Midstromal Isolated Bowman Layer Graft for Reduction of Advanced Keratoconus
A Technique to Postpone Penetrating or Deep Anterior Lamellar Keratoplasty

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Keratoconus (KC) is regarded as a noninflammatory disorder characterized by progressive ectasia, which is associated with compromised optical function. In the past, early KC stages were managed by hard contact lens wear to obtain a regular anterior optical surface until contact lens intolerance in advanced stages required penetrating keratoplasty (PK) or deep anterior lamellar keratoplasty (DALK). Since 2003, UV-A–induced collagen cross-linking became an alternative treatment option for KC in which the corneas measured at least 400 μm in thickness and preoperative maximum keratometry (Kmax) measured 58 diopters (D) or less. Additional developments have enabled treatment of thinner and steeper corneas. Nevertheless, treatment in cases with more advanced KC may be limited to PK or DALK, which may be complicated by suture-related problems, epithelial wound-healing abnormalities, and/or corneal curvature changes due to progression of KC in the peripheral host cornea, resulting in a cascade of secondary complications and disappointing visual outcomes.

Because fragmentation of the Bowman layer is a pathognomonic feature in advanced KC, we hypothesized that a partial restoration of the corneal anatomy might be obtained through a midstromal implant of an isolated Bowman layer graft to remodel (ie, to flatten) the corneal curvature. At the same time, stabilization of the ectasia may be obtained by the Bowman layer splint and through the wound-healing reaction between the host stroma and the Bowman layer graft.

In this article, we describe a new surgical approach using a midstromal implant of a donor-isolated Bowman layer graft to reduce ectasia in eyes with advanced KC (Kmax ≥70 D). This procedure should enable continued contact lens wear while avoiding most short- and long-term complications.

Methods
We performed midstromal dissection with implant of an isolated donor Bowman layer graft in the stromal pocket in 10 eyes of 9 patients (3 male and 6 female; age range, 17-71 years) with (relative) contact lens intolerance owing to progressive end-stage KC, defined as mean keratometry of at least 58 D and steepest Kmax of at least 70 D (Table 1 and Table 2). In all eyes, an unsuccessful attempt was made to fit a sclera-supported rigid contact lens. All patients signed an informed consent approved by the institutional review board of the Dutch Independent Ethics Committee; the study was conducted according to the Declaration of Helsinki.

Surgical Technique
We performed manual dissection of a stromal pocket using a technique previously described to create a lamellar dissection. The procedure should postpone penetrating or deep anterior lamellar keratoplasty. Ten eyes of 9 patients with progressive, advanced keratoconus and contact lens intolerance underwent the procedure with no intraoperative adverse events. Throughout the study period, we observed no complications related to stromal dissection and/or the Bowman layer graft. Maximum corneal power decreased from a mean (SD) of 74.5 (71) diopters (D) before to 68.3 (5.6) D after surgery (P = .002). Hence, implant of an isolated Bowman layer graft may offer a safe and effective new technique to reduce ectasia in eyes with advanced keratoconus, potentially allowing continued long-term contact lens wear. The low risk of complications may render the procedure suitable as a treatment to postpone penetrating or deep anterior lamellar keratoplasty in cases with impending contact lens intolerance and/or corneal scarring (clinicaltrials.gov Identifier: NCT01686906).

Donor Tissue
Donor corneas released for transplant were mounted on an artificial anterior chamber (Katena [distributed by Rockmed BV]). The epithelial layer was carefully removed using surgical spears. A 360° superficial incision was made using a 30-gauge needle in the clear part of the corneal periphery. With a custom-made stripper (DORC International BV), the Bowman layer was carefully isolated from the anterior stroma over the full 360° toward the central part of the cornea. After complete detachment, subsequent trephination resulted in a Bowman flap measuring 9.0 to 11.0 mm. Owing to the elastic properties of the Bowman membrane, a Bowman “roll” formed spontaneously, which was submerged in 70% ethanol to remove all epithelial cells. After rinsing the roll with balanced salt solution (BSS; Bausch & Lomb), it was stored in organ-culture medium (CorneaMax; Eurobio) at 31°C until transplant (Figure 1).

Surgical Technique
We performed manual dissection of a stromal pocket using a technique previously described to create a lamellar dissection.
plane in DALK.\textsuperscript{13,14} With the patient under local anesthesia, a side port was made using a blunt cannula at the 3 or the 9 o'clock position of the limbus to aspirate the aqueous and to fill the anterior chamber completely with air. At the 12 o'clock position of the limbus, the conjunctiva was opened and a superficial scleral frown incision, 5.0 mm in length and 1 to 2 mm outside the limbus, was made. With a dissection spatula (Melles spatula set; DORC International BV), a lamellar dissection was made to just within the

### Table 1. Preoperative and Postoperative Anterior and Kmax Data

<table>
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<tr>
<th>Patient No./Sex/</th>
<th>Eye/</th>
<th>Age, y</th>
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<th>1-mo FU</th>
<th>6-mo FU</th>
<th>Latest FU</th>
<th>Kmax, D</th>
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<th>1-mo FU</th>
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**All, mean (SD)**

| 16 (5)        | 65.9 (5.4) | 59.5 (4.6) | 60.8 (4.2) | 61.0 (5.4) | −4.8 (1.7) | 78.5 (6.3) | 69.9 (3.8) | 70.7 (3.8) | 71.6 (4.7) | −6.9 (4.3) |

**P value, PO to FU**

| .001 | <.001 | .001 | <.001 | <.001 |

**P value, 1 mo to latest FU**

| .25 |

**P value, latest FU**

| .37 |

### Abbreviations: D, diopter; FU, follow-up; K, keratometry value; Kmax, maximum keratometry value; L, left; NA, not available; PO, postoperative; Pre-op, preoperative; R, right; Δ, difference.

* Indicates postoperative values improved by at least a 5% decrease from preoperative values.

* Indicates postoperative values changed less than 5% from preoperative values.

Boldface indicates significant change.

### Table 2. Preoperative and Postoperative Posterior and Maximum Corneal Power Data

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</table>

**All, mean (SD)**

| 16 (5)         | −10.2 (0.8) | −9.0 (0.5) | −9.3 (0.7) | −9.5 (0.7) | 0.8 (0.6) | 74.5 (7.1) | 67.2 (3.0) | 67.8 (4.1) | 68.3 (5.6) | −6.2 (3.9) |

**P value, PO to FU**

| .005 | .006 | .003 | .004 | .002 | .002 |

**P value, latest FU**

| .15 |

**P value, 1 mo to latest FU**

| .47 |

**P value, 1 mo to latest FU**

| .55 |

### Abbreviations: D, diopter; FU, follow-up; K, keratometry value; L, left; NA, not available; PO, postoperative; Pre-op, preoperative; R, right; Δ, difference.

* Indicates postoperative values improved by at least a 5% decrease from preoperative values.

* Indicates postoperative values changed less than 5% from preoperative values.

Boldface indicates significant change.
superior cornea. At this point, the tip of the blade was slightly tilted downward to visualize the interface between the air bubble in the anterior chamber and the corneal endothelium; underneath the corneal dimple, the air-endothelium interface was seen as a specular light reflex localized at the tip of the blade (Figure 2A-F). Between the blade tip and the light reflex, a non-reflective dark band was seen, representing the nonincised corneal tissue between the blade and the air-endothelium interface. Because the dark band became thinner with advancement of the blade into the deeper stromal layers, the corneal depth of the blade could be judged from the thickness of the dark band to avoid perforation (Figure 2F).

After the 360° stromal pocket was created up to the limbus (Figure 2E and F), a surgical glide (BD Visitec [Fichman]; Beaver-Visitec International) was inserted into the pocket, and the air was removed from the anterior chamber. The Bowman roll was again immersed in 70% ethanol for 30 seconds to remove remnant cellular material, thoroughly rinsed with balanced salt solution, and stained with trypan blue (VisionBlue; DORC International BV). The Bowman roll was then carefully inserted into the stromal pocket, unfolded, and centered, using balanced salt solution to manipulate the tissue (Figure 2G and H). The eye was then pressurized by filling the anterior chamber with balanced salt solution. Postoperative medication included chloramphenicol eyedrops, 0.5%, 6 times daily and dexamethasone eyedrops, 0.1%, 4 times daily.

All surgical procedures were digitally recorded (DVR-RT601H-S; Pioneer). At standardized intervals before surgery and at 1 day, 1 week, and 1, 3, 6, 12, 18, and 24 months after surgery, we...
measured best spectacle-corrected visual acuity (VA) and best contact lens–corrected VA. Slitlamp biomicroscopy, anterior segment tomography (Pentacam HR; Oculus), and slitlamp optical coherence tomography (Heidelberg Engineering GmbH) images were obtained. The endothelium was photographed and evaluated in vivo using a noncontact autofocus specular microscope (SP-3000P; Topcon Medical Europe). Images were analyzed and manually corrected, and the mean of multiple measurements of endothelial cell density was calculated. Unless otherwise indicated, data are expressed as mean (SD).

Results
All surgical procedures were uneventful, and throughout the study period, we observed no complications related to stromal dissection and/or implant of the Bowman layer graft. Because the donor Bowman layer was intentionally stretched toward the corneal limbus, an intrastromal cavity was seen in some eyes within the first days after surgery (Figure 3). At longer intervals, the graft could be visualized within the recipient corneal stroma using biomicroscopy in all transplanted corneas (Figure 4).

Compared with preoperative measurements, all keratometry values decreased after surgery in all eyes. Mean anterior simulated keratometry values decreased from 65.9 (5.4) D before surgery to 59.5 (4.6) D at 1 month ($P = .001$); mean Kmax values, from 78.5 (6.3) to 69.9 (3.8) D ($P = .001$); mean posterior keratometry values, from −10.2 (0.8) to −9.0 (0.5) D ($P = .005$); and mean maximum corneal power, from 74.5 (7.1) to 67.2 (3.0) D ($P = .004$) (Figure 5 and Tables 1 and 2). From 1 to 12 months after surgery, the flattened curvature values remained stable ($P > .11$) (Tables 1 and 2).

Compared with preoperative measurements, mean central corneal thickness increased from 396 (42) to 417 (37) μm at 6 months and to 423 (38) μm at the most recent follow-up. The mean thickness of the thinnest point changed from 334 (61) to 360 (31) μm at 6 months and to 363 (49) μm at the most recent follow-up. None of the changes reached statistical significance ($P > .05$). Mean logMAR best spectacle-corrected VA and best contact lens–corrected VA showed no significant changes from preoperative to 6-month postoperative measurements ($P = .07$ and $P = .77$, respectively).

Before surgery, most of the eyes (cases 1, 2, 3, 4, 6, 8, and 9) could tolerate contact lens wear only for a few hours during the day owing to excessive corneal steepness with an impending corneal “touch” between the cone and the contact lens. After surgery, however, all eyes could be fitted with a sclera-supported rigid contact lens (developed by Visser Contact Lens Practice and Procornea). The lens materials used in this study have high oxygen permeability and were tolerated well during full daily wear. Mean endothelial cell density showed no significant change from preoperative (2571 [497] cells/mm²) to 12-month postoperative (2552 [263] cells/mm²) measurements ($P = .31$).

Discussion
In the past decade, the preferred treatment for progressive KC may have shifted from contact lens fitting for as long as tolerated, followed by PK or DALK, to UV-A–induced collagen cross-linking to stabilize corneal ectasia in the long term. Although techniques are being developed to treat thinner or steeper corneas, corneas thinner than 400 μm or steeper than 58 D may not be eligible for UV cross-linking. However, this group of patients would similarly benefit from stabilizing the cone to enable continued contact lens wear. In advanced cases of KC in particular that are managed by PK or DALK, the long-term clinical outcome of these procedures often may be complicated by a sequence of adverse effects and events, which may eventually reduce the final visual outcome. Clinical observation suggests that eyes with advanced KC are the most prone to various inflammatory reactions after surgery, possibly relating to a stronger atopnic constitution and therefore rendering any keratoplasty procedure as high risk owing to the possible long-term complications.

Therefore, our aim was to design a surgical procedure that would solve most of the clinical challenges in advanced KC.
Because fragmentation of the recipient's own Bowman layer is one of the pathognomonic features in pathological sections of corneas with KC \(^2, 3, 9\), management of KC with an isolated Bowman layer graft to restore its shape and tensile strength may be effective. If the donor Bowman layer graft were positioned inside the recipient cornea, the graft would be sandwiched between the stromal layers above and below, and no anterior corneal incisions or fixation means would be necessary. When fixed in this position, the donor Bowman layer would pull the anterior corneal surface flatter, creating a more homogeneous surface topography and

In the left eye (OS) of case 8, the anterior (A–C) and posterior (D–F) keratometric values show significant corneal flattening, whereas the pachymetry (G–I) remains unchanged. N indicates nasal; T, temporal.
The classification of keratoconus was developed by Krumeich et al.23 BL indicates Bowman layer; D, dioptr; DALK, deep anterior lamellar keratoplasty; ISCRS, intrastromal corneal ring segments; Kmax, maximum keratometry value; and PK, penetrating keratoplasty.

**ARTICLE INFORMATION**

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**Author Contributions:** Ms van Dijk and Dr Melles had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** van Dijk, Melles.

**Acquisition of data:** All authors.

**Analysis and interpretation of data:** van Dijk, Parker, Melles.

**Drafting of the manuscript:** van Dijk, Parker, Lie, Groeneveld-van Beek, Melles.

**Critical revision of the manuscript for important intellectual content:** van Dijk, Parker, Tong, Ham, Melles.

**Statistical analysis:** van Dijk, Parker.

**Administrative, technical, or material support:** Tong, Ham, Lie, Groeneveld-van Beek.

**Study supervision:** van Dijk, Melles.

**Conflict of Interest Disclosures:** Dr Melles is a consultant for DORC International/Dutch Ophthalmic USA. No other disclosures were reported.

**REFERENCES**


