# Pneumatic Dissection and Storage of Donor Endothelial Tissue for Descemet's Membrane Endothelial Keratoplasty

## *A Novel Technique*

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***Purpose:*** To investigate the feasibility of pneumatic dissection of donor endothelium and the effect of 7 days of storage in tissue culture medium on the endothelial cell count.

***Design:*** Experimental study.

***Participants:*** We used 20 human donor corneoscleral tissues deemed unsuitable for transplantation.

***Intervention:*** Donor corneas were mounted on an artificial anterior chamber and the anterior stroma removed with a 300-micron microkeratome head. Air was injected into the residual donor tissue with a 30-G needle from the endothelial side to detach Descemet's membrane. The "bubble" was expanded as far as possible into the periphery. A silicone weight was attached to the scleral ring and the prepared tissue was stored in tissue culture medium for 7 days.

***Main Outcome Measures:*** Complete detachment of Descemet's membrane, size of detachment, pre- and post-storage endothelial cell counts.

***Results:*** Complete detachment of Descemet's membrane was achieved in 19 of 20 (95%) cases. In 12 (60%) cases, this was achieved with a single injection; 7 (35%) cases required repeat injections. In 1 case, Descemet's membrane could not be detached despite repeat air injections. The average size of detachment was 8.11±2.0 mm. Endothelial cell loss after 7 days of tissue culture medium storage was 4.44±4.3%.

***Conclusions:*** Descemet's membrane and endothelium can be separated from the overlying stroma with a simple technique using air dissection. An adequate size of graft tissue is obtained without the need to manually handle the tissue. The technique allows storage of the tissue in tissue culture medium with low endothelial cell loss.

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Descemet's membrane endothelial keratoplasty (DMEK) represents the most recent development in posterior lamellar surgery. In contrast with Descemet's stripping endothelial keratoplasty, donor stromal tissue is not transplanted. This avoids formation of a corneal interface and therefore may optimize visual outcome and accelerate postoperative visual rehabilitation.[1]

However, to date, DMEK has not gained popularity, primarily because of the technical difficulties involved in both harvesting and delivering the donor endothelium. Various methods of mechanical dissection of donor tissue have been described.[2-5] These methods are highly dependent on the surgeon's skills and can cause a wastage of up to 16% of donor corneas during the preparation of the endothelial graft, as well as an endothelial cell loss of >8% immediately after preparation.[2,4] In addition, the delicate handling of the prepared donor tissue adds to the challenge.[4,5]

We describe herein a method that eliminates manual dissection of donor endothelium and report the effect of storage in tissue culture medium for 7 days on the dissected endothelium.

## Methods

Twenty human cadaver donor corneas with a scleral rim of ≥2 mm deemed unsuitable for transplantation were obtained from the eye bank (Fondazione Banca degli Occhi del Veneto, Venice, Italy). All tissues were free of corneal pathology and were unsuitable for transplantation owing to donor medical contraindication discovered after donation and retrieval. Endothelial cell density was determined by vital staining with 0.25% (weight/volume) trypan blue to visualize the nuclei of nonviable cells and subsequently exposed to hypotonic sucrose solution to visualize the swelling of the intercellular borders. The endothelium was then examined by light microscopy at ×100 magnification, and the cell density was estimated with the help of a 10 × 10-calibrated graticule mounted in the ocular of the microscope (fixed-frame analysis technique). The endothelial density was expressed as the mean (×100) of 5 different counts, each performed in a different region of the central corneal areas.[6,7]

Each cornea was then placed on an artificial chamber (ALTK System, Moria, Antony, France). Microkeratome-assisted removal of approximately two thirds of the anterior corneal stroma from the donor cornea was carried out using the 300-

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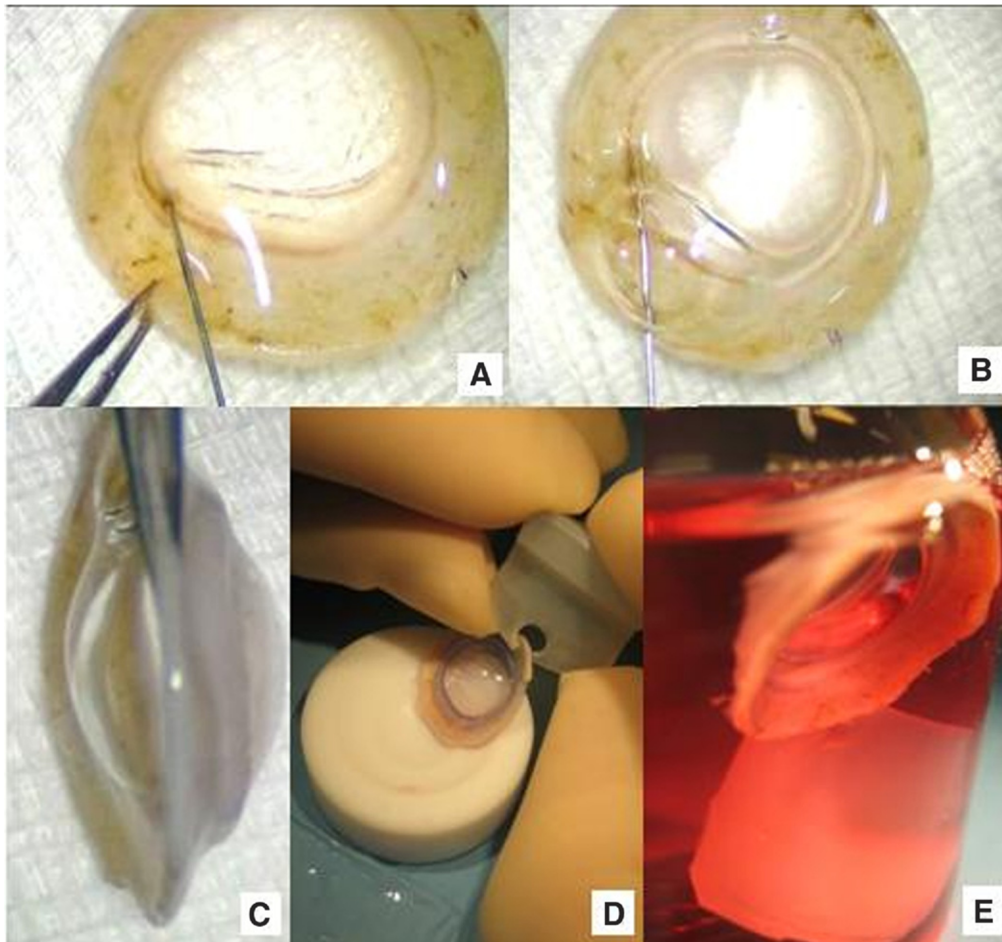


**Figure 1.** **A**, A 30-G needle inserted bevel up at the limbus. **B**, Needle advanced for 2 mm and injection of air results in formation of a "bubble." **C**, Side view of detached Descemet's membrane. **D**, Weight attached to the sclera ring. **E**, Prepared tissue stored in tissue culture medium.

micron microkeratome head (as for Descemet's stripping endothelial keratoplasty surgery). The rationale for this is described in the Discussion.

The corneas were placed on sterile gauze with the endothelial side up. A 30-G needle connected to a 10-cc, air-filled syringe was inserted bevel up into the peripheral cornea approximately 1 mm from the limbus and advanced in a tangential direction immediately beneath the endothelium for approximately 2 mm (Fig 1A). Air was subsequently injected to achieve detachment of Descemet's membrane and the bubble enlarged as far as possible into the corneal periphery (Video Clip 1; available online at http://aaojournal.org) (Fig 1B, C). Air was frequently seen to leak from the limbal area (irrespective of previous injection attempts). Persistent injection, however, ensured complete dissection. Calipers were used to mea-

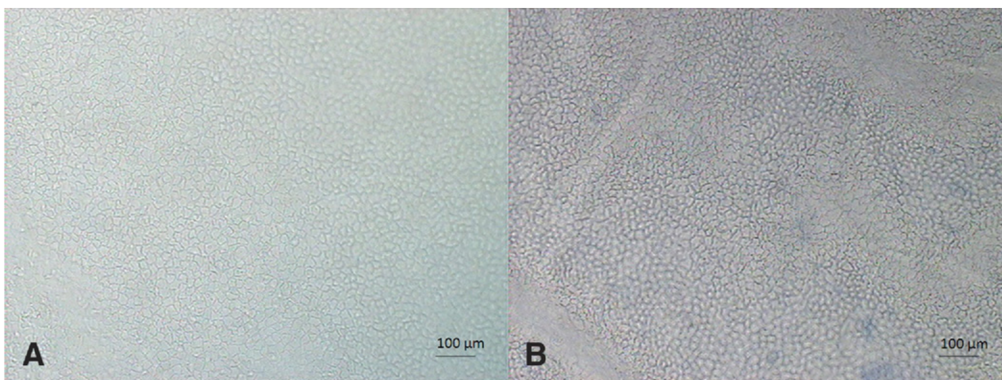


**Figure 2.** **A**, Light microscopy of endothelial cells before preparation. **B**, Folds and patchy loss of endothelial cells after 7 days of storage in tissue culture medium.

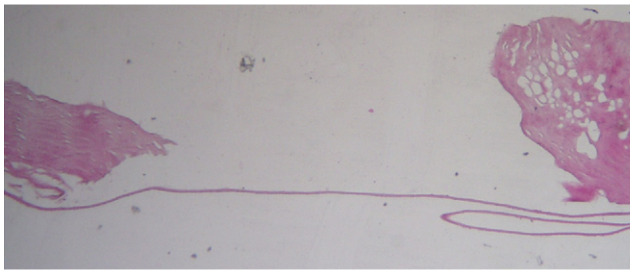

**Figure 3.** Hematoxylin and eosin staining of the preparation revealing endothelium and Descemet's membrane. No underlying stroma was noted (original magnification, ×20).

sure the diameter of the bubble and the size was determined using a ruler with 0.25-mm gradations.

A weight was created using a silicone stopper trimmed to allow it to pass through the neck of the storage bottle. A partial hole was created on the edge of the stopper to allow it to grip onto the scleral rim. This weight (Fig 1D) was attached to the rim to prevent the cornea from floating after immersion into the tissue culture medium and possibly dehydrating the "bubble." The donor tissue was preserved for 7 days (Fig 1E) and endothelial cell density was determined as described above by a single observer who was masked to the results of the previous evaluation. Figure 2A, 2B shows typical photomicrographs (taken with a light microscope equipped with a digital camera, at a magnification of ×100) of the prepared endothelial tissue before preparation and after 7 days of storage, respectively.

## Results

Complete detachment of Descemet's membrane was achieved in 19 of 20 cases (95%). In 12 cases (60%), this was achieved with a single injection; 7 cases (35%) required repeat injections with the needle being inserted at different sites. Four authors (MB, VS, GS, and DP) carried out 5 injections each. The only failure was obtained by the experienced corneal surgeon (MB). The detached layer consisted purely of endothelium and underlying Descemet's membrane (Fig 3).

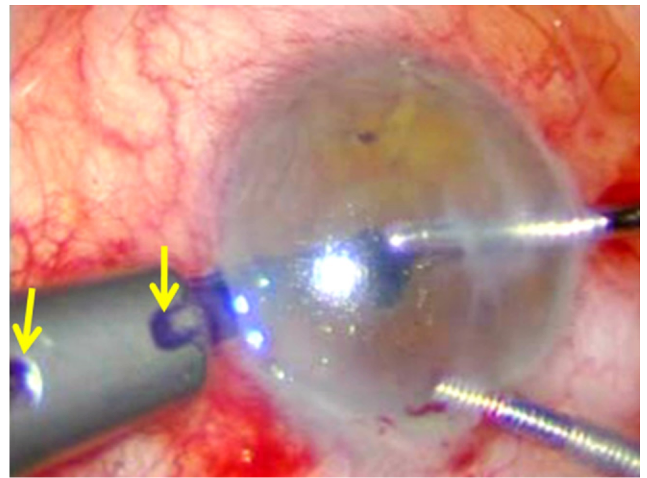

**Figure 4.** The stromal support prevents the endothelial graft from rolling and facilitates delivery while remaining within the glide (arrows).

The average diameter of the bubble achieved was 8.11±2.0 mm (Table 1). At the end of the storage period (7 days), the average loss in endothelial cell density was 4.44±4.3% (Table 1).

## Discussion

The possibility of separating Descemet's and endothelium from the overlying stroma by means of air injection is well

Table 1. Endothelial Cell Densities (ECD) before and after Pneumatic Dissection of Donor Corneoscleral Rims

| Donor Disc No. | Donor Age (yrs) | Donor Gender | Storage Time in TCM before Preparation (d) | ECD before Pneumatic Dissection (/mm$^2$) | No. of Injections | Bubble Diameter (mm) | ECD after 7 Days of Storage in TCM (/mm$^2$) | ECD Loss (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 53 | Female | 8 | 2600 | 1 | 8.50 | 2500 | 3.8 |
| 2 | 37 | Male | 8 | 2400 | 1 | 9.00 | 2400 | 0 |
| 3 | 62 | Male | 9 | 1800 | 1 | 7.50 | 1600 | 11.1 |
| 4 | 72 | Male | 8 | 1400 | 2 | 8.00 | 1200 | 14.3 |
| 5 | 60 | Female | 10 | 2100 | 3 | 8.00 | 2100 | 0 |
| 6 | 65 | Female | 19 | 2000 | 1 | 9.00 | 1900 | 5 |
| 7 | 60 | Male | 9 | 2100 | 1 | 9.00 | 2000 | 4.8 |
| 8 | 65 | Female | 16 | 2100 | 2 | 9.50 | 2100 | 0 |
| 9 | 72 | Male | 15 | 1900 | 1 | 7.75 | 1900 | 0 |
| 10 | 64 | Female | 7 | 1600 | 2 | 8.25 | 1400 | 12.5 |
| 11 | 67 | Male | 7 | 2400 | 1 | 7.50 | 2300 | 4.2 |
| 12 | 70 | Female | 15 | 2400 | 2 | 8.50 | 2300 | 4.2 |
| 13 | 70 | Male | 20 | 1800 | 1 | 8.00 | 1700 | 5.6 |
| 14 | 60 | Female | 15 | 2700 | 1 | 9.00 | 2700 | 0 |
| 15 | 66 | Male | 7 | 1900 | 1 | 9.00 | 1800 | 5.3 |
| 16 | 49 | Male | 20 | 2600 | 1 | 9.25 | 2400 | 7.7 |
| 17 | 68 | Male | 11 | 2800 | 2 | 8.50 | 2700 | 3.6 |
| 18 | 68 | Female | 7 | 1500 | 2 | 8.75 | 1400 | 6.7 |
| 19 | 66 | Male | 17 | 2800 | 1 | 9.25 | 2800 | 0 |
| 20 | 72 | Male | 8 | 2000 | 4 | 0.00 | — | - |

TCM = tissue culture medium.

known.[8] This method is routinely used to perform lamellar keratoplasty in patients with healthy endothelium (e.g., keratoconus). However, the success rate in creating the so-called big bubble depends highly on the surgical skills of the surgeon, because the needle used to inject the air must reach the critical depth immediately above the Descemet's membrane without perforating it.

Air dissection of Descemet's membrane is much easier in donor corneas, because the needle can be inserted from the endothelial side and advanced under direct observation to an appropriate depth immediately beneath the endothelium. The periphery of the endothelial graft prepared in this manner remains attached to the stroma and the entire dissected sclerocorneal rim can be stored in tissue culture medium (Fig 1E).

Because the prepared tissue remains adherent peripherally to the underlying stroma until the time of surgery, rolling of the tissue is prevented. Thus linear, focal, and irregular peripheral loss of endothelial cells, which has been documented after stripping of the endothelium, is avoided.[5,9] The stromal support also reduces the likelihood of "upside down" transplantation.[10] Furthermore, the stromal support is instrumental in providing a cushion whilst transporting the endothelium from the preparation site to the point of delivery into the eye (Fig 4).

In the rare case of failure to obtain a "big bubble," a Descemet's stripping endothelial keratoplasty procedure can still be performed with the same tissue thereby reducing wastage of donor tissue. The preliminary step of microkeratome-assisted removal of the anterior stroma with a 300-micron microkeratome head allows this, as well as the option to utilize this part of the donor tissue for anterior lamellar keratoplasty. This preliminary cut is not necessary, however, and units that do not have access to a microkeratome may bypass this step or obtain precut tissue from the eye bank.

Previously described preparation methods all involve handling the tissue with forceps or dissecting instruments, which cause mechanical damage to the endothelium and may eventually lead to graft failure.[2,4,5,10,11] This technique allows a touch-free preparation, thereby minimizing endothelial damage. Although the endothelial cell loss encountered in our study is lower than other preparation methods,[2,4] comparative studies and long-term follow-up are required to determine whether this is significant. It also allows for a larger area of graft tissue to be utilized than noncircular harvesting methods.[2]

For corneal surgeons and eye bank personnel who are accustomed to handling donor tissue, no particular additional skills are required to master the technique. The technique can therefore be used by a surgeon at the beginning of a DMEK procedure, or by a technician in the eye bank for storage in tissue culture medium and later delivery. Eye bank preparation eliminates the need for the surgeon to request spare tissue in case of failure to obtain a bubble and reduces surgical time.

Descemet's membrane and endothelium can be separated from the overlying stroma with a simple technique using air dissection. We expect that the above advantages and the ease with which the technique can be carried out would facilitate uptake of the DMEK procedure.

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## Footnotes and Financial Disclosures


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