

Case report

Extreme Descemet's membrane rupture with hydrops in keratoconus: Clinical and histological manifestations

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ABSTRACT

Purpose: To study the clinical and histological manifestations of an extreme Descemet's membrane rupture as a result of keratoconus.

Observations: Using Periodic acid-Schiff assay to study a keratoconic cornea with an extreme rupture showed that the ruptured Descemet's membrane had retracted and folded into scrolls and ridges. The dimensions of the rupture were estimated to be 3.7mm², and the central cornea was extremely thinned with a thickness of only 260µm. Stromal scarring and loosely packed lamellae were present anterior to the scrolls and ridges. Antibodies targeting the major components of Descemet's membrane, Laminin and type IV collagen, displayed intense labelling adjacent to the scrolls where the stroma was denuded and differential expression patterns lined the ridges. Environmental scanning electron microscopy showed possible collagen deposition at the site of rupture.

Conclusions and importance: The specific staining patterns of laminin and type IV collagen suggest these components have an important role in re-endothelialisation of the cornea. This is the first known report of spatial resolution of the topography of the Descemet's membrane rupture established by environmental scanning electron microscopic image montage.

1. Introduction

The cornea is a transparent and avascular tissue that comprises the anterior part of the eye. It consists of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium.¹ Descemet's membrane is the highly evolved basement membrane of the endothelium and plays a role in corneal hydration and maintenance of the endothelium after wounding and surgery. It has also been reported to play a role in mechanical support, filtration, and acts as a fluid barrier.² The thickness of Descemet's membrane increases from about 3µm at birth³ to about 10µm in adulthood.^{1,4}

Keratoconus is a degenerative ectatic disease, with both a genetic and environmental aetiology, characterized by progressive stromal thinning, focal disruption of Bowman's layer, and protrusion of the weakened cornea.^{1,5} Acute corneal hydrops is a well-known complication of keratoconus caused by sudden breaks in Descemet's membrane and endothelium, resulting in ingress of aqueous humour from the anterior chamber resulting in marked corneal oedema.^{1,6,7} As a result the corneal stroma swells and may cause the disrupted Descemet's membrane to fold onto itself. In most cases, the rupture is relatively small and localised at the site of most advanced thinning.⁸

As Descemet's membrane cannot regenerate itself,¹ the process of

repair relies upon re-endothelialisation. During the progression of repair, endothelial cells enlarge and migrate over the surface of the denuded stroma and ruptured Descemet's membrane. The endothelial cells subsequently lay down new basement membrane.⁹ Typically, the stromal oedema subsides with focal scarring and the hydrops generally resolves in 2–4 months.^{1,5,10–14}

We report a case of a giant rupture in Descemet's membrane caused by advanced keratoconus resulting in hydrops. We present the clinical observations with histological analysis and highlight the focal changes by environmental scanning electron microscopy images of the Descemet's membrane rupture.

2. Subject and methods

2.1. Subject

A 20-year-old New Zealand European male with keratoconus presented with a 6-week history of mild discomfort, watering, "whitening" of his cornea, and reduced vision in his right eye suggestive of hydrops corneae. Keratoconus had been diagnosed at 12 years of age with long term correction with rigid gas permeable contact lenses. There was a strong family history of keratoconus (father and two brothers) and he

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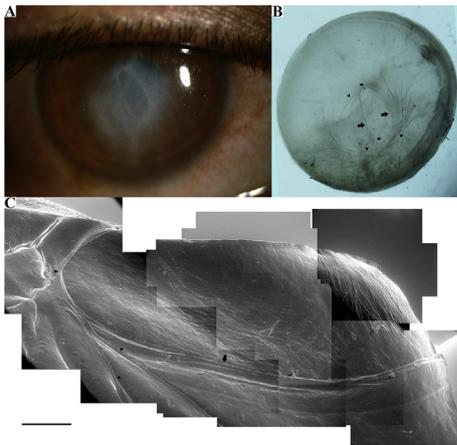


Fig. 1. a. Clinical Slit-lamp photograph of the affected cornea at 6-week post initial presentation, demonstrating the edges of the rupture in Descemet's membrane and surrounding whitening. b. A bright field image of the corneal button excised from the eye shown in 1a, demonstrating the torn edges and the folded creases (ridges) of Descemet's membrane. The scale bar is at 200 μ m. c. A montage of environmental scanning electron microscopic (ESEM) images of a denuded stroma due to the rupture in Descemet's membrane at 500x magnification.

For b and c, the ruptured Descemet's membrane retracted into scrolls (arrows), and ridges (arrowheads) rippled outwardly from the poles of the rupture (asterisk). Scattered aggregates possibly composed of collagen (fine arrows) are visible.

admitted to habitual eye-rubbing. His only significant past medical history was atopic rhinitis and asthma.

On examination, unaided vision was counting fingers in each eye, improving to 6/48 with pinhole right, and 6/9 with contact lens left eye. There was advanced stromal and epithelial corneal oedema in the right eye associated with an extensive infero-central Descemet's membrane rupture. The left eye exhibited moderately-severe keratoconus. The rest of the ocular examination was unremarkable.

The right eye was treated medically with frequent topical lubricants and Prednisolone acetate 1% four times per day and over the next 6 weeks the stromal and epithelial oedema gradually resolved leaving central corneal scarring and flattening. A large Descemet's membrane defect was still present after the corneal oedema had fully settled (Fig. 1a). Orbscan corneal tomography right revealed 6.60 dioptres (D) of astigmatism at 147° with relatively “flat” maximum simulated keratometry of 36.2 D (compared to maximum simulated keratometry of 54.5 D in the left eye.) Due to corneal surface irregularity corneal thickness could not be measured during or after the hydrops event.

Subsequently the patient underwent an uncomplicated 8.00mm diameter penetrating keratoplasty (the patient made an uneventful recovery regaining vision of 6/15 unaided, 6/9 corrected at three months). Due to the unusual size and persistence of the Descemet's membrane tear, informed consent was obtained for histological and immuno-histochemical analyses of the 8.00mm corneal host button.

2.2. Tissue preparation

The entire corneal tissue was immediately fixed in 10% formalin for 24 hours followed by 24 hours in 70% ethanol. The cornea was subsequently dissected into three pieces, one each for histology, environmental scanning electron microscopy (ESEM) and immunohistochemistry respectively. Each piece contained part of the Descemet's membrane rupture, the exposed stroma and the extended ridges from the two poles of the rupture.

2.3. Histology

Periodic acid-Schiff (PAS) labelling was done on 5 μ m corneal sections using standard routine protocols for histology. Briefly, the tissue sections were oxidized in 0.5% periodic acid solution for 5 min after de-paraffinisation and rehydration. Followed by a quick rinse in distilled water, the tissue sections were placed in Schiff reagent for 15min. After a 5min wash in lukewarm tap water, the tissue sections were counterstained in Mayer's hematoxylin for 1min. Tissue sections were washed in tap water for 5 min and dehydrated before mounting.

2.4. Environmental scanning electron microscopy

Fixatives were removed from the corneal tissue by washing multiple times in PBS over a 24-h period. Tissue was then rinsed several times with autoclaved MilliQ water for 3 hours, in order to remove residue from buffer that could precipitate out on top of the sample, prior to imaging.

The tissue sample was examined using a FEI Quanta 200 field emission Environmental SEM (FEI company, Hillsboro, OR).

2.5. Antigen retrieval and immunohistochemistry

Paraffin sections of 5 μ m thickness were de-paraffinized in 2 changes of xylene, 5 min each, and hydrated in 2 changes of 100% ethanol for 3min each, 95% and 80% ethanol for 1 min each. The slides were rinsed in distilled water for 10 sec and treated with 0.5% pepsin at 37 °C for 10 min. After cooling to room temperature, slides were rinsed in PBS-Tween for 3 washes of 5min each and treated with 20mM Glycine for 30 min at room temperature followed by 30min incubation with 2% goat serum in PBS-Tween. Slides were incubated with primary antibody in PBS-Tween for 2 hours at room temperature, washed 3 times for 10 min in 2% goat serum in PBS-Tween, and incubated with secondary antibody for 1 hour at room temperature in the dark. After 3 washes of 10 min each with 2% goat serum in PBS-Tween, slides were labelled with 0.1 μ g/ml 4', 6-diamidino-2-phenylindole (DAPI) for 10 min before sealing slides with coverslips.

Primary antibodies used were laminin (1:60, Rabbit anti-mouse polyclonal IgG, Sigma L-9393) and type IV collagen (1:1000, mouse anti-human monoclonal IgG, Sigma C-1926). Secondary antibodies used were goat anti-rabbit Cy3 (1:400, Jackson Immuno Research, #115-165-003) and goat anti-mouse IgG Alexa 546 (1:1000, Molecular Probes, A-11003).

2.6. Image analysis

The images of entire cornea were taken using bright field stereomicroscopy (Stereo Discovery V20., Zeiss, Germany) and of fluorescently labelled sections were collected using a fluorescence microscope with 10x, 20x and 40x lenses (Leica DR RA, Leica Microsystems, Heidelberg, Germany), both via a digital camera (Nikon DS-5Mc; Nikon Corporation, Tokyo, Japan) connected to a desktop computer (Dell Computer Corporation, Austin, TX) running Windows Vista (Microsoft Corporation, Seattle, WA) and NIS-Elements BR Imaging software (Nikon Corporation). These images were stitched together using Adobe Photoshop CS6 to give a full-width montage image. Histological staining was imaged with transmitted light using the same microscope and images were stitched together into a montage with Adobe Photoshop CS6.

Two hundred micron ESEM image sections of anterior, lateral and posterior cornea were collected and montaged using Photoshop CS6 to give an overall view of the corneal piece investigated.

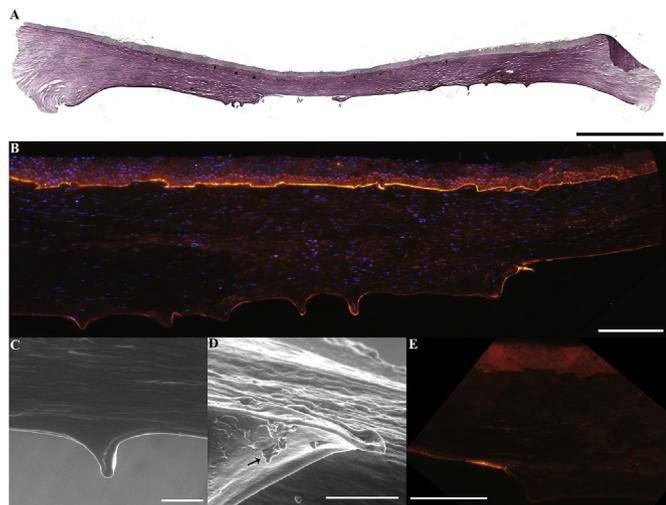


Fig. 2. a. Periodic acid-Schiff stained section of the excised corneal button demonstrating denuded stroma at the centre due to the Descemet's membrane rupture. The retracted Descemet's membrane curled into scroll-like structure at each side and ridges of Descemet's membrane were found further towards the peripheral edges of the cornea section. The cropped areas indicated the scrolls and ridges caused by Descemet's membrane rupture (scale bar = 1000 μ m). Arrows mark areas where stroma was pushed anteriorly towards epithelium and areas where Bowman's membrane becomes irregular. Arrowheads mark areas where Bowman's membrane is sandwiched interstitially to stroma lamellae. Stars label areas where posterior stroma is disorganised. The 1000 μ m bare region where Descemet's membrane is ruptured is labelled as *br*. Scrolls are marked as *s*, and ridges are as *r*. b. Immunolabelling of laminin (red) and DAPI (4', 6-diamidino-2-phenylindole, blue) at the scrolls and ridges of DM rupture. Most intense laminin staining was found at the Bowman's membrane, Descemet's membrane, particularly right next to the scrolls and lining both the endothelial and stromal sides of the ridges (scale bar = 100 μ m). c. ESEM of a ridge. (scale bar = 50 μ m). d. ESEM image of a scroll due to Descemet's membrane rupture. Possible collagen deposit was observed (arrow, scale bar = 50 μ m). e. Immunolabelling of type IV collagen (red). The most intense staining is on the surface of the denuded stroma where the Descemet's membrane was retracted, also around the stromal surface of the scroll was also partially labelled (scale bar = 100 μ m). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Histological observation

A clinical image of the right cornea is shown with an *ex vivo* stereomicroscopic imaging of the central corneal button in Fig. 1a and b, highlighting a large Descemet's membrane rupture. The location of the rupture was infero-nasal to the corneal apex and resulted in retraction of the Descemet's membrane causing “ripples” or folds to appear to fan outwardly from the poles of the rupture. The surface area of the rupture was estimated to be approximately 3.7mm² by 2D image analysis or roughly 5.4% of the entire 8.0mm corneal button. The length of the tear from the most distant poles is approximately one-third of the diameter of the corneal button.

PAS-staining of a section of the cornea (Fig. 2a) revealed 5–7 layers of epithelial cells, with regions where the stromal lamellae had been displaced anteriorly towards the epithelium. Bowman's layer was irregular across the width of the corneal section and was absent in multiple areas, leaving stroma directly in contact with the epithelium (Fig. 2a). In some sections, Bowman's layer appeared to be “sandwiched” interstitially between lamellae of the anterior stroma, rather than directly underneath the epithelium as in a normal cornea. In contrast to the tightly organised lamellae of the normal corneal stroma, the posterior stroma of this corneal button revealed more loosely packed lamellae

due to previous hydrops oedema. At the centre of the corneal section (Fig. 2a) Descemet's membrane was absent and had retracted approximately 500 μ m from the edge of the tear leaving a bare region of almost 1000 μ m. The retracted Descemet's membrane was curled and folded inwardly, forming a scroll-like structure at the edge. Further towards the periphery of the corneal button, the retracted Descemet's membrane was rippled into ridge-like structures. The posterior stroma adjacent to these ridges was more disorganised than the regions directly adjacent to the scroll-like elements of Descemet's membrane and the areas of rupture where the stroma had been denuded. The thickness of the cornea at the centre of the Descemet's rupture was approximately 260 μ m, increasing to around 430 μ m where the ridges were identified. Further towards the mid-peripheral cornea, the thickness was approximately 450 μ m and at the very edge of the corneal button approximately 880 μ m.

Environmental SEM imaging (Fig. 1c) from the posterior face of a quarter piece of the cornea revealed microstructural changes of the Descemet's membrane observed at the site of the rupture. There is a clear demarcation of the edge of the rupture, where Descemet's membrane forms scrolls (arrowheads in Fig. 1c, and Fig. 2d) and is further distorted into ridges (arrows in Figs. 1c and 2c). The collagen fibres present in the denuded stroma appear stretched at the site of rupture. Along the scrolls and ridges, scattered aggregates, possibly of collagen, are also visible (Fig. 2d, marked with arrow).

3.2. Immunohistochemistry

Laminin staining was observed in basal epithelium, stromal lamellae and Descemet's membrane (Fig. 2b). Laminin was present predominantly on the endothelial side of Descemet's membrane. To a lesser extent, Laminin also appeared to line the stromal side of Descemet's membrane where ridges were found. In addition, laminin was identified on the stromal side of the Descemet's membrane scroll where the endothelial side of Descemet's membrane was in direct contact with the stroma.

Similar to laminin, the most intense collagen type IV labelling (Fig. 2e) was localised immediately adjacent to the scroll and less so along the denuded stroma where Descemet's membrane had ruptured. Of note, there was almost no type IV collagen labelling found in the scroll of retracted Descemet's membrane, whereas, type IV collagen was found in the central and posterior stroma above the retracted DM where the thickness of the cornea increased.

4. Discussion

In 1998, Stone noted the observation of Descemet's membrane retraction into scrolls and ridges.¹⁵ In the current case we report more detailed observations with wide-field montaged ESEM images of the Descemet's membrane rupture and in-depth descriptions of these associated structures with PAS staining. The clinical history and histological observations of partial thinning and irregularity of epithelium, breaks in Bowman's layer and dramatic thinning of the central stroma correlate well with descriptions of keratoconus.¹⁶

It has been reported that the average thickness of the keratoconic cornea is reduced to approximately 437 μ m centrally and 559 μ m towards the periphery,¹⁷ whereas, in cases with unresolved oedema following Descemet's membrane rupture, the central cornea can be 878 μ m¹⁸ or more.¹⁹ However, our subject with keratoconus and resolved oedema provided much thinner corneal measurements at 260 μ m centrally and 450 μ m at 8mm diameter. This extreme thinning and flattening of the central cornea might be a result of the extensive rupture of Descemet's membrane. Indeed, the overall dimension of the rupture was approximately 3.7mm² (5.4% of the 8.00mm corneal button), with the longest axis of the tear being one-third of the diameter of the corneal button.

Laminin is a well-known basement membrane component and

laminin staining was observed mainly on the endothelial side of Descemet's membrane. Laminin staining was also found on the stromal side of both the ridges and scrolls. This may mean that as Descemet's membrane retracted and recoiled into scrolls, the endothelial side of the membrane temporarily became the new stromal side. Previous observations of repair mechanisms suggest that retracted edges of Descemet's membrane can unravel and redistribute to cover the site of rupture. Interestingly, the denuded stroma exhibited very intense laminin staining and intense laminin staining was also noted in the stroma anterior to the scrolls and ridges, revealing that the stromal scar persisted after the oedema had resolved. The stromal lamellae of the central cornea showed the overall compact formation of normal cornea, however, towards the periphery the posterior corneal stroma adjacent to the Descemet's ridges was very loosely packed and somewhat disorganised. This latter may relate to tearing and retraction of the innermost lamellar thus compromising the structural integrity of the cornea.

In addition to laminin, type IV collagen is a major component of Descemet's membrane, indeed, type IV collagen forms the backbone for the attachment of other basement membrane components.^{1,8,20–22} Multiple studies have found that $\alpha 1$ - $\alpha 2$ type IV collagen is present on the stromal face of Descemet's membrane.^{23–25} Our monoclonal type IV collagen antibody recognized an epitope located on $\alpha 1$ and/or $\alpha 2$ chains and, through immunohistochemical analysis, was found on the stromal face of Descemet's membrane and to a very limited extent in the scrolled membrane (Fig. 2e).

The limited immunolocalisation may be a result of the disrupted collagen IV assembly at the site of rupture. This correlates with the observation of Kenney et al. (1997)²⁴ that the change in type IV collagen expression in keratoconic tissue was only triggered after scar formation. Ljubimov et al.,²³ also found similar results with scarred regions of the cornea showing greater than normal staining, paradoxically, the staining of the epithelial basement membrane for IV collagen and laminin appeared decreased in non-scarred regions, whereas the posterior stroma outside of the scarred areas did not show significant changes in the staining pattern. In keratoconic corneas that had morphologic folds and irregularities of Descemet's membrane but no scarring, there were no consistent alterations in its immunohistochemical patterns for basement membrane components such as type IV collagen, laminin and fibronectin. It was proposed that the differential staining pattern may reflect locally increased protease activity and ongoing wound healing.²⁴

The endothelium controls corneal hydration and nutrition using the apical gap and macula occludens junctions together with an ATPase-dependent metabolic pump located in the lateral plasma membranes to form a barrier that restricts water flowing into the stroma but allows nutrients to pass. During wound healing, endothelial cells flatten and enlarge to maintain an intact monolayer whilst also depositing abnormal collagenous material on the posterior surface of Descemet's membrane.²⁶ Several researchers have described the process of endothelial wound healing.^{3,27–29} It begins when the endothelial cells adjacent to the wound undergo endothelial polymegathism and pleomorphism by becoming flattened to migrate to the wounding site. When the cells reach the wound, further migration is stopped by contact inhibition and the large elongated cells return to nearer their normal size and morphology. Therefore peripheral cells will be pulled further towards the wound. When the endothelial cells cover the exposed the wound, they will start to deposit a new Descemet's membrane.³⁰

5. Conclusions

In this case report, a cornea with an extreme Descemet's membrane rupture of approximately 3.7mm^2 was observed. The torn Descemet's membrane retracted into scrolls at the edge of the rupture and into ridges further towards the mid-periphery. As the oedema resolved clinically, the central cornea became extremely thinned and flattened

reduced to a thickness of $260\mu\text{m}$. The corneal stroma remained scarred and lamellae loosely packed above the ridges. It seems that the extensive rupture, extreme corneal flattening and persistent scrolls and ridges of the rupture might have delayed or compromised the wound healing process and limited rehabilitation of the eye to surgical options.

The intense staining of laminin and type IV collagen at the edge of the rupture where the Descemet's membrane retracted into scrolls may indicate the beginning of re-endothelialisation. This case study was limited by tissue availability and processing, however, it would be interesting to investigate the involvement of basement membrane components and cell proliferation and migration markers to further understand the process of re-endothelialisation.

We have presented a montage ESEM image of the Descemet's membrane rupture. To the best of our knowledge, this is the first time that ESEM imaging has been reported and been correlated with clinical, histological and immunohistochemical results.

Patient consent

Written consent to publish case details was obtained from the patient.

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Conflicts of interest

The following authors have no financial disclosures: I-PL, JCFG, TS, CNJM.

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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