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Eccentric Lamellar Keratolimbal Grafts Harvested with a Manually Guided Microkeratome

Technical Report

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Key Words
Limbus · Stem cells · Transplantation · Lamellar keratoplasty · Microkeratome · Artificial anterior chamber

Abstract
Background: To perform lamellar keratolimbal allograft transplantation in a one-step procedure with a single graft, we investigated the feasibility of harvesting eccentric lamellar keratolimbal grafts from conventionally processed corneoscleral buttons using a manually guided microkeratome in conjunction with an artificial anterior chamber system. ***Methods:*** We used the Moria LSK-One microkeratome and the automated lamellar therapeutic keratoplasty (ALTK) system (Antony, France). Ten human donor eyes were used to obtain single-piece lamellar keratolimbal grafts. Specimens were processed for light and electron microscopy. ***Results:*** Eccentric keratolimbal grafts could be obtained from all human donor buttons. Grafts include a crescent-shaped limbal and a large corneal portion. No visible damage to the limbal region was discernible. ***Conclusion:*** Our data show that the LSK-One microkeratome in conjunction with the ALTK system allows harvesting eccentric keratolimbal grafts from donor corneoscleral buttons.

Limbal stem cells are the ultimate source of corneal epithelial regeneration and are exclusively located at the corneal-conjunctival junction, i.e. the limbus [1–6]. Destruction of the limbal epithelium leads to conjunctival overgrowth (conjunctivalization) on the clear cornea, a condition termed limbal stem cell deficiency (LSCD) [7–11]. The conventional therapy in this situation is transplantation of healthy limbal epithelium to ensure a transparent and smooth corneal surface [12–16]. In situations where corneal scarring requires additional lamellar corneal transplantation, Sundmacher and Reinhard [17] proposed a technique called lamellar central limbokeratoplasty.

Freehand donor limbal tissue harvest is time consuming, creates a relatively irregular stromal interface and requires some experience to prevent traumatic alteration

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Fig. 1. A human corneoscleral donor button is eccentrically placed on the artificial chamber. Note the crescent-shaped limbal area (white tissue on the left side, black asterisks). The black arrow shows the direction of the microkeratome advancement.

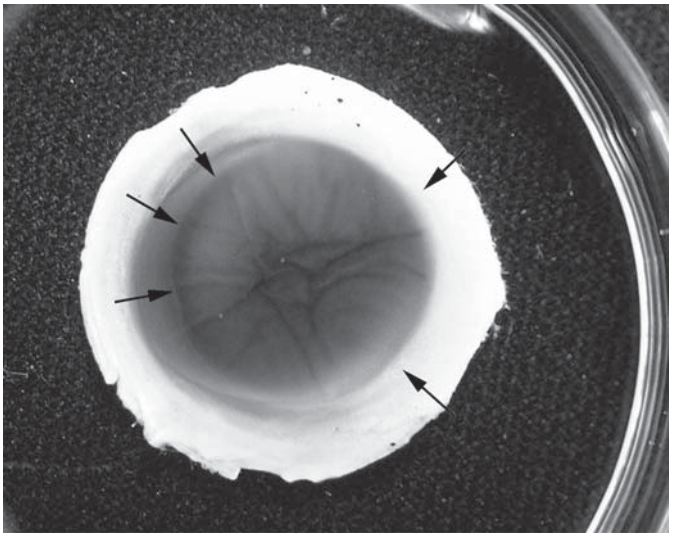

Fig. 3. The human donor button shows an eccentric donor bed which clearly includes the optical axis (black arrows left).

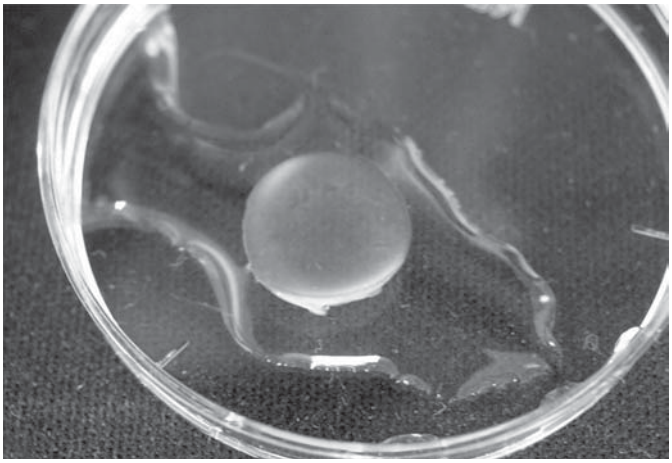

Fig. 2. The obtained human lamellar graft comprises a large clear corneal portion and a crescent-shaped limbal area measuring 3–4 clock hours.

of the delicate stem cell containing limbal epithelium and adjacent stroma. Therefore, the feasibility of using a microkeratome to harvest limbal tissue for ocular surface transplantation has been investigated in the past [18–20]. However, all investigators used whole donor globes. Since most of the donor corneas in modern eye banks are stored as corneoscleral buttons under either cold storage or tissue culture conditions, we believe it is important to study the option of harvesting limbal tissue from such corneoscleral buttons using a manually guided microkeratome and an anterior chamber device.

In the following study, we present a modified approach of harvesting eccentric lamellar keratolimbal grafts from donor corneoscleral buttons using a manually guided microkeratome in conjunction with an artificial anterior chamber system to circumvent some of the technical and conceptional problems encountered in the past.

Ten human donor corneoscleral buttons not suitable for transplantation were kindly provided by the eye bank of the Ludwig Maximilians University, Munich, Germany. Corneoscleral buttons were routinely stored in organ culture at 37°C. The mean culture duration was 18 ± 5 days; the mean age was 46.6 ± 10 years.

For the presented study, we used a manually guided microkeratome (Moria LSK-One; Moria, Antony, France), and an artificial anterior chamber system (ALTK; Moria). Both instruments, which are routinely used for standard lamellar keratoplasty and LASIK procedures, did not undergo any kind of modification to perform our experiments. The adjusting ring was locked in the lowest position and no stop ring was used. A precalibrated microkeratome head of 350 μm was used to create eccentric lamellar keratolimbal grafts. The standard oscillation

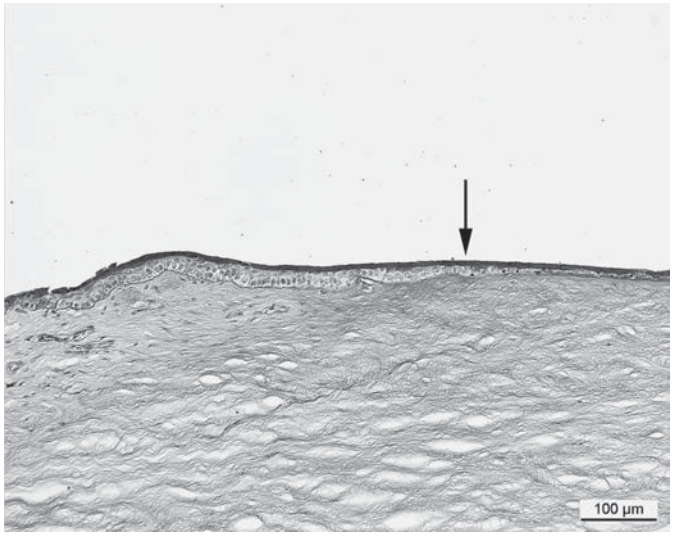

Fig. 4. PAS staining of the limbal region. Note the absence of Bowman's layer and the loose stromal texture compared to the compact corneal stroma. The black arrow marks the limbal area. On the left, the limbal epithelium shows a higher stratification compared to the corneal epithelium. The basal epithelial layers show typical undulations.

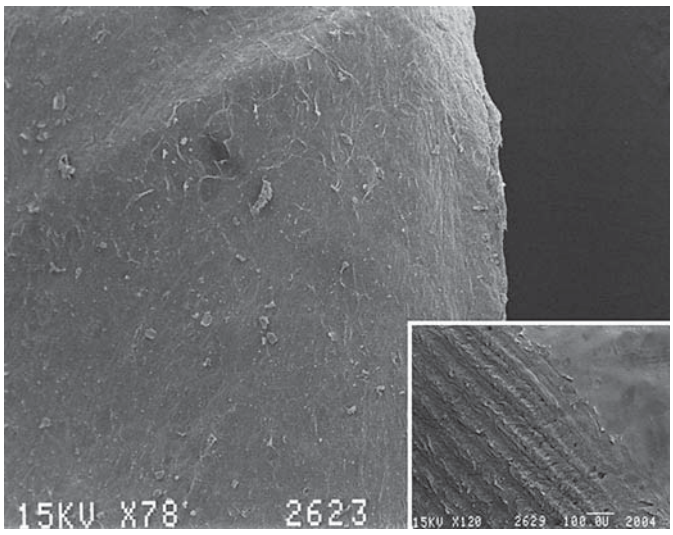

Fig. 5. Electron microscopy of the stromal plane of the obtained graft shows a smooth cutting plane. The edge corresponds to the limbal portion of the graft and does not show scatter lines compared to the central corneal portion (insert). Scatter lines represent contusion forces at the area where the microkeratome enters the tissue.

rate was 15,000 rpm. The pressure of the anterior chamber was set at 60 mm Hg using a height-adjustable infusion bottle. Pressure was controlled by tonometry (Barraquer tonometer). We started the microkeratome pass at the center of the cornea to minimize excessive tissue contusion at the limbus when the blade enters the tissue. Specimens were further processed for light and electron microscopy.

The ALTK system in conjunction with the Moria LSK-One microkeratome allowed reproducible preparation of eccentric lamellar keratolimbal grafts. In all performed cases, eccentric positioning of the corneoscleral buttons on the artificial anterior chamber was feasible when a minimum of 3 mm scleral rim was present (fig. 1). In all 10 human samples, the macroscopically discernible limbal area measured 3–4 clock hours (fig. 2). The average horizontal diameter was 11.0 ± 0.5 mm. The average vertical diameter was 10.5 ± 0.5 mm as measured with a Castroviejo caliper. The donor bed of the corneoscleral button shows that the optical axis of the cornea is well within the excised graft (black arrows; fig. 3).

PAS-stained sections (fig. 4) showed a large corneal portion with a two-layered corneal epithelium, Bowman's layer and a compact corneal stroma. The number of epithelial layers increased towards the limbal region (fig. 4, black arrow). Anatomically the limbal region is characterized by the absence of Bowman's layer, a loose subepithelial stroma and a more undulated basal epithelium. No damage of the epithelial and underlying stromal structures potentially caused by the preparation was noticed. Scanning electron microscopy of the obtained human disks showed a smooth stromal plane (fig. 5).

Our approach of creating a large clear corneal lamellar graft with a crescent-shaped limbal portion allows us to cover the optical axis of the recipient and place the limbal donor tissue on limbal recipient stroma at the same time. Hypothetically this appears to be crucial for proper limbal stem cell survival. Such large-diameter grafts can be obtained using the presented technique. Because we know that the function of epithelial stem cells is substantially modulated by its surrounding stromal environment, i.e. the stem cell niche [21–25], we believe it is important to place limbal transplants to their original location, i.e. the conjunctival-corneal junction and not to the midperipheral clear cornea as proposed by Reinhard et al. [26], Sundmacher and Reinhard [27] and Sundmacher et al. [28]. A potential downside of this technique is the fact that we place the limbal tissue in a vascularized bed with a high-

er risk of immunoreactions. With our technique, only 3–4 clock hours of limbal tissue can be obtained, therefore transplants can only be used for partial LSCD with superficial scarring.

We were able to show excellent morphological preservation of the limbal corneal transition zone as well as a smooth cutting plane for optimal graft/donor apposition. Improvement of graft/donor tissue apposition might control some of the problems encountered by Shimmura et al. [29] who transplanted 360° lamellar keratolimbal grafts in patients with total LSCD and scarring of the anterior corneal stroma. In a high percentage of cases, they noticed vascularization of the interface between the donor and recipient tissue causing ultimate graft failure.

Another major advantage of using the ALTK system for limbal graft preparation is the fact that we used corneoscleral donor buttons as provided by modern eye banks to ensure proper tissue quality and serological testing and not whole donor globes. However, for any kind of keratolimbal transplantation the providing eye bank should be notified prior to sending out the cornea to avoid excessive resection of conjunctival tissue.

Based on our results, further investigations should be made to prepare the recipient bed in the same way using a large suction ring (H ring) of the Moria system to allow optimal donor graft/recipient bed apposition. One could speculate that this proceeding may prevent some of the complications described by Shimmura et al. [29]. Regarding apposition of donor and recipient tissue it has to be taken into account that disks obtained by mechanical microkeratomes show differences in thickness depending on the area of the disk. The edges are thinner than the mid portion of the disk. This will result in a more diagonal cutting plane in the center of the cornea but should not affect the apposition between donor and recipient. The optical quality of such a diagonal cutting plane compared to a more parallel LASIK flap might be compromised. However, patients undergoing eccentric keratolimbal transplantation do not have the same expectations regarding the quality of visual rehabilitation as patients undergoing refractive surgery.

Our study provides baseline information for further studies on microkeratome-assisted eccentric keratolimbal transplantation for partial LSCD with superficial stromal scarring using an artificial chamber system.

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