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Quantified Histopathology of the Keratoconic Cornea

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Abstract

Purpose—The present study systematically investigated and quantified histopathological changes in a series of keratoconic (Kc) corneas utilizing a physiologically formulated fixative to not further distort the already distorted diseased corneas.

Methods—Twelve surgically removed Kc corneal buttons were immediately preserved and processed for light and transmission electron microscopy using an established corneal protocol. Measurements were taken from the central cone and peripheral regions of the host button. The sample size examined ranged in length from 390–2608um centrally and 439–2242um peripherally.

Results—The average corneal thickness was 437um centrally and 559um peripherally. Epithelial thickness varied centrally from 14–92um and peripherally from 30–91um. A marked thickening of the epithelial basement membrane was noted in 58% of corneas. Centrally, anterior limiting lamina (ALL) was thinned or lost over 60% of the area examined, while peripheral cornea was also affected, but to a lesser extent. Histopathologically, posterior cornea remained undisturbed by the disease. Anteriorly in the stroma, an increased number of cells and tissue debris were encountered and some of these cells were clearly not keratocytes.

Conclusions—It is concluded that Kc pathology, at least initially, has a distinct anterior focus involving the epithelium, ALL and anterior stroma. The epithelium had lost its cellular uniformity and was compromised by the loss or damage to the ALL. The activity of the hitherto unreported recruited stromal cells may be to break down and remove ALL and anterior stromal lamellae leading to the overall thinning that accompanies this disease.

Keywords

keratoconus; epithelium; anterior limiting lamina; stroma; histopathology; transmission electron microscopy

Keratoconus (Kc) is a non-inflammatory condition characterized by corneal ectasia, steepening, thinning and scarring.^{1–3} Reports in the literature have described a number of pathological changes within the Kc cornea. Featured prominently in these reports are observations of “breaks/interruptions/fragmentations/dehiscences/ruptures” of the anterior limiting lamina (ALL).^{4–19} This observation appears to describe a ‘crack’ or brief discontinuity of the ALL. Although most sources agree that Kc is a disease of the stromal layer, Chi et al. (1956)⁵ presented some evidence of an epithelial contribution to this ocular pathology. Teng (1963) even postulated that the origin of this disease was to be found in the epithelium,²⁰ and this hypothesis has later received further scientific support.^{6,7,12,21–23}

Stromal changes in the Kc cornea have been noted in the literature but in limited detail. Leibowitz and Morello (1998) stated that “collagen fibers are said to be normal, with thinning attributable to a decrease in the number of collagen lamellae”¹⁰. The loss of lamellae as an explanation to corneal thinning is also proposed by other sources.^{14,24–26} In addition, and according to Morishige, the Kc cornea showed “less lamellar interweaving and marked reduction or loss of [anterior] inserting lamellae”.²⁵

Most corneal alterations reported appear to have an anterior location,^{1,4,14,17–21} which seems to be confirmed by *in-vivo* corneal confocal microscopy. However, histopathological changes can occur in the posterior cornea in this condition as a result of hydrops. However, hydrops is viewed as an uncommon complication in this disease.²⁷ Rupture of the posterior limiting lamina (PLL), leading to a discontinuity of endothelial cell coverage of the posterior corneal surface, allows aqueous free entry into the corneal stroma.^{14,24,27} Although the induced corneal edema mostly clears up, resultant scarring may prevent patients with history of hydrops from regaining acceptable visual acuity.²⁷

The current understanding of the pathophysiology of Kc is incomplete and eyecare practitioners managing Kc patients would greatly benefit from improvement of our knowledge base. Detailed information on tissue changes within the cornea may provide indications on the ability of the unhealthy cornea to handle the presence of a contact lens or where lens bearing is undesirable. More precise knowledge of the location and focus of the disease may better direct the surgeon to the optimal choice of procedure, which, in transplant surgery, may be lamellar or penetrating keratoplasty.

The literature contains only a modest number of histopathological reports on Kc involving tissue samples from a larger case series. However, with few exceptions, they lack morphometry and in some cases, are limited to light microscopy and a protocol using a high osmolality fixative that further distorts an already distorted cornea.^{12,23,28–30} The purpose of this study was to systematically investigate and quantify pertinent histopathological changes in a series of Kc corneas, at both the apex of the cone and the adjacent mid-peripheral corneal region, using an established fixative with physiological osmolality.

MATERIALS AND METHODS

Twelve keratoconic corneal buttons were surgically removed by one author (JG), with eight corneas removed by penetrating keratoplasty (PKP) and four by deep lamellar keratoplasty (LKP). The corneas were from 12 different individuals, aged 18 to 70 years, and all manifested at least one clinical sign for Kc, such as Fleischer ring, Vogt striae, Munson sign, and had vision compromised by the disease. Four apparently disease free corneas obtained from eye banks and the National Disease Research Interchange, were utilized as control tissue. All corneas were handled in accordance with the guidelines of the Declaration of Helsinki regarding research on human tissue. Informed consent was obtained from all subjects after first explaining that there was no risk involved in donating their tissue. This study received appropriate institutional review board approval.

Immediately following the removal of the Kc corneal button, the surgeon placed the tissue into a fixative solution of 2% glutaraldehyde in 80mM sodium cacodylate (pH 7.4, 320 to 340 mOsm/kg). Control eyes were stored in the eyebank medium (Optisol GS, Bausch & Lomb, Irvine, CA) and arrived on ice. These corneas were fixed within 72 hours after death. For all corneas, the fixative and tissue preparation followed an established protocol developed to minimize tissue distortion and dimensional change.^{31–32} Small pieces (1 × 3 mm) were cut from the visibly thinnest area of the cone apex and within the Fleischer ring, and the periphery, defined as the area most peripheral to the Fleischer ring, of each corneal

button. The pieces were thereafter placed in a freshly prepared 1% solution of osmium tetroxide (OsO_4) in 0.1M cacodylate buffer for post-fixation in subdued lighting. Thereafter, the samples were washed in buffer and then dehydrated through a graded alcohol series (30% to 100% in 6 steps) at room temperature. Then the samples were infiltrated with propylene oxide (3 changes at 10 min intervals) and subsequently with a 2:1 v/v mixture of propylene oxide and Araldite resin (product number 4300, Electron Microscopy Sciences, Fort Washington, PA, USA) for 3 hours. This was followed by overnight immersion in a 1:1 v/v mixture of propylene oxide and Araldite resin, followed by 4–8 hours in 1:3 v/v mixture of propylene oxide and Araldite resin and then transferred to 100% Araldite resin. The tissue samples were oriented in embedding molds and left for overnight polymerization at 60°C.

All corneal samples were first prepared for light microscopy by cutting thick (0.5–1µm) transverse sections and then staining with toluidine blue. The area examined microscopically is expressed as a length measure along the epithelial basement membrane (BM). Centrally, tissue samples ranged in length from 389.6µm to 2607.9µm and measured on average 1363.0µm, while peripherally these measurements were 439.3µm, 2242.3µm and 1182.0, respectively. The length of the tissue examined depended in part on how much of the tissue had suffered from physical damage during the surgery and subsequent handling. For electron microscopy, parallel bar copper grids (SPI Supplies, West Chester, PA, USA; product number 2440C-XA) with a 80 µm spacing were used, and the ultrathin sections (approx. 60–90nm) were stained first in 3.5% uranyl acetate (dissolved in water) for 20min at 60°C, followed by Reynold's lead citrate for 10min at room temperature. The tissue was examined ultrastructurally in a Tecnai G2 12 twin transmission electron microscope (FEI Company, Hillsboro, OR) operating at 100kV.

Using light microscopy (Olympus BX51 digital light microscope), a variety of measurements were taken from Kc corneas in two different transverse sections, separated by at least 200–300µm, and then averaged. These measurements were taken at three locations (central and at each extreme) along the tissue length and included: epithelial thickness (both LKP & PKP), epithelial cell layer counts (both LKP & PKP), ALL pathological involvement (both LKP & PKP), stromal thickness (only PKP), and total corneal thickness (only PKP). Length measurements were obtained using the NIH image software at the light microscope.

Tissue from PKP corneas was also evaluated ultrastructurally utilizing transmission electron microscopy for general and high resolution observations, photography and measurement of epithelial BM thickness. Control tissue was used for general observations and epithelial BM measurements but could not be utilized for other length or thickness measurements because of post-mortem tissue swelling. For obvious reasons, the eyebank eye almost invariably has postmortem ocular surface defects and, therefore, could not be used in the epithelial thickness and cell layer counts.

RESULTS

Total Kc corneal thickness and stromal thickness could only be measured in those corneas removed by PKP (n=8). Central stromal thickness was, on average, 379.2µm and ranged from 236.2µm to 633.1µm. Total corneal thickness centrally averaged 436.5µm and peripherally averaged 558.6µm (Table 1, 2). Epithelial thickness was quite variable for both the central cone region and the peripheral region, ranging from 13.5µm to 91.6µm (avg: 42.2µm) and from 29.8µm to 90.9µm (avg: 54.5µm), respectively (Figure 1). There were, on average, 6 epithelial cell layers centrally (range: 4–9), and 7 cell layers peripherally (range: 5–8) (Table 1, 2). The epithelium also showed both elongated and compressed basal cells and granular deposits within the cytoplasm (Figure 2, 3). Frequently epithelial cells had not taken up the stain in a characteristically uniform manner with some cells appearing

strikingly densely stained while others were pale. Light microscopically, it was recorded that 7 of the 12 corneas had developed a noticeably thickened BM (Figure 2). Ultrastructural observations of the PKP central buttons (n=8) confirmed an extremely abnormal BM thickness, which averaged 1.46 μ m and ranged from 0.05–3.67 μ m (Table 3). It was often stratified and contained large cracks on the side facing the basal epithelial cells. In contrast, the control corneas (n=4) were thinner with more uniform BM's measuring 0.05–0.65 μ m with an average of 0.41 μ m.

Large areas of ALL were thinned or completely missing centrally in 10 of 12 Kc corneas, but peripherally 8 of 12 corneas manifested abnormalities in this respect (Table 4, 5). Out of these, four Kc corneas had completely lost ALL over significant areas centrally, while peripherally, only two corneas exhibited such a loss. In these corneas, the center of the cone showed that ALL was affected over 60% of tissue examined. ALL was only affected peripherally if it was also affected centrally. In no instance was the peripheral corneal portion of the button affected without central involvement. In the central cone region, areas of extensive ALL loss ranged in length from 31.4 μ m to 790.0 μ m and areas of thinned ALL ranged from 32.4 μ m to 2602.4 μ m. Peripherally, areas of lost ALL and thinned ALL ranged from 16.28 to 363.9 and 53.0 to 767.9, respectively. Control tissue had ALL of uniform thickness and no areas with ALL missing.

In all specimens examined, a striking feature of the disease was its anterior corneal focus. Other than the already mentioned epithelial changes and the gradual destruction of the ALL, the anterior stroma, immediately posterior to ALL, was severely affected and in places even lost. An increased number of stromal cells and tissue debris were typically observed in this region and clearly some of these cells were not keratocytes, showing lighter stained cytoplasm (Figure 4, 5). None of these features were recorded in the control tissue.

Interestingly, we did not observe the clinically well established phenomenon of prominent nerves in the central Kc tissue. However, the button contained, on occasion, slender stromal unmyelinated fiber bundles that were abnormal in shape (Figure 6).

Posteriorly, the corneal morphology of the Kc buttons examined were similar to normal control tissue. On occasion, an abrupt kink was observed in the Kc lamellar outline. Physical trauma to the tissue and its handling during the surgical procedure produced endothelial damage, but away from such areas the endothelial cells appeared healthy and similar to those of the control corneas. The overall impression was that this disease does not involve the endothelium and its BM until a more advanced stage that is associated with hydrops.

DISCUSSION

This study attempted to morphometrically characterize Kc-induced pathological changes in a case series of 12 corneas from 12 patients. Furthermore, our data were generated from tissue that was preserved in a fixative formulated to better match corneal physiology.³¹ Our Kc tissue protocol, together with the immediate post-surgical fixation, avoided the dilemma of further distortion of an already pathologically distorted tissue. Therefore, it is here argued that the data and observations presented are accurate and generally free of artifacts. In the present study, control tissue was utilized for general observations and comparison with Kc specimens, but with the limitation of eye bank corneas, they could not be assessed for many of the measurements carried out on the Kc corneas. Further work on the normal human cornea conducted in our laboratory has been published separately.^{32,33}

The ALL is intimately related to the epithelium and provides the foundation that the epithelium needs to remain firmly attached to the cornea.^{34,35} In the absence of ALL, it is not surprising that the epithelium becomes irregular and less well organized, as was noticed

in the present study. In our samples, the central Kc cornea showed an almost 80um (range: 13.5–91.6um) variation in epithelial thickness. Indeed, this variation was greater than the normal thickness (50um) of the epithelium, which typically has an extremely uniform outline in transverse sections.^{4,35–38} In PRK, where ALL invariably is ablated, similar variations in the epithelial thickness has been described.³⁹ The peripheral cornea also showed epithelial thickness variations, but to a lesser extent. Somewhat surprisingly, the observed thickness variation was not explained by a simple variation in layers of cells. On average, the epithelium was formed by 6 layers of cells, which is within the normal range,³⁵ but where the epithelium was thick, cells were also relatively taller. This observation suggested that pathological epithelial thickness changes in Kc were the result of changes in size rather than cellular proliferation. *In vivo* corneal confocal microscopy has also revealed dimensional epithelial cell variations and varying thicknesses of the epithelial layer in keratoconic corneas.^{40–42}

Contact lens (CL) wear, which with Kc patients is typically of the gas permeable variant, may have exaggerated intra-corneal epithelial thickness variation as may have eye rubbing. For visual reasons, some patients may have worn their CL's sufficiently close to the surgery date to have had an effect on the tissue examined histopathologically. Indeed, patients, who were CL wearers, were not discouraged to wear their lenses prior to the surgery. However, many patients had become intolerant to their CL's and were not wearing them. Therefore, we would argue that the observed intra-corneal epithelial thickness variations were mainly a product of the disease itself and to a lesser extent, a result of CL wear.

Although epithelial thickness variation may, to a degree, be the result of an adaptation to a pathological relationship with underlying tissue, there was still distinct evidence of abnormal epithelial behavior. Especially the basal cells often stained densely and had adopted peculiar stances or positions, which is an observation also reported by earlier literature.^{17,20} Interestingly, the BM, which should be up to 0.20um in the healthy eye⁴³, and measured 0.40um in our control tissue, was in some of our Kc specimens substantially thicker measuring up to 3.67um in thickness and occurred without the presence of scar tissue. Others have made similar observations ultrastructurally, reporting BM thickening and fragmentation, but offering no measurements.^{20,44} Teng (1963), in observing basal cell and BM pathological changes, arrived at the conclusion that Kc had an epithelial etiology.²⁰ The findings in the present study cannot confirm Teng's hypothesis. However, the epithelium clearly appeared to be an involved participant in the Kc disease process, but it was not apparent whether it is an instigator or a passive responder.

A striking finding was the involvement of ALL in the disease process in all central Kc corneas except two. In the majority of the Kc corneas examined, ALL was affected in more than 50 percent of the area assessed (Table 4). In four of the twelve Kc corneas evaluated, ALL was completely missing in places. The variation between the individual corneas is most likely due to a combination of factors, such as disease severity and different disease expressions or types. Furthermore, the area, from which the data was collected, may not have been from the corneal region most severely afflicted, although central cornea tissue sampling came from within the Fleischer ring. The discovery of the significant and widespread effect of the disease on the ALL is in contrast to the common belief that Kc leads to breaks, ruptures, cracks, etc.^{4–19} This observation, supported by the accompanying morphometry, clearly uncovered more extensive pathological changes than simple breaks or ruptures of ALL. The removal of parts or all of ALL, often together with loss of anterior stromal tissue, may contribute to the weakening of the cornea leading to subsequent corneal ectasia. However, there must be other factors involved in the provocation of corneal ectasia since photorefractive keratectomy, which invariably removes ALL, only rarely leads to ectasia.⁴⁵ As mentioned above, the effect of a defective or removed ALL also has epithelial

consequences, which includes deficient adhesion and inability to maintain a normal, uniform thickness. Clinically, it is known that the epithelium in keratoconus is more fragile and more often will show surface defects.⁴⁶

An increased number of cells, compared to control corneas, were noted in the Kc anterior stroma, often adjacent to ALL. Many of these cells were clearly not keratocytes, but were cells that had either been differentiated or were of an entirely different lineage. They could be recognized primarily on their lighter staining cytoplasm, but also on their organelle content. Hyperreflective cells in the anterior stroma have been observed using *in vivo* confocal microscopy. In one study combining confocal and light microscopy, cells with *in vivo* hyperreflectivity were correlated to fibroblastic cells viewed, after tissue fixation, with the light microscope.⁴⁰ In the present study the atypical stromal cells, not present in the control corneas, appeared to be involved in the removal or destruction of ALL and anterior stromal lamellae but did not share cytological characteristics with inflammatory cells. Typically these cells were agranular, contained lysosomes and mitochondria and often were intimately aligned to a keratocyte. Furthermore, surrounding stromal tissue harbored a large amount of debris. We postulate that these cells are involved in the breaking down and phagocytosis of corneal tissue (Figure 5). An elevation in cells in Kc stroma has been reported and it was postulated that this abnormal cell behavior may be related to the ALL defects.^{47–49} The prominent nerve fibers often described as a Kc clinical hallmark,^{9,10,17,50} were conspicuously absent in our Kc specimens. Interestingly, *in vivo* confocal microscopy has also been unable to describe the prominent nerve fibers observed biomicroscopically.^{40–42} However, nerve fibers in the Kc cornea appear to follow an abnormal course, which is in distinct contrast to the healthy cornea as seen by the *in vivo* confocal microscope.^{41,42,51} These nerve fibers have not been described in the histopathological literature concerning this disease. The nerve trunks encountered in our Kc corneal button specimens showed signs of abnormalities such as abnormal Schwann cell and axon relationship. They were no larger than nerve fibers normally seen in central cornea. Perhaps the prominent nerves are a feature of more peripheral corneal regions. The abnormal neurology of the Kc cornea appears to explain the reduced corneal sensitivity in patients with this pathology.^{46,52}

The peripheral region of the host Kc button examined was closer to the mid-peripheral cornea and, therefore, was not representative of the extreme peripheral cornea. However, the peripheral button examined did show similar changes as seen in the central samples, but to a much lesser extent. This observation suggested that this disease involves corneal regions beyond the ectatic cone portion, which may provide an explanation to recurrence of Kc reported in literature^{53–59} since pathological tissue remains in the host cornea after the transplant procedure. However, with an incidence of one in 2000,¹ Kc is hardly a rare disease, and together with the existence of form fruste Kc, an alternative explanation to disease recurrence may well be found in the laws of probability dictating the frequency with which the donor cornea will have an undetected or form fruste Kc.

In summary, the present and first morphometric approach to characterize Kc histopathologically has strongly established Kc as an anterior corneal disease that, in mild to modest cases, leaves the posterior portions apparently structurally unharmed. The epithelium appears to be involved in the pathophysiology of Kc, but it remains uncertain whether this corneal layer is the origin of the disease. Kc severely affects the ALL and the anterior stroma to a greater degree than earlier descriptions suggested. Recruited cells, not normally residents of the stroma, are apparently involved in the breakdown and removal of ALL and anterior stromal lamellae. Future research directed towards determining the exact origin of this disease and the true nature of invading stromal cells, would be most welcome.

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Figure 1.

(A) Keratoconus – Anterior cornea with distinct epithelial thickness variations and abnormal cells. Across the width of this view, the ALL shows variable thickness, ranging from thinning to absent. (B) Control - Internally, the epithelium is adherent to a linear basement membrane while its surface exhibits post-mortem changes not related to tissue preparation. The ALL maintains a smooth outline and uniform thickness. Approx mag 100X. Light micrographs. Stained with toluidine blue.

Figure 2.

The epithelial, ALL and stroma interface. **(A)** Keratoconus – The grossly thickened basement membrane (BM) (*) is prominently evident. In this view, the ALL (A) and the anterior stroma appears normal and unaffected by the disease. The epithelium (E) consists of apparently normal and abnormal cells that produced an electron dense substance (arrow). Scale bar = 2um. Inset: an extremely distorted vacuolated and electron dense basal cell (arrow) is lodged between two neighboring basal cells, which are not of normal appearance either. Scale bar = 2um. **(B)** Keratoconus – High magnification of the abnormal, thickened epithelial BM (*), which shows partial discontinuities (arrow) and granular material (*) laid down on top of an irregular, electron dense layer (triangle). Scale bar = 2um. **(C)** Control – basal cells are adherent to a relatively linear BM (arrow) of normal thickness. ALL (A) is of normal texture and thickness (approx. 10um) and anterior stroma shows normal interweaving anterior lamellae. Keratocytes (triangle) are present in the anterior stroma. Scale bar = 5um. Inset: high magnification of the normal, uniform and uninterrupted BM (arrow) onto which epithelial basal cells (E) are adherent. Scale bar = 2um. Electron micrographs. Stained with uranyl acetate and Reynold's lead citrate.

Figure 3.
Keratoconus – Anterior cornea showing modest epithelial irregularities and loss of ALL. The ALL (arrow) is seen to become thinner and eventually lost (arrowhead). Approx mag 100X. Light micrograph. Stained with toluidine blue.

Figure 4.

Keratoconus – Thinning of ALL. The ALL (A) is less than half its normal thickness and immediately adjacent are activated stromal cells (*). Material debris are scattered across ALL and anterior stroma (arrows). Inset: Area with ALL loss. A thin strand of ALL (arrow) is projecting to an area of complete ALL loss (*). Scale bars = 5µm. Electron micrograph. Stained with uranyl acetate and Reynold's lead citrate.

Figure 5.

(**A**) Keratoconus – Mid-stroma with elevated number of cells. An increased number of cells were present in mid and anterior stroma. Many of them appeared activated or showed signs of fragmentation (arrows). (**B**) Control – Mid-stroma showing normal keratocyte density and none appearing activate. Scale bars = 5 μ m. Electron micrographs. Stained with uranyl acetate and Reynold's lead citrate.

Figure 6.

Keratoconus – Stromal terminal nerve fiber. A single unmyelinated nerve fiber bundle (arrow) embedded in the stromal tissue contains terminal axon varicosities containing electron dense bodies. Multiple axons, with abnormal appearing axoplasm, are enclosed by the Schwann cell in an atypical manner. Scale bar = 5 μ m. Electron micrograph. Stained with uranyl acetate and Reynold's lead citrate.

Table 1

Average Central Corneal Thickness & Epithelial Morphometry in Keratoconus.

Cornea	Tissue Length (um)	Full Thickness (um)	Epithelial Thickness (um)	Epithelial Cell Layer Count	Stromal Thickness (um)
1	1176 ± 15	406 ± 59	50 ± 9	5.5 ± 0.5	338 ± 61
2	1010 ± 44	380 ± 24	32 ± 7	5.2 ± 0.6	328 ± 16
3	860 ± 5	LKP	48 ± 4	6.6 ± 0.5	LKP
4	1212 ± 51	451 ± 31	36 ± 12	6.2 ± 1.1	383 ± 20
5	2005 ± 23	414 ± 6	46 ± 2	6.0 ± 0.0	339 ± 4
6	1364 ± 23	650 ± 103	39 ± 14	5.5 ± 0.9	598 ± 83
7	2193 ± 349	434 ± 44	52 ± 23	7.4 ± 1.0	368 ± 56
8	2602 ± 8	387 ± 71	42 ± 16	5.5 ± 1.3	377 ± 31
9	2465 ± 32	370 ± 20	49 ± 6	7.3 ± 0.6	303 ± 21
10	633 ± 2	LKP	49 ± 5	6.4 ± 0.5	LKP
11	391 ± 3	LKP	46 ± 7	6.5 ± 0.7	LKP
12	444 ± 20	LKP	18 ± 4	4.1 ± 0.3	LKP

Table 2

Average Peripheral Corneal Thickness & Epithelial Morphometry in Keratoconus.

Cornea	Tissue Length (um)	Full Thickness (um)	Epithelial Thickness (um)	Epithelial Cell Layer Count	Stromal Thickness (um)
1	1009 ± 29	656 ± 158	60 ± 4	6.0 ± 0.6	598 ± 197
2	1267 ± 87	485 ± 5	62 ± 4	6.8 ± 0.5	398 ± 9
3	910 ± 2	LKP	54 ± 4	7.4 ± 0.5	LKP
4	1233 ± 11	560 ± 66	57 ± 10	7.1 ± 1.0	488 ± 76
5	2068 ± 30	476 ± 66	45 ± 2	6.8 ± 0.4	414 ± 67
6	768 ± 11	914 ± 94	64 ± 29	6.3 ± 0.9	738 ± 26
7	1309 ± 1	509 ± 18	54 ± 2	5.9 ± 0.3	426 ± 22
8	2209 ± 48	443 ± 37	40 ± 12	6.0 ± 0.0	369 ± 38
9	1433 ± 24	427 ± 16	58 ± 4	7.9 ± 0.7	346 ± 15
10	807 ± 7	LKP	59 ± 14	6.0 ± 1.0	LKP
11	729 ± 10	LKP	49 ± 1	6.5 ± 0.5	LKP
12	443 ± 5	LKP	52 ± 2	6.8 ± 0.6	LKP

Table 3

Basement Membrane (BM) Morphometry in Keratoconus.

Cornea	BM Thickness (um)
1	0.6 ± 0.2
2	1.933 ± 0.115
3	LKP
4	3.667 ± 0.305
5	0.863 ± 0.180
6	2.933 ± 0.503
7	0.05 ± 0
8	1.608 ± 0.378

Table 4

Central Anterior Limiting Lamina (ALL) Morphometry in Keratoconus.

Cornea	Average Tissue Length (um)	Area Affected (um)	% of Total Area Examined	ALL Lost (um)	% of Area Examined
1	1176	0	0	0	0
2	1010	832	82	443	44
3	860	177	21	0	0
4	1212	1176	97	0	0
5	2005	2005	100	0	0
6	1364	1002	74	574	42
7	2193	2193	100	1458	67
8	2602	2602	100	364	14
9	2465	622	25	0	0
10	633	410	65	0	0
11	391	0	0	0	0
12	444	256	58	0	0

Table 5

Peripheral Anterior Limiting Lamina (ALL) Morphometry in Keratoconus

Cornea	Average Tissue Length (um)	Area Affected (um)	% of Total Area Examined	ALL Lost (um)	% of Area Examined
1	1009	0	0	0	0
2	1267	283	22	0	0
3	910	210	23	0	0
4	1233	89	7	0	0
5	2068	67	3	0	0
6	768	768	100	66	9
7	1309	0	0	364	28
8	2209	389	18	0	0
9	1433	0	0	0	0
10	807	162	20	0	0
11	729	0	0	0	0
12	443	0	0	0	0