Clinicopathologic Correlations of Retrocorneal Membranes Associated With Endothelial Corneal Graft Failure

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• PURPOSE: To provide clinicopathologic correlations for retrocorneal membranes associated with Descemet stripping automated endothelial keratoplasty (DSAEK) failure.

• DESIGN: Retrospective case series.

• METHODS: The specimens and medical records of the patients diagnosed with clinically significant retrocorneal membranes associated with DSAEK failure at the Bascom Palmer Eye Institute or the University of Miami Veterans Hospital between October 2015 and March 2020 were reviewed for demographics, clinical presentation, comorbidities, and surgeries performed. Histopathologic analysis was performed on hematoxylin-eosin and periodic acid–Schiff sections. Immunohistochemical studies were performed for smooth muscle actin (α-SMA), pancytokeratin, and CK7. Immunofluorescence was performed for vimentin, N-cadherin, ROCK1, RhoA, ZEB1, and Snail.

• RESULTS: A total of 7 patients (3 male and 4 female) were identified to have a clinically significant retrocorneal membranes at the time of graft failure. The average age at the time of first DSAEK was 70 years (range: 55-85 years). All patients were pseudophakic and had a glaucoma drainage device in place; 1 had a history of failed DSAEK. Ranging from 0 to 47 months after surgery, a variably thick retrocorneal fibrous membrane was observed, eventually leading to graft failure. Four patients underwent subsequent penetrating keratoplasty and 3 underwent repeat DSAEK. On histopathologic evaluation, a pigmented fibrocellular tissue was identified along the posterior margin of the corneas and DSAEK buttons in all cases. Further characterization with immunohistochemistry and immunofluorescence demonstrated membranes to be negative for pancytokeratin and positive for α-SMA, vimentin, CK7, N-cadherin, ZEB1, Snail, ROCK1, and RhoA.

• CONCLUSIONS: Fibrocellular retrocorneal membrane proliferation may be associated with DSAEK failure in patients with previous glaucoma drainage device surgery. Our results demonstrate myofibroblastic differentiation and a lack of epithelial differentiation. Positivity for markers of an endothelial-to-mesenchymal transition indicates possible endothelial origin and could be the hallmark for future targeted pharmacotherapy. (Am J Ophthalmol 2021;222:24–33. Published by Elsevier Inc.)

DESCEMET STRIPPING AUTOMATED ENDOTHELIAL keratoplasty (DSAEK) has become one of the preferred techniques for patients with endothelial cell dysfunction and severe anterior segment disease.[1,2] It has gained popularity owing to the decreased risk of rejection and more predictable refractive outcomes when compared to penetrating keratoplasty.[3] However, despite its popularity, complications occur that can lead to graft failure, including graft detachment, allograft rejection, and infection. Histopathologic findings in failed DSAEK grafts vary and include endothelial attenuation, retained host Descemet membrane, and/or fibrocellular membranes. These fibrocellular membranes can be found in the interface between the DSAEK graft and the host anterior stroma or posterior to the graft's Descemet membrane; the latter of these are termed retrocorneal membranes.[4,5]

Retrocorneal membranes were first described in 1901 by Fuchs as a complication of penetrating keratoplasty (PKP) and were postulated to occur secondary to iritis.[6] Further histopathologic studies demonstrated that up to 50% of failed PKP have tissue behind the Descemet membrane, most often being fibrocellular in nature.[6,7] Postulated causes of retrocorneal membranes include epithelial downgrowth, keratocyte ingrowth, and endothelial cell metaplasia.[2,8,9] Most retrocorneal membranes are not appreciated clinically and are identified solely on histopathologic analysis.

Retrocorneal membranes have been described to a lesser extent in the setting of DSAEK, but a few studies have reported on retrocorneal membranes in DSAEK and Descemet membrane endothelial keratoplasty (DMEK).[10–12] In

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1 retrospective study, histopathologic examination with light microscopy of failed grafts found retrocorneal fibrous membranes in 4 of 13 cases (31%) of early failed DSAEK grafts.[2] One case report described a clinically significant retrocorneal membrane causing DSAEK failure 1 month after surgery in a 76-year-old woman. In this case, peripheral anterior synechiae were noted with a retrocorneal membrane extending from the iris to the cornea, with loss of anterior chamber volume demonstrated on anterior segment optical coherence tomography. Unfortunately, no histopathologic analysis was performed, as the patient did not undergo further surgery.[10] The mechanisms underlying DSAEK-associated retrocorneal membranes are postulated to be similar to those found in PKP, with epithelial downgrowth, keratocyte ingrowth, or/and fibrous metaplasia of the corneal endothelium as the main hypothetical contributors to the formation of these membranes.[12–14]

We identified a subset of patients who presented with clinically significant, progressive retrocorneal membranes that led to DSAEK failure. Given the limited data on the nature of retrocorneal membranes in DSAEK, the purpose of this study was to perform a clinicopathologic assessment and characterization of these membranes with the goal of identifying their source and providing a rationale for future targeted pharmacotherapy.

METHODS

• **STUDY POPULATION:** Under approval of the institutional review boards of the University of Miami and the Miami Veterans Affairs Hospital, specimens and medical records of the patients diagnosed with clinically significant, progressive retrocorneal membranes associated with DSAEK failure at the Bascom Palmer Eye Institute and the University of Miami Veterans Hospital between October 2015 and March 2020 were reviewed. Patients were identified based on pathology records from the Florida Lions Ocular Pathology Laboratory at the Bascom Palmer Eye Institute. Data extracted from the clinical record included demographics, clinical presentation, comorbidities, and surgeries performed.

• **HISTOLOGIC ANALYSIS:** Three mechanisms have been proposed for retrocorneal membrane formation: epithelial downgrowth, keratocyte (fibroblastic) ingrowth, and/or fibrous metaplasia of the corneal endothelium.[12–14] We thus chose our immunohistochemistry and immunofluorescence probes to evaluate each of these possibilities.

Epithelial downgrowth is characterized by 1-3 layers of stratified nonkeratinized squamous epithelium extending over the posterior cornea.[15] These cells are cytokeratin positive[16] and thus, we performed immunohistochemistry for pancytokeratin to evaluate an epithelial derivation of the membranes.

Unfortunately, there are no unique markers to discern stromal and endothelial origins, but immunoreactivity for a combination of markers can be highly suggestive of a cell of origin within these lineages. Keratocytes express CD34 under normal physiologic conditions; however, this marker is lost when cells are perturbed and thus this marker was not tested for in our membranes.[14] Following injury, keratocytes can transform into either fibroblasts or myofibroblasts,[17] the latter of which leads to contraction and scarring as part of the normal wound-healing process. Myofibroblasts express alpha-smooth muscle actin (α-SMA), an element of the contractile unit in cells, which mediate pseudopodia retraction,[18,19] and vimentin, an intermediate filament that strengthens and maintains the integrity of the myofibroblast cell body.[20]

However, as with keratocytes, endothelial cells can also undergo a transformation toward mesenchymal phenotypes, including myofibroblasts, and thus α-SMA and vimentin positivity alone cannot differentiate between a keratocyte[21] or endothelial cell of origin.[14] The process whereby an endothelial cell changes in phenotype towards a mesenchymal cell is termed endothelial-to-mesenchymal transition (EndoMT)[17] and has been mostly described in vitro with cultured corneal endothelial cells (CECs).[22–24] During this process, the endothelial cells change morphology and acquire a fibroblastic, spindle-like appearance.[24]

Numerous extracellular signals have been experimentally used to drive EndoMT, including transforming growth factor-β (TGF-β). TGF-β stimulation leads to activation of the transcription factors Snail and ZEB1,[25] which subsequently drive the phenotypic changes characteristic of EndoMT, such as disassembly of tight and gap junctions and reversal of endothelial cell polarity. Molecularly, expression of E-cadherin[25–27] is reduced during EndoMT, while N-cadherin is upregulated.[28,29] ZEB1 activation also upregulates α-SMA and vimentin.[25,27,30] Another pathway activated by TGF-β is the GTPase RhoA[31] and its downstream effector, ROCK1,[24,32] which are involved in actin cytoskeletal reorganization and mediate the extension of cell projections and stress fiber formation (contractile actin bundles).[24,31,33] We thus evaluated the immunofluorescence of ZEB1, Snail, N-cadherin, RhoA, and ROCK1 as evidence of an activated EndoMT process in the membranes. Finally, as CK7 has been described to be positive in diseased corneal endothelium, we performed CK7 staining to confirm endothelial metaplasia.[14,34]

Microscopic glass slides prepared from paraffin-embedded tissue sections were stained with hematoxylin-eosin and periodic acid–Schiff. Immunohistochemical studies were performed in the Immunohistochemistry Department of the University of Miami for pancytokeratin, CK7, and α-SMA. Immunohistochemistry was performed by incubating samples in α-SMA (Catalog #PA0943;

Leica, Buffalo Grove, Illinois, USA), CK7 (Catalog #PA0942; Leica), or pancytokeratin cocktail consisting of AE1/AE3 (1:200; Dako, Santa Clara, California, USA), Cam 5.2 (1:1500; Becton Dickinson, Franklin Lakes, New Jersey, USA), and HMW (1:50; Dako) for 15 minutes; post primary for 8 minutes; polymer for 8 minutes; peroxide block for 8 minutes; and red chromogen for 10 minutes; and counterstaining with hematoxylin for 10 minutes. Pretreatment with Epitope Retrieval Solution 1 (Leica) low pH 6 was performed for 20 minutes for CK7 and pancytokeratin.

For immunofluorescence analysis, paraffin-embedded sections were initially deparaffinized using xylene, followed by rehydration in serial alcohol dilutions. Afterward, antigen retrieval with citrate buffer, permeabilization, and blocking were performed. The samples were incubated overnight with primary antibody (rabbit anti-ZEB1 primary antibody, 1:50; Santa Cruz, Dallas, Texas, USA), rabbit anti-Snail primary antibody (1:50; Cell Signaling, Danvers, Massachusetts, USA), rabbit anti-N-cadherin primary antibody (1:100; Abcam, Cambridge, Massachusetts, USA), mouse anti-vimentin primary antibody (1:100; Santa Cruz), mouse anti-RhoA primary antibody (1:50; Santa Cruz), or mouse anti-ROCK1 primary antibody (1:50; Santa Cruz). Samples were then washed 3 times in phosphate-buffered saline and incubated for 2 hours in the appropriate secondary antibody (goat anti-rabbit AlexaFluor594, donkey anti-mouse AlexaFluor488, or donkey anti-mouse AlexaFluor594 secondary antibody [1:200; Abcam]). Samples were washed 3 times with phosphate-buffered saline and counterstained with 1× PureBlu DAPI (BioRad, Hercules, California, USA) for 20 minutes, and mounted. Slides were then imaged using a laser scanning confocal microscope (Leica SP8; Leica Microsystems, Buffalo Grove, Illinois, USA).

RESULTS

A TOTAL OF 7 PATIENTS WERE IDENTIFIED WITH CLINICALLY significant progressive retrocorneal membranes that eventually led to DSAEK graft failure. Three individuals were male and 4 were female (Table). The average age at time of initial DSAEK was 70 years (range 55-85 years). At the time of initial DSAEK, all patients were pseudophakic: 1 with anterior chamber intraocular lenses (IOLs) and 6 with posterior chamber IOLs, 4 in bag and 2 scleral fixated. One patient had a history of previous DSAEK. All patients had a history of glaucoma, 5 had primary open-angle glaucoma (POAG), one had neovascular glaucoma, and 1 had chronic angle-closure glaucoma. All patients had a history of glaucoma drainage device surgery. Five had 1 Baerveldt implant with the tube in the anterior chamber, 1 had both a Baerveldt and an Ahmed implant with the tubes in the anterior chamber, and 1 had a Molteno3 implant with the tube in the vitreous cavity.

TABLE. Demographics and Characteristics of Failed Descemet Stripping Automated Endothelial Keratoplasty Caused by Retrocorneal Membranes

Patient No.	Sex	Age (Years)	Race	Ethnicity	Preoperative Diagnosis	Intraocular Lens at Time of DSAEK	Glaucoma Implant at Time of DSAEK	Time to Graft Failure (Months)	Second Procedure
1	F	55	W	H	Failed DSAEK	PCIOL (bag)	Baerveldt (tube in AC)	3	DSAEK
2	F	66	B	NH	Corneal edema	PCIOL (bag)	Baerveldt (tube in AC)	5	PKP
3	M	54	B	NH	Corneal edema	PCIOL (bag)	Baerveldt (tube in AC), CPC	10	PKP, removal of lens, shunt revision, vitrectomy
4	M	69	B	NH	Corneal edema	ACIOL	Baerveldt (tube in AC)	12	PKP, ACIOL removal + sutured PCIOL, shunt revision, vitrectomy
5	F	78	W	NH	Corneal edema	PCIOL (scleral fixated)	Ahmed, Baerveldt (tubes in AC)	7	DSAEK
6	M	81	W	NH	Corneal edema	AKREOS scleral sutured lens	Molteno3 (tube in vitreous)	1	PKP
7	F	85	W	H	Corneal edema	PCIOL (bag)	Baerveldt (tube in AC)	47	DSAEK

AC = anterior chamber; ACIOL = anterior chamber intraocular lens; B = black; CPC = cyclophotocoagulation; DSAEK = Descemet stripping automated endothelial keratoplasty; H = Hispanic; NH = Non-Hispanic; PCIOL = posterior chamber intraocular lens; PKP = penetrating keratoplasty; W = white.

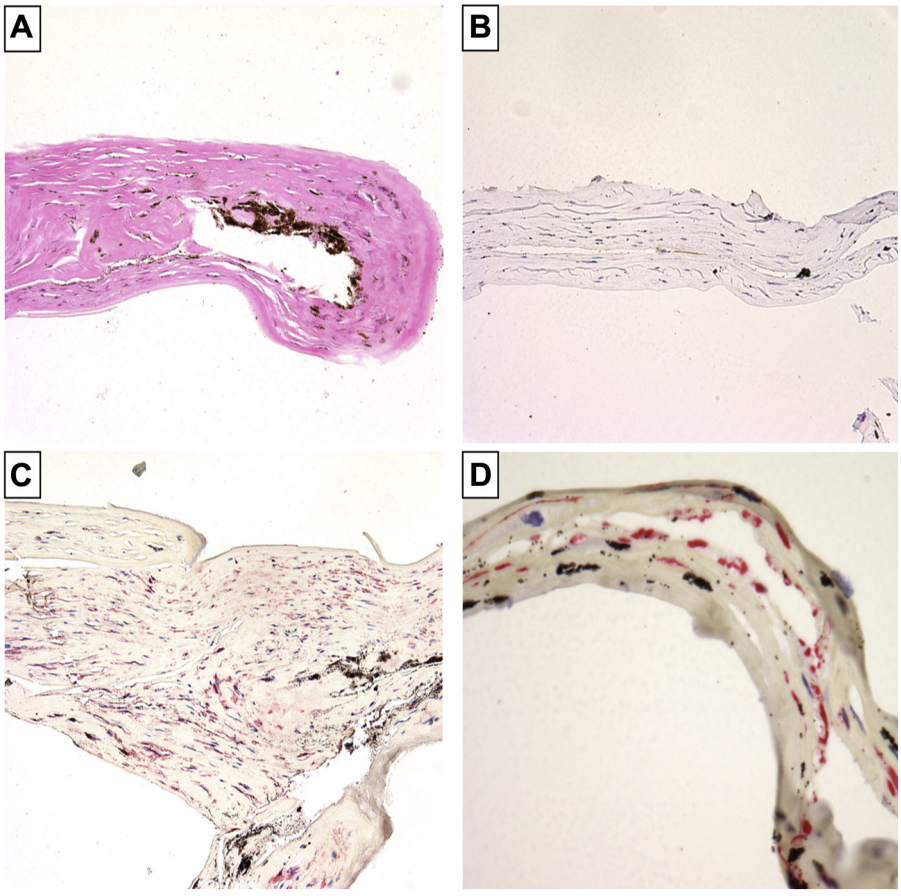

FIGURE 1. A. Pigmented fibrocellular tissue (Hematoxylin-eosin, Original magnification x 200). B. Pancytokeratin negative fibrocellular tissue (Pancytokeratin, Original magnification ×200). C. Fibrocellular tissue with myofibroblastic differentiation (Smooth muscle actin (SMA), Original magnification ×200). D. Cytokeratin 7 (CK7) positive fibrocellular tissue (CK7, Original magnification ×400)

Initial postoperative visits showed a clear graft, with no signs of infection. However, between 0 and 47 months after DSAEK surgery, a variably thick retrocorneal fibrous membrane was observed which proliferated and eventually led to graft failure. After graft failure, 4 patients underwent penetrating keratoplasty and 3 underwent repeat DSAEK. The membranes were removed during surgery and sent for histopathologic evaluation. During repeat DSAEK or PKP, 2 patients underwent concurrent procedures, including vitrectomy, tube repositioning, and IOL removal.

On histopathologic evaluation, a pigmented fibrocellular tissue was identified along the posterior margin of the corneas and DSAEK buttons in all cases on hematoxylin-eosin (Figure 1, A). Further characterization and immunohistochemical studies demonstrated all membranes to be negative for pancytokeratin (Figure 1, B) and positive for α-SMA (Figure 1, C). Four of the membranes demonstrated positivity for CK7 (Figure 1, D) and 3 were noncontributory. Immunofluorescence showed all membranes to be positive for vimentin, N-cadherin, ZEB1, Snail, ROCK1, and RhoA (Figure 2).

Following are representative case narratives of 3 of the 7 individuals.

• **CASE 1:** A 56-year-old man with a history of cataract extraction (CE) with posterior IOL placement and POAG, treated with Baerveldt tube and cyclophotocoagulation, presented with corneal edema (Figure 3, A). A DSAEK was performed and, simultaneously, a second Baerveldt implant was placed with both tubes positioned in the sulcus. After 8 months, a dense fibrovascular membrane was noted that covered the anterior IOL and involved the inferior cornea and iris, with peripheral anterior synechiae (PAS) (Figure 3, B). However, the central cornea was still clear. An Nd:YAG laser was used to open the anterior lens capsule in an attempt to clear the membrane. Within 2 months, the cornea became cloudy with progressive fibrosis that caused further contraction of the iris onto the cornea (Figure 3, C). As such, a full vitrectomy was performed under a temporary keratoprosthesis; the tubes were repositioned into the vitreous cavity; the IOL, membranes, and involved iris were removed; and the graft was

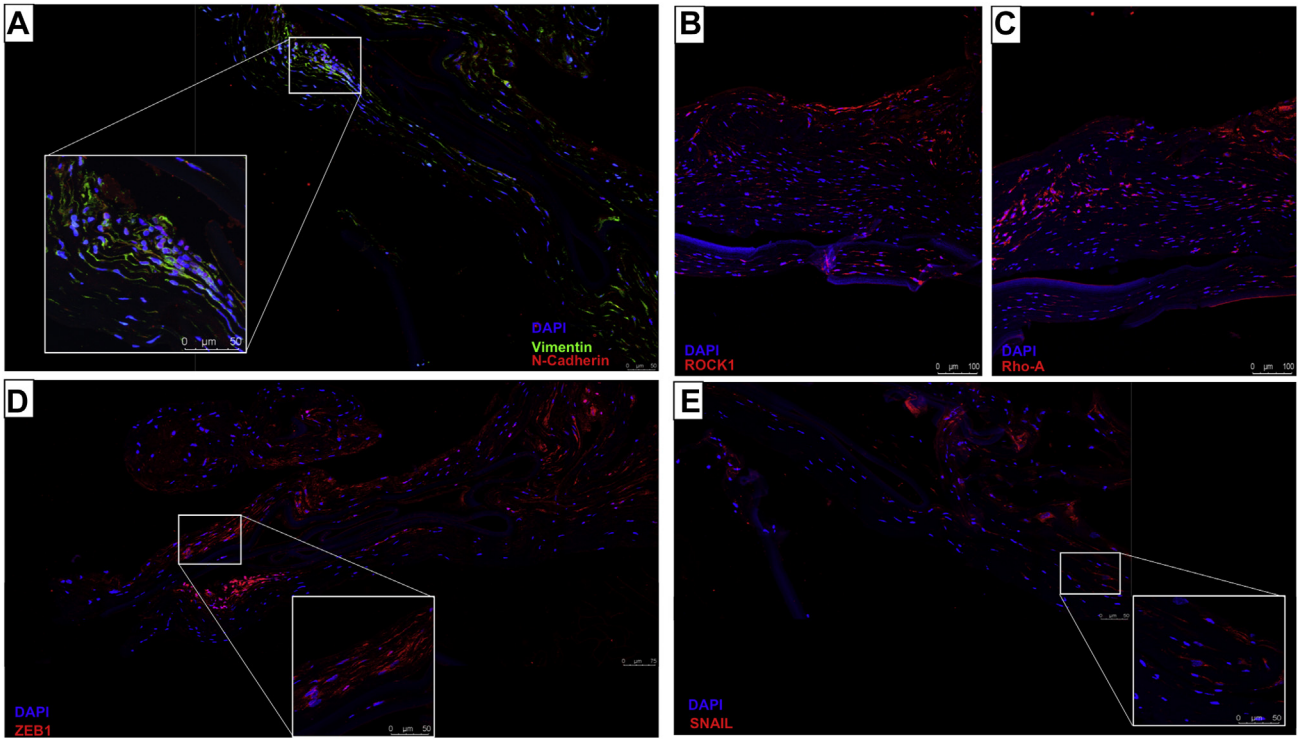

FIGURE 2. Retrocorneal membrane immunofluorescence on paraffin-embedded sections. Membranes demonstrate positivity for Vimentin (A), N-Cadherin (A), Rock1 (B), Rho-A (C), Zeb1(D), and Snail (E). All nuclei were counterstained with DAPI (blue).

sewn into place. The graft was initially clear (Figure 3, D and E) but eventually failed 2.5 years after PKP (Figure 1, F), with no signs of recurrent membranes.

• **CASE 2:** A 69-year-old man with a history of complex CE necessitating an anterior chamber IOL (ACIOL) and POAG treated with a Baerveldt drainage device and anterior chamber tube (Figure 4, A) presented with corneal edema 17 months after cataract surgery (Figure 4, B). Eight months later, a DSAEK was performed owing to worsening edema. Three months after DSAEK, a fibrous membrane was noted on the ACIOL (Figure 4, C) but the angle was still open. Six months after DSAEK, the angle was noted to be closed inferiorly with new PAS but the central cornea remained clear (Figure 4, D). However, 1 year after DSAEK, the cornea became opaque and the lens, iris, and cornea were all contracted owing to a proliferative membrane with 360-degree PAS (Figure 4, E) As such, a full vitrectomy was performed under a temporary keratoprosthesis, the ACIOL was removed, the glaucoma tube was moved to the vitreous cavity, the membranes were peeled off the iris, a 3-piece acrylic lens was sutured to the iris, and the graft was sewn into place. One year post PKP, the corneal graft remained clear, with no signs of recurrent membranes (Figure 4, F).

• **CASE 3:** An 81-year-old man with a history of complex CE necessitating an ACIOL followed by retinal detachment repair developed iris neovascularization and underwent a Molteno implant with the tube placed in the vitreous cavity. One year later, corneal edema was noted (Figure 5, A) and the ACIOL was removed and an intraocular lens was sutured to the sclera (Figure 5, B). Eight months later, a DSAEK was performed and intraoperatively, thick membranes were found that connected the endothelium, angle, and iris superiorly. During their removal bleeding occurred, with residual blood in the anterior chamber at the time of graft placement (Figure 5, C). The graft was initially attached but a month later was found to be completely detached (Figure 5, D). Given our past experience with retrocorneal membranes as a poor prognostic sign for long-term DSAEK survival, we chose to proceed with PKP instead of attempting to rebubble the graft. As such, 3 months after DSAEK, a PKP was performed along with membrane and superior iris removal (Figure 5, E). Six months later the graft remains clear, with no recurrent membranes noted (Figure 5, F).

DISCUSSION

ENDOTHELIAL KERATOPLASTY PERMITS SELECTIVE replacement of diseased corneal endothelium.[35] This allows for earlier visual recovery, earlier refractive stability, more predictable postoperative refractive outcomes,

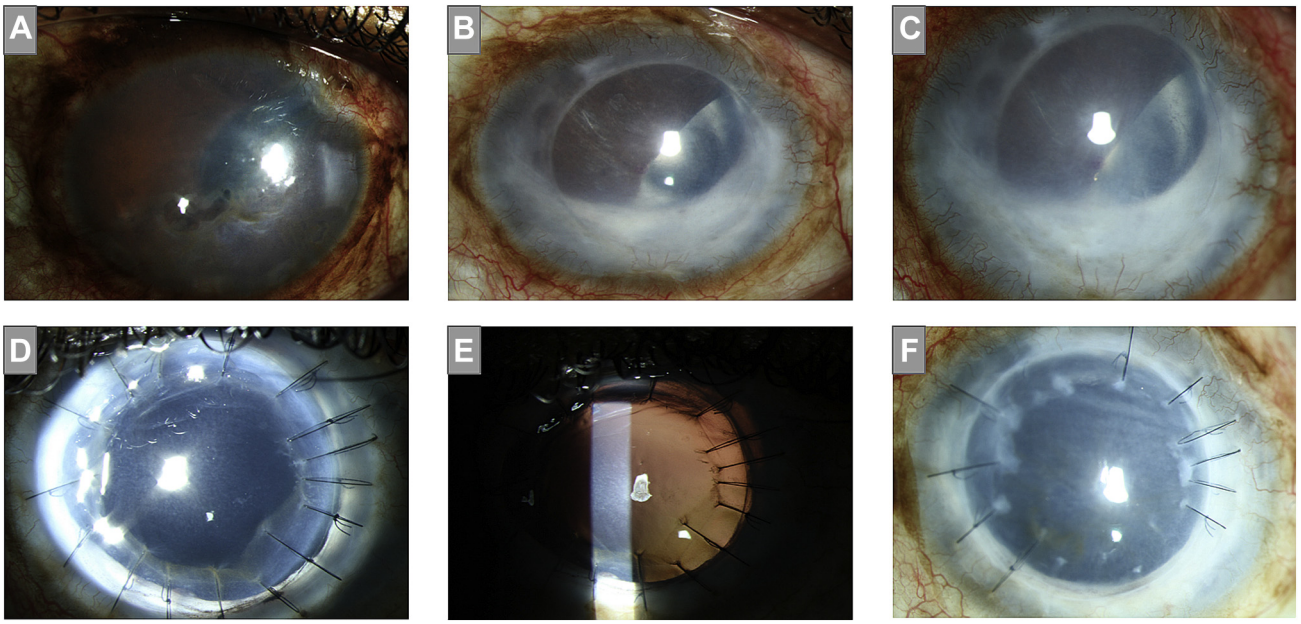

FIGURE 3. A. Slit-lamp picture demonstrating corneal edema in a 56-year-old male with a clinical history of primary open-angle glaucoma (POAG) treated with Baerveldt implant and cyclophotocoagulation. B. A Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) was performed and a dense fibrovascular membrane was noted eight months after DSAEK. C. Within two months, the cornea became cloudy with progressive fibrosis causing contraction of the iris onto the cornea. D-E. A penetrating keratoplasty (PKP) was performed and tubes were repositioned into the vitreous. F. The graft eventually failed 2.5 years after PKP with no signs of recurrent membranes.

avoidance of wound- and suture-related complications, shorter surgical time, easier postoperative follow-up and reduced risk of intraoperative and late suprachoroidal hemorrhage.[36] Despite the advantages of DSAEK, studies have reported a 5% frequency of graft failure (range 0%-29%).[36] The main causes of graft failure include graft dislocation, endothelial rejection, and primary graft failure.[4,37] In our series, we identified a poorly described cause of graft failure, namely progressive anterior chamber membranes leading to formation of PAS and, ultimately, DSAEK failure.

Interestingly, all individuals had a history of prior surgery, including placement of a glaucoma drainage device and cataract extraction. It is well known that eyes with glaucoma drainage devices have a worse prognosis after DSAEK than eyes without glaucoma drainage devices. Even when surgery is successful, grafts in eyes with glaucoma drainage devices typically fail within 3-5 years.[38] Endothelial damage owing to mechanical stress; increased blood-aqueous permeability to oxidative, apoptotic, and inflammatory proteins; and nutritional depletion are thought to underlie this clinical finding.[39]

Regardless of etiology for failure, the most common histopathologic finding in failed DSAEK grafts is endothelial cell loss,[35,40] and this was seen in all of our specimens. Additionally, histopathologic analysis with hematoxylin-eosin demonstrated a pigmented fibrocellular membrane with elongated, spindle-shaped cells that varied in thickness and cellularity on the posterior surface of the DSAEK in all cases. Some membranes were adhered to the button and some were detached. All membranes were positive for the same markers and thus all appear to be derived from the same cell of origin and/or pathologic process.

None of the specimens were positive for pancytokeratin, and thus an epithelial origin could be ruled out. On the other hand, the membranes were all α-SMA and vimentin positive, indicating a myofibroblastic and mesenchymal nature of these membranes. Previous studies on retrocorneal membranes have found similar results. In an interventional cases series, histopathologic analysis was performed on corneal buttons removed at the time of secondary PKP in 2 cases of primary graft failure after DMEK (PKP performed 6 months post DMEK). Hematoxylin-eosin staining revealed a retrocorneal membrane composed of collagen and elongated fibroblast-like cells, which was positive for α-SMA.[11] A similar finding was described in a series of 11 eyes with fibrous retrocorneal membranes associated with perforating injury and ulceration studied by light and electron microscopy. On histopathology, spindle-shaped cells consistent with myofibroblasts were identified, and electron microscopy showed the presence of fibroblasts and myofibroblasts. The retrocorneal membranes were positive for α-SMA and vimentin, indicating a myofibroblastic identity.[21]

Our novel contribution to the field is in the evaluation for markers of an EndoMT process in these membranes.

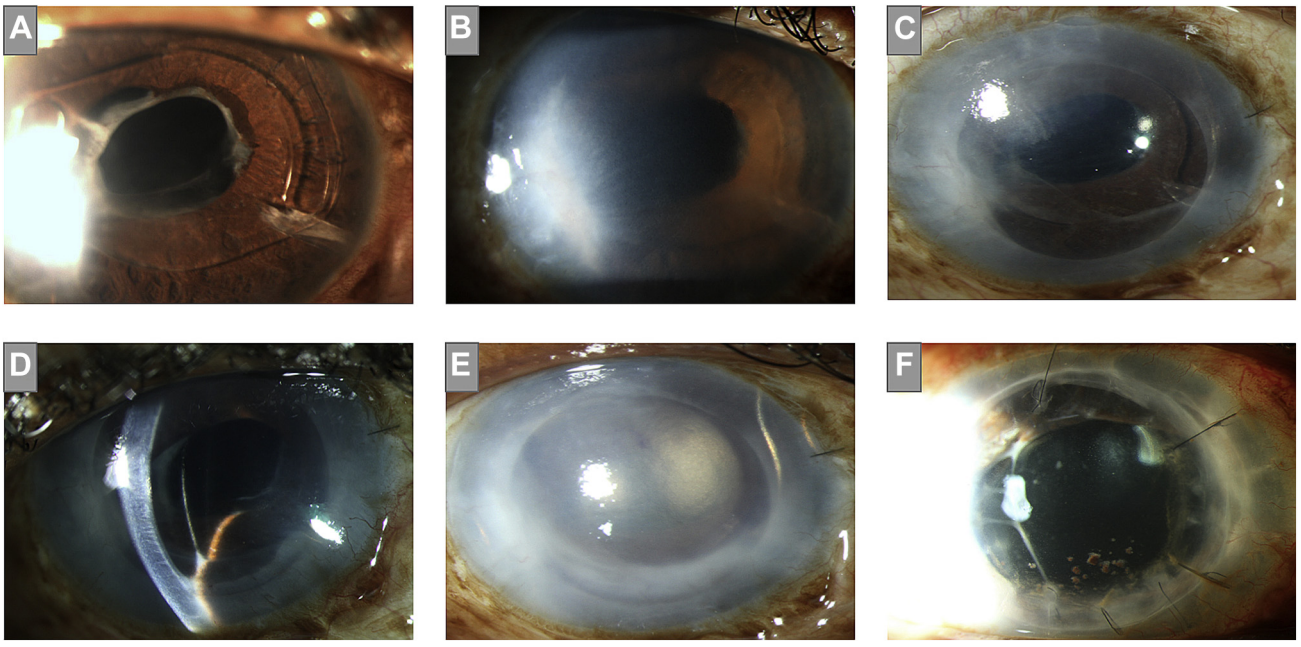

FIGURE 4. A. Slit-lamp picture of a 69-year-old male with a history of complex cataract extraction (CE), anterior chamber intraocular lens (ACIOL) and POAG treated with a Baerveldt drainage device and anterior chamber tube. B) Corneal edema was noted 17 months after cataract surgery. C. DSAEK was performed due to worsening edema and three months post-DSAEK, a fibrous membrane was noted. C) Six months after DSAEK, the angle was noted to be closed inferiorly with new PAS. D. One year after DSAEK, the cornea started to become opaque. E. The proliferation of the membrane eventually caused the lens, iris and cornea to contract together. F) One-year post-PKP, the corneal graft remained clear with no signs of recurrent membranes.

In fact, all of the membranes in our cohort stained positive for Snail, ZEB1, N-cadherin, RhoA, and Rock1. This suggests that the myofibroblastic membranes originate from remnant host endothelial cells that undergo a mesenchymal transformation. A similar study was performed by Jakobiec and Bhat on the histopathology of retrocorneal membranes of failed grafts (32 PKP, 6 DSAEK). Their group also stained for α-SMA, vimentin, and CK7 as markers of endothelial origin.[14] In their study, they identified 5 different membranes: epithelial, keratocytic, endothelial metaplasia, indeterminate, and mixed. The keratocytic membranes were thicker and were positive for α-SMA and vimentin, while negative for CK7, whereas thinner membranes that were positive for α-SMA, vimentin, and CK7 were considered of endothelial origin.[14] In comparison, our membranes were of similar thickness to the membranes Jakobiec deemed to have a keratocytic origin.

Based on prior and current findings, we postulate that multiple mechanisms may contribute to the observed membranes, including EndoMT and/or keratocytic fibrous downgrowth. However, the positivity for EndoMT markers and CK7 seen in our study leads us to postulate that EndoMT is an important contributor to membrane formation. Furthermore, as all retrocorneal membranes were clinically observed to start in the periphery and extend centrally, we hypothesize that the membranes originate from host cells, compromise the angle and iris, and proliferate toward the center of the cornea, causing graft failure. Unfortunately, on histopathology, we cannot identify the exact location of the membranes in relation to the cornea, as many membranes separate during specimen processing and thus their original position cannot be determined with certainty.

It is interesting that all individuals in our series had glaucoma drainage devices, and we postulate that their presence may be a risk for retrocorneal membrane formation. That is because myofibroblastic differentiation can be driven by a range of molecules, including TGF-β, inflammatory cytokines, and oxidative stress proteins.[41,42] It is well described that individuals with glaucoma drainage devices have increased pro-inflammatory cytokines and oxidative stress markers in their aqueous humor.[39] The combination of an inflammatory milieu, coupled with the stress of DSAEK, exposure of stroma, and damage to adjacent endothelial cells via the Descemet stripping procedure, may constitute the ideal context for endothelial metaplasia and membrane formation. Iris injury and damage that may occur at the time of DSAEK may have also contributed to increased anterior chamber cytokine levels and, thus, membrane formation.[43] Additionally, we believe the iris contributed the pigment present in these membranes as a result of direct injury or via iridocorneal adhesion after membrane formation.

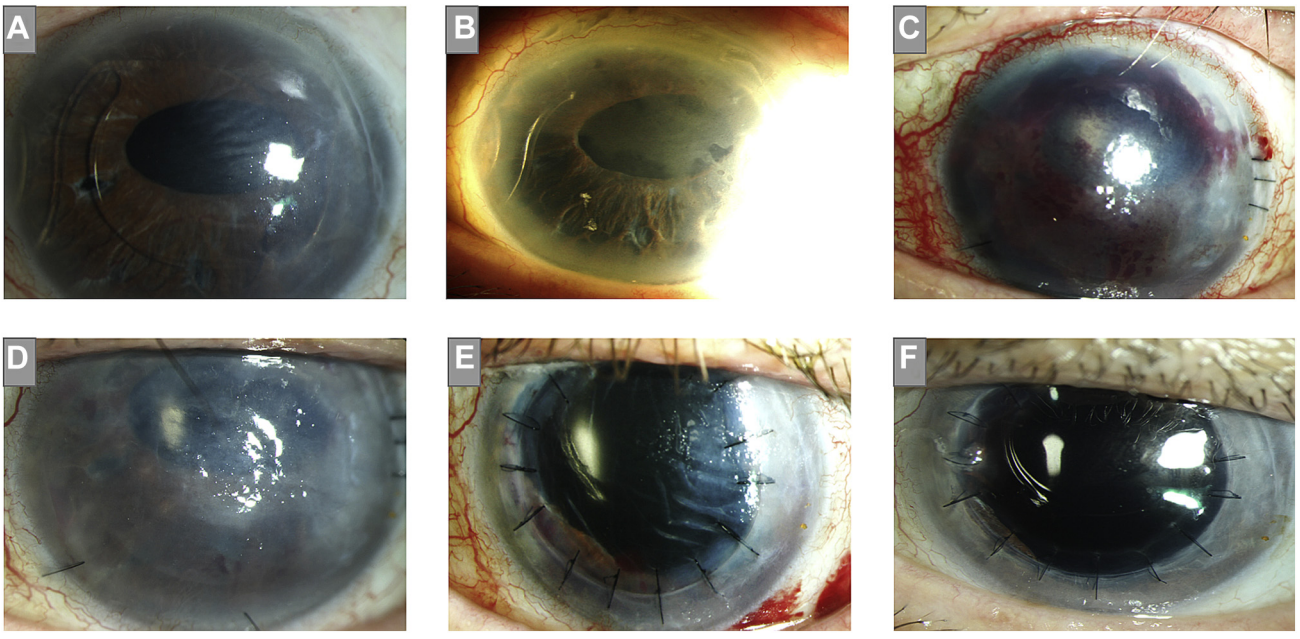

FIGURE 5. A. Slit-lamp picture of an 81-year-old man with a history of complex CE, ACIOL and Molteno implant with the tube placed in the vitreous cavity with corneal edema one year after surgery. B. ACIOL was removed and an intraocular lens was sutured to the sclera. C. Eight months later, a DSAEK was performed and a thick membrane that connected the iris, corneal endothelium and angle was removed. D. Graft detached one month later and recurrent membranes in the anterior chamber were noted. E. Three months after DSAEK, a PKP was performed along with membrane and superior iris removal. F. Graft remains clear with no recurrent membranes noted at six months follow up.

The findings of this study should be considered within the constraints of its limitations, which include a limited number of cases and defined histopathologic markers. Despite these limitations, our findings set the ground for future targeted pharmacotherapy in retrocorneal membranes. The fact that our cells were positive for markers of EndoMT indicate that this pathway may be manipulated therapeutically. Animal models have demonstrated that a RhoA/ROCK1 pathway inhibitor, Y27632, promotes CEC adhesion and preserves endothelial morphology.[44,45] In a rabbit model, the corneal endothelium was mechanically scraped with a 20 gauge silicone needle and rabbit CECs were injected concomitantly with and without Y-27632. The inhibitor-treated eyes presented with a monolayer of hexagonal-shaped cells, whereas the eyes in which rabbit CECs were injected without Y-27632 exhibited a stratified fibroblastic phenotype positive for α-SMA.[44] The TGF-β pathway represents another potential therapeutic target in light of our findings. Studies have demonstrated that SB431542, an inhibitor of TGF-β pathways, halts the spontaneous occurrence of EndoMT in vitro.[46] When human and primate CECs were cultured with SB431542, there was inhibition of morphologic changes to a fibroblastic phenotype, and endothelial cells were able to retain expression of the endothelial functional markers Na+/K+-ATPase and ZO-1.[46] Similarly, bone morphogenetic protein-7 (BMP-7), a member of the TGF-β superfamily that is known to antagonize the effects of TGF-β1 mesenchymal transformations via a smad-dependent mechanism,[47] not only inhibited EndoMT, but also reversed the process. The elongated, fibroblastic phenotype was reversed to the polygonal cell morphology and cells maintained functional marker expression in a BMP-7 concentration-dependent manner.[46] Although promising, these therapies have not been studied in human corneas and have only been used in vitro and in animal studies. Further in vitro and animal studies are needed to elucidate their safety and potential in preventing or treating DSAEK-associated membranes.

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