1524-475X.2003.11110.x.
2. Fantes FE, Hanna KD, Waring GO III, Pouliquen Y, Thompson KP, Savoldelli M. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. *Archives of Ophthalmology*. 1990;108(5):665-675. doi: 10.1001/archophth.1990.01070070051034.
3. Connon CJ, Tandon A, Sharma A, Rodier JT, Klibanov AM, Rieger FG, et al. BMP7 Gene Transfer via Gold Nanoparticles into Stroma Inhibits Corneal Fibrosis In Vivo. *PLoS ONE*. 2013;8(6):e66434. doi: 10.1371/journal.pone.0066434.
4. Yam GHF, Riau AK, Funderburgh ML, Mehta JS, Jhanji V. Keratocyte biology. *Experimental Eye Research*. 2020. 196 DOI 10.1016/j.exer.2020.108062

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: February 08, 2025

Last Modified: February 10, 2025

Protocol Integer ID: 119834

Keywords: collagen reconstruction; anterior stroma; posterior stroma; corneal opacity; corneal wound healing; basement membrane regeneration., thickness corneal injury, corneal injury, term alterations of collagen reconstruction, collagen reconstruction, injury in rabbits purpose, injury in rabbit, corneal scarring, transmission electron microscopy for collagen fiber, basement membrane regeneration, epithelial basement membrane, collagen fiber, left eye of new zealand white rabbit, collagen type, cornea, basement membrane, incomplete regeneration, incomplete regeneration of dm, regeneration, penetrating injury, anterior stroma, persistent fibrosis within the posterior stroma, persistent fibrosi, undetectable in the anterior stroma, posterior stromal fibrosi, penetrating injury model

Abstract

Purpose: To investigate the long-term alterations of collagen reconstruction and basement membrane (BM) regeneration after corneal full-thickness penetrating injury in rabbits.

Methods: The corneal full-thickness penetrating injury model was established in the left eye of New Zealand White rabbits using a 2.0 mm trephine. All corneas were evaluated using slit-lamp photography, hematoxylin and eosin staining, immunofluorescent staining for collagen types I and III (Col I, III), and transmission electron microscopy for collagen fibers and basement membrane.

Results: Between 3 days and 3 weeks, Col I and III expression were documented, exhibiting a largely disorganized distribution throughout the stromal thickness. At 3 weeks, the epithelial basement membrane (EBM) partially regenerated. From 3 weeks to 2 months, Col III was undetectable in the anterior stroma but present in the posterior stroma; Col I was disorganized in the posterior stroma. At 2 months, Descemet's membrane (DM) exhibited incomplete regeneration. From 3 to 4 months, Col I was disorganized in only a small part of the posterior stroma; Col III persisted in the posterior stroma; the EBM fully regenerated while DM exhibited incomplete regeneration.

Conclusions: Following full-thickness corneal injury, persistent fibrosis within the posterior stroma appears to be primarily responsible for the persistence of corneal scarring. Notably, regeneration of the EBM coincides with remodeling of the anterior stroma, whereas incomplete regeneration of DM is associated with posterior stromal fibrosis.

Attachments



Supporting informat...

2.8MB

Image Attribution

 Sample

Guidelines

Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research

Materials

sterile 2.0 mm trephine (Storz, USA)
0.5% compound tropicamide eye drops (SHENYANG XINGQI PHARMACEUTICAL CO, LTD, China)
1% pentobarbital sodium (AG, Germany)
0.5% proparcaine hydrochloride ophthalmic anesthetic solution (Alcon-COUVREUR, USA)
0.3% Levofloxacin gel
0.5% Levofloxacin eye drops (YABANG Pharma, China)
4% paraformaldehyde (Solarbio, China)
3% EDTA, 0.1 M phosphate-buffered saline (PBS) and permeabilized with 0.3% Triton X-100
anti-COL1A1 antibody (NB600-450, Mouse anti-Collagen I alpha 1; Novus)
anti-COL3A1 antibody (sc-271249, Mouse monoclonal antibody, 1:100, Santa Cruz)
1% bovine serum albumin (BSA)
secondary antibody (2284614, Alexa Fluor 488 goat anti-mouse antibody; Thermo Fisher Scientific)
secondary antibody (4408S, Alexa Fluor 488 goat anti-mouse antibody; Cell Signaling Technology),
4',6-diamidino-2-phenylindole (DAPI) (C0065-10ml, Solarbio, China)
Leica TCS SP8 microscope (Leica, Wetzlar, Germany) equipped with a Fluoview FV1000 confocal system (Olympus, Japan).
Image analysis was performed using ImagePro software (Leica)
2.5% glutaraldehyde and 4% paraformaldehyde
1% osmium tetroxide
graded ethanol and acetone series
ultramicrotome (Leica EM UC7, Germany)
transmission electron microscope (Hitachi H-7650, Japan)
scanning electron microscopy (TESCAN VEGA3, Czech Republic)



Safety warnings

- ! All procedures adhered to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research (protocol number: 2024E50301), approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Ethics statement

All procedures adhered to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research (protocol number: 2024E50301), approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Material and methods

- 1 Forty-eight New Zealand White rabbits were randomly assigned to eight groups ($n = 6/\text{group}$) representing different post-operative time points. The left eyes of each rabbit received a penetrating injury using a sterile 2.0 mm trephine (Storz, USA) to create the model, while the right eyes served as controls. The injury model creation followed established protocols. Briefly, each rabbit underwent left eye trephination at the central cornea using the aforementioned sterile trephine. Prior to the procedure, the experimental eyes were dilated with 0.5% compound tropicamide eye drops (SHENYANG XINGQI PHARMACEUTICAL CO, LTD, China) to prevent iris incarceration in the trephined area during the operation. Following pupil dilation in the rabbits, a precise weighing procedure was conducted. General anesthesia was subsequently administered via intravenous infusion of 1% pentobarbital sodium (at a dose of 3ml/kg) through the marginal ear vein. A 0.5% proparacaine hydrochloride ophthalmic anesthetic solution (Alcon-COUVREUR, USA) was then applied to the ocular surface of the operative eye. After confirming the effectiveness of both general and local anesthesia, a pediatric eyelid speculum was carefully positioned over the surgical eye to fully expose the rabbit's cornea. The eye was stabilized using forceps, and a sterile trephine with a 2.0 mm diameter was precisely aligned at the center of the cornea. Using the rotary trephine, the entire layer of corneal tissue was meticulously excised, revealing the aqueous humor. Next, 0.3% Levofloxacin gel was applied to the corneal wound to prevent anterior chamber collapse and iris adhesion. Notably, the rabbits regained consciousness within 30 minutes post-procedure. Within 15 minutes, fibrin clots formed on the corneal wound, effectively sealing it and re-establishing the anterior chamber. The rabbit corneal perforation injury model was successfully established. Postoperatively, one drop of 0.3% Levofloxacin gel was administered every 8 hours for the first three days, followed by 0.5% Levofloxacin eye drops (YABANG Pharma, China) every 8 hours for two weeks. Mitomycin C and topical corticosteroids were not utilized in this study. Any instances of corneal ulceration or neovascularization were excluded from the study.
- 2 Standardized broad-beam illumination corneal slit-lamp photographs were obtained at various time points: 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, and 4 months post-surgery. Euthanasia was performed by intravenous injection of pentobarbital sodium 100 mg/kg (AG, Germany). Corneoscleral rims from both wounded and unwounded eyes were excised using forceps and scissors, ensuring no contact with the cornea itself, for subsequent histopathology and corneal ultrastructural examination.
- 3 **Slit-Lamp Microscopic Examination**
At each time point following corneal injury, all rabbit eyes were evaluated using slit-lamp microscopy. The corneal epithelial defect area was measured using ImageJ software following the instillation of 1% sodium fluorescein solution and photography with cobalt blue light. Corneal opacity was graded on a modified Fantes' scale. (0 = no opacity, 4 =

complete opacity) as previously described. Briefly, grade 0.5 indicates slight opacity with visible pupillary margin and iris details, grade 1 indicates observable opacity with both details visible, grade 2 signifies partial pupillary margin visibility, and grade 3 represents moderate opacity without a visible pupillary margin.

4 **Histopathologic Examination**

Histopathological changes in injured and control corneas were assessed using hematoxylin and eosin (H&E) staining. At each designated time point, rabbits were euthanized under deep anesthesia, and eyes were immediately enucleated. Following fixation in 4% paraformaldehyde (Solarbio, China) for 48 hours, the eyes were paraffin-embedded, sectioned into 4- μ m slices, and dewaxed with xylene. These sections were then subjected to H&E staining for histological analysis.

5 **Immunofluorescence Analysis**

Immunofluorescence (IF) staining was employed to assess the expression and localization of type I collagen and type III collagen, markers for ECM protein deposition, respectively. The preparation of the wax blocks and the slicing procedures was performed in strict accordance with the previously described methods. Paraffin-embedded corneal sections were subjected to dewaxing in xylene followed by rehydration through a graded ethanol series. After rehydration, the slides were immersed in 3% EDTA and heated in a microwave oven for antigen retrieval. Following natural cooling, the slides were washed three times with 0.1 M phosphate-buffered saline (PBS) and permeabilized with 0.3% Triton X-100 at room temperature for 10 minutes. To minimize non-specific binding, the slides were blocked with 30% normal goat serum in PBS for 1 hour. The slides were then incubated overnight at 4°C with anti-COL1A1 antibody (NB600-450, Mouse anti-Collagen I alpha 1; Novus) at 1:100 dilution in 1% BSA, anti-COL3A1 antibody (sc-271249, Mouse monoclonal antibody, 1:100, Santa Cruz) at 1:30 dilution in 1% bovine serum albumin (BSA). Slides were then incubated for 30 minutes with the secondary antibody (2284614, Alexa Fluor 488 goat anti-mouse antibody; Thermo Fisher Scientific) at dilution of 1:200 (COL1A1), and with the secondary antibody (4408S, Alexa Fluor 488 goat anti-mouse antibody; Cell Signaling Technology) at dilution of 1:50 (COL3A1). Then counterstained with 4',6-diamidino-2-phenylindole (DAPI) (C0065-10ml, Solarbio, China). Tissue sections were visualized using a Leica TCS SP8 microscope (Leica, Wetzlar, Germany) equipped with a Fluoview FV1000 confocal system (Olympus, Japan). Image analysis was performed using ImagePro software (Leica).

6 **Transmission Electron Microscopy (TEM)**

Corneal tissue processing followed previously established protocols.^[32] Briefly, specimens were fixed in a 2.5% glutaraldehyde and 4% paraformaldehyde mixture for 24 hours, followed by incubation in 1% osmium tetroxide for 2 hours at 4°C. Dehydration was achieved through a graded ethanol and acetone series. Subsequently, the central corneal block was embedded in epoxy resin and sectioned using an ultramicrotome (Leica EM UC7, Germany) to 1 μ m thickness. Toluidine blue staining facilitated the



identification of the corneal injury area. Ultrastructural changes in the corneal tissue during the repair process were observed under a transmission electron microscope (Hitachi H-7650, Japan). Additionally, scanning electron microscopy (TESCAN VEGA3, Czech Republic) was employed to capture micrographs of the tissue.

Protocol references

1. Wilson SE, Sampaio LP, Shiju TM, Hilgert GSL, de Oliveira RC. Corneal Opacity: Cell Biological Determinants of the Transition From Transparency to Transient Haze to Scarring Fibrosis, and Resolution, After Injury. *Investigative Ophthalmology & Visual Science*. 2022;63(1):22. doi: 10.1167/iovs.63.1.22.
2. Espana EM, Birk DE. Composition, structure and function of the corneal stroma. *Experimental Eye Research*. 2020;198:108137.doi: 10.1016/j.exer.2020.108137.
3. Bukowiecki A, Hos D, Cursiefen C, Eming S. Wound-Healing Studies in Cornea and Skin: Parallels, Differences and Opportunities. *International Journal of Molecular Sciences*. 2017;18(6):1257. doi: 10.3390/ijms18061257.
4. Ljubimov AV, Saghizadeh M. Progress in corneal wound healing. *Progress in Retinal and Eye Research*. 2015;49:17-45. doi: 10.1016/j.preteyeres.2015.07.002.
5. Kempuraj D, Mohan RR. Autophagy in Extracellular Matrix and Wound Healing Modulation in the Cornea. *Biomedicines*. 2022;10(2):339. doi: 10.3390/biomedicines10020339.
6. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nature Reviews Molecular Cell Biology*. 2014;15(12):786-801. doi: 10.1038/nrm3904.
7. Mutoji KN, Sun M, Elliott G, Moreno IY, Hughes C, Gesteira TF, et al. Extracellular Matrix Deposition and Remodeling after Corneal Alkali Burn in Mice. *International Journal of Molecular Sciences*. 2021;22(11):5708. doi: 10.3390/ijms22115708.
8. Bianchetti L, Barczyk M, Cardoso J, Schmidt M, Bellini A, Mattoli S. Extracellular matrix remodelling properties of human fibrocytes. *Journal of Cellular and Molecular Medicine*. 2012;16(3):483-495. doi: 10.1111/j.1582-4934.2011.01344.x.
9. García-de-Alba C, Becerril C, Ruiz V, González Y, Reyes S, García-Alvarez J, et al. Expression of Matrix Metalloproteases by Fibrocytes. *American Journal of Respiratory and Critical Care Medicine*. 2010;182(9):1144-1152. doi: 10.1164/rccm.201001-0028OC.
10. de Oliveira RC, Wilson SE. Fibrocytes, Wound Healing, and Corneal Fibrosis. *Investigative Ophthalmology & Visual Science*. 2020;61(2):28. doi: 10.1167/iovs.61.2.28.
11. Meng N, Wu J, Chen J, Luo Y, Xu L, Li X. Basement membrane regeneration and TGF- β 1 expression in rabbits with corneal perforating injury. *Molecular Vision*. 2023;29:58-67.
12. Cro S, Partington G, Cornelius VR, Banerjee PJ, Zvobgo TM, Casswell EJ, et al. Presenting clinical characteristics of open globe injuries in ocular trauma: baseline analysis of cases in the ASCOT national clinical trial. *Eye*. 2022;37(8):1732-1740. doi: 10.1038/s41433-022-02206-z.
13. Goodman WM, Raj NS, Garone M, Arffa RC, Thoft RA. Unique Parameters in the Healing of Linear Partial Thickness Penetrating Corneal Incisions in Rabbit: Immunohistochemical Evaluation. *Current Eye Research*. 2009;8(3):305-316. doi: 10.3109/02713688908997573.
14. Wilson SE. Fibrosis Is a Basement Membrane-Related Disease in the Cornea: Injury and Defective Regeneration of Basement Membranes May Underlie Fibrosis in Other Organs. *Cells*. 2022;11(2):309. doi: 10.3390/cells11020309.
15. Marino GK, Santhiago MR, Santhanam A, Torricelli AAM, Wilson SE. Regeneration of Defective Epithelial Basement Membrane and Restoration of Corneal Transparency After Photorefractive Keratectomy. *Journal of Refractive Surgery*. 2017;33(5):337-346. doi: 10.3928/1081597X-20170126-02.
16. Torricelli AAM, Singh V, Santhiago MR, Wilson SE. The Corneal Epithelial Basement Membrane: Structure, Function, and Disease. *Investigative Ophthalmology & Visual Science*. 2013;54(9):6250-6256. doi: 10.1167/iovs.13-12547.

17. Torricelli AAM, Singh V, Agrawal V, Santhiago MR, Wilson SE. Transmission Electron Microscopy Analysis of Epithelial Basement Membrane Repair in Rabbit Corneas With Haze. *Investigative Ophthalmology & Visual Science*. 2013;54(6):4026-4033. doi: 10.1167/iovs.13-12106.
18. Torricelli AAM, Santhanam A, Wu J, Singh V, Wilson SE. The corneal fibrosis response to epithelial–stromal injury. *Experimental Eye Research*. 2016;142:110-118. doi: 10.1016/j.exer.2014.09.012.
19. Wilson SE, Marino GK, Torricelli AAM, Medeiros CS. Injury and defective regeneration of the epithelial basement membrane in corneal fibrosis: A paradigm for fibrosis in other organs? *Matrix Biology*. 2017;64:17-26. doi: 10.1016/j.matbio.2017.06.003.
20. Sampaio LP, Shiju TM, Hilgert GSL, de Oliveira RC, DeDreu J, Menko AS, et al. Descemet's membrane injury and regeneration, and posterior corneal fibrosis, in rabbits. *Experimental Eye Research*. 2021;213:108803. doi: 10.1016/j.exer.2021.108803.
21. Medeiros CS, Saikia P, de Oliveira RC, Lassance L, Santhiago MR, Wilson SE. Descemet's Membrane Modulation of Posterior Corneal Fibrosis. *Investigative Ophthalmology & Visual Science*. 2019;60(9):3308-3316. doi: 10.1167/iovs.18-26451.
22. Wilson SE. The corneal fibroblast: The Dr. Jekyll underappreciated overseer of the responses to stromal injury. *The Ocular Surface*. 2023;29:53-62. doi: 10.1016/j.jtos.2023.04.012.
23. Saikia P, Medeiros CS, Thangavadivel S, Wilson SE. Basement membranes in the cornea and other organs that commonly develop fibrosis. *Cell and Tissue Research*. 2018;374(3):439-453. doi: 10.1007/s00441-018-2934-7.
24. de Oliveira RC, Tye G, Sampaio LP, Shiju TM, DeDreu J, Menko AS, et al. TGFβ1 and TGFβ2 proteins in corneas with and without stromal fibrosis: Delayed regeneration of apical epithelial growth factor barrier and the epithelial basement membrane in corneas with stromal fibrosis. *Experimental Eye Research*. 2021;202:108325. doi: 10.1016/j.exer.2020.108325.
25. Connon CJ, Meek KM. Organization of corneal collagen fibrils during the healing of trephined wounds in rabbits. *Wound Repair and Regeneration*. 2003;11(1):71-78. doi: 10.1046/j.1524-475X.2003.11110.x.
26. Fantes FE, Hanna KD, Waring GO III, Pouliquen Y, Thompson KP, Savoldelli M. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. *Archives of Ophthalmology*. 1990;108(5):665-675. doi: 10.1001/archopht.1990.01070070051034.
27. Connon CJ, Tandon A, Sharma A, Rodier JT, Klivanov AM, Rieger FG, et al. BMP7 Gene Transfer via Gold Nanoparticles into Stroma Inhibits Corneal Fibrosis In Vivo. *PLoS ONE*. 2013;8(6):e66434. doi: 10.1371/journal.pone.0066434.
28. Miosge N. The ultrastructural composition of basement membranes in vivo. *Histology and Histopathology*. 2001;16(4):1239-1248. doi: 10.14670/HH-16.1239.
29. Santhanam A, Marino GK, Torricelli AAM, Wilson SE. EBM regeneration and changes in EBM component mRNA expression in stromal cells after corneal injury. *Molecular Vision*. 2017;23:39-51. PMID: 28210236.
30. Liu R, Li J, Guo Z, Chu D, Li C, Shi L, et al. Celastrol Alleviates Corneal Stromal Fibrosis by Inhibiting TGF-β1/Smad2/3-YAP/TAZ Signaling After Descemet Stripping Endothelial Keratoplasty. *Investigative Ophthalmology & Visual Science*. 2023;64(3):9. doi: 10.1167/iovs.64.3.9.
31. Tang Y, Du E, Wang G, Qin F, Meng Z, Dai L, et al. A negative feedback loop centered on SMAD3 expression in transforming growth factor β1-induced corneal myofibroblast differentiation. *Experimental Eye Research*. 2023;236:109654. doi: 10.1016/j.exer.2023.109654.
32. Yam GHF, Riau AK, Funderburgh ML, Mehta JS, Jhanji V. Keratocyte biology. *Experimental Eye Research*. 2020;196:108062. doi: 10.1016/j.exer.2020.108062.

33. Wilson SE. Corneal myofibroblast biology and pathobiology: Generation, persistence, and transparency. *Experimental Eye Research*. 2012;99:78-88. doi: 10.1016/j.exer.2012.03.018.
34. Hassell JR, Birk DE. The molecular basis of corneal transparency. *Experimental Eye Research*. 2010;91(3):326-335. doi: 10.1016/j.exer.2010.06.021.
35. Ishizaki M, Shimoda M, Wakamatsu K, Ogro T, Yamanaka N, Kao CWC, et al. Stromal fibroblasts are associated with collagen IV in scar tissues of alkali-burned and lacerated corneas. *Current Eye Research*. 2009;16(4):339-348. doi: 10.1076/ceyr.16.4.339.10684.
36. Marino GK, Santhiago MR, Santhanam A, Lassance L, Thangavadivel S, Medeiros CS, et al. Epithelial basement membrane injury and regeneration modulates corneal fibrosis after pseudomonas corneal ulcers in rabbits. *Experimental Eye Research*. 2017;161:101-105. doi: 10.1016/j.exer.2017.05.003.
37. Medeiros CS, Lassance L, Saikia P, Santhiago MR, Wilson SE. Posterior stromal cell apoptosis triggered by mechanical endothelial injury and basement membrane component nidogen-1 production in the cornea. *Experimental Eye Research*. 2018;172:30-35. doi: 10.1016/j.exer.2018.03.025.
38. Sampaio LP, Hilgert GSL, Shiju TM, Santhiago MR, Wilson SE. Topical Losartan and Corticosteroid Additively Inhibit Corneal Stromal Myofibroblast Generation and Scarring Fibrosis After Alkali Burn Injury. *Translational Vision Science & Technology*. 2022;11(7):9. doi: 10.1167/tvst.11.7.9.
39. Gallego-Muñoz P, Lorenzo-Martín E, Fernández I, Herrero-Pérez C, Martínez-García MC. Nidogen-2: Location and expression during corneal wound healing. *Experimental Eye Research*. 2019;178:1-9. doi: 10.1016/j.exer.2018.09.004.
40. Santhanam A, Torricelli AAM, Wu J, Marino GK, Wilson SE. Differential expression of epithelial basement membrane components nidogens and perlecan in corneal stromal cells in vitro. *Molecular Vision*. 2015;21:1318-1327. PMID: 26692758.
41. Netto MV, Mohan RR, Sinha S, Sharma A, Dupps W, Wilson SE. Stromal haze, myofibroblasts, and surface irregularity after PRK. *Experimental Eye Research*. 2006;82(5):788-797. doi:10.1016/j.exer.2005.09.021.
42. Wilson SE. Corneal wound healing. *Experimental Eye Research*. 2020;197:108089. doi:10.1016/j.exer.2020.108089.
43. de Oliveira RC, Wilson SE. Descemet's membrane development, structure, function and regeneration. *Experimental Eye Research*. 2020;197:108090. doi:10.1016/j.exer.2020.108090.
44. Lassance L, Marino GK, Medeiros CS, Thangavadivel S, Wilson SE. Fibrocyte migration, differentiation and apoptosis during the corneal wound healing response to injury. *Experimental Eye Research*. 2018;170:177-187. doi:10.1016/j.exer.2018.02.018.
45. Galligan CL, Fish EN. The role of circulating fibrocytes in inflammation and autoimmunity. *Journal of Leukocyte Biology*. 2013;93(1):45-50. doi:10.1189/jlb.0712365.
46. Gallego-Muñoz P, Ibares-Frías L, Valsero-Blanco MC, et al. Effects of TGFβ1, PDGF-BB, and bFGF on human corneal fibroblasts proliferation and differentiation during stromal repair. *Cytokine*. 2017;96:94-101. doi:10.1016/j.cyto.2017.03.011.
47. Wilson SE. Corneal myofibroblasts and fibrosis. *Experimental Eye Research*. 2020;201:108272. doi:10.1016/j.exer.2020.108272.
48. Barbosa FL, Chaurasia SS, Cutler A, et al. Corneal myofibroblast generation from bone marrow-derived cells. *Experimental Eye Research*. 2010;91(1):92-96. doi:10.1016/j.exer.2010.04.007.
49. Medeiros CS, Marino GK, Santhiago MR, Wilson SE. The Corneal Basement Membranes and Stromal Fibrosis. *Investigative Ophthalmology & Visual Science*. 2018;59(8):4044-4053. doi:10.1167/iovs.18-24428.

50. de Oliveira RC, Sampaio LP, Shiju TM, Santhiago MR, Wilson SE. Epithelial Basement Membrane Regeneration After PRK-Induced Epithelial-Stromal Injury in Rabbits: Fibrotic Versus Non-fibrotic Corneal Healing. *Journal of Refractive Surgery*. 2022;38(1):50-60. doi:10.3928/1081597X-20211007-02.
51. Göhring W, Sasaki T, Heldin C-H, Timpl R. Mapping of the binding of platelet-derived growth factor to distinct domains of the basement membrane proteins BM-40 and perlecan and distinction from the BM-40 collagen-binding epitope. *European Journal of Biochemistry*. 1998;255(1):60-66. doi:10.1046/j.1432-1327.1998.2550060.x.
52. Wilson SE. The Cornea: No Difference in the Wound Healing Response to Injury Related to Whether, or Not, There's a Bowman's Layer. *Biomolecules*. 2023;13(5):771. doi:10.3390/biom13050771.
53. Lipshitz I, Loewenstein A, Varssano D, Lazar M. Late Onset Corneal Haze after Photorefractive Keratectomy for Moderate and High Myopia. *Ophthalmology*. 1997;104(3):369-374. doi:10.1016/S0161-6420(97)30306-6.
- Mohan RR, Hutcheon AEK, Choi R, et al. Apoptosis, necrosis, proliferation, and myofibroblast generation in the stroma following LASIK and PRK. *Experimental Eye Research*. 2003;76(1):71-87. doi:10.1016/S0014-4835(02)00278

Acknowledgements

We are grateful to Professor Xia Li for her invaluable guidance in article writing. We also extend our appreciation to

Yuqing Luo and Jinling Wu for their technical assistance, Luxing Xu for his transmission electron microscopy expertise, Na Meng for her assistance with data organization, and Luting Xie for her immunofluorescence technical support. We acknowledge the co-equal contributions of Jingjing Chen and Yuqing Luo to this article.