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Regenerative medicine in Fuchs' endothelial corneal dystrophy

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Abstract:

The management of Fuchs' endothelial corneal dystrophy (FECD) has evolved rapidly since the introduction of endothelial keratoplasty (EK). In recent years, advances in our understanding of endothelial cell biology, in particular with respect to the regenerative capacity of endothelial cells, have opened the door to novel therapeutic options that stray from the traditional paradigm of allograft transplantation. We review the development of descemetorhexis without EK (DWEK) as a primary treatment for FECD and discuss the lessons learned to date about the mechanism of wound healing, surgical technique, patient selection, and refractive outcomes. Multiple randomized clinical trials are currently underway to evaluate the potential for pharmacological supplementation with rho-associated kinase inhibitors to increase the success rate of corneal clearance following DWEK. Biologic supplementation with intracameral endothelial cell injection and acellular Descemet's membrane transplantation are other avenues of adjuvant therapy. DWEK is a promising surgical option for management of a subset of FECD patients.

Keywords:

Descemet's stripping only, descemetorhexis without endothelial keratoplasty, Fuchs' endothelial corneal dystrophy, regenerative medicine, rho-associated kinase inhibitor

Introduction – A Historical Perspective on Endothelial Keratoplasty

For most of the twentieth century, penetrating keratoplasty was the only therapeutic option in the management of medically recalcitrant corneal edema and scars. In the last few decades, interest in partial-thickness transplantation as a potential method to achieve better visual outcomes, shorten recovery time, and reduce rates of rejection and failure has led to the development and popularization of anterior and posterior lamellar techniques.[1]

In particular, the management of corneal endothelial disorders has rapidly evolved over the last two decades with the advent of endothelial keratoplasty (EK), credited largely to the pioneering work of Melles, Terry, and Gorovoy, who since 1998 have successively introduced posterior lamellar keratoplasty, deep lamellar EK, Descemet's stripping (automated) EK (DSAEK), and Descemet's membrane EK (DMEK).[2] While each iteration refined the technique to strive for improved outcomes, EK is fundamentally based on the idea that replacement of endothelium with donor tissue is necessary because corneal endothelium does not have the regenerative capacity to repopulate the central cornea, and therefore, contact between donor endothelium and a denuded host posterior stroma is required to restore transparency.

This idea is derived from classic teaching about corneal endothelial cell biology, which states that while both proliferation and migration drive the formation of the endothelial monolayer from neural crest-derived progenitor cells during development, mature cells are arrested in the G1 phase of mitosis and remain functionally

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nonproliferative.[3] This state of cell cycle arrest is mediated *in vivo* by mechanisms of contact inhibition and a relative paucity of local mitogenic, compared to antimitogenic and growth factors.[3] Endothelial cell wound healing is thought to occur primarily by migration of the remaining healthy cells. In posttransplant corneas, this wound healing paradigm is presumed to explain the observed repopulation of donor endothelium by host cells[4] and the clinical observation that centripetal host cell migration likely contributes toward postoperative endothelial stability.[5]

However, *in vitro* and *ex vivo* studies have definitively demonstrated that corneal endothelial cells do have the capacity to proliferate,[3,6] and there is evidence to suggest *in vivo* regeneration potential as well. For example, a subpopulation of adult corneal endothelial cells from both normal and diseased tissues was recently shown to express the transcription profile of neural crest-derived progenitor cells, suggesting the local presence of cells with the potential for proliferation and differentiation.[7] The evidence for corneal endothelial regeneration is extensively summarized in recent reviews[8,9] and will not be revisited here.

These burgeoning basic science insights into endothelial regeneration have raised the potential for new therapies in the treatment of corneal endothelial disorders. These therapies would refine and challenge the current standard of EK and continue the momentum of the past two decades that advanced the field from the seemingly draconian days of penetrating keratoplasty to the present. This review will trace the evolution of descemetorhexis without EK (DWEK) as one such clinical correlate in the management of Fuchs' endothelial corneal dystrophy (FECD).

Serendipitous Precursors to Descemetorhexis without Endothelial Keratoplasty

DWEK, known alternately in published literature as Descemet's stripping only among other names,[10] describes a surgical technique in which central guttae is deliberately removed without subsequent graft transplantation. The formalization of this technique as an option for the primary treatment of FECD was preceded by anecdotal reports of spontaneous corneal clearance following iatrogenic Descemet's membrane (DM) defects.

Iatrogenic descemetorhexis in patients without Fuchs' endothelial corneal dystrophy

Inadvertent descemetorhexis in patients with no history of endothelial disease was initially reported in isolated case reports of patients aged 65–96 years in the setting of cataract extraction[11-18] [Table 1]. In seven of the eight cases,

Table 1: Clinical outcomes following iatrogenic descemetorhexis in patients without Fuchs' endothelial corneal dystrophy

Study	Procedure	Success	Age	Descemetorhexis		Preoperative			Months to clear	Postoperative (at LFU)			LFU (months)
				Size (mm)	Technique	BCVA	CCT (µm)	ECD (cells/mm^2)		BCVA	CCT (µm)	ECD (cells/mm^2)	
Braunstein *et al.*, 2003[11]	Phaco	Yes	79	5	Viscodissect	-	-	2114*	6	20/30	540	827	12
Patel *et al.*, 2004[12]	Phaco	Yes	90	6	Viscodissect	-	-	-	< 9	20/50	553	794	45
Zvi *et al.*, 2005[13]	Phaco	Yes	68	5	Viscodissect	-	-	2446*	1.5	20/30	520	834	8
Pan and Eong, 2006[14]	Phaco	No	96	5.5	Viscodissect	LP	-	2100*	>14	20/80[§]	-	Unable	14
Agarwal *et al.*, 2006[15]	Phaco/Trab	Yes	65	~6	Viscodissect	20/30	-	-	1	20/25	556	1301	24
Srivastava *et al.*, 2010[16]	Phaco	Yes	76	3.5x4.5	Forceps trauma	-	-	2344*	4.5	20/80[¶]	-	1222	4.5
Choo *et al.*, 2010[17]	ECCE	Yes	86	~8	-	20/200	-	-	10	20/120	-	-	15
Ullienne, 2015[18]	Phaco	Yes	85	5	Viscodissect	-	-	2084*	5	20/25	559	1344	22

-Not available, *ECD of contralateral eye as proxy for preoperative ECD in operative eye, [¶]Vision limited by 4D postoperative astigmatism, [§]Vision limited by age-related macular degeneration. BCVA=Best-corrected visual acuity, CCT=Central corneal thickness (i.e. pachymetry), ECD=Endothelial cell density, LFU=Last follow-up, LP=Light perception, Phaco=Phacoemulsification, Trab=Trabeculectomy

patients experienced complete resolution of corneal edema without intervention – two within 6 weeks, three within 6 months, and another two within 12 months – supported by normal postoperative pachymetry and increasing central endothelial cell density (ECD) that remained stable at last follow-up (4.5 months to 4 years postoperatively). Confocal imaging of the patient with the longest follow-up duration revealed improvement in polymegathism (coefficient of variance decreased from 70% to 32%) and polymorphism (hexagonal cells increased from 29% to 50%) at postoperative year 3, suggesting ongoing remodeling and repair.[12]

With the exception of one report of a central 3.5 mm × 4.5 mm Descemet's defect induced by forceps trauma,[16] and a second report which omitted information regarding the mechanism of injury,[17] the remainder of cases occurred secondary to "capsulorhexis" of the central DM following unrecognized viscodissection of DM during injection of viscoelastic. The size of the ensuing descemetorhexis was 5–6 mm in all cases.

In the oldest patient of the eight cases, spontaneous clearing was noted gradually over 5 months in a 96-year-old woman, with concomitant improvement in visual acuity from hand motion on postoperative day 1 to 20/80 at month 14. The subnormal best-corrected visual acuity (BCVA) was attributed to macular degeneration. However, specular microscopy was unable to record a central ECD, no pachymetric measurements are reported, and peripheral Descemet's folds were noted on examination at 14 months, which suggests that successful endothelial repopulation and complete corneal deturgescence were likely not achieved.[14]

These observations are interesting for three reasons that later resonate when confronted with patient selection criteria and optimal surgical technique for DWEK. First, there was a wide distribution of time to clearance of 6 weeks to 12 months. Second, the lone patient whose Descemet's defect was the result of forceps trauma experienced 4 diopters of oblique astigmatism resulting in BCVA of 20/80, while the remainder of the patients whose defects were caused without inducing stromal trauma (excluding one whose vision was limited by retinal pathology[14]) all attained BCVA better than 20/50. Third, the patient in whom clearance was most rapid (1 month) occurred in the youngest patient, while the patient in whom we surmise lack of clearance at 14 months postoperatively was the oldest of the cohort.

Iatrogenic descemetorhexis in patients with Fuchs' endothelial corneal dystrophy

The authors of the preceding case reports speculated that the ability of the cornea to repair large defects in DM may have been predicated on healthy preoperative tissue, and hence, this degree of wound healing might not occur with diseased corneas.[15] Subsequent reports of spontaneously clearing corneal edema in patients with FECD refute this hypothesis [Table 2]. In particular, two cases of traumatic descemetorhexis during phacoemulsification in patients with FECD showed complete clearance within 1 and 4 months, with BCVA of 20/20 and 20/25, respectively.[24,25] In one of these patients, visual acuity and corneal clarity were maintained for 16 years postoperatively.[24]

With the popularization of DMEK, reports emerged of endothelial repopulation in spite of areas of prolonged graft detachment. In 2009, the Melles group reported two cases of patients who underwent standard DMEK[28] with 360° circumferential scoring and removal of 9-mm central DM ("9-mm descemetorhexis") in the host cornea followed by graft placement. In both cases, over 75% of the graft detached in the immediate postoperative period, but spontaneous clearance and endothelial repopulation occurred in the host cornea within 3 months. Visual acuity by 9 months was excellent in spite of persistent graft detachment.[19] This was followed by a retrospective series of 27 persistent graft detachments from 150 consecutive DMEK eyes. In 14 patients in whom <30% of the graft detached, corneal edema cleared in 100% of cases, most within 3 months. Excluding two cases in which final BCVA was explained by other pathology, 75% were better than 20/25. In ten patients with >30% partial detachment, edema cleared in 70% of cases by 6–12 months, of which 71% achieved BCVA better than 20/40. In three patients with either no graft placement or 100% graft detachment, no discernible clearance or re-endothelialization was noted at 4.5 months, and all patients underwent repeat EK.[20]

With the startling evidence that total graft apposition might not be a requirement for successful DMEK in the treatment of FECD, the term DM endothelial transfer (DMET) was coined to describe a potential simplified surgical technique in which the donor tissue is only partially affixed to the recipient stroma after a 9-mm descemetorhexis is created.[22] To elucidate whether the mechanism of repair is migration/proliferation from the host peripheral endothelium or seeding from the donor graft, a prospective nonrandomized study compared DMET in seven FECD patients to five patients with bullous keratopathy.[23] All FECD patients showed progressive clearance and repopulation of the central endothelium at 6-month follow-up, while no improvement was seen in any patient with bullous keratopathy (confirmed on histopathology to have no endothelial cell repopulation anterior to the descemetorhexis margin[29]). While 100% of the FECD patients who initially demonstrated corneal clearance in this study ultimately decompensated and

Table 2: Clinical outcomes following iatrogenic descemetorhexis in patients with Fuchs' endothelial corneal dystrophy

Study	Procedure	Success	Number cases	Age	Descemetorhexis		Preoperative		Months to clear	Postoperative (at LFU)			LFU (months)
					Size (mm) [% detached]	Technique	BCVA	CCT (µm)		BCVA	CCT (µm)	ECD (cells/mm²)	
Balachandran *et al.*, 2009[19]	DMEK	Yes	1	69	9 [75]	Scoring	20/150	632	3	20/28	614	380	9
	DMEK	Yes	1	47	9 [100]	Scoring	20/50	674	3	20/20	571	440	9
Dirisamer *et al.*, 2011[20]	DMEK	Yes	14	-	9 [<33]	Scoring	-	-	<12	20/26*	542*	1458*	12
	DMEK	Yes	7	-	9 [33-99]	Scoring	-	-	<12	20/53*	629*	1514*	12
	DMEK	No¶	3	-	9 [33-99]	Scoring	-	-	-	20/63*	831*	740*	6
	DMEK	No¶	3	-	9 [100]	Scoring	-	-	-	-	-	988*	6
Shah *et al.*, 2012[21]	DSAEK	Yes	1	34	8 [100]	Scoring	20/50	595	5	20/20		-	12
Dirisamer *et al.*, 2012[22,23]	DMET	No¶	10	-	9 [100]	Scoring	20/67*	636*	-	-	-	-	4-31§
Koenig 2013[24]	Phaco	Yes	1	84	4.5	Viscodissect	20/60		4	20/25	604	464	192
Moloney *et al.*, 2015[25]	FLACS	Yes	1	76	-	-	-		1	20/20	-	753	1
Sánchez *et al.*, 2017[26]	DMET triple	Yes	1	73	3	-	-	751	3	-	653	653	
Daravagka *et al.*, 2019[27]	DMEK triple	No¶	1	81	9 [100]	Scoring	20/100	687	3	20/50	572	-	12
	DMEK triple	No¶	1	69	9 [100]	Scoring	20/70	606	3	20/30	556	-	12
	DMEK	No¶	1	56	9 [100]	Scoring	20/200	832	3	20/100	580	-	12

-Information not available, *BCVA, CCT, and ECD for series of more than 1 patient listed here are average values. BCVA average is computed by converting reported Snellen acuity to logMAR, calculating an average, and then converting back to Snellen representation, ¶Patients were offered secondary EK due to either lack of clearance or poor visual recovery, §Long-term outcomes were reported in a separate publication (Birbal 2019). BCVA=Best-corrected visual acuity, CCT=Central corneal thickness (i.e. pachymetry), DMEK=Descemet's membrane endothelial keratoplasty, DMET=Descemet's membrane endothelial transfer, ECD=Endothelial cell density, FLACS=Femtosecond laser-assisted cataract surgery, LFU=Last follow-up, Phaco=Phacoemulsification

required secondary EK within a range of 4–31 months postoperatively,[30] the disparate short-term responses in patients with bullous keratopathy compared to FECD suggest that the capacity for the cornea to "re-endothelialize" in the absence of an attached graft depends primarily on the recipient endothelium as opposed to donor cell repopulation from the detached graft. From here, it is a small leap of logic to question whether a descemetorhexis alone without graft placement would be sufficient in the treatment of FECD.

Deliberate Descemetorhexis without Grafting – The First Descemetorhexis without Endothelial Keratoplasty

The first report of DWEK to our review dates to 1955. In his book chapter on lamellar keratoplasty, Rycroft wrote that he "had the idea of trying posterior peeling of the cornea…to see whether a regeneration of the posterior plane was possible." He then described a technique, attributed to Paufique, of scraping the posterior surface of the cornea with a Chalazion curette from limbus to limbus and stripping the posterior surface of DM and endothelium. Although he reports attaining "at least partial" spontaneous regression of edema within 1–3 months, the details of his cases are not available, nor do there appear to be further allusions to this technique until 2012.[31]

The theoretical benefits of DWEK are manifold. A descemetorhexis alone would significantly simplify EK surgery, with attendant lower rates of postoperative complications.[32] The absence of a transplanted allograft eliminates the risk of allograft rejection, lowers the potential risk of disease transmission, reduces postoperative immunosuppressive eye drop burden, and may be a more feasible treatment strategy in countries with limited donor tissue supply. All published cases and case series of DWEK are summarized in Tables 3a and 3b.

Early successes and failures: 2012–2017

The first report of DWEK in the modern era was of a 34-year-old female patient with possible FECD (versus posterior polymorphous corneal dystrophy) whose right eye underwent DSAEK with 8-mm descemetorhexis and was complicated by total graft detachment and subsequent removal of the donor lenticule. Her cornea spontaneously cleared by postoperative month 5 with 20/20 BCVA. Bolstered by this result, DWEK was performed in the contralateral eye (presumably using the same technique of scoring to create an 8-mm descemetorhexis but not explicitly stated). Complete corneal clearance was noted by postoperative month 6 and BCVA was 20/25; both remained stable at 2.5 years.[21,46]

Table 3a: Clinical outcomes after descemetorhexis without endothelial keratoplasty (reports with <3 cases)

Study	Procedure	Success	Number cases	Age	Descemetorhexis		Preoperative		Months to clear	Postoperative (at LFU)			LFU (months)
					Size (mm)	Technique	BCVA	CCT (µm)		BCVA	CCT (µm)	ECD (cells/mm²)	
Shah *et al.*, 2012[21]	DWEK	Yes	1	34	-	Scoring	20/50	597	6	20/25	-	-	6
Arbelaez *et al.*, 2014[33]	DWEK	No	3	45	6.5	Scoring	20/30	469	DNC	20/60	Unable	769	4
		No		46	6.5	Scoring	-	582	DNC	20/50	704	-	1
		No		40	6	Scoring	20/40	540	24	20/25	530	847	24
Koenig 2015[34]	DWEK triple	No	2	68	6	Scoring	20/50	669	DNC	20/40	811	Unable	12
	DWEK triple	No		67	6	Scoring	20/70	623	DNC	20/150	Unable	Unable	12
Galvis *et al.*, 2016[35]	DWEK triple	No	1	67	5	Scoring	-	-	DNC	20/70	-	-	4.5
Moloney *et al.*, 2015[25]	DWEK	Yes	1	54	4-5	Scoring	20/40		1	20/20		731	6
Kaufman *et al.*, 2017[36]	DWEK	Yes	1	58	4	Strip/Peel	20/25	671	1.5	20/25	489	1471	24
Astakhov *et al.*, 2018[37]	DWEK/CCL	Yes	1	61	5	Scoring	20/200	767	4.5	20/20	553	1546	4.5
Ploysangam and Patel, 2019[41]	DWEK triple + netarsudil	Yes	1	51	4	Strip/Peel	20/30	595	1	20/25	555	700	9

-Information not available. BCVA=Best-corrected visual acuity, CCL=Corneal cross-linking, CCT=Central corneal thickness (i.e. pachymetry), DWEK=Descemetorhexis without endothelial keratoplasty, DNC=Did not clear, ECD=Endothelial cell density, LFU=Last follow-up, Phaco=Phacoemulsification

Table 3b: Clinical outcomes after descemetorhexis without endothelial keratoplasty (series with >3 cases)

Study	Procedure	Success	Number cases	Age*	Descemetorhexis		Preoperative		Months to clear*	Postoperative (at LFU)			LFU* (months)
					Size (mm)	Technique	BCVA*	CCT* (µm)		BCVA*	CCT* (µm)	ECD* (cells/mm²)	
Bleyen *et al.*, 2012[39]	DWEK triple	No	7	-	-	-	-	-	DNC	-	-	-	-
		Yes	1	-	-	-	-	-	18	20/20	-	-	-
Borkar *et al.*, 2016[40]¶	DWEK triple	Yes	4	56	4	Scoring	20/35	591	1	20/19	544	788	13.3
		Yes	4	66	4	Scoring	20/31	577	3	20/32	542	644	7.8
		Yes	2	84	4	Scoring	20/126	618	6	20/35	667	603	8
		No	3	64	4	Scoring	20/62	669	DNC	Unable	Unable	Unable	3
Malyugin *et al.*, 2017[41]	DWEK triple	Yes	13	-	4	Scoring	-	-	<12§	~20/30	569	634	12
		No	5	-	4	Scoring	-	-	DNC	-	-	-	12
Iovieno *et al.*, 2017[42]	DWEK±Phaco	Yes	4	71	4	Scoring	20/81	552	3.1	20/38	532	1000	9.5
	DWEK triple	No	1	65	4	Scoring	20/60	773	7	20/200	632	Unable	7
Moloney *et al.*, 2017[43]	DWEK	Yes	9	-	4	Scoring	20/36	620	3.1	20/24	586	860	14.75
	DWEK + ripasudil	Yes	2	-	4	Scoring	20/40	589	3.7	20/27	549	580	5.7
	DWEK + Y-27632	No	1	-	4	Scoring	20/32	651	DNC	20/63	Unable	568	9
Davies *et al.*, 2018[44]¶	DWE	Yes	5	72	4	Strip/Peel	-	608	<2	13/14	577	425	-
		Yes	8	67	4	Strip/Peel	-	656	3-5	better	580	619	-
		Yes	1	70	4	Strip/Peel	-	560	6-8	than 20/25	506	901	-
		No	3	64	4	Scoring	-	644	D C	20/62	675	994	-
Huang *et al.*, 2018[32]	DWEK triple	Yes	12	67	4	Scoring	20/40	613	3.4	20/30	-	-	6.1
Macsai and Shiloach, 2019[45]	DWEK±Phaco	Yes	10	-	<5	Strip/Peel	20/56	686	2.1	20/22	587	736	12
	DWEK±Phaco + ripasudil	Yes	7	-	<5	Strip/Peel	20/55	691	1.1	20/21	566	1241	12
		No	1	-	<5	Strip/Peel	20/60	753	DNC	20/200	712	Unable	12

- Information not available,*Age, BCVA, CCT, ECD, months to clear, and LFU listed here are average values. BCVA average is computed by converting reported Snellen acuity to logMAR, calculating an average, and then converting back to Snellen representation, ¶Results listed for these studies are stratified by time to clearance (categorized as fast responders, slow responders, responders, and nonresponders per Borkar *et al.*). §26% cleared in <1 month. BCVA=Best-corrected visual acuity, CCT=Central corneal thickness (i.e. pachymetry), DWEK=Descemetorhexis without endothelial keratoplasty, DNC=Did not clear, ECD=Endothelial cell density, LFU=Last follow-up, Phaco=Phacoemulsification, logMAR: Logarithm of the Minimum Angle of Resolution

Multiple early reports also emerged of failed DWEK, either due to nonresolving edema or unacceptable visual outcomes in spite of edema regression.[33-35,39] The largest series of negative outcomes described eight consecutive patients who underwent DWEK with simultaneous phacoemulsification and intraocular lens implantation ("DWEK triple"). After 18 months of follow-up, only one patient maintained corneal clarity and BCVA 20/20, while the remaining seven patients required secondary EK. Unfortunately, the age, severity of FECD, size of descemetorhexis, surgical technique, preoperative pachymetry, and postoperative ECD were not reported.[39] Three other groups collectively described six cases of DWEK or DWEK triple with descemetorhexis sizes of 5–6.5 mm in patients ranging from 40 to 68 years old with suboptimal results at last follow-up (average: 1.5 years, range: 4.5 months–4 years).[33-35] All cases in which surgical technique was described were performed with 360° scoring of central DM. Five of the six patients (83%) had persistent corneal edema and were offered secondary EK. The last patient cleared after 2 years but suffered from significant oblique astigmatism and poor visual quality.[33]

A turning point in our understanding of DWEK came from a series of 13 eyes by Borkar *et al*., all with central guttae only, which underwent DWEK triple with a 4-mm descemetorhexis. With these parameters, 77% of cases (patients aged 51–91 years) had resolution of edema within 6 months: 31% within 1 month, 31% within 3 months, and 15% after 3 months. Excluding one patient with preexisting macular pathology and a second who developed postoperative cystoid macular edema, all patients with corneal clearance had BCVA of 20/20 or better at last follow-up (6–24 months).[40] The three patients who showed no improvement in corneal edema at 3 months underwent uncomplicated secondary DMEKs.[47]

This study stratified patients into categories of fast responders (clearance within 1 month), responders (clearance in 1–3 months), slow responders (clearance >3 months), and nonresponders. Taken together with the conflicting evidence presented thus far regarding the viability of DWEK, this stratification helped frame the question of what factors account for the observed variability in wound healing in FECD eyes after DWEK.

Lessons learned: 2017 – Present

With the main concern about DWEK being the predictability of results – i.e. whether endothelial repopulation occurs, and if so, within what timeframe and with what long-term outcomes – subsequent case series more rigorously analyzed (retrospectively) or imposed (prospectively) patient selection criteria to define the optimal population for DWEK. Since Borkar's study in 2016, six case series enrolling between 5 and 19 patients have reported successful clearance rates between 65% and 100% at an average of approximately 3 months after intervention.[32,41-45] The lessons learned from these studies are summarized below.

The mechanism of endothelial repopulation is likely a combination of migration and proliferation

Repopulation of the central endothelium after descemetorhexis may occur due to a combination of endothelial cell migration and proliferation. In two series that reported pre- and postoperative peripheral ECD,[43,45] a statistically significant decrease in peripheral ECD of 10%–40% was noted in the postoperative group at an average follow-up of 12–15 months, which suggests a role for migration. On the other hand, creating a defect in the endothelial layer relieves cells of the contact inhibition that plays a role in its cell cycle arrest. Bilobed nuclei representing possible mitoses have been reported on confocal microscopy at 3 months following DWEK, which supports potential proliferative activity.[43] The mechanism of endothelial repopulation is not yet fully understood.

Larger descemetorhexis is associated with lower rates of corneal clearance

Guttae have been shown *in vitro* to create a microenvironment that induces senescence and apoptosis.[48] A descemetorhexis that removes the maximum amount of guttae without compromising wound healing is logical for both immediate endothelial cell repopulation and long-term stability following DWEK. An argument has also been made for a larger descemetorhexis to incite proliferation, as peripheral cells have been shown to harbor greater proliferative potential than central cells.[3] On the other hand, if migration was the predominant mechanism of repopulation, a smaller surface area to repopulate might be more likely to succeed. While no study has compared outcomes stratified by descemetorhexis size, all of the recent case series with success rates above 65% have performed a 4–5-mm descemetorhexis. In contrast, all cases of complete or near-complete graft detachment in the DMEK/DMET studies with 8–9-mm descemetorhexis described in the section "Iatrogenic descemetorhexis in patients with Fuchs' endothelial corneal dystrophy" failed, as did all DWEK reports with 6–6.5-mm descemetorhexis. Interestingly, patients with iatrogenic descemetorhexis following cataract extraction (described in "Iatrogenic descemetorhexis in patients with Fuchs' endothelial corneal dystrophy" section) were able to clear in spite of a larger defect up to 6 mm, perhaps reflecting a larger pool of healthy peripheral endothelial cells in otherwise healthy corneas. In FECD, patient

selection with guttae confined to the central 4–5 mm of the endothelium seems prudent to increase the likelihood of success with DWEK.

Surgical descemetorhexis technique influences both the rate of clearance and visual outcomes

In early reports of failed DWEK, the authors noted that the sites of persistent edema corresponded to areas of stromal irregularity[33] and suggested that successful outcomes may require care to avoid traumatizing the posterior stroma during scoring. *In vitro* work has shown that scoring results in a stromal trench and cell loss on both sides adjacent to the wound, further supporting a technique that minimizes stromal contact.[49] This hypothesis is corroborated in the Davies *et al*. series of 17 patients[44] in which all patients who failed to clear had a descemetorhexis created with a scoring technique, while all of those in whom a stripping/tearing technique (as in a "capsulorhexis") was used did successfully clear. A subsequent prospective trial on the use of ROCK inhibitors after DWEK[45] in which every descemetorhexis was created using the stripping/tearing technique found that 94% of patients successfully cleared.

Stromal trauma might account for visually significant astigmatism even if the cornea does clear. In their series of five DWEK patients in whom 360° scoring was performed, Iovieno *et al*. reported corneal clearance in 80% of patients but only 20% with BCVA better than 20/25. The poor visual outcomes were associated with the presence of posterior stromal nodules in the area of the descemetorhexis.[42] Maloney *et al*. similarly observed posterior nodules at the descemetorhexis margin in 25% of patients; in their cohort, a higher proportion (58%) of patients attained BCVA better than 20/25.[43] On the other hand, series in which the capsulorhexis technique was used found that >90% achieved BCVA better than 20/25 and had no evidence of irregular astigmatism on postoperative topography.[44,45] These findings suggest that the stripping/tearing technique may be associated with better and more consistent visual outcomes.

Patient demographic factors predictive of successful descemetorhexis without endothelial keratoplasty remain elusive

Our series of 17 DWEKs included 8 that were performed in both eyes of 4 patients. In all eight cases, contralateral eyes cleared within 1 month of the other. This concordance suggested that patient-specific factors influence DWEK outcomes.[44] Two series[40,42] found that nonclearing patients had the highest preoperative pachymetry, but two larger series found no association between pachymetry and time to clearance.[32,44]

Our study also showed a trend toward lower preoperative peripheral ECD with increased time to clearance that was not statistically significant ($P = 0.22$). Moloney *et al*. included peripheral ECD > 1000 as an inclusion criterion for enrollment in their prospective study of 12 patients and found no correlation between peripheral ECD and rate of clearance.[43] This is surprising given the DMET trial finding that no eyes with bullous keratopathy demonstrated clearance after DMET, whereas FECD eyes all initially cleared. If the authors' hypothesis is accurate – i.e. that this disparity is due to the lack of a depot of healthy peripheral cells in bullous keratopathy compared to FECD – then there is likely a peripheral ECD threshold below which post-DWEK eyes are less likely to clear. The peripheral ECD floor of 1000 in two prospective studies[43,45] is based on the theoretical number needed to repopulate a central descemetorhexis of 4 mm and retain central ECD >500 (a number below which corneal decompensation is more likely to occur).

Finally, neither our series nor Moloney *et al*. detected a statistically significant difference between age and rate of clearance, which is again surprising given *ex vivo* evidence for increased proliferative potential in younger patients.[3] As the effect of confounding variables such as surgical technique and size of descemetorhexis diminishes in the future with standardization of the surgical procedure, multivariate analyses of larger patient cohorts might be helpful to unveil potential predictive factors to help guide patient selection.

Successful descemetorhexis without endothelial keratoplasty is associated with a small hyperopic shift

To evaluate the refractive outcomes following successful DWEK, we retrospectively reviewed 25 cases of DWEK triple in which corneal clearance was documented with at least 6 months of follow-up. Refraction at 1 month following corneal clearance revealed an average hyperopic shift of +0.38 D, and only 48% of cases were within 0.5 D of their refractive target.[50] The refractive data presented in the 11 cases of corneal clearance following DWEK by Moloney *et al*. similarly reveal an average +0.32 D hyperopic shift at last follow-up.[43] Intraocular lens calculations for a DWEK triple should incorporate a +0.5 D adjustment for optimal results, similar to DMEK surgery.

Future of Descemetorhexis without Endothelial Keratoplasty: Rho-Associated Kinase Inhibitor Supplementation and Beyond

The trend toward a more standardized surgical technique in preselected patients has improved the clearance rate of DWEK. For patients who fail to demonstrate clearance, secondary EK has been performed successfully.

Supplementation with exogenous agents that can modulate the endothelial wound healing response is currently a topic of great interest as a method of improving the success rates of primary DWEK and potentially expanding on the indications for DWEK.

Role of rho-associated kinase inhibitors

The rho family of proteins plays many important roles, including the regulation of cell adhesion and motility, cell cycle progression, apoptosis, and smooth muscle contraction. Rho-mediated signaling pathways have been studied as potential therapeutic targets for systemic diseases.[51] Rho-associated coiled-coil-containing protein kinase (ROCK) was the first downstream effector to be discovered and exists as two isoforms – ROCK1 and ROCK2 – that have significant homology but variable distribution across different tissues.[52] There are two commercially available ophthalmic ROCK inhibitors, both approved for the management of ocular hypertension or glaucoma: ripasudil, approved in Japan in 2014, and netarsudil, approved in the United States in 2017. In addition, multiple agents have been synthesized and used in research settings, including a number of so-called "Y-compounds" such as Y-27632. All of these agents nonselectively inhibit both ROCK isoforms, albeit with different relative potency. Ripasudil has higher affinity for ROCK2,[53] Y-27632 has higher affinity for ROCK1,[54] and netarsudil has equal affinity for both.[55]

Over the last decade, Kinoshita's group has studied the effect of ROCK inhibition with Y-27632 in corneal endothelial cells. They demonstrated that ROCK inhibition promotes endothelial wound healing by increasing cell adhesion and proliferation and decreasing apoptosis. These findings were first reported in 2009 in monkey-derived corneal endothelial cell culture and subsequently with *in vivo* rabbit and primate models.[56-58] In 2011, they reported the results of a pilot clinical trial that enrolled 8 patients with FECD or bullous keratopathy who underwent a transcorneal freezing model of endothelial wound creation followed by 6 times/day administration of Y-27632.[58] While all FECD patients had reduction in edema and pachymetry after 1 week, there was no control group (i.e. transcorneal freezing without ROCK inhibitor) to prove that the result was due to the intervention as opposed to the injury itself. Following commercial availability of ripasudil, a Phase I clinical trial was performed in 2015 to assess safety of ripasudil in healthy controls. Transient morphological changes of the corneal endothelium were noted in all six enrolled patients but resolved without sequelae, and no adverse events were otherwise reported.[59]

The applications of Kinoshita's findings to DWEK are clear – here we have an inhuman endothelial wound model and a surgery whose success depends on complete and timely wound healing. In 2017, Moloney *et al*. were the first to report the use of ROCK inhibitors in three patients who had failed to demonstrate edema regression at 2–4 months after DWEK. Two patients were treated with ripasudil 6 times daily for 2 weeks and dramatically achieved complete clearance after 10–14 days of therapy. The third patient, treated with Y-27632, did not improve.[43] Huang *et al*. similarly reported one case in which persistent edema 3 months after DWEK was "rescued" with initiation of ripasudil.[32]

In vitro studies of ROCK inhibitors show that cell cycle progression is greatest close to the wound edge and stops when the wound is healed, presumably due to contact inhibition.[60] Aside from their potential role in salvaging nonclearing corneas, ROCK inhibitors could promote faster time to clearance if started earlier when the wound is larger. One report of a patient who underwent DWEK and started netarsudil on postoperative day 1 cleared in 1 month.[38] The only prospective interventional clinical trial on the use of ripasudil after DWEK found that the treatment group cleared at an average of 4.6 weeks compared to the 6.5 weeks in the observation group.[45]

Clearly, more prospective studies are needed to better understand the use of ROCK inhibitors in DWEK surgery and the stability of long-term outcomes. To our search of the clinicaltrials.gov database, there are seven prospective single-center Phase I/II studies in the USA and Europe currently enrolling patients to investigate the use of ripasudil or netarsudil in FECD patients after DWEK. We eagerly await the results of these trials.

Role for biologic supplementation

Since 2018, two important proof-of-concept studies investigating novel ways of promoting endothelial cell regeneration have been reported. First, Kinoshita *et al* reported the first clinical trial of intracameral cultured corneal endothelial cell injection combined with Y-27632 in 11 patients with bullous keratopathy. At 24 weeks of follow-up, 100% of patients had repopulation of central endothelium with ECD >500 and significantly decreased corneal thickness. All patients retained corneal transparency after 2 years.[61] While many questions have been raised about the study design and safety,[62] better understanding of the mechanism by which the procedure results in endothelial cell repopulation will have significant implications for future treatment options for corneal endothelial disease. For example, if regeneration in this scenario is primarily due to paracrine effects from the injected cells, then a combination of DWEK with intracameral cultured cell injection might lead to superior outcomes compared with either of the interventions alone.

Second, Mehta's group reported a case of a first inhuman trial of acellular DM transplantation (DMT) for the

treatment of FECD. In their report, a DWEK triple was performed with a 5-mm descemetorhexis, followed by implantation of a decellularized cadaveric graft donor DM.[63] At postoperative month 6, the patient had visual acuity of 20/25, normal central corneal thickness, and normal ECD. Animal studies have shown that the presence of DM as scaffolding promotes endothelial wound healing and decreases the rate of retrocorneal fibrosis.[64] A larger series of DMT would be informative to assess if outcomes of DWEK are superior in the presence of acellular extracellular matrix scaffolding.

Conclusion

The concept of healing by secondary intention for the treatment of corneal opacities is sometimes ascribed to Erasmus Darwin, who wondered in 1796: "could not a small piece of the cornea be cut out…and would it not heal with a transparent scar?"[65] The answer to this query with respect to DWEK in FECD, supported by a preponderance of clinical evidence reviewed here, is affirmative – albeit with qualifications. The extent to which these qualifications impact outcomes, and whether pharmacologic or biologic interventions can increase rates of success, are the subject of ongoing work. In parallel, advances in our understanding of mechanisms of endothelial cell regeneration continue to challenge the treatment paradigm for corneal endothelial disorders.

In the context of corneal transplantation history, we would do well to remember that the first penetrating keratoplasty in a human was accomplished in 1905 following almost 100 years of animal experimentation, and refinement of the surgery and postoperative management required many more decades of work. Surveying the rapidly growing field of therapeutic options for corneal endothelial disorders just two decades since the popularization of EK, we have every reason to be excited for the future.

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Conflicts of interest
The authors declare that there are no conflicts of interests of this paper.

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