



Ultra-thin DSAEK using an innovative artificial anterior chamber pressuriser: a proof-of-concept study

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Abstract

Purpose To report the impact of establishing and maintaining a high intracameral pressure (ICP) of 200 mmHg on UT-DSAEK graft preparation using an artificial anterior chamber pressuriser (ACP) control unit (Moria SA, Antony, France).

Method Retrospective laboratory and clinical study. Four paired donor corneas were mounted on an artificial anterior chamber and subjected to 70 mmHg (“low”) and 200 mmHg (“high”) ICP using an ACP system. The central corneal thinning rate was measured after 5 min using AS-OCT and the endothelial cell viability was analysed using trypan blue and live/dead staining following 70 mmHg and 200 mmHg ICP. Visual outcomes and complications in a clinical case series of nine patients with bullous keratopathy who underwent UT-DSAEK using 200 mmHg ICP during graft preparation are reported.

Results Laboratory outcomes showed $2 \pm 1\%$ and $2 \pm 2\%$ dead cells following 70 mmHg and 200 mmHg ICP respectively. Percentage viability in the 70 mmHg group ($52.94 \pm 5.88\%$) was not found to be significantly different ($p = 0.7$) compared to the 200 mmHg group ($59.14 \pm 10.43\%$). The mean corneal thinning rate after applying 200 mmHg ICP was $27 \pm 13 \mu\text{m}/\text{min}$ centrally ($7.2\%/\text{min}$). In the clinical case series, two cases were combined with cataract surgery. Re-bubbling rate was 11%. At the last follow-up (259 ± 109 days), graft thickness was $83 \pm 22 \mu\text{m}$ centrally, endothelial cell density was $1175 \pm 566 \text{ cell}/\text{mm}^2$ and the BCVA of $0.08 \pm 0.12 \text{ logMAR}$ was recorded with no episodes of rejection.

Conclusion ACP control unit for UT-DSAEK graft preparation helps in consistently obtaining UT-DSAEK grafts without compromising endothelial cell viability.

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Key Message:What Was Known

- High pressure inside the artificial anterior chamber (AAC) for a short time (<5 minutes) does not damage the endothelial cells or its pump functions during DSAEK preparations.
- Corneal thinning rate is important to prepare an UT-DSAEK graft with a microkeratome using a single cutting calibrated head of 350 microns.
- Thick grafts are responsible for the creation of stromal folds on connection between the donor and the patient tissue. Achieving a minimum corneal thickness of 500 microns before the cut helps to obtain an UT-DSAEK graft in 97% of cases.

What This Paper Adds

- A constant high intracameral pressure during the UT-DSAEK graft preparation does not affect the endothelial cell viability when compared to a lower pressure.
- Elevated anterior chamber pressures (200 mmHg) led to a faster corneal thinning rate i.e. 7.2%/min when compared to clamp and dry technique i.e. 11 µm/min.
- The artificial anterior chamber pressuriser (ACP) unit helps in the standardisation and preparation of a uniform, reliable and reproducible UT-DSAEK graft.

Keywords Ultrathin DSAEK · Intracameral pressure · Thickness · Eye bank · Endothelial keratoplasty · Endothelial cells · Corneal thinning

Introduction

Although a continued increase in uptake of Descemet membrane endothelial keratoplasty (DMEK) has been observed in the last few years [1], the overall numbers of endothelial keratoplasty (EK) carried out are still higher for Descemet stripping automated endothelial keratoplasty (DSAEK) [2]. DSAEK is popular for EK procedures due to a standardised preparation of the corneal donor tissue using microkeratome and reproducibility of the technique. Ultra-thin DSAEK (UT-DSAEK) is a type of DSAEK where the thickness of the prepared donor tissue is less than 100 µm centrally [3]. Reports show that better visual outcomes are achieved with UT-DSAEK compared to DSAEK [4].

The microkeratome has simplified DSAEK graft preparation by standardising the procedure using a fixed blade oscillation rate and speed control. It lowers the number of irregularly cut tissues, which reduces the tissue thickness and improves the visual outcomes [5]. However, the pressure inside the artificial anterior chamber (AAC, Moria SA, Antony, France) is one of the key parameters that has not yet been fully standardised. High intracameral pressure (ICP) inside the AAC has been shown to produce better results in terms of corneal thinning rate, corneal graft thickness

and profile [5–7]; however, it is difficult to maintain equal pressure throughout the preparation/cutting phase due to the corneal appplanation induced during the microkeratome pass. Moria SA (Antony, France) has developed the artificial chamber pressuriser (ACP) in order to standardise the pressure inside the AAC during corneal graft preparation. The ACP is able to establish, then constantly obtain the desired AAC pressure from 35 to 250 mmHg throughout the corneal graft preparation procedure; however, the effect of a high pressure, such as 200 mmHg, on corneal endothelial cells and on the preparation performance has only partly been reported [8].

This study reports the evaluation of corneal thinning rate of the donor tissue and related endothelial cell viability using a constantly high AAC pressure of 200 mmHg to prepare UT-DSAEK grafts in an eye bank setting, and clinical outcomes after UT-DSAEK delivery prepared using the same settings.

Materials and methods

Ethical approval

Human corneo-scleral discs maintained in tissue culture medium (TCM—prepared by FBOV) at 31 °C were obtained from Fondazione Banca degli Occhi del Veneto

(FBOV, Venice, Italy) to be used for research, with written consent from the donor's next-of-kin. In this donor-matched, randomised study, the tissues were utilised and discarded in accordance with the guidelines set by Centro Nazionale Trapianti, Rome, Italy. All surgeries included in this retrospective case series, undergoing UT-DSAEK, were performed at Azienda USL della Romagna, Emilia Romagna, Italy. The study was approved by the local IRB and the patients were consented for the surgeries performed between September 2016 and October 2017 by one surgeon (LA).

Donor characteristics and baseline data

Donor characteristics were retrospectively obtained from the FBOV database. All the tissues were free of corneal pathology but unsuitable for transplantation because of low donor endothelial cell count after donation (< 2200 cells/mm²). In a donor-matched study (bilateral corneas from the same donor were obtained and one cornea was used in one arm of the study and the other cornea was used in another arm), four pairs of tissues were used to measure the endothelial cell viability after 70 mmHg (low) and 200 mmHg (high) ICP. The endothelial cells were counted by placing the tissue with endothelial side facing the petri dish in 1.8% sucrose solution. The cells were counted manually at 5 different regions using an in-built reticule in the eye piece of an inverted microscope. The endothelium was stained with trypan blue to count the number of necrotic cells by counting the number of trypan blue positive cells (TBPCs) as previously described [9, 10]. Corneal thinning rate was also measured from the grafts that were treated at 200 mmHg ICP.

Laboratory investigation

Corneal thinning rate evaluation

The residual epithelium was scraped from the corneal surface, and corneas were placed on the AAC that was connected to a bottle of TCM on a IV pole positioned 120 cm from the base of the laminar flow hood. Via a three-way stopcock, the ACP control unit was connected to its dedicated single-use ACP tubing set (Moria SA, Antony, France) (Fig. 1). We chose 70 mmHg (low) and 200 mmHg (high) ICP based on our previous work [11] and also because they are both commonly used. All corneas were maintained in this set-up and the central corneal thickness (CCT) was measured at baseline and after 5 min. Each measurement was performed with a swept-source anterior segment optical coherence tomography (AS-OCT) (Casia 1, Tomey, Erlangen-Tennenlohe, Germany).

Endothelial cell viability

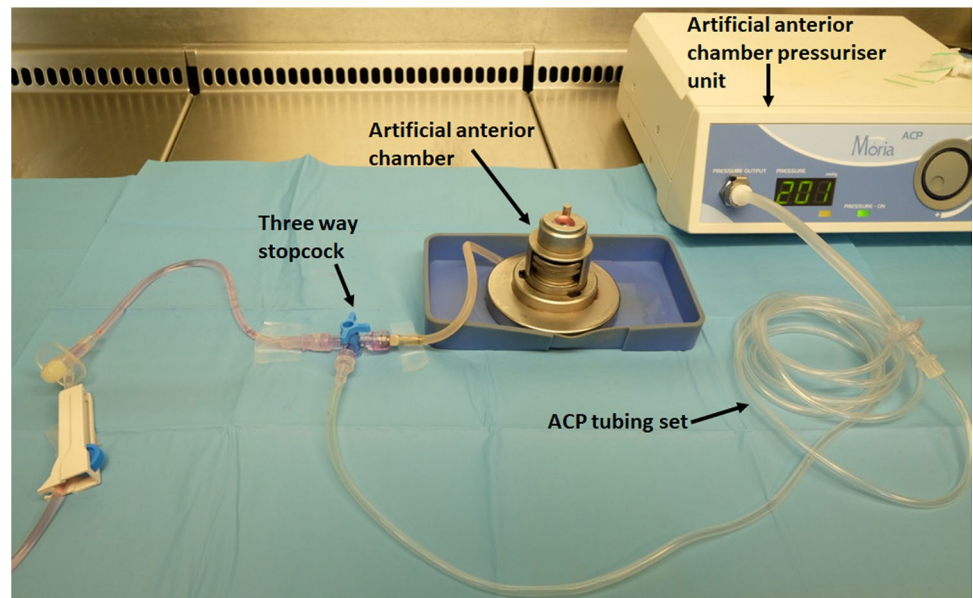
All corneas were mounted on a single-use AAC (Moria SA, Antony, France) filled with the TCM. Four paired corneas were subjected to either 70 mmHg or 200 mmHg ICP for a period of 20 min. After that time, the corneas were stained with trypan blue for approximately 30 s and washed with phosphate-buffered saline (PBS). The endothelial cells were evaluated as previously described [9, 10].

The effect of ICP on endothelial cell viability was then analysed. The corneas were mounted on the AAC (Moria SA, Antony, France) filled with TCM. Three paired corneas were subjected to either 70 mmHg or 200 mmHg for a period of 20 min ($n = 6$). After that time, the corneas were washed with PBS and a solution of 200 µl of PBS containing calcein AM (0.4 µM) and Hoechst 33342 (3 µg/ml) was added to the endothelial surface and incubated at RT in the dark for 45 min. Hoechst is a cell-permeant nuclear counterstain. It acts as a vital nucleic acid dye. Calcein acetoxymethyl (calcein AM) is a non-fluorescent, hydrophobic compound that permeates into live cells and when hydrolysed, it is retained in the cells. Compromised cells do not retain calcein. Therefore, this staining was mainly used as an alternative confirmatory analysis to trypan blue staining and to determine the viability of the graft. A flat mount was obtained by making relaxing radial cuts at 3 positions before mounting and protecting the tissue with a coverslip. The staining was viewed with an LSM 800 confocal microscope. A tile scan was performed using a $\times 5$ objective and the whole surface reconstructed using the ZEN software. Trainable Weka segmentation on Fiji was used to analyze the percentage area covered by viable cells as previously described by Romano et al. [12].

For tiled image processed through Weka segmentation, it was difficult to identify the apoptotic cells. Therefore, the acquired images at higher magnification ($\times 100$) (average of five random areas) were split in two colours and using ImageJ, the original image was converted into a binary image. A copy was made and all the cells were automatically counted using measure option followed by which the copied image was overlapped to get the cells with overlapped numbers. Only the cells showing Hoechst positivity and calcein AM negativity were manually counted to determine the number of cells with compromised cytoplasm or apoptotic cells. Areas showing no cells or those at iatrogenic folds showing faded calcein positive cells that were difficult to identify were excluded from the analysis.

A Mann–Whitney U test was used to compare differences between two independent groups. A probability value (p) of < 0.05 was considered statistically significant. Analysis was performed using the SPSS statistical software V.20.0 (IBM, Armonk, New York, USA) [12].

Fig. 1 ACP control unit connected to the AAC with its dedicated ACP tubing set, with the intracameral pressure established then maintained at 200 mmHg



Clinical investigation

A retrospective case series of nine patients affected by bullous keratopathy underwent UT-DSAEK, as previously described [8, 13]. Visual outcomes, postoperative central graft thickness, endothelial cell density, complications and adverse events were recorded. All endothelial corneal grafts were prepared by a single surgeon at the time of surgery using a constant high ICP i.e. with ACP established at 200 mmHg.

Results

Laboratory investigation

Average age of the donor corneas was 72 ± 4 years. Average post-mortem time was 7 ± 2 h and the storage time of the tissue in the TCM was 29 ± 3 days at 31°C before experimental use. Endothelial cell density before preparation was 1811 ± 127 cells/mm² with $1 \pm 1\%$ mortality/TBPCs. For the corneal thinning rate evaluation, the mean initial CCT using AS-OCT was 1001 ± 129 μm without epithelium. There was a reduction of corneal thickness in all corneas after 5 min, and this was presumed to be predominantly due to stromal thickness as the epithelium had been removed before initiating the experiments. The mean corneal thinning rate after the application of 200 mmHg pressure was found to be 26.6 ± 12.9 $\mu\text{m}/\text{min}$ centrally (7.2%/min). Mean endothelial cell counts after applying the ICP were 1766 ± 141 cells/mm² and 1722 ± 164 cells/mm² in the 70 mmHg and 200 mmHg groups respectively. TBPCs/mortality was recorded at $2 \pm 1\%$ and $2 \pm 2\%$ in the 70 mmHg and 200 mmHg groups respectively. Calcein AM and Hoechst 33342 staining were used to identify the areas (%) covered by viable

cells. Percentage viability in the 70 mmHg group (52.94%, SD 5.88) (Fig. 2a) was not found to be statistically significantly different ($p=0.7$) compared to the 200 mmHg group (59.14%, SD 10.43) (Fig. 2b). Apoptotic cells from the 70 mmHg group (0.7%, SD 0.2) (Fig. 2c—marked with red arrow) did not show statistical difference ($p=0.2803$) compared with the 200 mmHg group (0.9%, SD 0.3) (Fig. 2d—marked with red arrow).

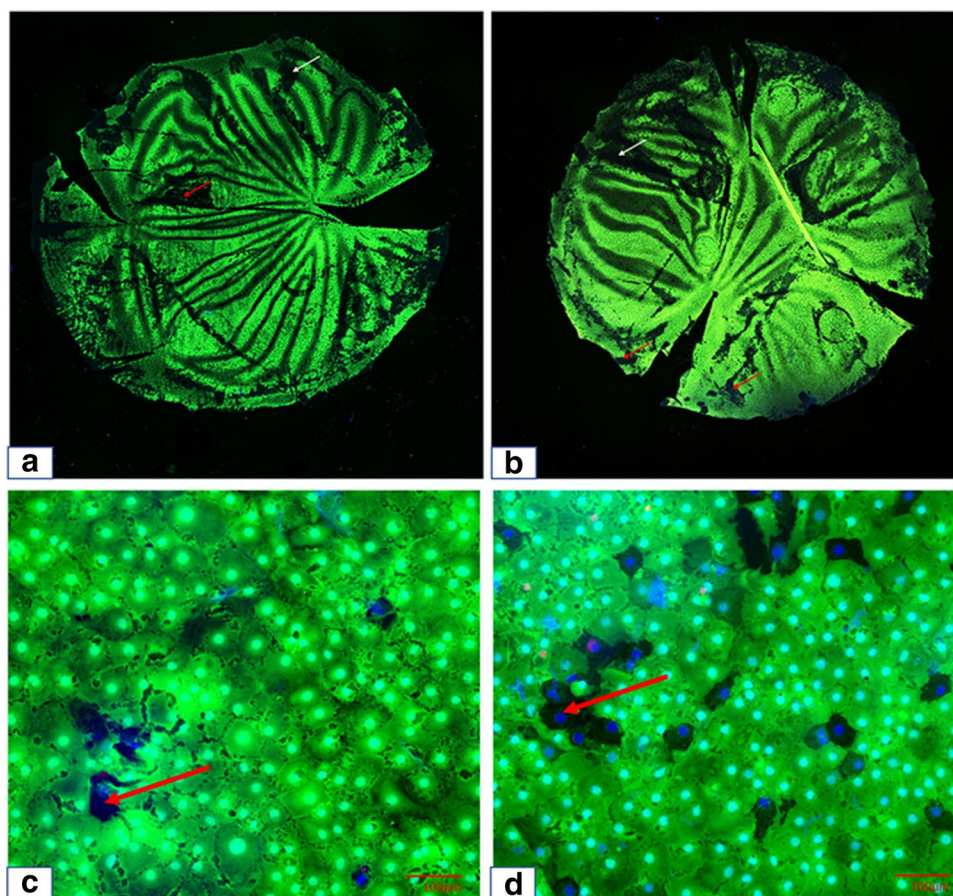
Clinical outcomes

Mean age of the donor corneas was 57 ± 25 years. Average post-mortem time was 10 ± 9 h and the storage time of the tissue in the TCM was 23 ± 10 days before the experiments. Endothelial cell count was 2500 ± 63 cells/mm². Nine patients with bullous keratopathy underwent UT-DSAEK with the donor tissues being prepared at 200 mmHg ICP using ACP system. The mean follow-up was 259 ± 109 days. Two cases were combined with cataract surgery and both of them (11%) required re-bubbling at early follow-up. No rejection was noticed (Fig. 3a). Best-corrected visual acuity at last follow-up was 0.08 ± 0.12 logMAR. Central corneal graft thickness measured by swept-source AS-OCT (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) was 83 ± 22 μm (Fig. 3b), with endothelial cell density (Konan Medical, Inc., Hyogo, Japan) of 1175 ± 566 cell/mm² (Fig. 3c), at last follow-up.

Discussion

Corneal clarity is heavily dependent on the health of its endothelium. DSAEK has served as a gold standard to treat endothelial dysfunction and, therefore, its standardisation

Fig. 2 Live/dead analysis on a flat-mounted cornea using HEC staining after the tissues were treated with **a** 70 mmHg and **b** 200 mmHg pressure. Higher magnification images of cells treated with HEC following **c** 70 mmHg and **d** 200 mmHg pressure. White arrow indicates degenerated area and red arrow indicates apoptotic cells (green = calcein Am-live cells; blue = Hoechst-nuclei)



becomes important for each step, one of which includes the maintenance of pressure in AAC. Earlier, we have observed that high pressure inside the AAC for a short time does not damage the corneal endothelial cells during DSAEK graft preparation. Similar studies conducted by Melamed et al. reported that increased AAC pressure for a short time (<5 min) does not damage the corneal endothelium or endothelial pump function [14]. A continuously maintained pressure in the AAC could therefore be advantageous in order to obtain a reliable corneal graft with a high

reproducibility rate and low endothelial cell damage. Our laboratory and clinical results highlight the advantages of using the ACP control unit for UT-DSAEK graft preparation. We found that a constant high ICP of 200 mmHg during the UT-DSAEK graft preparation does not appear to significantly affect the endothelial cell viability when compared to a lower ICP of 70 mmHg. One limitation of this study is due to the difficulty in obtaining human donor tissue for research, the number of samples available was limited and therefore, statistical analysis was not conclusive.

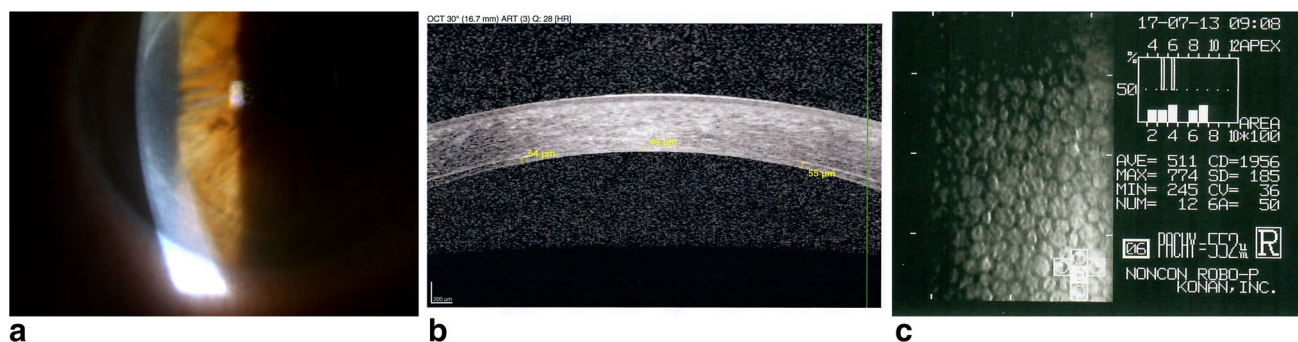


Fig. 3 Presentation of the clinical results of the same patient showing **a** no rejection episode and clearing of the graft at last follow-up. **b** Central corneal graft thickness at AS-OCT. **c** Endothelial cell density at last follow-up

It has been shown that the visual gain is significantly correlated with the central graft thickness post DSAEK [4]. Bhogal et al. showed that reducing the transition speed during microkeratome dissection produces thinner donor lenticules without any difference in mean asymmetry [15]. Thomas et al. [16] interestingly reported that anterior stromal dehydration reduces donor corneal thickness to obtain thin grafts more frequently using standard DSAEK microkeratomes without causing significant additional damage to the corneal endothelium. Corneal thinning rate is particularly important for surgeons or technicians who aim to prepare an UT-DSAEK graft with a microkeratome using a single cutting calibrated head of 350 microns by a single pass. Achieving a minimum corneal thickness of 500 microns before the cut helps to obtain an UT-DSAEK graft (< 100 microns centrally) in 97% of cases [5, 6, 17].

In the present study, the central corneal thickness was measured only 5 min after donor tissue being mounted into an AAC and established ICP. This time point was chosen based on our prior surgical experience. Corneal tissues that are received for transplant are preserved in a deturgescent media and, therefore, their initial central thickness is usually below 600 microns, and as our target was to achieve 500–510- μ m thickness centrally for surgery [8], 5 min is usually sufficient to achieve this. In order to be applicable in the surgical theatre, only a 5-min time point was used for corneal thickness measurements. However, as the tissues for laboratory investigation were preserved in TCM, the initial thickness was over 1000 microns and so to reduce that thickness to physiological levels, we maintained ICP for 20 min.

A limitation of the study is that the data may vary when comparing tissues that are preserved in cold storage, TCM or deturgescent medium. All three have different properties and can allow swelling/de-swelling of the tissue under their respective preservation conditions. The laboratory study reflects tissues that are preserved in the TCM, while the clinical study reflects those that are preserved in deturgescent medium.

In our report, we found that a high ICP of 200 mmHg led to a faster central corneal thinning rate ($26.6 \pm 12.9 \mu\text{m}/\text{min}$ (7.2%/min)) when compared to clamp and dry technique ($11 \mu\text{m}/\text{min}$, 1.5%/min) [11]. We thus showed that using the AAC connected to the ACP helps to establish, maintain and standardise this process. As a consequence, we can conclude that the ACP control unit with AAC helps to achieve an UT-DSAEK graft using a fully standardised process. A study by Nishisako S et al. also concluded that ICP of 200 mmHg, maintained constantly using ACP, resulted in high predictability of cut depth and uniformity of graft thickness without endothelial cell damage [18]. This study partially supports our data; i.e. a high (200 mmHg) ICP established and maintained by the ACP leads to a faster central corneal thinning rate, and does not damage the endothelial cells significantly,

shown in both our laboratory study and clinical follow-ups. Thus, incorporating the ACP control unit helps in standardised preparation of a uniform, reliable and reproducible UT DSAEK graft.

Author contribution

1. Substantial contributions to:
 - a) Conception and design—AR, LA, LD, DP, VR
 - b) Acquisition of data—AR, MP, GW, LA, HL
 - c) Analysis—AR, MP, GW, LA, SF, HL
 - d) Interpretation of data—AR, MP, GW, LA, LD, HL, DP, SF, VR
2. Drafting the article or revising it critically for important intellectual content—AR, MP, GW, LA, LD, HL, DP, SF, VR
3. Final approval of the version to be published—AR, MP, GW, LA, LD, HL, DP, SF, VR

Data availability All the raw data are available in the FBOV repository.

Declarations

Consent to participate For laboratory study, consent from the donor's next-of-kin was obtained for the tissues to be used for research. For clinical study, consent from the patient was obtained before surgery.

Competing interests The authors declare no competing interests.

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