Microkeratome Versus Femtosecond Laser Predissection of Corneal Grafts for Anterior and Posterior Lamellar Keratoplasty

Olan Suwan-apichon, MD,† Johann M. G. Reyes, MD,* Neil B. Griffin, MD,‡ Jerry Barker, BS,‡ Patrick Gore, RN,§ and Roy S. Chuck, MD, PhD**

Purpose: To compare 2 different techniques for predissection of human anterior and posterior lamellar corneal grafts for eye bank storage.

Methods: A mechanical microkeratome (group 1, N = 5) and a femtosecond laser (group 2, N = 5) were used to dissect intended 350-μm-deep lamellar planes in deepithelialized donor corneas mounted on an artificial anterior chamber. These corneas were replaced in Optisol GS at 4°C postoperatively and examined 2 days later to simulate a clinical scenario. Ultrasonic pachymetry of corneal lamellar sections was measured before and after separation of the lamellar grafts. Group 1 sections were separated by the mechanical microkeratome, whereas group 2 sections were manually separated 2 days after laser dissection. Endothelial cell viability was evaluated in posterior grafts.

Results: Total corneal thicknesses immediately before dissection were 559 ± 61 (group 1) and 578 ± 79 μm (group 2; $P = 0.46$). Immediate postdissection anterior and posterior graft thicknesses were 361 ± 68 and 203 ± 74 μm (group 1), respectively. Achieved anterior and posterior graft thicknesses 2 days later were 282 ± 44 and 413 ± 35 μm (group 1) and 324 ± 112 and 397 ± 51 μm (group 2), respectively. Percentage of devitalized endothelial cells were 3.4% ± 1.6% (group 1) and 1.6% ± 1.2% (group 2; $P = 0.35$).

Conclusions: Centralized predissection by both techniques, cold storage, and shipping by airmail results in viable grafts without significant endothelial cell loss 2 days later.

Key Words: lamellar, cornea, transplant, anterior, posterior, endothelium, microkeratome, femtosecond, laser

(*Cornea* 2006;25:966–968)

Received for publication October 18, 2005; revision received March 14, 2006; accepted for publication March 25, 2006.

From the *Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore, MD; †Department of Ophthalmology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; ‡Carolina Eye Associates, Albemarle, NC; and §Central Florida Lions Eye and Tissue Bank, Tampa, FL.

Supported by NIH Grant EY000412-04, Stark-Mosher Center for Cataract and Corneal Diseases, Research to Prevent Blindness.

The authors state that they have no proprietary interest in the products named in this article.

Reprints: Roy S. Chuck, MD, PhD, Wilmer Ophthalmological Institute, Johns Hopkins University, 255 Woods Building, 600 North Wolfe Street, Baltimore, MD 21287-9278 (e-mail: rchuck1@jhmi.edu).

Although full-thickness, penetrating keratoplasty is by far the most commonly performed type of corneal graft surgery performed today, there has been renewed interest in developing new procedures for both anterior[1,2] and posterior lamellar keratoplasty.[3–7] Recent efforts by our laboratory have focused on developing both mechanical[1,2,8–15] and femtosecond laser[12,16] based techniques for lamellar grafting.

Although there are many potential tectonic and refractive advantages to the application of modern microkeratome technology to the reemerging field of lamellar corneal transplantation, the instrumentation is often cost prohibitive to maintain in many surgical facilities and may also require a new skill set to operate. To obviate these problems, we propose that corneal lamellar grafts could possibly be precut in a centralized facility and stored in an eye bank before distribution for clinical transplantation. Here we describe our preliminary results and provide proof of principle for this proposed eye banking scenario.

MATERIALS AND METHODS

Ten corneoscleral buttons stored in Optisol GS at 4°C and not suitable for clinical transplantation, but with cell counts greater than 2500 cells/mm^2 by specular microscopy, were obtained (Central Florida Lions Eye and Tissue Bank, Tampa, FL) and divided into 2 groups. In accordance with the manufacturer's instructions, after mounting the corneas on an artificial anterior chamber instructions (ALTK system; Moria, Antony, France), pressurized with syringe-filled Optisol GS to 65 mm Hg as measured by tonometry, ultrasonic pachymetry (AccuPach; Accutome, Malvern, PA) was used to measure the preoperative central corneal thickness after mechanical deepithelialization and after either mechanical microkeratome or femtosecond laser sectioning.

The mounted corneas were sectioned using either a mechanical microkeratome (LSK-1; Moria, Antony, France; group 1, N = 5) or a femtosecond laser (Intralase, Irvine, CA; group 2, N = 5), both set for 350-μm lamellar depth from the anterior deepithelialized surface. Group 1 sections were separated by the mechanical microkeratome, whereas group 2 sections were manually separated 2 days after laser dissection. After this processing step, to simulate an eye bank storage scenario, each cornea was replaced in its original eye bank Optisol-filled storage container and subjected to cold storage while being shipped by express air courier service from North Carolina to Maryland. Two days later, the cut corneas were

TABLE 1. Total and Lamellar Corneal Thicknesses on Days 0 and 2 After Microkeratome Dissection (Mean ± SD, μm)

Microkeratome	Day 0	Day 2	*P* (Day 0 vs. 2)
Mechanical (N = 5)			
Total	559 ± 61		
Anterior	361 ± 68	282 ± 44	0.043
Posterior	203 ± 74	413 ± 35	0.043
Laser (N = 5)			
Total	578 ± 79		
Anterior		324 ± 112	
Posterior		397 ± 51	

brought to room temperature, the anterior and posterior grafts were separated, and each section was remeasured by ultrasound pachymetry while mounted on the artificial anterior chamber, bathing both surfaces in Optisol.

Endothelial cell damage after graft harvest was assessed by staining with trypan blue 0.25% (Sigma-Aldrich, St. Louis, MO) and alizarin red S 0.2% (Sigma-Aldrich).[17]

RESULTS

Total corneal thicknesses immediately after deepithelialization and before microkeratome dissection were 559 ± 61 (group 1) and 578 ± 79 μm (group 2; $P = 0.46$). Immediately after dissection, anterior and posterior graft thicknesses were 361 ± 68 and 203 ± 74 μm (group 1), respectively. The group 2 anterior and posterior halves of the laser-dissected corneas were not separated until 2 days later. Achieved anterior and posterior graft thicknesses 2 days later after sectioning and cold storage in Optisol were 282 ± 44 and 413 ± 35 μm (group 1) and 324 ± 112 and 397 ± 51 μm (group 2), respectively (anterior sections, $P = 0.92$; posterior sections, $P = 0.46$). The pachymetry data are summarized in Table 1. Percentages of devitalized endothelial cells on day 2 were 3.4% ± 1.6% (group 1) and 1.6% ± 1.2% (group 2; $P = 0.35$; Fig. 1).

DISCUSSION

Here we describe techniques for predissection of donor tissue for anterior and posterior lamellar keratoplasty in which corneoscleral sections are mounted onto an artificial anterior chamber and stromal dissection is accomplished using either a mechanical microkeratome or femtosecond laser. In this study, after 2-day storage in cold Optisol, the endothelial cell loss associated with the predissections seemed low and comparable to that previously described immediately after dissection.[3,7] In our experimental design, we chose to not separate the laser-cut sections until day 2 (>48 hours later), to reduce the exposure time of the stromal surfaces to media to limit induced swelling. However, the laser device did not show any significant difference from the steel blade instrument in terms of accuracy and reproducibility after the 2-day postdissection storage period. Although the laser cut is known to have a finer precision and accuracy than the mechanical microkeratome,[16,18] after 2 days of postdissection swelling in Optisol media, the difference disappeared.

On closer examination of the observed changes in lamellar thickness of the mechanical microkeratome–cut sections on day 2, there appeared to be significant thinning of the anterior lamellar sections ($P = 0.043$). In contrast, there was also significant swelling of the posterior sections ($P = 0.043$), despite reapposition of the anterior and posterior sections while in storage. However, these findings are in agreement with previous reports of human corneal swelling in Optisol GS corneal storage media. Namely, it has previously been reported that removal of the epithelium before storage results in increased stromal hydration.[19] Also, it is known that, although hydration of the stroma increases during swelling through the posterior surface, it paradoxically decreases during swelling through the anterior surface, in agreement with our own experimental findings.[20]

Because we did not separate the laser-cut lamellar sections immediately after the procedure, we could not compare the swelling at day 2 to that at day 0. However, average total corneal thickness did increase similarly by the end of the study period, using both instruments (mechanical microkeratome [group 1], 559 to 695 μm; laser [group 2], 578 to 721 μm).

Although it seems that the femtosecond laser offers results comparable to those of its mechanical microkeratome counterpart, there still exist concerns that will need to be addressed before its clinical implementation for lamellar keratoplasty. First, despite centralization of the unit for eye

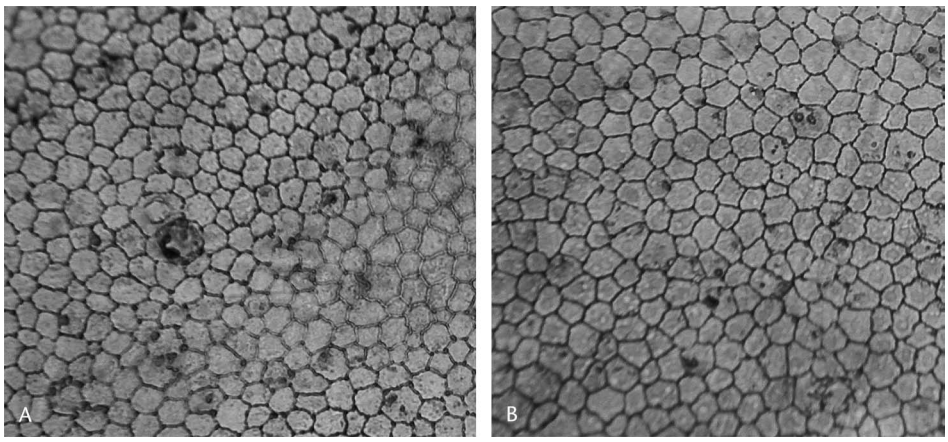

FIGURE 1. Vital staining of the endothelium reveals low cell loss in both techniques: (A) mechanical microkeratome and (B) femtosecond laser.

banking, it is still significantly more costly to both purchase and maintain than the classic microkeratome. Second, it has been shown recently that, in current form, the Intralase femtosecond laser produces posterior grafts that contain imperfect ridges in the periphery of the produced sections.[21] However, these same authors state that new hardware and software are currently in development to overcome this problem.

Despite these concerns, however, we are optimistic about eye bank implementation of the femtosecond laser. The posterior corneal sections obtained here were achieved using close to the deepest settings available with both instruments. In some cases, the thickness achieved after 2 days of cold storage may be more than is desired. Using hardware modifications currently in development,[17] the femtosecond laser may be able to cut much thinner posterior grafts more accurately and reproducibly than its mechanical microkeratome counterpart.[1,10]

Using modern microkeratome technology, we propose that, at the eye bank level, one donor cornea may be divided into at least 2 transplantable lamellar grafts. Here we showed that centralized predissection, cold storage, and shipping by airmail results in viable grafts without significant endothelial cell loss 2 days later.

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