Single-Pass Dissection of Ultrathin Organ-Cultured Endothelial Lamellae Using an Innovative Microkeratome System

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Purpose: To determine the feasibility, dissection accuracy, and endothelial viability of ultrathin endothelial lamellae harvested from organ-cultured corneas using a single-pass with an innovative motor-driven linear microkeratome system.

Methods: Forty-eight (n = 48) paired organ-cultured human corneas were randomly assigned to dissection (study eyes, n = 24) with fellow eyes serving as the control (fellow eyes, n = 24). After organ culture and deswelling in a medium containing 6% dextran, endothelial lamellae with a target thickness ≤100 μm were dissected using a motor-driven linear microkeratome system (SLc, Gebauer, Neuhausen, Germany) equipped with 400-μm (n = 4), 450-μm (n = 10), 500-μm (n = 5), or 550-μm (n = 5) heads. Central corneal thickness (CCT) and posterior and anterior lamellar thicknesses were measured using ultrasound pachymetry (Pachette 3; DGH Technology Inc, PA) and anterior segment optical coherence tomography (Casia SS-1000; Tomey, Nagoya, Japan). Endothelial viability [endothelial cell density (ECD)] was measured using trypan vital staining.

Results: CCT measured 595 ± 66 μm (n = 48) on arrival, 846 ± 131 μm (n = 48) after organ culture, and 565 ± 58 μm (n = 48) after deturgescence. CCT did not differ between study and control eyes. Posterior lamellar thickness measured 88 ± 18 μm (n = 24) immediately after dissection, 126 ± 30 μm (n = 24) 1 hour after dissection, and 131 ± 41 μm (n = 24) 2.3 ± 0.6 days after dissection. ECD measured 2637 ± 264 cells per square millimeter (n = 48) on arrival, 2524 ± 232 cells per square millimeter (n = 48) after organ culture, 2493 ± 253 cells per square millimeter (n = 48) after dissection, and 2311 ± 218 cells per square millimeter (n = 48) 2.3 ± 0.6 days after dissection. ECD did not differ between study and control eyes at all time points.

Conclusions: Single-pass motor-driven linear microkeratome dissection provides an accurate and safe alternative for harvesting ultrathin endothelial lamellae from organ-cultured donor corneas.

Key Words: endothelial keratoplasty, ultrathin Descemet stripping automatic endothelial keratoplasty, eye banking, precut tissue, single-pass dissection

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Precut tissue for endothelial keratoplasty has replaced surgeon-cut tissue as the method of choice for donor preparation in the United States.[1,2] The main benefits of precut tissue include postcut endothelial assessment, avoiding intraoperative complications associated with donor tissue preparation, saving valuable operating theater time, and eliminating the costs associated with purchasing and maintaining a microkeratome system.[3–7]

Recent reports on faster and better visual recovery after transplantation of ultrathin (UT) grafts have led surgeons to request cornea banks to harvest UT endothelial grafts for intraoperative use.[8,9] However, dissection of UT grafts has been shown to result in increased endothelial cell damage and high rates of donor perforation.[10] In Europe and other parts of the world where organ culture is the prevailing preservation method, significant donor swelling up to twice the original thickness[11] makes harvesting UT grafts even more challenging.

The unpredictability of current microkeratome technology has been shown to be a function of both slit width and transition time.[12,13] Several methods have previously been suggested to overcome these obstacles, including double-pass mechanical dissection and femtosecond laser photodisruption.[14,15] However, these techniques may result in high rates of donor perforation, increased endothelial cell damage, stromal irregularities limiting visual recovery, and increased costs.[10,16,17]

In light of the unique challenges associated with harvesting UT lamellae from organ-cultured corneas, we set out to determine the accuracy and safety of single-pass dissection of UT lamellae using a novel motor-driven linear microkeratome system. A paired laboratory study was performed to provide a suitable control group. From each pair, 1 cornea was dissected while the fellow cornea served as the control. A series of measurements of these corneas was

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performed, including ultrasound pachymetry, optical coherence tomography, and endothelial cell viability analysis.

METHODS

Twenty-four pairs (n = 48) of human corneoscleral buttons unsuitable for transplantation were obtained from the Euro Cornea Bank, Beverwijk, the Netherlands. Consent for use for research in the event of unsuitability for transplantation was obtained in all cases in accordance with Dutch legislation and the tenets of the Declaration of Helsinki.

Donor tissue was preserved according to conventional eye bank techniques.[11] After a short hypothermic storage between recovery and arrival at the eye bank, corneoscleral buttons were dissected and stored in organ culture comprising minimum essential medium (Biowest, France) supplemented with 25 mM HEPES, 26 mM sodium bicarbonate, 5.5 mM glucose, 2 mM L-glutamine, 1 mM pyruvate, 2% (vol/vol) newborn calf serum, 10 IU/mL of penicillin, 0.1 mg/mL of streptomycin, and 0.25 μg/mL amphotericin at 31°C. To allow deturgescence, corneoscleral buttons were transferred to a transport medium supplemented with 6% dextran (Sigma Aldrich, MO) before and immediately after dissection. Paired donor corneas from the same donor were randomly assigned for dissection (study eye) while the fellow eye served as the control. A flowchart summarizing the study design is shown in Figure 1.

Study eyes (n = 24) were mounted on an artificial anterior chamber (SLc, Gebauer, Neuhausen, Germany) pressurized to 65 mm Hg using a minimum essential medium infusion raised to a height of 95 cm above the artificial anterior chamber and clamped 15 cm from the entrance to the chamber. The epithelium was removed before dissection, eliminating differences among corneas induced by variation in epithelial swelling. For lamellar dissection, the SLc microkeratome equipped with 4 gap widths: 400-μm (n = 4), 450-μm (n = 10), 500-μm (n = 5), or 550-μm (n = 5) heads was used with a target lamellar thickness ≤100 μm.

Central corneal thickness (CCT) was measured at 6 time points: (1) on arrival at the eye bank, (2) after organ culture, (3) after deswelling, (4) immediately after dissection (study eyes only), (5) 1 hour after dissection (study eyes only), and (6) 2.3 ± 0.6 days after dissection. Corneal thickness was measured using ultrasound pachymetry (Pachette 3, DGH Technology Inc). Before and immediately after dissection, thickness measurements were obtained using anterior segment optical coherence tomography (AS-OCT) (Casia SS-1000; Tomey, Nagoya, Japan).

Endothelial viability was evaluated using trypan blue vital staining to determine endothelial cell density (ECD) at 4 time points: (1) on arrival at the eye bank, (2) after organ culture, (3) immediately after dissection, and (4) 2.3 ± 0.6 days after dissection. Briefly, after swelling of the intercellular space using a hypotonic sucrose solution, trypan blue (0.25% wt/vol) was applied to detect the nuclei of nonviable cells. Evaluation was performed at a magnification of ×100 using the Gundersen method with a 10- × 10-calibrated reticule mounted on the microscope ocular lens, and ECD was expressed as the mean of 5 different counts, each performed in a different region.[18]

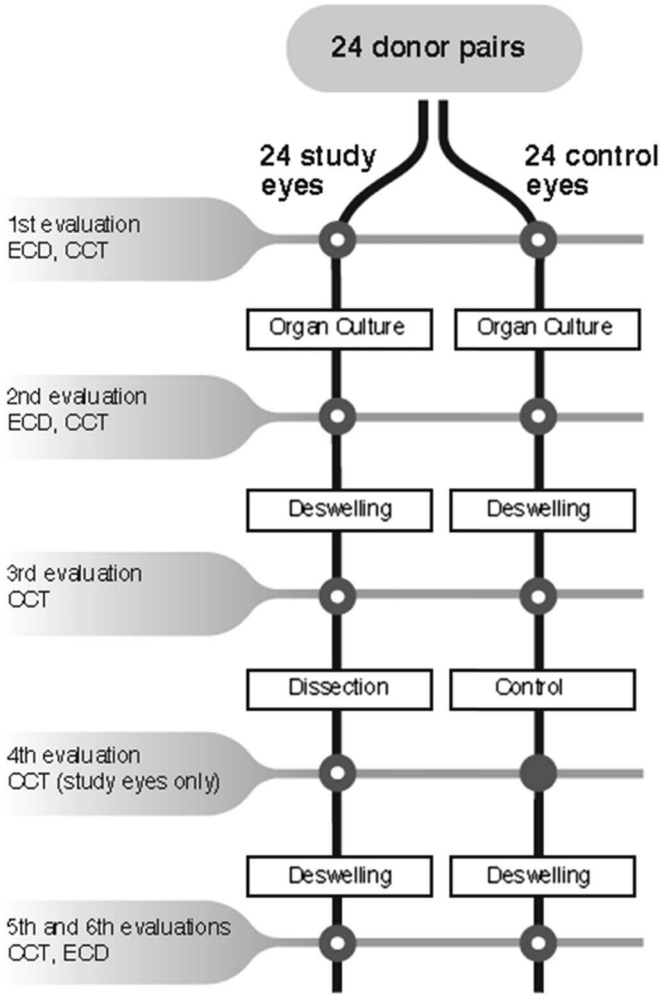

FIGURE 1. Flow diagram illustrating the study design. Twenty-four donor pairs (n = 48) underwent organ culture followed by deswelling in organ culture supplemented with 6% dextran. From each pair, 1 cornea was randomly assigned for dissection while the other served as the control. Measurements, including central corneal, anterior and posterior lamellar thickness (CCT), and ECD were performed on arrival at the bank (first evaluation), after organ culture (second evaluation), after deswelling (third evaluation), immediately after dissection (fourth evaluation), and 1 hour and 2.3 ± 0.6 days in the transport medium (fifth and sixth evaluations, respectively).

Sample size calculation based on previous data, indicating a mean difference of 99 ± 179 cells per square millimeter between premicrokeratome and postmicrokeratome dissection,[19] yielded a sample size of 48 corneas (24 pairs) to allow detection of a mean difference of 100 cells per square millimeter with 80% power and 0.05 probability of a type I error.

Statistical analysis was performed using SPSS for Windows (version 20.0; SPSS Inc, Chicago, IL). To quantify changes in CCT and ECD in time, a linear mixed-model

analysis was performed with CCT and ECD as dependent variables, donor as grouping factor, and study group as covariate. For all statistical tests performed, statistical significance was set at 0.05.

RESULTS

Baseline Donor Characteristics and Storage Intervals

Donor age averaged 72 ± 8 (58–78) years with a 3:1 male:female ratio. The mean interval between death and recovery and between recovery and organ culture measured 12 ± 6.3 hours and 12 ± 8.0 hours, respectively. Mean CCT and ECD on arrival at the bank did not differ between study and control eyes and measured 595 ± 66 μm (n = 48) and 2637 ± 264 cells per square millimeter (n = 48), respectively. Mean storage in organ culture and transport medium measured 23 ± 8.5 days and 26 ± 6.3 hours, respectively, with a mean postdissection storage interval of 2.3 ± 0.6 days (Table 1).

Central Corneal Thickness

Mean CCT did not differ between study and control eyes from the same donor on arrival and after organ culture and deswelling. Compared with CCT on arrival (595 ± 66 μm, n = 48), corneas swelled significantly during organ culture (846 ± 131 μm, n = 48) ($P < 0.001$), followed by significant deswelling (565 ± 58, n = 48) ($P < 0.001$) after storage in the transport medium (Fig. 2).

Multivariate analysis demonstrated a significant relationship between CCT and the number of days in organ culture ($\beta = 10$, $P < 0.001$) and hours in the deswelling medium ($\beta = -9$, $P < 0.001$). None of the other factors examined was found to predict corneal thickness in either media: death to recovery time, recovery to storage time, CCT on arrival, ECD on arrival, and donor age and sex.

Posterior and Anterior Lamellar Thickness

Mechanical dissection was successful in all cases. Mean posterior lamellar thickness measured 88 ± 18 μm (n = 24), significantly thinner than 100 μm ($P = 0.003$). AS-OCT images taken immediately before and after dissection of an UT lamella are shown in Figure 3. One hour after dissection, posterior lamellar thickness increased significantly to 126 ± 30 μm ($P < 0.001$), remaining stable during 2.3 ± 0.6 days in the transport medium supplemented with 6% dextran (Fig. 2). Dissection accuracy did not differ between the 4 gap widths examined (Table 2). Anterior lamellar thickness measured 476 ± 54 μm (n = 24) immediately after dissection, increasing significantly to 551 ± 67 μm ($P < 0.001$) 1 hour after dissection, and 605 ± 70 μm ($P < 0.001$) after 2.3 ± 0.6 days in the transport medium supplemented with 6% dextran (Fig. 2).

TABLE 1. Mean (±SD) Storage Intervals of Study and Control Eyes

	Storage Medium (Temperature)	Mean ± SD
Death to recovery	Room temperature	12 ± 6.3 h
Recovery to preservation	Ice (4°C)	12 ± 8.0 h
Organ culture preservation	Organ culture (31°C)	23 ± 8.5 d
Deswelling	Organ culture + 6% dextran (31°C)	26 ± 6.3 h
Dissection to last evaluation	Organ culture + 6% dextran (31°C)	2.3 ± 0.6 d

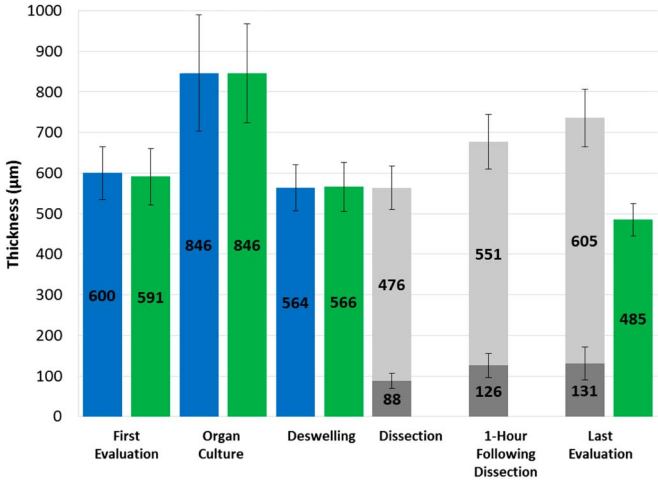

FIGURE 2. Mean (±SD) central corneal, posterior (dark gray), and anterior (light gray) lamellar thicknesses of study eyes (blue) and paired nondissected control eyes (green).

Endothelial Cell Density

Mean ECD (cells/mm^2) is given in Table 3. There was no difference in ECD between study and fellow eyes at all measured time points. Compared with ECD on arrival, ECD decreased significantly during organ culture ($P < 0.001$). Lamellar dissection was not associated with ECD loss, either immediately or 2.3 ± 0.6 days after dissection, compared with paired controls from the same donor. Multivariate analysis showed a significant relationship between ECD decline during organ culture and both number of days in organ culture ($\beta = -5$, $P < 0.001$) and number of hours from death to recovery ($\beta = -0.5$, $P < 0.001$). None of the other factors examined were found to predict ECD loss in either media: recovery to storage interval, ECD on arrival, and donor age and sex.

DISCUSSION

In the United States, precut tissue has replaced surgeon-cut tissue as the method of choice for donor preparation for Descemet stripping automated endothelial keratoplasty.[1,2] Reports on better visual outcomes using thinner donor tissues have led many surgeons to request cornea banks harvest UT grafts for their surgeries.[8,9] However, harvesting such grafts from edematous donor corneas remains a challenging procedure, particularly in Europe and other parts of the world where organ culture preservation is used.

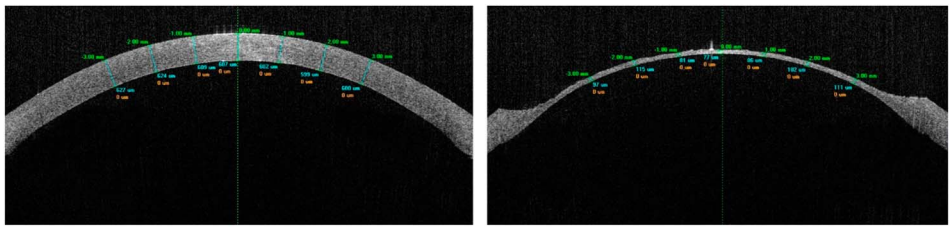

FIGURE 3. Spectral domain AS-OCT (SS-1000 Casia; Tomey) image of a 77-μm thick endothelial lamella (right) harvested in a single-pass from a 607-μm thick donor cornea (left) using a motor-driven linear microkeratome (SLc, Gebauer Medizintechnik GmbH) equipped with a 550-μm head.

The current laboratory study sets out to evaluate the feasibility, dissection accuracy, and endothelial viability associated with single-pass dissection of UT lamellae using a novel motor-driven linear microkeratome system. A paired donor design was chosen to provide an appropriate control group. Serial measurements of these corneas, including ultrasound pachymetry, AS-OCT, and endothelial cell analysis were performed, providing useful information for timing dissection and choosing a suitable microkeratome head, which will result in UT lamellae without donor perforation.

Under normal physiological conditions, fluid driven into the corneal stroma by imbibition and swelling pressures is counterbalanced by active ion transport.[20,21] However, organ culture results in significant swelling and loss of transparency of the donor cornea due to diminished endothelial and epithelial pump function and loss of intraocular pressure.[11] In our study, corneas reached a mean thickness of 846 ± 131 μm. This increase in corneal thickness was strongly related to the number of days in the culture with paired eyes from the same donor swelling to a similar extent. Swelling was however not related to other factors recorded during conventional eye banking such as death to recovery time, recovery to storage time, initial CCT, ECD on arrival, and donor age and sex.

Swelling of donor corneas during organ culture requires deswelling with a hyperosmolar medium (ie, organ culture enriched with 6% dextran) exerting colloid osmotic pressure to extract excess fluid accumulated in the stroma before dissection and transplantation. In our study, a complete reversal of donor swelling was achieved using dextran in a time-dependent fashion in both experimental and control eyes from the same donor. However, this should be weighed against the known toxicity of dextran to endothelial cells and keratocytes.[22–25]

Harvesting UT lamellae from edematous organ culture corneas is complicated by the SD of mechanical microkeratomes, particularly when using heads with wider gap widths.[13] Gradual corneal applanation with nonlinear microkeratomes requires application of increasing force resulting in deviation from the indented dissection depth. Double-pass dissection has been suggested to overcome this obstacle.[14] However, this technique has been shown to result in a high rate of donor perforation, endothelial cell damage, and a smaller graft diameter.[10]

Our study demonstrates that single-pass dissection using a motor-driven linear microkeratome system provides an accurate and safe alternative for harvesting UT lamellae. Dissection accuracy in our study can be explained by the design of the cutting head of the linear microkeratome, which applanates the entire cornea before dissection. In addition, motor-driven dissection standardizes the dissection time and speed, eliminating variability inherent to manual dissection.[12] Indeed, dissection accuracy in our study was independent of the microkeratome gap width and donor thickness, and no perforations occurred despite a mean lamellar thickness of 88 ± 18 μm.

The significant swelling of UT lamellae within an hour of dissection in our study is consistent with previous studies that showed that corneal swelling is depth dependent, occurring mostly in the posterior stroma, which has been attributed to different glycosaminoglycan composition and less interweaving of corneal lamellae in the posterior stroma.[26] Although extreme swelling could potentially result in endothelial trauma during graft insertion, moderate swelling has the potential to facilitate surgical handling. Further stabilization of graft thickness during storage affords flexibility in planning the operation but should be weighed against the toxicity of dextran.

One of the major challenges in assessing whether UT tissue is in fact better is the inconsistency on whether graft thickness is being measured preoperatively or postoperatively. Furthermore, correlation between preoperative and postoperative pachymetry, at least on the scale that differentiates average versus UT tissues, has not been well established. In our study, postdissection posterior lamellar thickness positively correlated with posterior lamellar thickness measured 3 days after storage in organ culture on a tissue-by-tissue basis ($r = 0.6$, $P < 0.05$). Further studies are needed to assess this relationship in vivo and its relevance to transplant outcomes.

Compared with posterior lamellae, anterior lamellae swelled to a lesser extent in our study. This may be ascribed to several factors, including the barrier function of the Bowman layer, anterior stromal architecture and glycosaminoglycan composition, medium hyperosmolarity, and the action of the endothelial pump. When properly repositioned after dissection, the anterior lamellar cap has been shown to

TABLE 2. Cutting Depth (in Microns) With the Gebauer SLc Microkeratome System Using 4 Different Slit Widths

	400-μm SLc (n = 4)	450-μm SLc (n = 10)	500-μm SLc (n = 5)	550-μm SLc (n = 5)
Mean ± SD	400 ± 24	461 ± 19	502 ± 31	542 ± 13
95% CI	362–439	432–491	452–552	521–562

CI, confidence interval.

TABLE 3. Mean (±SD) ECD (in Cells per Square Millimeter) of Study and Control Eyes

	Study Eyes (n = 24)	Fellow Eyes (n = 24)	P*
Arrival at the bank	2650 ± 277	2626 ± 256	0.6
After organ culture	2536 ± 234	2513 ± 234	0.7
After dissection	2514 ± 235	2474 ± 271	0.7
Last evaluation	2300 ± 209	2322 ± 230	0.6

*Paired *t*-tests were used to compare means between study and control eyes.

reduce stromal swelling, prevent Descemet membrane detachment, and minimize endothelial cell damage.[27] The similarity in endothelial viability between study and control eyes after storage after dissection in our study supports a protective role for the anterior cap.

Given the substantial perioperative endothelial cell loss, most eye banks select donors with the highest ECD for endothelial keratoplasty. Similar to previous studies, endothelial cell loss in our study was time dependent, favoring a short storage interval.[18] Our results also suggest that longer recovery times increase endothelial cell loss during organ culture. Interestingly, none of the other factors recorded during conventional eye banking was found to influence endothelial cell loss. These findings should be verified in future studies with a larger sample size.

Importantly, this study showed that single-pass using a motor-driven linear microkeratome is not associated with endothelial cell loss, compared with paired nondissected controls, either directly or several days after dissection. Although the small observed difference (50 cells/mm^2) in postdissection ECD limits the power of our study, the clinical significance of such a difference is questionable. Previous studies showed no relationship between donor and posttransplantation ECD across a larger range of donor endothelial counts.[28,29]

In conclusion, this study demonstrated the accuracy and safety of a novel motor-driven linear microkeratome system for harvesting UT lamellae. Further studies are needed to determine the clinical outcomes of endothelial keratoplasty performed with single-pass cut UT endothelial lamellae.

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