Cohesive Tensile Strength of Human LASIK Wounds With Histologic, Ultrastructural, and Clinical Correlations

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ABSTRACT

PURPOSE: To measure the cohesive tensile strength of human LASIK corneal wounds.

METHODS: Twenty-five human eye bank corneas from 13 donors that had LASIK were cut into 4-mm corneoscleral strips and dissected to expose the interface wound. Using a motorized pulling device, the force required to separate the wound was recorded. Intact and separated specimens were processed for light and electron microscopy. Five normal human eye bank corneas from 5 donors served as controls. A retrospective clinical study was done on 144 eyes that had LASIK flap-lift retreatments, providing clinical correlation.

RESULTS: The mean tensile strength of the central and paracentral LASIK wounds showed minimal change in strength over time after surgery, averaging 2.4% (0.72±0.33 g/mm) of controls (30.06±2.93 g/mm). In contrast, the mean peak tensile strength of the flap wound margin gradually increased over time after surgery, reaching maximum values by 3.5 years when the average was 28.1% (8.46±4.56 g/mm) of controls. Histologic and ultrastructural correlative studies found that the plane of separation always occurred in the lamellar wound, which consisted of a hypocellular primitive stromal scar centrally and paracentrally and a hypercellular fibrotic stromal scar at the flap wound margin. The pathologic correlations demonstrated that the strongest wound margin scars had no epithelial cell ingrowth—the strongest typically being wider or more peripherally located. In contrast, the weakest wound margin scars had epithelial cell ingrowth. The clinical series demonstrated the ability to lift LASIK flaps without complications during retreatments up to 8.4 years after initial surgery, correlating well with the laboratory results.

CONCLUSIONS: The human corneal stroma typically heals after LASIK in a limited and incomplete fashion; this results in a weak, central and paracentral hypocellular primitive stromal scar that averages 2.4% as strong as normal corneal stroma. Conversely, the LASIK flap wound margin heals by producing a 10-fold stronger, peripheral hypercellular fibrotic stromal scar that averages 28.1% as strong as normal corneal stromal, but displays marked variability. [*J Refract Surg.* 2005;21:433-445.]

Wound healing in the human cornea is slower and less complete when compared to most other tissues in the body—presumably because the cornea is avascular—resulting in permanent weak areas in the cornea after surgery. For instance, two cases have been reported where spontaneous late-onset dehiscence of traumatic full-thickness corneal lacerations occurred 17 and 56 years after surgical repair.[1] Similarly, another series of cases found a 2.5% prevalence of traumatic, late-onset wound dehiscence in eyes with penetrating keratoplasty; although the majority of these cases occurred before 4 years after surgery, one third were between 6 and 13 years postoperatively.[2] Another case series reported that 7.2% of penetrating keratoplasty corneas developed spontaneous partial or complete wound dehiscence within 3 weeks of suture removal, despite an average postoperative time of 25 months.[3] Similarly, corneas have been documented to rupture along the radial keratotomy scars from blunt trauma in cases 10 years postoperatively.[4] At least 16 cases of traumatic, late-onset flap dehiscence occurring in corneas out to 38 months after LASIK have been reported.[5] The long-term weakness of the LASIK interface wound is further supported by the fact that LASIK flaps have been successfully re-lifted out to 11 years after initial surgery by one author (G.O.W.).

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TABLE 1

Demographics of Human LASIK Eye Bank Corneas

Donor No./ Sex/Age (y)	Time Between Death and Preservation (h)	Time Between Death and Testing (d)
1/M/20	20.4	7
2/F/39	14.3	5
3/M/34	20.1	5
4/M/50	5.7	4
5/F/48*	16.2	4
6/M/55	7.3	4
7/M/30	4.5	3
8/M/58	5.2	8
9/F/67	14.6	2
10/F/31	11.4	10
11/M/42	7.1	3
12/F/54	2.5	6
13/F/45	9.8	4
Mean±SD Age 44.1±13.1	10.7±5.7	5.0±2.1

SD = standard deviation
**Laser microkeratome corneas*

TABLE 2

Demographics of Human Control Eye Bank Corneas

Donor No./ Sex/Age (y)	Time Between Death and Preservation (h)	Time Between Death and Testing (d)
1/F/30	13.0	5
2/F/22	3.0	5
3/F/61	6.3	5
4/M/55	16.3	5
5/M/54	10.9	5
Mean±SD Age 44.4±17.2	9.9±5.3	5.0±0

SD = standard deviation

Our previous laboratory work concurs with the clinical information described above, as it showed that approximately one third of the histologically fixed human LASIK corneas developed either a partial or complete artifactual flap dehiscence during tissue processing or tissue sectioning.[6]

The purpose of the current study was to quantify the biomechanical strength of the LASIK interface wound by separating the wound with a motorized pulling device with an attached tensiometer. Correlative histology and ultrastructure along with a retrospective clinical case series helped interpret the results.

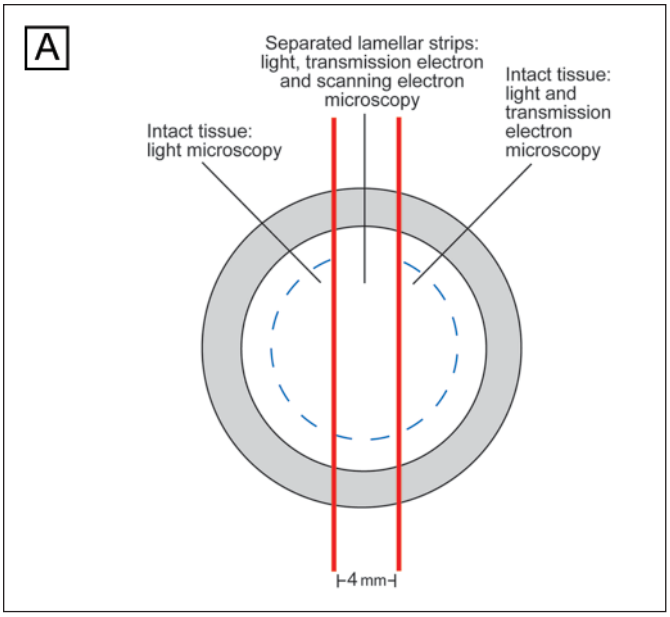
PATIENTS AND METHODS

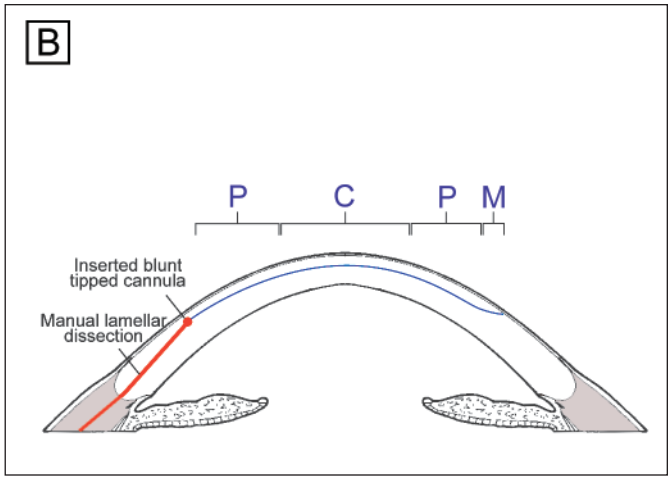
Tissue Samples

After approval by the Emory University Institutional Review Board, 25 corneoscleral specimens from 13 donors with a history of LASIK (23 mechanical microkeratome, 2 laser microkeratome) from 5 months to 6.5 years after surgery were obtained from various eye banks in North America (Table 1). Five age-matched and time-in-preservation-matched normal corneoscleral specimens from 5 patients served as controls (Table 2). All specimens were stored in Optisol-GS (Bausch & Lomb Surgical, Irvine, Calif) at 4°C within 24 hours of death and were tested in our laboratory within 10 days of death. Review of the donor's ophthalmic history was performed when available.

Tissue Preparation

The corneoscleral specimens were evaluated for signs of previous LASIK surgery by identifying a semicircular ring of haze present at the LASIK flap margin using a dissecting microscope (Möller-Wedel Optical, Wedel, Germany). The flap hinge was identified and marked with a wax pencil. A corneoscleral strip was prepared using a method described previously with slight modification (Fig 1A).[6-8] The corneoscleral specimens were placed anterior side up on a convex polyethylene surface and cut into 4-mm limbus-to-limbus strips centered on the LASIK flap hinge using a specially designed double-bladed knife. A 30-gauge needle was inserted through the scleral rim to stabilize and anchor the tissue in place. A crescent blade was used to perform a manual lamellar dissection of sclera on the side of the hinge at a depth of approximately 50% until the limbus was reached. A blunt-tipped cannula was inserted into the LASIK interface wound at the hinge and was left in place so that lamellar dissection could proceed until eventually reaching the interface wound (Fig 1B). The cannula was then removed and the dissection was stopped. A hole was made with a 16-gauge needle into the scleral rim of the anterior and posterior lamellar layers so that 5-mm-long anchoring hooks with attached metal chains were inserted into the holes. The chain connected to the posterior lamellar layer was then attached to the mobile portion of the pulling device via an isometric force transducer (Gould Metrigram BG-500, Valley View, Ohio) and the chain connected to the anterior lamellar layer was attached





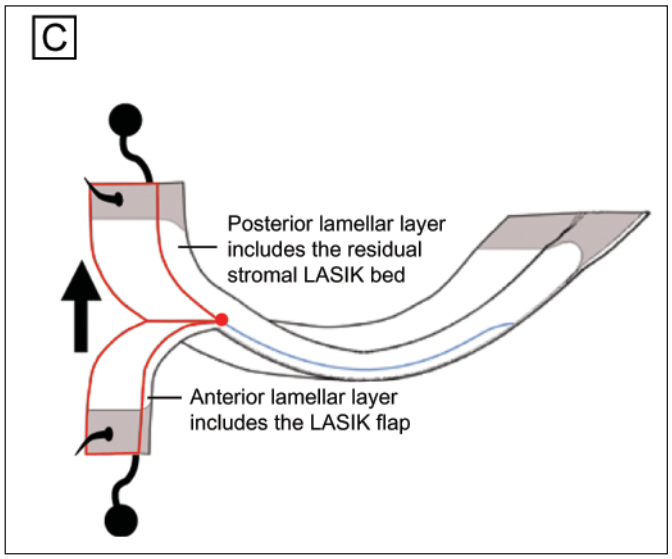


Figure 1. A) Diagram shows how the LASIK corneoscleral specimens were sectioned to obtain 4-mm-wide strips and then subsequently processed. **B)** Cross-sectional diagram of the middle strip in (A) showing how the manual lamellar dissection was connected to the LASIK interface wound at the hinge. C = central wound, P = paracentral wound, M = peripheral flap wound margin. **C)** Diagram demonstrates how the flap and residual stromal bed were separated using the motorized pulling device. Blue lines = LASIK wound, red lines = manual lamellar dissection, red dots = point where blunt tipped cannula was inserted.

to the immobile base plate via a protruding hook. The flap was separated along the length of the LASIK wound from the site of the hinge to the wound margin by setting the pulling device at a constant speed of 1.6 mm/s (Fig 1C). The force transducer readings measured in grams were recorded on the vertical y-axis of the line graph as the flap was separated. The horizontal x-axis of the line graph corresponded to time with the paper speed set at 5 mm/s. Marks were placed on the line graphs to identify initial wound separation, the center of the flap, the flap margin, and when the flap became completely dislocated from the residual stromal bed. Using the measured width of the strip in millimeters, the tensile cohesive strength measurements were converted to the unit grams per millimeters of tissue separated so that comparative analysis could take place. The force transducer was calibrated before each experiment using a 50- or 100-g weight (Fig 2). The two separated portions of the lamellar strip (flap and residual stromal bed) and the two intact lateral portions of the original corneoscleral specimen were processed for routine light microscopy, transmission electron microscopy, and scanning electron microscopy.

The procedure was performed in a similar fashion for normal control specimens (n=5). The notable differences being that the lamellar strips were always cut along the vertical meridian (shortest measured anterior corneal meridian) and the manual dissection was only carried out into the anterior third of sclera before separating and measuring the interlamellar cohesive tensile strength of normal tissue.

Conventional Light Microscopy

The formalin-fixed specimens were dehydrated in a graded series of alcohol solutions, cleared with xylene solution, infiltrated, and embedded in paraffin for sectioning. Five-micron-thick sections were cut and stained with hematoxylin-eosin or periodic acid Schiff (PAS) according to routine protocols. Qualitative and quantitative histopathologic findings from light microscopy evaluations (Olympus BH-2 microscope, Tokyo, Japan) were recorded.

Scanning Electron Microscopy

The glutaraldehyde-fixed specimens were rinsed in 0.1 mol/L cacodylate buffer and post-fixed in 1%

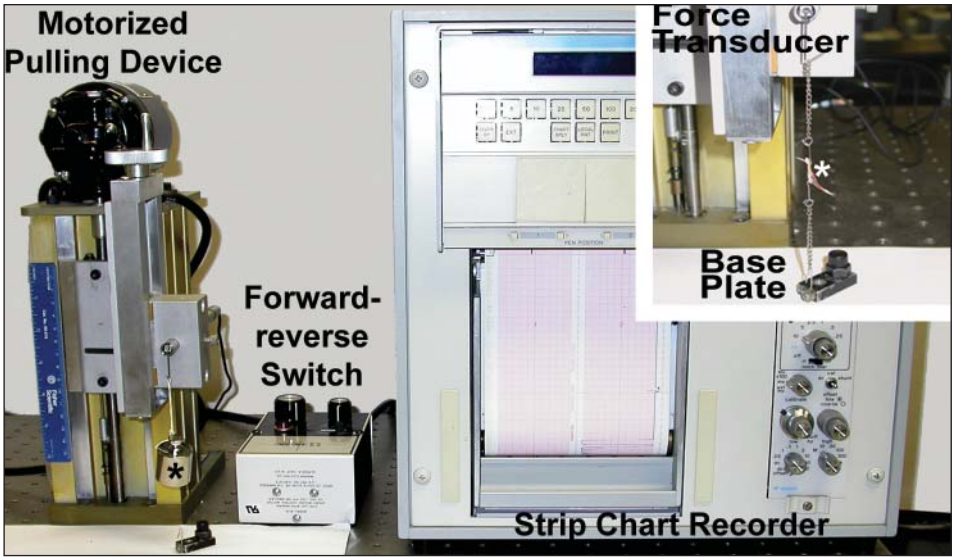


Figure 2. Motorized pulling device with labels. The main picture shows calibration of the device using a 100-g weight (black asterisk). The inset is a magnified view of the motorized pulling arm with a central 4-mm LASIK corneal strip (white asterisk) in place, just before separating it along the wound from the hinge to the flap wound margin.

osmium tetroxide. After dehydration, the samples were mounted on stubs, sputtered with gold-palladium, and observed with JSM-35CF scanning electron microscope (JEOL Ltd, Tokyo, Japan) at magnifications ranging from 100× to 1000×. Images were taken from the posterior surface of the flap and the anterior surface of the residual stromal bed in central and peripheral locations in LASIK specimens, or from similar locations in controls.

Transmission Electron Microscopy

The glutaraldehyde-fixed specimens were rinsed in 0.1 mol/L cacodylate buffer and post-fixed in 1% osmium tetroxide. After dehydration, the samples were embedded in LX 112 epoxy resin according to standard techniques. Thick sections (1.5 µm) were then stained with toluidine blue and evaluated by light microscopy to find the lamellar LASIK wound. The blocks were trimmed around areas of interest and thin sections (70 to 80 nm) were cut, placed in a copper grid, double-stained with uranyl acetate and lead citrate, and examined with a JEM-100 CXII transmission electron microscope (JEOL Ltd) at magnifications ranging from 3500× to 90,000×.

Clinical Case Series

A retrospective review of consecutive patient records from one author's clinics (G.O.W.) identified 144 eyes that received a LASIK flap-lift retreatment. The initial procedure in all cases was performed with the Hansatome microkeratome (Bausch & Lomb, Rochester, NY) making a superior hinge, and either the NIDEK EC-5000 (NIDEK Inc, Fremont, Calif) or the Alcon Autonomous LADARVision (Alcon Laboratories, Ft Worth, Tex) excimer laser was used for refractive ablation between May 3, 1996 and July 15, 2004. The retreatment procedures were done under the operating microscope of the excimer laser ranging from 2.7 months to 8.4 years after the initial LASIK surgery.

During the retreatment procedures, a Sinskey hook was pulled gently on the epithelial surface peripheral to the flap inferiorly. The tip of the instrument fell into the wound in all cases and a 1 to 2 o'clock length of the flap margin was opened by blunt dissection. A blunt tying forceps was used to grasp this area of the flap and lift the flap by peeling it in the superior direction, parallel to the corneal surface. A laser retreatment ablation was carried out before replacing the flap back onto the bed.

Statistical Analysis

All results are presented as mean±standard deviation (SD). When data groups were compared to determine whether a statistically significant difference occurred, a Wilcoxon rank-sum test was performed with the level of significance set at $P \leqslant .05$.

RESULTS

Demographics of LASIK and Control Cases

The demographics of the LASIK and control specimens tested and evaluated are given in Tables 1 and 2. Average age of the 13 LASIK donors (6 female and 7 male) was 44.1±13.1 years (range: 20 to 67 years) with an average time after LASIK of 3.1±1.6 years (range: 5 months to 6.5 years). Average age of the 5 control donors (3 female and 2 male) was 44.4±17.2 years (range: 22 to 61 years).

Interlamellar Cohesive Tensile Strength of Controls

The tensile strength was greatest in the sclera, averaging 47.13±6.69 g/mm (range: 40 to 60 g/mm; n=5). The tensile strength gradually decreased, typically

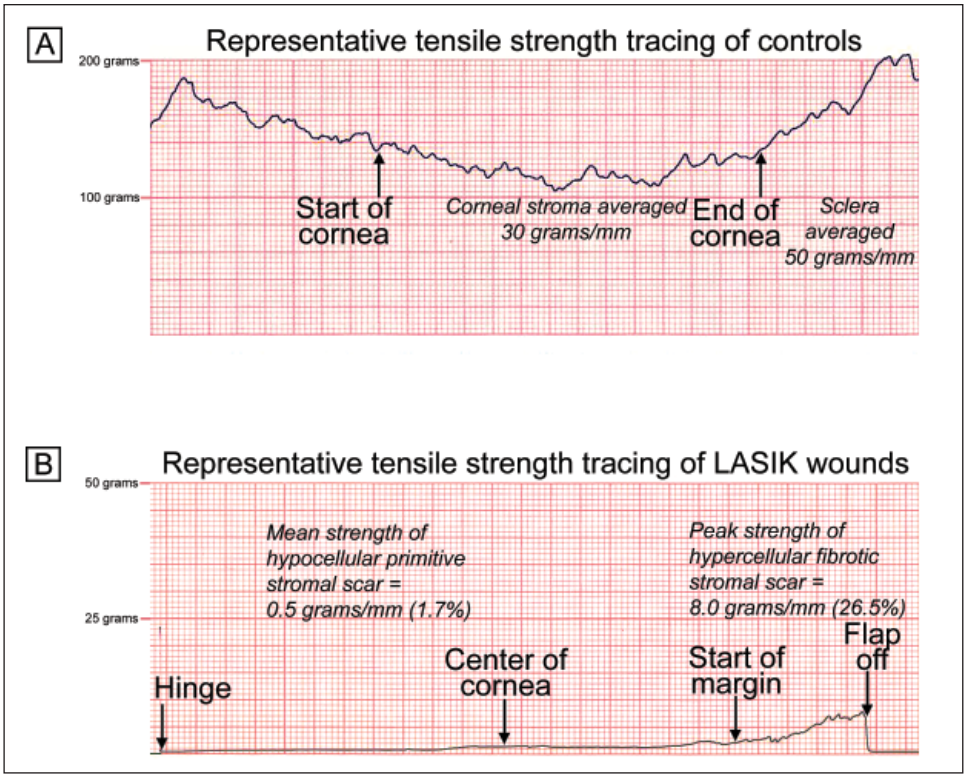


Figure 3. Representative cohesive tensile strength line graphs of a control cornea and LASIK cornea. Both tracings are plotted from left to right. **A)** The tracing of the interlamellar cohesive tensile strength of a normal cornea mechanically separated in a lamellar fashion. All control corneas were similar to the graph shown. The cornea from limbus to limbus is measured between the arrows, and the tracings outside the arrows represent the sclera. **B)** Cohesive tensile wound strength tracing of a LASIK cornea 5 years after surgery. All LASIK corneas had tracing similar to this one. The location of separation in the LASIK wound is labeled in the graph with accompanying arrows, and the quantitative results are labeled in italic print.

reaching a minimum value near the center of the cornea before gradually increasing again. The average tensile strength of the normal corneal stroma was 30.06±2.93 g/mm (range: 22.5 to 36.5 g/mm; n=5). Line graphs of control corneas showing this raw data displayed irregular tracings (Fig 3A).

Cohesive Tensile Strength of LASIK Wounds

Central and Paracentral Wounds. The mean tensile strength in the central and paracentral LASIK wounds averaged 0.72±0.33 g/mm (range: 0.25 to 1.5 g/mm; n=25) (Table 3) (Fig 3B). No observable gain in wound strength was noted in these wound regions over time after LASIK (Fig 4). Statistical analysis showed that the average strength of these LASIK wound regions was significantly different compared to controls ($P<.0001$). Statistical analysis also found no significant difference between mechanical and laser microkeratome LASIK corneas ($P=.4667$). Line graphs of these wound regions typically displayed relatively smooth, horizontal tracings, such as the one shown in Figure 3B. A few corneas had rare small focal spikes in their tracings up to tensile strengths of 2.4 g/mm.

Peripheral Flap Wound Margin. The peak tensile strength in the peripheral flap wound margin averaged 7.18±4.15 g/mm (range: 2.5 to 15 g/mm; n=20) (see Fig 3B). A gradual increase was noted in these values up to 3.5 years after LASIK before maximum values were reached (see Fig 4). Between 3.5 and 6.5 years after LASIK, the average peak tensile wound strength was 8.46±4.56 g/mm (range: 2.7 to 15 g/mm; n=11). Statistical analysis showed that both the overall and the ⩾3.5 years postoperative wound margin results were significantly weaker than controls, but significantly stronger than the central and paracentral wounds ($P<.0001$). Statistical analysis also demonstrated no significant difference between mechanical and laser microkeratome LASIK corneal wounds ($P=.4286$). The line graphs of these wound regions typically resulted in fine undulating, positively sloped tracings (see Fig 3B). Some had focal larger undulations.

Light Microscopy

Light microscopy of the intact (non-separated), lateral portions of the control specimens showed no pathologic findings. The intact, lateral portions of the LASIK corneas demonstrated a hypocellular primitive stromal scar present in the regions of the central and paracentral wounds, and a hypercellular fibrotic stromal scar at the peripheral flap wound margin.

Light microscopy of the separated, central strips in controls showed that the separation consistently occurred between corneal stromal lamellae, resulting in a moderately rough, irregular surface caused by conspicuous lamellar surfaces with protruding ruptured ends of interweaving and crossing lamellae (Fig 5A). In contrast, the mechanical (Fig 5B) and laser microkeratome LASIK (Fig 5C) corneas showed that the separation

TABLE 3

Summary of Wound Strength Data in Human LASIK Eye Bank Corneas

				Cohesive Tensile Wound Strength		Histopathology at the Flap Margin		
Donor No.	Eye	Time After LASIK (y)	Central Flap Thickness (μm)	Central and Paracentral Wound (g/mm)	Flap margin (g/mm)	Epithelial Ingrowth (μm)	Bowman's Layer Gap (μm)	Location of Wound Margin
1	OD	0.4	85	0.44	2.00	—	20	P
	OS		83	0.44	*	*	*	*
2	OD	0.5	106	0.25	3.00	—	8	P
	OS		106	0.33	*	*	*	*
3	OD	1.5	160	1.0	2.50	—	70	P
	OS		145	0.75	*	*	*	*
4	OD	2.0	244	0.75	10.75	—	12	P
	OS		196	1.25	9.50	—	40	P
5	OD†	2.0	137	0.75	*	*	*	*
	OS†		123	1.0	5.00	90	8	P
6	OD	2.5	127	0.75	4.00	400	50	P
	OS		113	0.5	*	*	*	*
7	OD	3.5	226	0.5	7.25	—	4	P
	OS		242	0.5	14.00	—	60	P
8	OD	3.5	200	0.33	3.67	150	80	P
	OS		178	0.5	13.00	—	4	L
9	OD	4.0	187	1.5	4.00	224	16	P
	OS		149	1.0	2.75	140	84	P
10	OD	4.5	187	0.75	4.00	60	56	P
	OS		153	0.75	15.00	—	106	P
11	OD	5.0	205	0.4	5.75	216	84	P
	OS		180	0.5	8.00	—	2	P
12	OD	5.5	145	1.25	12.00	—	40	P
13	OD	6.5	167	0.75	7.50	40	82	P
	OS		187	0.5	10.00	—	90	P
Mean±SD		3.1±1.6	168±51	0.72±0.33	7.18±4.15	165±116	46±35	

OD = right eye, OS = left eye, P = peripheral cornea, L = limbus, SD = standard deviation
**Hinge/partial hinge*
†Laser microkeratome LASIK corneas

always occurred along the interface wound (central, paracentral, and wound margin); these wound surfaces were much smoother in appearance on cross-sectional views than the controls.

Pertinent correlative light microscopy findings from LASIK wound regions measured for cohesive tensile strength are summarized in Table 3. Of the corneas that were maximally healed (3.5 and 6.5 years after LASIK), the wound margin scars with epithelial cell ingrowth (46% of cases; n=6) were found to be approximately one-third as strong (4.61 g/mm vs 11.32 g/mm, P=.0023) as those without epithelial cell ingrowth (54% of cases; n=7). Additionally, of the corneas without epithelial ingrowth, the wound margins that had wider gaps in Bowman's layer measured by the horizontal separation distance and wounds that went within the corneal limbus had the strongest tensile strengths.

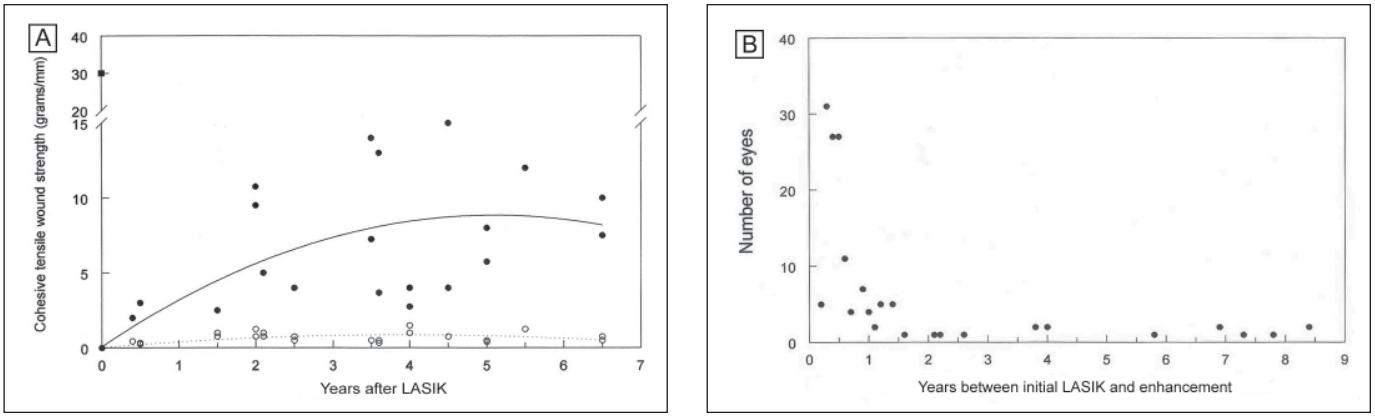


Figure 4. A) Scattergraph of the cohesive tensile strength measurements in the region of the hypocellular primitive stromal scar in the central and paracentral wound (open circles) and the hypercellular fibrotic stromal scar at the flap wound margin (black solid circles) plotted versus the time after LASIK. Best curve fit lines (second order polynomial) were made for each of the two data sets. No evidence of a gain in tensile wound strength over time was measured in the hypocellular primitive scar best fit curve (dotted line), whereas a gradual increase was measured up to 3.5 years after LASIK in the hypercellular fibrotic scar (solid line) before reaching a plateau. Square = mean value of the 5 normal controls. **B)** Scattergraph of 144 consecutive clinical eyes that had LASIK flap-lift enhancements, plotting the number of eyes reoperated versus time after initial LASIK.

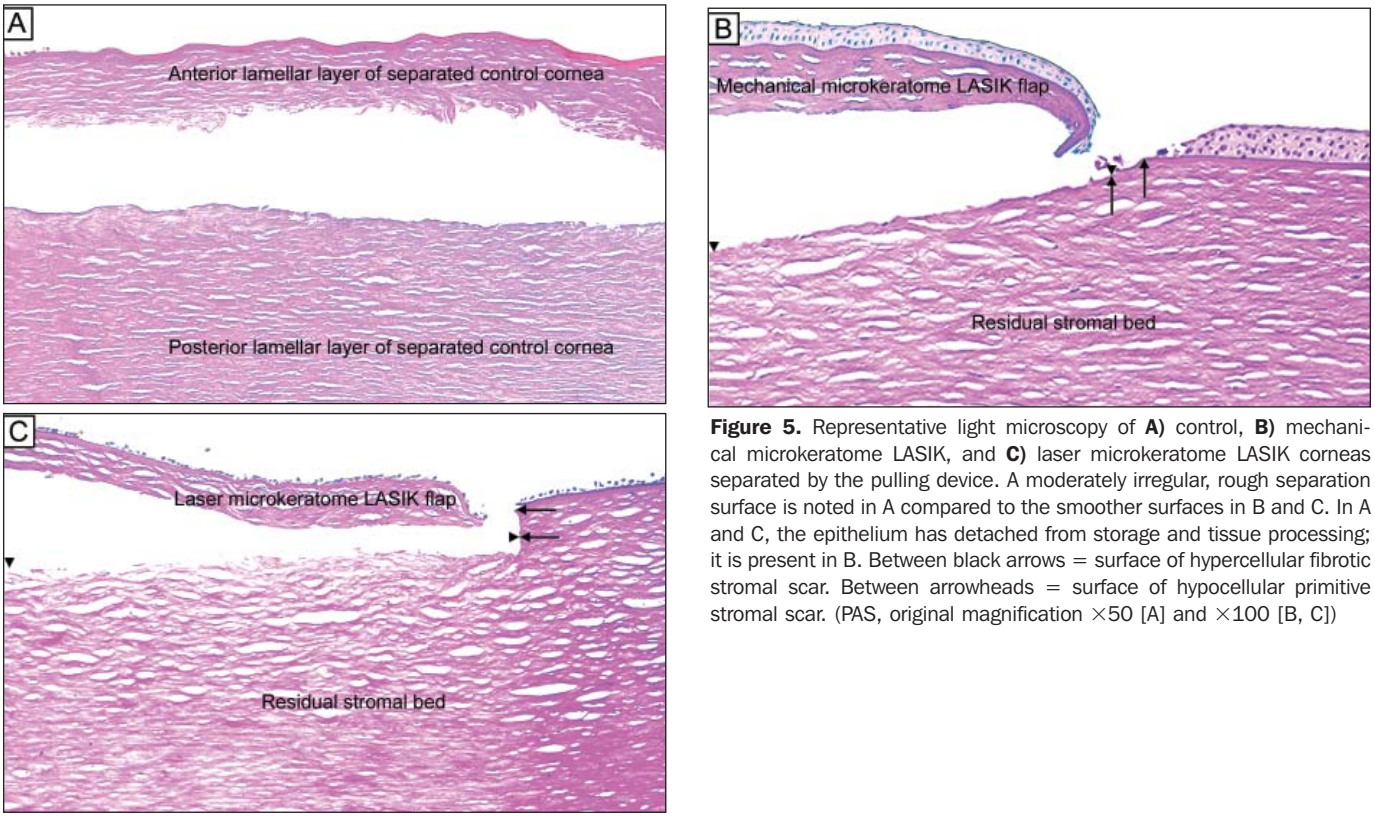


Figure 5. Representative light microscopy of **A)** control, **B)** mechanical microkeratome LASIK, and **C)** laser microkeratome LASIK corneas separated by the pulling device. A moderately irregular, rough separation surface is noted in A compared to the smoother surfaces in B and C. In A and C, the epithelium has detached from storage and tissue processing; it is present in B. Between black arrows = surface of hypercellular fibrotic stromal scar. Between arrowheads = surface of hypocellular primitive stromal scar. (PAS, original magnification ×50 [A] and ×100 [B, C])

Electron Microscopy

Transmission Electron Microscopy. Cross-sectional ultrastructural views of intact, lateral portions of controls showed normal morphology. In contrast, the LASIK corneas (mechanical and laser microkeratome) demonstrated a hypocellular primitive stromal scar in the central and paracentral wounds, which consisted of electron dense granular material, intermixed with scattered loose networks of small diameter (21 nm) collagen fibrils and infrequent keratocytes. Previous analysis by our laboratory has shown that this electron dense granular material is predominantly a type of abnormally large, non-fibril-bound proteoglycan.[6] In contrast, the hypercellular fibrotic stromal scar found at the LASIK flap wound margins consisted of a dense irregular network of normal diameter (26 nm) collagen fibrils, many interspersed keratocytes, and occasional myofibroblasts. Surgically cut ends of corneal stromal

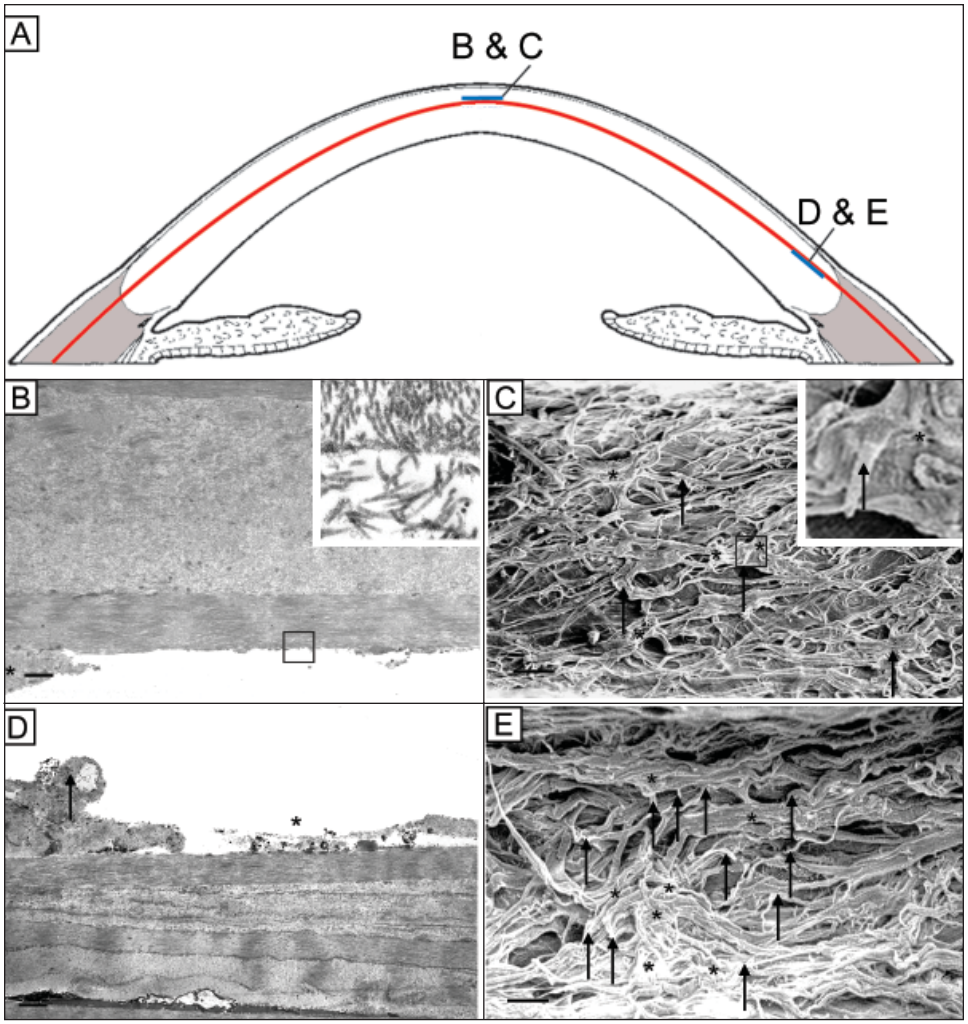


Figure 6. A) Diagram showing the location of transmission electron microscopy (B, D) and scanning electron microscopy (C, E) from separated control corneas. **B, C)** Central posterior surface of the anterior lamellar layer and **D, E)** peripheral anterior surface of posterior lamellar layer. The separated surfaces of the control corneas show a moderately irregular, rough contour from conspicuous corneal lamellae. Protruding from the surface are ruptured ends of interweaving lamellae (arrows) and foci of bridging filaments (asterisks) that are located in areas where lamellae crossed one another (B, C); the density of these ruptured fibrils and filaments was higher in the peripheral areas of the cornea (D, E). Boxes correspond to insets. Bar in transmission electron microscopy = 1 μm and scanning electron microscopy = 100 μm (transmission electron microscopy, original magnification ×3500; scanning electron microscopy, original magnification ×100).

lamellae were observed adjacent to both scar types. Higher magnification showed that the newly formed collagen fibril networks in the stromal scar intercalated between or around the surgically cut ends of lamellar and Bowman's layer collagen fibrils, but never reconnected to them.

Cross-sectional ultrastructural views of the mechanically separated strips were obtained on the central and peripheral regions of control (Fig 6A), mechanical microkeratome LASIK (Fig 7A), and laser microkeratome LASIK (Fig 8A) corneas. The control specimens demonstrated that the primary structures in the corneal stroma affected by mechanical separation were the interweaving stromal lamellae and the areas where adjacent lamellae crossed one another (Figs 6B, 6D). Both of these areas had structures that showed evidence of traumatic rupture resulting in collagen fibril or filament ends that protruded into the plane of mechanical separation, being markedly more numerous in the periphery of the cornea (see Fig 6D) compared to the center of the cornea (see Fig 6B). Conversely, the LASIK corneas demonstrated predominantly cut interweaving and non-interweaving corneal lamellae (Figs 7B, 7D, 7F; Figs 8B, 8D, 8F). In the laser microkeratome LASIK cases, scant, regularly distributed, ruptured, interweaving lamellae and foci of bridging filaments where adjacent lamellar crossed one another were present (see Figs 8B, 8F).

Scanning Electron Microscopy. Tangential ultrastructural surface evaluations of the separated, central strips in control corneas showed that the central posterior surface of the anterior lamellar layer was moderately rough and irregular from conspicuous corneal lamellae that had frequent protruding ruptured ends of collagen fibril bundles or ruptured groups of interlamellar bridging filaments (Fig 6C). In contrast, the central posterior surfaces of both the mechanical (Fig 7C) and the laser microkeratome LASIK corneas (Fig 8C), which are the surfaces not treated with excimer laser, were relatively smooth, because the hypocellular primitive scar (that contained large amounts of proteoglycans) covered most

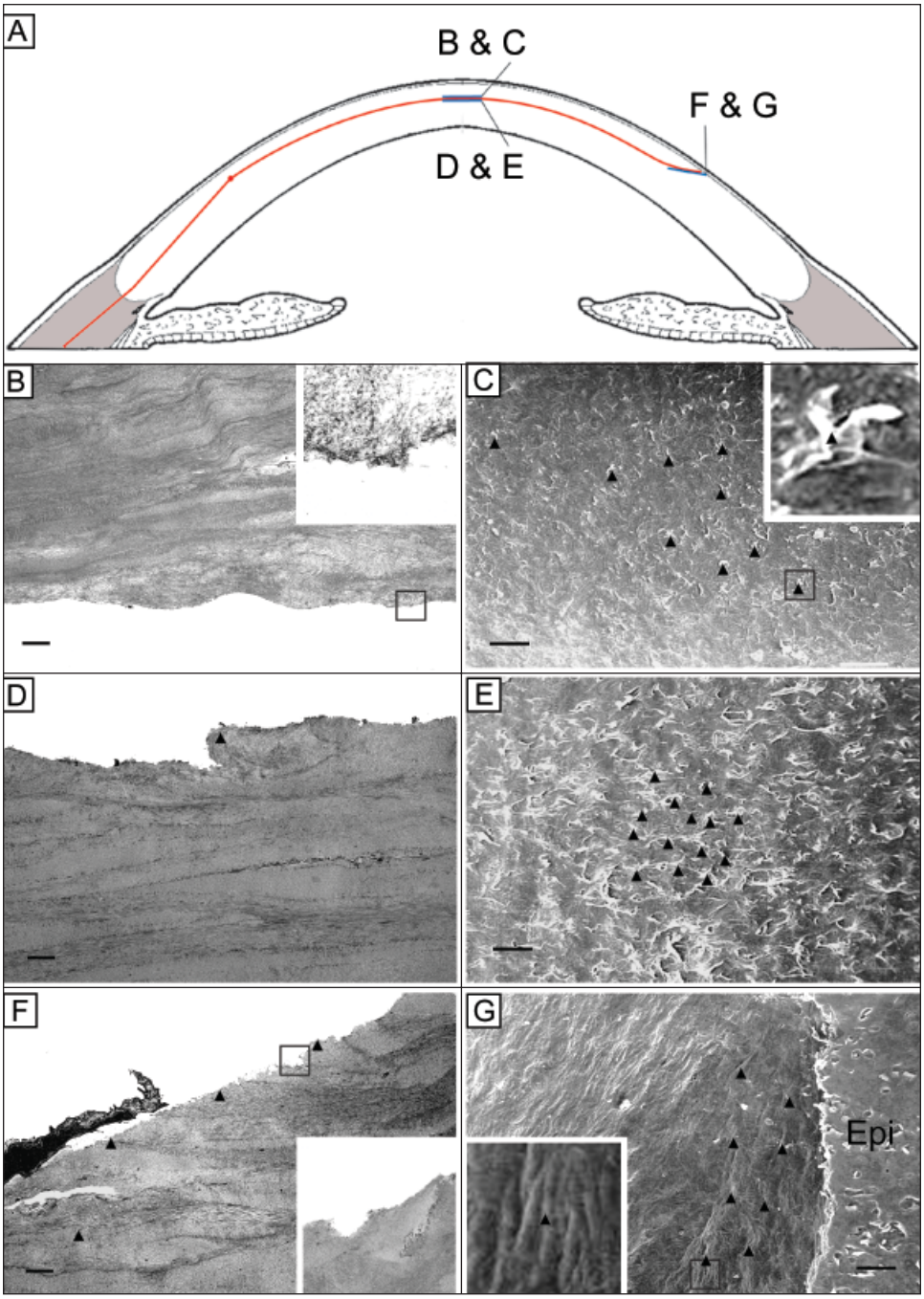


Figure 7. A) Diagram showing the location of transmission electron microscopy (B, D, F) and scanning electron microscopy (C, E, G) from separated mechanical microkeratome LASIK corneas. **B, C)** Central posterior surface of the LASIK flap. **D, E)** Central anterior surface of the residual stromal LASIK bed. **F, G)** Peripheral anterior surface of the residual stromal LASIK bed. The red line (in A) shows both the peripheral manual dissection and the LASIK lamellar bed. The separated surfaces of these LASIK corneas are smoother than the surfaces of the control corneas because the hypocellular primitive scar provides a smooth coating of proteoglycans over the corneal lamellae. Occasionally, protruding cut ends of lamellae (black arrowheads) are seen scattered throughout this surface, being less dense on the non-excimer laser ablated surfaces (B, C) than on the excimer laser ablated surfaces (D, E). The wound margin is similar in appearance to the other non-excimer laser ablated surfaces, the only notable difference being that many concentric ripples are present because the oblique side cut severs more corneal lamellae per length of cut (F, G). Boxes correspond to insets. Epi = corneal epithelial surface. Bar in transmission electron microscopy = 1 μm and scanning electron microscopy = 100 μm (transmission scanning microscopy, original magnification $\times$3500; scanning electron microscopy, original magnification $\times$100).

of these stromal surfaces. Occasional irregularities poked through this smooth surface from protruding cut or ruptured ends of stromal lamellae, or ruptured groups of interlamellar bridging filaments. The posterior surface of the laser microkeratome flap (see Fig 8C) was always found to be slightly rougher and more irregular than that of the mechanical microkeratome cases (see Fig 7C).

In control corneas, the central anterior surface of the posterior lamellar layer was similar to the central posterior surface of the anterior lamellar layer. In contrast, the central anterior surface of the residual stromal bed, which was treated with the excimer laser, in the mechanical (Fig 7E) and laser microkeratome (Fig 8E) cases were both mildly rough and irregular from protruding cut lamellae, being rougher compared to that of the non-excimer ablated posterior surfaces of the flap (see Figs 7C, 8C). No difference was seen between the excimer-ablated mechanical and laser microkeratome LASIK surfaces.

The peripheral anterior surface of the posterior lamellar layer of control corneas (Fig 6E) was similar

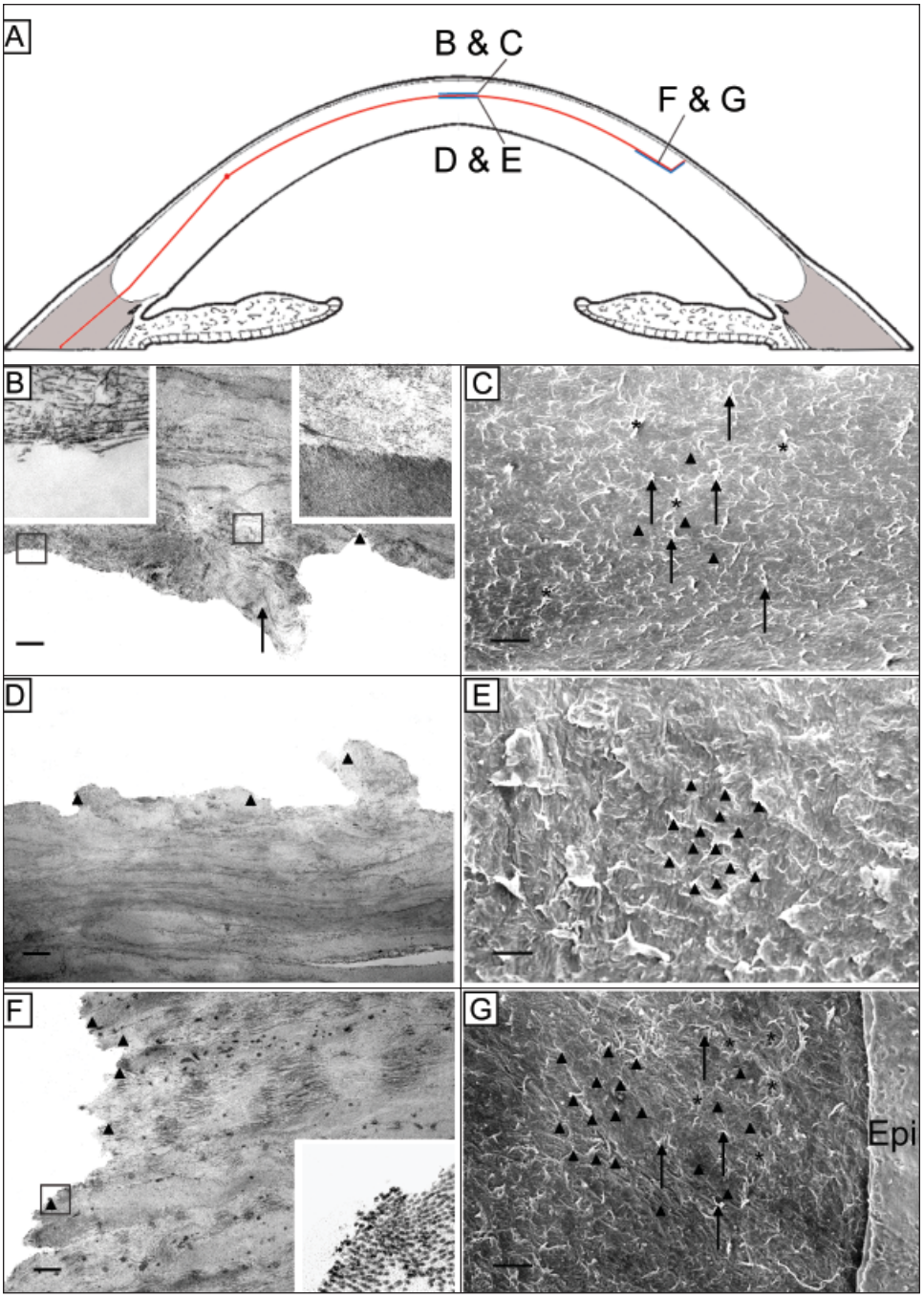


Figure 8. A) Diagram showing the location of transmission scanning microscopy (B, D, F) and scanning electron microscopy (C, E, G) from separated femtosecond laser microkeratome LASIK corneas. **B, C)** Central posterior surface of the LASIK flap. **D, E)** Central anterior surface of the residual stromal LASIK bed. **F, G)** Peripheral anterior surface of the residual stromal LASIK bed. The red line (in A) shows both the peripheral manual dissection and the LASIK lamellar bed. These corneas had wound strengths similar to mechanical microkeratome corneas, averaging 0.9 g/mm in the central and paracentral wound regions and 5.0 g/mm in the wound margin. The non-excimer laser ablated surfaces of these corneas (B, C) typically are more irregular and rough compared with the mechanical microkeratome corneas (see Figs 7B, 7C), presumably because the femtosecond laser leaves small uncut areas between treatment spots. The excimer laser ablated surfaces (D, E) were no different from the mechanical microkeratome corneas (see Figs 7D, 7E). Although the side cut of the laser microkeratome corneas (F, G) was vertical as opposed to oblique to the corneal surface, it also was more irregular and rougher than the mechanical microkeratome corneas (see Figs 7F, 7G). Boxes correspond to insets. Epi = corneal epithelial surface. Black arrows = ruptured interweaving lamellae. Black asterisks = ruptured foci of bridging filaments. Black arrowheads = cut ends of stromal lamellae. Bar in transmission scanning microscopy = 1 μm and scanning electron microscopy = 100 μm (transmission scanning microscopy, original magnification $\times$3500; scanning electron microscopy, original magnification $\times$100).

in roughness and irregularity to the central areas of control corneas (see Fig 6C), but had a higher density of ruptured interweaving lamellae and foci of bridging filaments on its surface than the central areas (see Fig 6E). In LASIK specimens, the peripheral anterior surfaces of the residual stromal bed, which had not received excimer laser treatment, in both the mechanical (Fig 7G) and laser microkeratome (Fig 8G) cases were not substantially different in appearance to that of the posterior non-excimer ablated surfaces of their respective flaps (see Figs 7C, 8C).

Clinical Cases Series

The preoperative demographics and data on the clinical eyes that received LASIK flap-lift retreatments were: 144 patients (88 women and 56 men); mean age at initial LASIK surgery 41.5±8.1 years (range: 23 to 64 years); and mean refraction prior to initial LASIK surgery −5.2±3.0 diopters (D) (range: −11.0 to +4.6 D). At the time of retreatment, the mean time after initial LASIK surgery was 12.3±19.3 months (range: 2.7 months to 8.4 years) (see Fig 4B) and the mean refraction prior to enhancement was −0.8±0.9 D (range: −2.5 to +5.1 D).

In all cases during retreatment surgery, after the wound margin was broken focally with the hook, the flap was lifted smoothly and easily. Although the flap typically separated from the residual stromal bed with minimal resistance regardless of the postoperative time, some eyes with retreatments performed more than 1 year after initial surgery showed slight resistance in lifting the flap from the bed, predominantly at the wound margin. In no case was it necessary to stop lifting the flap and defer to sweeping the interface to enhance the separation. Flap margins with visible areas of epithelial ingrowth were easier to lift than those without epithelial ingrowth. In eyes where the flap edge was close to the limbus, resistance was increased compared to more centrally located flap edges, requiring direct manual dissection with the hook or forceps. In all cases, when the hinge was encountered, the flap lift stopped abruptly because of a marked increase in resistance to further separation. Although one case developed a partially dehisced hinge, no cases were complicated by flap tears or buttonholes.

DISCUSSION

The issue of corneal cohesive tensile wound strength after LASIK is important because it affects the long-term stability of the cornea and the integrity of the LASIK flap. Because our previous work only qualitatively assessed cohesive tensile wound strength, this study was specifically designed to quantitatively measure the cohesive tensile strength of the LASIK interface wound.[6,9]

The corneal stroma is composed predominantly of a framework of insoluble collagen fibrils, water-soluble proteoglycans, and keratocytes. The collagen fibrils function primarily to maintain the shape and strength of the tissue by resisting tensile (expansile) forces. Proteoglycans serve primarily as space fillers that resist compressive forces as this is where the incompressible water resides.[10] Keratocytes form an organized syncytium and primarily serve to maintain the extracellular matrix of collagen fibrils and proteoglycan; these cells also have another role under certain conditions: they can differentiate into a tensile stress-resisting contractile cell, the myofibroblast.[11]

A variety of objective tests have been used to investigate the biomechanical properties of corneal wounds and most tests measure indirectly or directly cohesive tensile wound strength over the entire area of the wound (g/mm^2).[12-14] A disadvantage of those techniques is that they do not provide detailed information about the strength of the wound in specific areas. The advantage of the technique used in this study is that it can accurately measure the tensile breaking force (g/mm) required to separate wounded or normal tissue at each point over an advancing line across the entire sample.[7,8,15] This technique, therefore, provides functionally useful information to correlate with the histology, ultrastructure, and clinical behavior of the tissue. Its main disadvantage is that it does not provide useful information on the elastic stress or strain measurements of the wound or tissue.

The interlamellar cohesive tensile strength results on normal control corneas averaged 30.06 ± 2.93 g/mm, which is similar to previously published values.[8,15] The irregular wavy tracings on line graphs of the controls suggest that the ascents and descents of each wave correspond to bundles or groups of collagen fibrils and/or filaments undergoing maximal stress and subsequently rupturing. The ultrastructural studies on normal control corneas support this concept because they show ruptured bundles of collagen fibrils from interweaving lamellae and ruptured groups of filaments from areas where adjacent lamellae crossed one another.[15] The highest density of ruptured collagen fibrils and filaments was in the more peripheral areas of the control corneas, which explains the anisotropy of the control line graphs.

The cohesive tensile strength of corneal stromal wounds has been measured predominantly in animal models, which in comparison to human corneal wounds heals much more strongly and more completely than human corneal wounds.[7,12,16-18] Thus, although animal studies are practical and useful, particularly when evaluating early stages of wound healing or when studying many treatment groups that require large numbers, human corneal wound healing studies are needed to understand more completely the long-term clinical outcome. The present study exemplifies this fact as human central and paracentral LASIK wounds were found to contain a hypocellular primitive stromal scar that regained on average 2.4% of normal stromal strength and displayed no evidence of remodeling. This is notably weaker than similarly located rabbit corneal wounds that were found to heal up to 25% to 50% of normal strength by 2 months after surgery.[7] In contrast, the flap wound margin in humans was found to contain a hazy, hypercellular fibrotic stromal scar that showed evidence of remodeling up to 3.5 years after LASIK before reaching a maximum tensile strength that averaged 28.1% of normal stromal strength. These data are again different compared to animal data, but generally are in agreement with one long-term adult human corneal wound healing study, which found that the tensile strength of corneal cataract wounds increased up to 4 years after surgery before reaching a plateau.[7,14]

The ultrastructural correlations performed in this study demonstrated that the newly created collagen

fibrils in the stromal scar intercalate between and around the adjacent old cut fibrils and entrap themselves into their networks. This presumably is why the cohesive tensile strength of adult human corneal stromal wounds never returns to normal values and is similar to what Maurice[17] proposed in 1987—new collagen fibrils in the stromal scar do not reconnect end-to-end with old cut fibril ends. The contribution provided by myofibroblasts to the total tensile wound strength has not been determined directly, but may factor in because the strongest wound margin scars were typically those variants found to have a higher number of persistent myofibroblasts in their hypercellular fibrotic scars, especially those without epithelial ingrowth that had the widest gaps in Bowman's layer or were located in the limbus.[6] Animal wound healing studies have also shown that these two wound variants were typically the strongest.[16,18]

A marked change was observed in the morphology of the stromal scar as it approached the epithelial surface at the LASIK flap wound margin (transition from a hypocellular primitive to a hypercellular fibrotic stromal scar), which has been previously described.[16,19,20] This presumably occurs because of epithelial–stromal interactions by way of chemotactic cytokines and growth factors that come from epithelial cells, keratocytes, and possibly the tears.[21,22] The wound strength results presented in this study are the first to isolate and directly measure the tensile strength from these two different wound regions in human corneas after LASIK. The histological correlations are also the first to show that fully healed human corneal wounds with epithelial cell ingrowth (4.61 g/mm) have on average one-third the tensile strength as those without epithelial cell ingrowth (11.32 g/mm). This was previously only calculated from theoretical data in radial keratotomy specimens.[23] This difference probably occurs because the depth of the hypercellular fibrotic scar is less in eyes with epithelial ingrowth (5 to 20 µm deep vs 50 to 75 µm deep) and because epithelial cells provide markedly less tensile strength to the wound than a fibrotic scar.[6]

The clinical knowledge gained from the LASIK flap-lift retreatment cases correlated well with the laboratory results. The tip of the Sinskey hook typically fell into the LASIK wound margin with minimal effort correlating with the gap in Bowman's layer seen histopathologically. Most of the resistance when lifting the flap occurred at the flap margin, particularly the cases >1 year after surgery and those with the wound in the corneal limbus, correlating with the area of hypercellular fibrotic stromal scarring and its greater measured tensile strength. Conversely, the resistance to lifting the flap in the central and paracentral regions of the interface wound was always minimal, correlating with the area of the hypocellular primitive stromal scarring and its lesser tensile strength. In some eyes, after the flap was lifted, the surface of the residual stromal bed in the central interface wound showed visible circular zones from previous broad area excimer laser ablation, further attesting to the minimal healing described pathologically in the central and paracentral LASIK bed.

This study shows that the primary structural reason for the high cohesive tensile strength of normal corneal stroma is the collagen fibrils from interweaving corneal lamellae and the groups of bridging collagen filaments where stromal lamellae cross one another. Corneal stromal LASIK wounds were found to heal weaker than normal because these structures were not regenerated during the healing response. Moreover, the central and paracentral stromal LASIK wounds were found to heal by producing a hypocellular primitive stromal scar that is very weak in tensile strength, averaging 2.4% of normal, and displays no evidence of remodeling over time in specimens out to 6.5 years after surgery. In contrast, the more superficial, flap margin stromal LASIK wound, which is adjacent to the surface epithelium, was found to heal by producing a 10-fold stronger, hypercellular fibrotic stromal scar that reaches maximum tensile strength by approximately 3.5 years after surgery, averaging 28.1% of normal. Histologic and ultrastructural studies add to our understanding of these results as they show that the amount of densely packed, intercalating collagen fibrils and possibly the number of myofibroblasts contained in the scar are most indicative of strong scars (30% to 50% of normal strength). In comparison, flap wound margin stromal scars that contain epithelial cells or central and paracentral stromal scars that contain non-fibril bound proteoglycans were found to be the weakest scars (8% to 15% and 1% to 5% of normal strength, respectively).

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