Therapeutic Penetrating Keratoplasty using Full-Thickness Gamma Irradiated Corneal Tissue

Gustavo Corrales
MedStar Washington Hospital Center
Sina Sabet
MedStar Washington Hospital Center
Bhaskar Kallakury
MedStar Washington Hospital Center
Eileen Rusnock
MedStar Georgetown University Hospital
Cameron Sabet (cameron.j.sabet.24@dartmouth.edu)
Dartmouth College
Esen Akpek
Johns Hopkins Hospital

Research Article

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1; Gustavo A. Corrales, M.D. 2; Sina J. Sabet M.D., F.A.C.S 3; Bhaskar Kallakury, M.D. 4; Eileen Rusnock 5; Cameron J. Sabet 6; Esen K. Akpek, M.D.

1 Cornea and External Disease Service, Medstar - Washington Hospital Center, Washington, DC, USA

2 Departments of Ophthalmology and Pathology, Medstar - Georgetown University Medical Center, Washington DC, USA and Neuro-Ophthalmology Section, Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

3 Department of Pathology, Medstar - Georgetown University Medical Center, Washington DC, USA

4 Department of Pathology, Medstar - Georgetown University Medical Center, Washington DC, USA

5 Dartmouth College, Hanover, New Hampshire, USA

6 Ocular Surface Disease and Dry Eye Clinic, Cornea and External Disease Service, The Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Running Head: Penetrating Keratoplasty Using VisionGraft®

Corresponding Author
Gustavo A. Corrales, M.D.
Cornea and External Disease Service, Medstar
Washington Hospital Center
110 Irving St NW, Washington, DC 20010
Phone: 703-532-0728
Fax: 888-972-9036
Email: gcorrales@novavcs.com

Highlights:

- VisionGraft® is a commercially available acellular gamma-irradiated sterile cornea.

- It is used clinically as a tectonic graft in cases of severe infectious keratitis.
- It can be a primary substitute for allogenic transplantation.
- It decreases antigen load and risk of rejection.

Abstract:

Purpose: This paper is intended to report the ultrastructural and biological features of VisionGraft®, a commercially available acellular gamma-irradiated sterile cornea. This is the first known ultrastructural examination of the VisionGraft® with electron microscopy after in-vivo explanation.

Observations: This graft was initially placed for tectonic purposes in a non-responsive culture positive Aspergillus keratitis in a 50 year-old diabetic male, unresponsive to maximal medical therapy. Five months later, a second penetrating keratoplasty with fresh tissue was performed and the Visiongraft® was submitted for histopathologic evaluation. This study reports that there is minimal regrowth of nerves and endothelial cells into the graft, and corneal clarity appears to be preserved even in the absence of endothelium.

Conclusions and Importance:
Examination of the acellular cornea showed no significant epithelial regrowth, no nerve regeneration, no infiltration by leukocytes or antigen presenting cells, no significant endothelial regrowth, and yet, surprisingly, no interstitial edema. We offer some hypotheses for these observations based on the histopathologic evaluation and offer some suggestions for future avenues of research.

Keywords:
acellular cornea; VisionGraft; infectious keratitis; penetrating keratoplasty; corneal transplant.

Introduction:
Impending or frank corneal perforations in the setting of infectious keratitis pose a challenge to the ophthalmic surgeon. The treatment options for these cases include management of the area of corneal thinning with tissue adhesives,[1-5] conjunctival flaps,[6,7] amniotic membrane grafting,[8-11] patching with scleral lamellae,[12,13] or fresh[14-18] or glycerin-preserved corneal tissues.[19, 20] In the presence of active/ongoing infection, management can be particularly difficult, especially if there is resistance to drug therapy, (e.g., cases of fungal or acanthamoeba keratitis).

The VisionGraft Sterile Cornea® is a sterile gamma-irradiated cornea with a shelf-life of one year at room temperature. It has been used in such cases of actively inflamed eyes where it is necessary to surgically excise the infected tissue and quiet the eye before a donor graft is placed. In such situations, no viable endothelium is necessary, similar to lamellar keratoplasty[21] or Boston type 1 keratoprosthesis[22] surgeries. It has shown no long-term risks to the recipient and has excellent outcomes. Currently, the tissue is provided pre-cut by the manufacturer in

various shapes and sizes with full or partial thickness stroma for use as patch grafts or lamellar transplant procedures.

We herein report clinical outcomes as well as light microscopic and ultrastructural findings of an explanted full-thickness VisionGraft Sterile Cornea® in a patient with a history of severe fungal keratitis with impending perforation.

Case Report

A diabetic 50-year-old Hispanic male was referred for a non-healing corneal ulcer. His problem started with mild trauma to the eye with vegetative matter while mowing the lawn about a month prior to being referred to us. What started as a foreign body sensation after the trauma rapidly progressed to worsening pain, inflammation, and decreased vision. He had no history of contact lens wear or previous eye problems.

On presentation, his visual acuity was hand motions. Slit-lamp examination of the left eye demonstrated a central mid-stromal ring infiltrate, with an overlying epithelial defect measuring 1.5 mm in diameter as well as several satellite infiltrates. The left eye was promptly cultured and the patient was started on fortified vancomycin (25mg/ml), tobramycin (15mg/ml) and voriconazole 1% every hour due to the high suspicion of a fungal or polymicrobial infection. The initial corneal culture was negative for any microorganisms. However, a subsequent culture grew Aspergillus flavus.

Despite aggressive medical therapy, including oral itraconazole, hourly topical voriconazole 1%, and topical fortified antibiotics, the patient's condition deteriorated over the next few weeks. He developed a hemorrhagic hypopyon, iris neovascularization and corneal thinning with impending perforation or microperforation. The hypopyon increased in size and became organized, raising the suspicion of intraocular invasion (Figure 1). The decision was made to proceed with surgical intervention. The use of a sterile acellular cornea versus a fresh donor cornea was discussed with the patient. Owing to sterility and lack of antigenicity it was recommended to perform an initial transplantation using Visiongraft®. The patient provided informed consent.

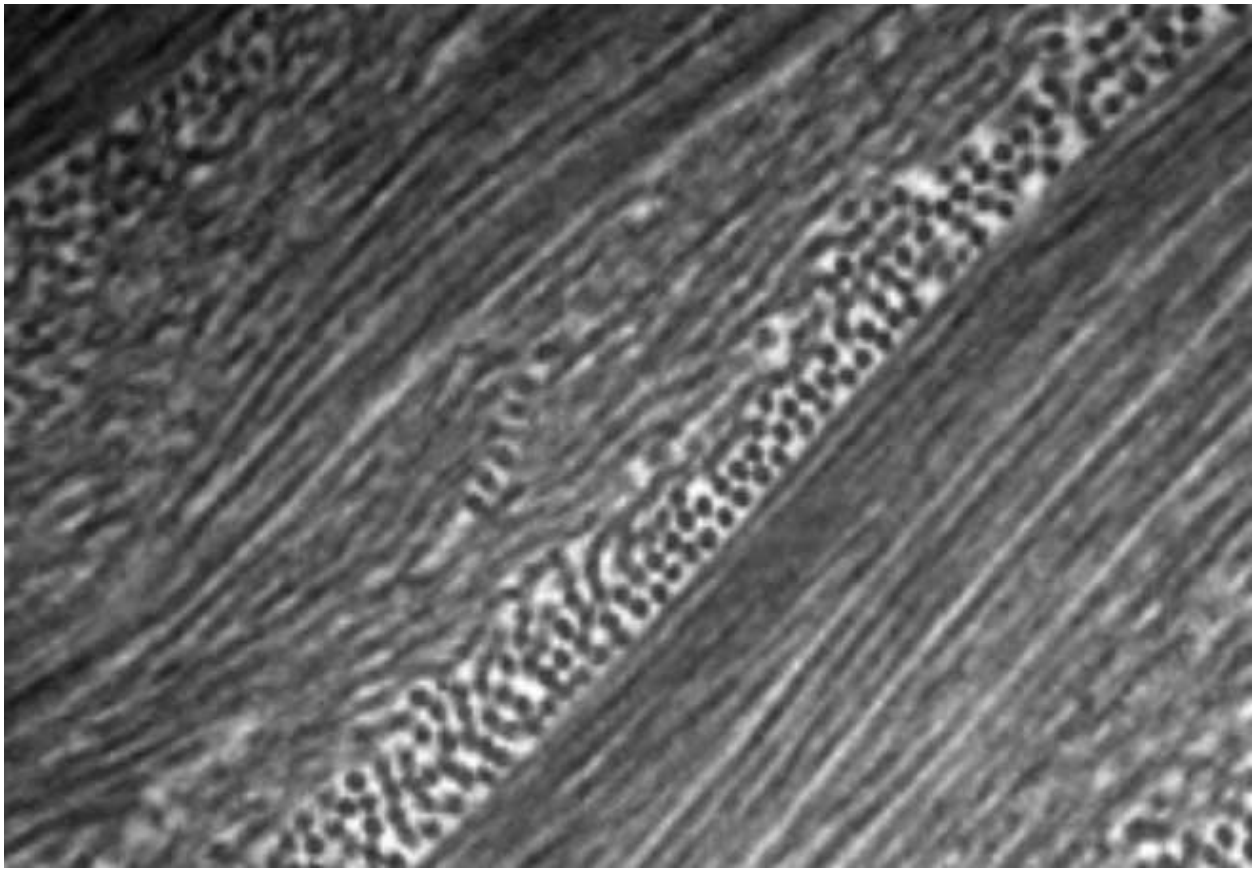

Fig. 1: Collagen fibers showing evidence of cross-linking.

A therapeutic penetrating keratoplasty was performed using a 8.75 mm VisionGraft cornea oversizing the graft by 0.25mm. The donor was sutured in place with interrupted 10-0 nylon sutures. The aqueous fluid sample was sent for culture and intracameral injection of 0.2 ml of 100 mcg/100microliter voriconazole was given at the end of the procedure.

In the immediate postoperative period, the patient developed a hyphema due to bleeding from the iris. The intraocular pressure increased to 40 mmHg. Despite maximal medical therapy using topical and oral agents the patient had to be taken back to the operating room for an anterior chamber wash-out due to high intraocular pressures. Following the anterior chamber wash-out, the patient's anterior chamber remained quiet and intraocular pressures were normal for the rest of the follow-up. The topical antifungals were continued for 3 weeks after keratoplasty.

The Visiongraft integrated very well with the recipient bed. The graft-host junction healed well, there was full re-epithelialization, and the graft looked clear. However, after about 2 weeks a central epithelial defect that slowly gained the appearance of a sterile neurotrophic defect developed. This central defect persisted despite aggressive use of lubricants and a bandage contact lens (Figure 2).

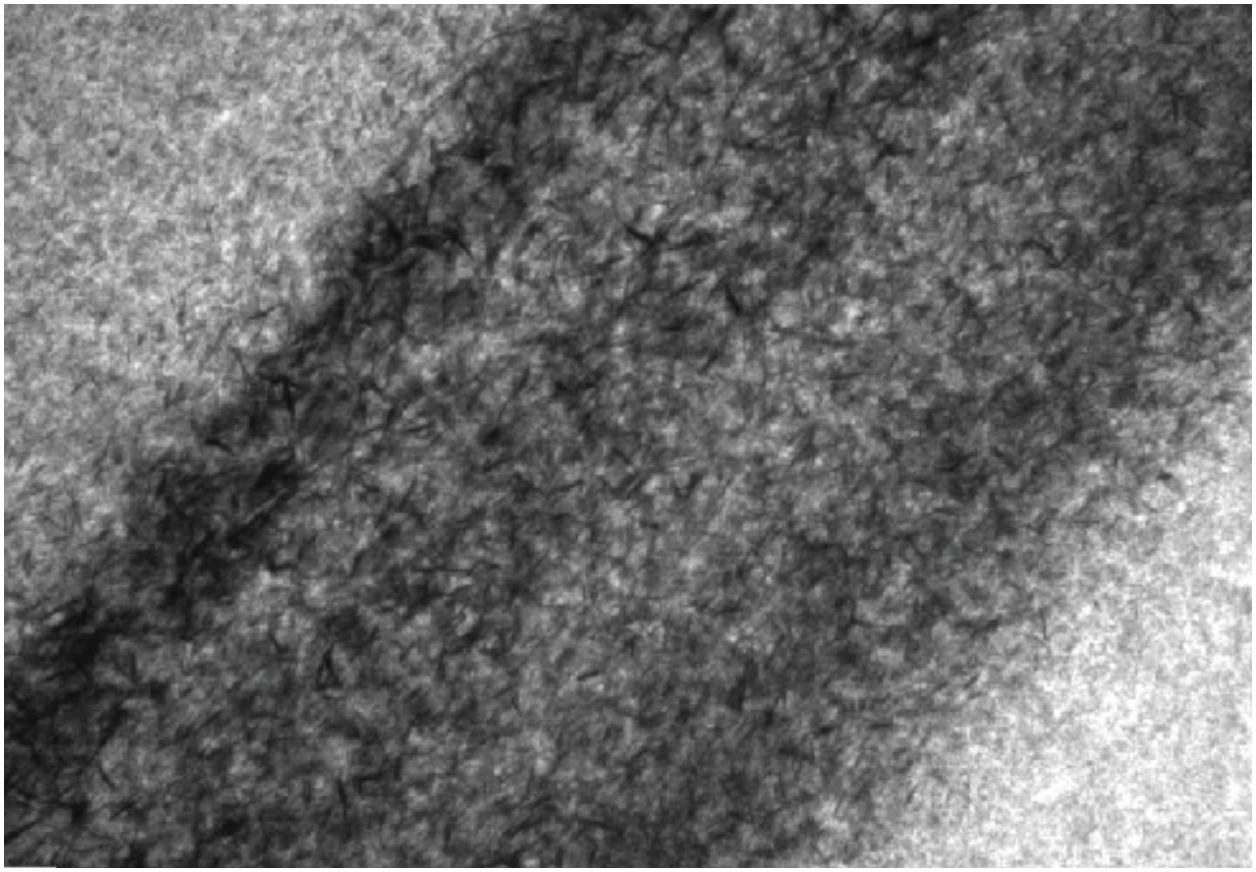

Figure 2: Electron-dense band in Descemet’s membrane.

In addition to this epithelial defect, a dense, white cataract developed. The decision was made to proceed with a triple procedure with cataract extraction, intraocular lens implantation and a full-thickness penetrating corneal transplantation using fresh donor tissue. The Visiongraft® was explanted and submitted for histopathologic and ultrastructural evaluation.

The fresh donor tissue healed as expected with the host epithelium covering the entire donor within 10 days. The last ocular examination was performed 9 months after the second keratoplasty and demonstrated a clear corneal graft. The final visual acuity was 20/200 caused primarily by diabetic macular edema.

Microscopic examination of the explanted Visiongraft® showed corneal tissue with complete absence of the endothelium. The epithelium was present only in the periphery of some sections, but largely absent over the rest (Figure 3). The stroma was predominantly devoid of keratocytes. However, in the periphery of the graft, a few stromal keratocytes were visible with irregular nuclei. The lamellae of the stroma appeared regular, but lacked normal artifactitious clefting throughout most of the sections. Bowman's and Descemet's membranes appeared normal, but no endothelial cells were present. Keratocytes were largely absent, except some irregular cells

in the periphery of the graft. No inflammatory cells were present. Immunohistochemical staining with neurofilament for axonal nerve fibers, and S-100 for Schwann cells, were both negative.

Electron microscopy showed the maintenance of the typical cross-sectional hexagonal arrangement of corneal stromal collagen fibers arranged in lamellae. There was some evidence of cross-linking between the collagen fibrils (figure 4). Some of the keratocytes present in the periphery of the graft were apoptotic, while others appeared viable (figure 5). Some of them showed highly pleomorphic inclusions in their cytoplasm, which gave them a granular appearance on lower magnification (figure 6 and 7). The identity of these inclusions could not be ascertained. Also present were areas of long spacing collagen (Luse bodies) scattered sporadically and very sparsely throughout the stroma (figure 8). No electron microscopic evidence of axonal regeneration was found. Bowman's membrane showed the typical haphazard arrangement of small-diameter collagen fibrils. However, Descemet's membrane showed a highly electron dense band of unclear composition on its inner aspect (figure. 9). The presence of some epithelial cells on the graft periphery (figure 10) and the complete absence of endothelium was confirmed.

Discussion

Emergency keratoplasties are often performed in situations where it is not possible to wait for inflammation or infection to remit, with impending micro or frank perforations such as resistant infectious keratitis, corneal melts, and acute trauma. In these situations an emergent penetrating keratoplasty is performed to remove the offending infection (therapeutic) or restore the integrity of the eye (tectonic).[15]

Grafts following emergency keratoplasty are more likely to fail and suffer more immune rejections compared to non-emergency keratoplasties. Maier et al found that the failure risk for emergency keratoplasties is equivalent to scheduled high risk penetrating keratoplasties.[23] The clear graft survival at 4 years was 67.9% for emergency keratoplasties, 70.2% for high-risk keratoplasties and 86.9% for non-high risk keratoplasties. Another retrospective study by Ang et al.[24] reviewed the outcomes of tectonic keratoplasties, finding that the Kaplan-Meier probability for survival at 10 years was 44.2% for penetrating keratoplasties performed for tectonic purposes. Active corneal inflammation and recipient graft sizes >9mm were significant risk factors for graft failure.

As in high risk keratoplasties, several approaches have been suggested to improve the outcome of emergency keratoplasties, including the use of systemic immunosuppression[23, 25] and – if possible, waiting until the inflammation has subsided before performing the corneal transplant.[15, 26] Systemic immunosuppression is usually not effective in preventing an allograft rejection in these grafts.[23] Additionally well-known side effects make them less than ideal for some patients.

Glycerin-preserved corneas can be used in emergency procedures, but they are not sterile, may not be clear initially, and are thick and rubbery, making them difficult to handle. Preservation in a -78°C freezer may be necessary to obtain transparent and pliable glycerol-preserved corneas.[27]

Visiongraft® has been reported to be easy to use, remains clear in packaging without increase in thickness with excellent tensile strength during suturing.[28] Postoperative outcomes were also favorable with epithelialization, excellent biological incorporation and long-term clarity. Therefore, we preferred Visiongraft® over glycerine-preserved tissue for our surgery.

VisionGraft®Sterile Cornea is a patent-pending sterile gamma-irradiated human donor corneal tissue produced and distributed by Tissue Banks International (Baltimore, Maryland) that is indicated for use in corneal procedures that do not require a viable endothelium and where is desirable to minimize tissue antigenicity in the graft. The tissue has previously been used for surgeries such as lamellar keratoplasty,21 glaucoma patch graft,[28] and Boston type 1 keratoprosthesis surgeries.[28] Due to the gamma irradiation these tissues offer additional patient safety compared to fresh corneal tissues and eliminate the risk for bacterial, viral or fungal disease transmission. Gamma irradiation is known to deplete antigen presenting cells in the donor tissue, preventing sensitization of the host immune system by donor antigens and thereby reducing the likelihood of future allograft rejection.[29-33]

Light microscopic, immunohistochemical, and electron microscopic studies of the VisionGraft® offered interesting insights, many of which point the way for further research and work in this area. The ingrowth of keratocytes into the periphery of the graft was a promising finding. These keratocytes are presumably of host origin, something which can be confirmed in future studies with HLA typing of these keratocytes and comparing them to the host and donor haplotypes. There was also some peripheral epithelial growth, although this was quite modest. This can perhaps be explained by the histopathologic finding of a lack of axonal regeneration to provide neurotrophic support. It is not clear if the VisionGraft® can support axonal regeneration, or whether the absence of such fibers in this case was simply due to insufficient time for regeneration and host factors, like diabetes mellitus in our patient. Clinically, there was some initial epithelial regrowth, but this appeared to be tenuous at best. Previous cases reporting the use of VisionGraft® tissue have not reported difficulty of the graft sustaining healthy epithelium.

One interesting finding in this study was the corneal clarity observed clinically in the absence of endothelium observed histopathologically. We offer two theories. One is that the preservation process may have led to a chemical modification of the stromal collagen, such as cross-linking noted on ultrastructural studies,, that protected the graft against edematous distortion and therefore opacification. Another possibility is that the electron dense band visible in Descemet's membrane provided a chemical/structural barrier to aqueous humor transgression into the cornea. Future studies will need to focus on clarifying the nature of this band and any possible role it may have in preserving corneal clarity. It would be intriguing to start evaluating VisionGraft® as more than just a tectonic graft, and more as a possible substitute for a living allograft.

Conclusion:

VisionGraft Sterile Cornea® should be considered in lieu of fresh donor corneas, cryo- or glycerin-preserved tissues for emergency tectonic full thickness keratoplasty because of the availability, ease of handling, lack of immunogenicity and sterility. This study shows that there is minimal regrowth of nerves and endothelial cells into the graft, but that corneal clarity appears to be preserved despite the absence of endothelium. This finding offers intriguing possibilities for the use of VisionGraft® as more than just a tectonic graft and as a substitute for allogenic transplantation.

Patient Consent:
Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

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Conflicts of Interest:
The following authors have no financial disclosures: GC; SS; BK; ER; CS; EA

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

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Conflict of Interest: None

Disclosures: A version of this case report is available online in a non-peer-reviewed communication.
Link:
https://www.semanticscholar.org/paper/Therapeutic-Penetrating-Keratoplasty-Using-Sterile-Corrales-Sabet/a35c7aea3cfea2a0a1a5c51f9ffe555a7ccb4de0

Figure Titles and Captions:

Figure 1: Corneal Collagen Cross-Linking. Electron micrograph presenting evidence of cross-linking between collagen fibers.

Figure 2: Electron micrograph of Decemet's membrane showing an electron-dense band of unclear composition.

Figures

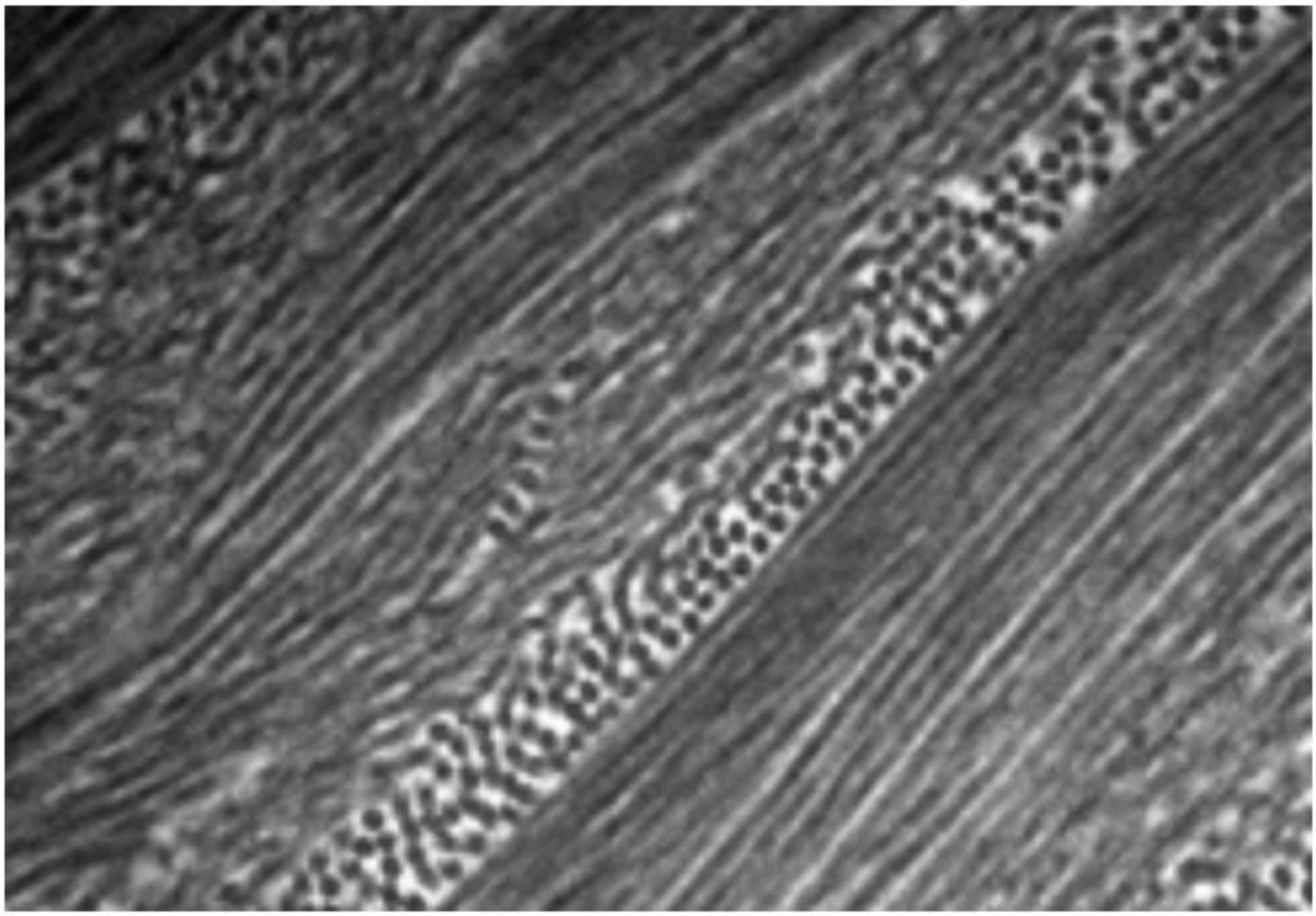

Figure 1

Corneal Collagen Cross-Linking. Electron micrograph presenting evidence of cross-linking between collagen fibers.

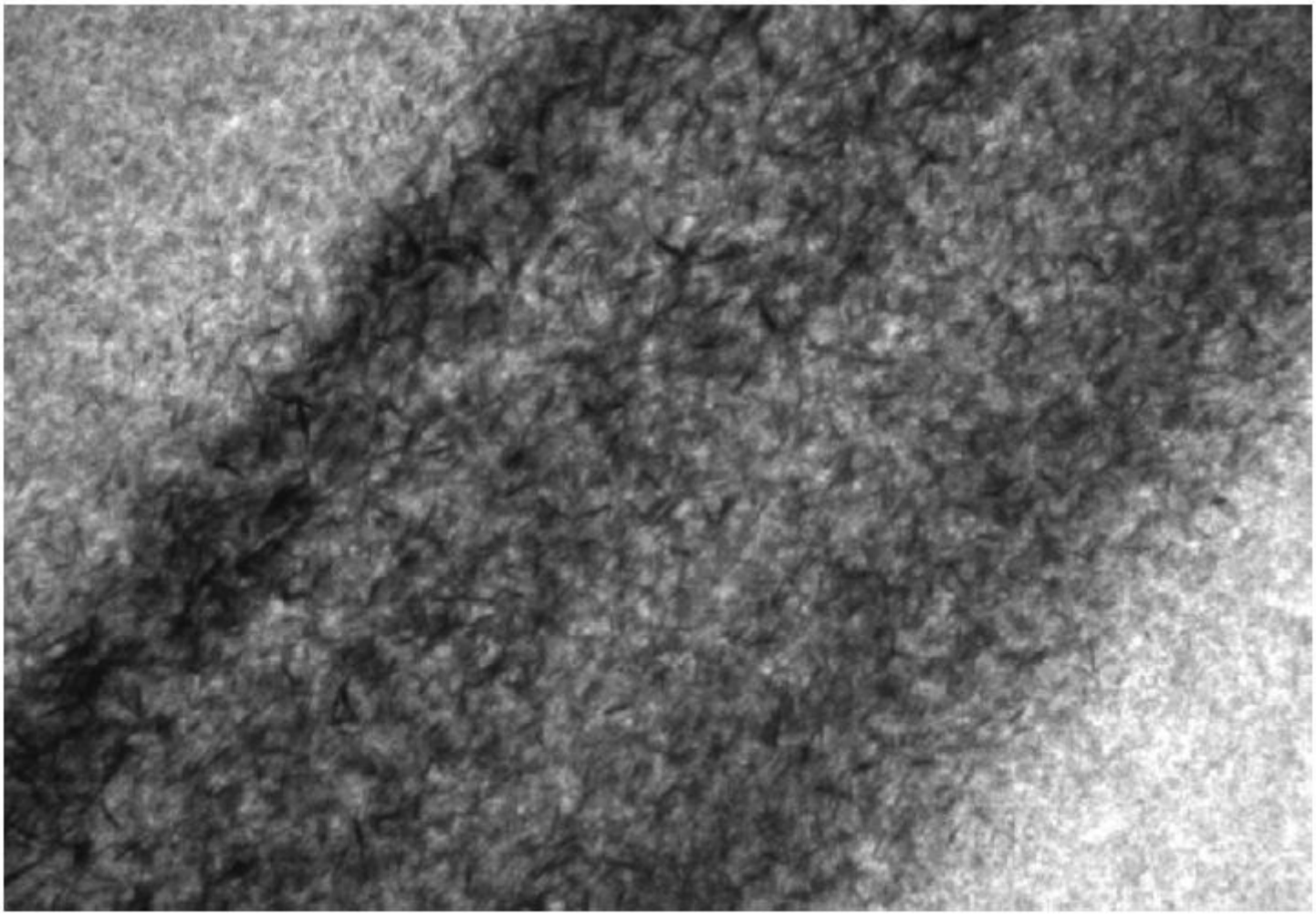

Figure 2

Electron micrograph of Decemet’s membrane showing an electron-dense band of unclear composition.