# LONG-TERM EVALUATION OF CORNEAL ENDOTHELIAL CELL TRANSPLANTATION*

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**ABSTRACT**

*Purpose:* This report describes the clinical course, refractive changes, confocal microscopic and histological evaluation of corneal endothelial cell transplantation in rabbits with long-term follow-up.

*Methods:* Transplantation of corneal endothelial cells using a cell/carrier device was performed in 19 rabbits. Clinical evaluation between 1-25 months included slit-lamp examination, keratometry, retinoscopy and surface topography. Two grafts in rabbits with 12 and 24 month survivals were evaluated in vivo by 3D tandem scanning confocal microscopy. The same grafts were then processed for transmission electron microscopy. BrdU labeling of the grafted cells in one transplant was performed in order to distinguish between host and grafted endothelial cells.

*Results:* All grafts cleared and remained clear for an average of one year without signs of rejection or inflammation. Postoperative refraction data and topography of the transplants showed progressive development of myopia and steep corneas compared to the unoperated eyes in each case. Confocal microscopy *in vivo* demonstrated a regular hexagonal pattern of the transplanted endothelial cells and a thickened Descemet's membrane, which correlated with the light and electron microscopic findings. BrdU labeling of the grafted endothelial cells showed a homogenous labeling of cell nuclei 6 months after the transplantation.

*Conclusions:* This study demonstrates that corneal endothelial cells grown on a biomaterial can be replaced and remain functional for a long period of time.

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## INTRODUCTION

Replacement of the endothelial monolayer without full-thickness corneal transplantation has been a long-term goal in ophthalmology.[1,2] Such a replacement procedure uses cultured corneal endothelial cells grown on a carrier that is capable of maintaining the fluid-pump system across the endothelium. The advantages of such a replacement procedure would be the elimination of any immunogenic responses from donor stromal and epithelial antigens and the ability to use only cultured cells with well-characterized cell-surface antigens, which may minimize the possibility of graft rejection. Since the number of available intact donor corneas may become limited in the very near future owing to the increase in popular refractive surgeries, it will be important to develop such an alternative replacement procedure.

In the past, we have shown that corneal endothelial cells seeded onto a coated hydrogel carrier can be successfully transplanted into rabbits and cats for an average of 40 days.[3] This manuscript reports the clinical and laboratory evaluation of grafted rabbit endothelial cells with long-term survival rates.

## METHODS

### CELL CULTURE

Cell cultures of New Zealand white rabbit corneal endothelial cells were obtained by a modification of the methods previously described.[3] Descemet's membrane with endothelium attached was peeled off and incubated in EDTA-Trypsin (0.05% trypsin and 0.35 mM EDTA 4 Na) (GIBCO BRL, Grand Island, NY) for 20 minutes. After centrifugation at 200 g, endothelial cells were resuspended in Medium 199 containing 10% fetal bovine serum, 100 U/mL streptomycin, 50 µg/mL gentamycin, and 0.25 µg/mL amphotericin B (GIBCO BRL, Grand Island, NY) in a 24-well cell culture cluster (COSTAR) in a cell density of 4 x $10^5$/mL. Cells reached confluency in 5 to 7 days. After trypsinization, subcultured endothelial cells were seeded onto the coated hydrogel lenses with a cell density of 5 x $10^5$. Culture medium was changed twice a week. Corneal endothelial cells established a complete monolayer on the coated surface of the hydrogel lens by 7 days with a final cell density of 3,000 cells/$mm^2$.

### CELL CARRIERS

Ten hydrogel lenses from Chiron Ophthalmics and 9 hydrogel soft contact lenses (Lidofilcon A and B) commercially available from Bausch & Lomb (B & L) were used for transplantation. Three B & L lenses were used for the control surgeries and 7 additional B & L lenses were used for testing

cell viability and cell morphology. Parameters of the lenses were as follows: water content 70% to 80% and lens diameter 5.5 mm. Refractive powers of the carrier lenses were as follows: Chiron lenses 0 to -4.5 D (10 grafts), Lidofilcon A lenses -1.00 to -6.0 D (8 grafts), and Lidofilcon B lenses +10.00 D (1 graft). All the carrier lenses were coated on their concave surfaces with extracellular matrix proteins including fibronectin and poly-L-lysine and varied from 100 to 250 $\mu$m in thickness when hydrated.

#### ENDOTHELIAL CELL VIABILITY

The carriers with the cell suspension were maintained in tissue culture dishes (6 wells; Costar, Cambridge, Mass) for 7 days at 37°C in 95% air and 5% $C0_2$, 95% relative humidity. Endothelial cell viability was tested by using 1% Trypan-blue vital staining (GIBCO BRL) on confluent monolayers maintained in tissue culture conditions with 10% serum. The cells were counted in a Neubauer hemocytomer, and the ratio of viable (unstained) and nonviable (stained) cells was determined. Ninety-six percent of the cells were living after 1 week in culture. This number dropped to 63% after 21 days and was less than 20% after 28 days.

#### SURGICAL PROCEDURE

For this study, 19 New Zealand white rabbits were used for cell/carrier transplantations. The housing and care of the animals were in accordance with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research and with federal, state, and local regulations. The surgical procedure has been described earlier.[3] Briefly, to prepare animals for the transplantation procedure, the corneal endothelium was removed using the following procedure. Balanced salt solution (BSS, Alcon) was injected into the anterior chamber while a 1.5-mm corneal incision was made 1 mm from the limbus. The entire endothelium was then scraped off with a diamond-dusted Jensen capsule polisher, leaving an intact Descemet's membrane. No suture was used for the incision, and 1% prednisone eye drops (Inflamase Forte; IOLAB, Claremont, Calif) were applied twice a day to the operated eye for 1 week. Corneas became cloudy within 24 hours after surgery and remained cloudy throughout the whole time before transplant surgery. After endothelial cell scraping, animals were maintained for 5 weeks to ensure that no intraocular inflammation and no regeneration of host endothelium were present.

The transplantation procedure was performed as follows: A 7.5-mm trephine was used to cut one third of the depth of the stroma, and after a blunt lamellar dissection, the middle corneal stroma was removed. The remaining lamellar portion of the cornea was cut into the anterior chamber using a 5.0-mm trephine and discarded. The opened anterior cham-

ber was irrigated with 100 U/mL heparin, and 0.5 mL Healon (Pharmacia, Monrovia, Calif) was injected to maintain the anterior chamber. The carrier covered by a monolayer of endothelium was rinsed in BSS and inserted into the stromal pocket created by the first trephine cut. The outer corneal flap was sutured back into place using continuous 10/0 nylon (Alcon).

#### IMMUNOHISTOCHEMISTRY OF DONOR CELLS

To distinguish between host and donor cells, corneal endothelium was cultured and seeded onto lenses as already described, except that 10 μg/mL bromo-deoxyuridine (BrdU) SIGMA, St Louis) was added to the culture medium at a subconfluent stage of the culture for 3 days before transplantation. The BrdU-labeled cell-carrier was then transplanted as described. At postoperative day 180, the rabbit was sacrificed and the cornea was prepared for immunostaining as follows: The graft and host cornea was prepared as a flat mount preparation, fixed in 10% buffered formaldehyde for 30 minutes then washed in PBS. After hydrolyzing with 1 N HCI, and rising with 1 N NaOH, the specimen was incubated with a 1:100 dilution of anti BrdU monoclonal antibody (Becton Dickinson, San Jose, Calif) overnight at 4°C. FITC-conjugated antimouse antibody in a dilution of 1:25 was then applied for 2 hours at room temperature. The specimen was mounted in Vectashield medium and examined by epiflurescence microscopy.

#### POSTOPERATIVE CARE

All animals were treated by subconjunctival gentamicin (gentamicin sulfate, 0.2 mg/mL, Elkins-Sinn, Inc, Cherry Hill, NJ) and 8 mg subconjunctival methylprednisolone acetate suspension (DepoMedrol, 4 mg/mL, Upjohn, Kalamazoo, Mich) immediately after the surgery. Further treatment included 0.05% dexamethasone sodium phosphate ointment (Rugby Lab Inc, Rockville, NY) and 0.3% gentamicin sulfate eye drops (Genoptic, Allergan Pharmaceuticals) twice daily. Sutures were removed at day 15, and no more local or general immunosuppressant or other treatment was used thereafter. Between 1 and 12 months, keratometry (Bausch & Lomb), retinoscopy (Welch Allyn, Streak Retinoscope), and slit-lamp examination were performed on a weekly basis. Intraocular pressure was measured with TonoPen weekly from the first to the fourth month. Comparative dioptric maps of surface topography (TMS-1) were also used. Attempts were made to visualize the grafted endothelial cells by specular microscope and to measure the graft thickness by ultrasound pachymetry, but none of these instruments provided any reading or measurement, probably owing to the different physical properties of the carrier biomaterial.

### SCANNING CONFOCAL MICROSCOPY

Two transplanted animals with 1- and 2-year survival times were evaluated in vivo using confocal microscopy to visualize the grafted endothelial cells.

### LIGHT AND ELECTRON MICROSCOPY

Seventeen corneas from transplanted animals were fixed in 10% formalin for 24 hours and embedded in paraffin. Serial and step sections of the grafts were processed for hematoxylin-eosin (H & E), periodic acid–Schiff (PAS), and Masson trichrome staining. Two grafts with 1-year and 2-year follow-up (the same two grafts that were evaluated by confocal microscopy) were fixed in buffered 4% glutaraldehyde and prepared for transmission electron microscopy. Rhodamine-phalloidin (Molecular Probes Inc, Eugene, Ore) staining of the endothelial cells from the 2-year graft was also performed to label cytoskeletal actin.

## RESULTS

A complete summary of the observations for each lens type used in the grafted animals is included in Table 1.

### MORPHOLOGY OF ENDOTHELIAL CELLS ON THE CARRIER

Light microscopy of the rabbit corneal endothelial cells growing on the carrier before transplantation demonstrated a normal monolayer with a cell density of 3,000 cells/mm$^2$ (Fig 1).

### CLINICAL OBSERVATIONS

All grafts cleared within 5 days (Fig 2, A and B) and remained clear (Fig 2B) for an average of 362 days in the Chiron lens group (n=10) and an average of 279 days in the Bausch & Lomb group (n=9). The longest survival was 728 days. There were no clinical signs of infection or rejection any time after the surgery in any of the cases. Intraocular pressures were always normal (< 20 mm Hg). The host cornea remained cloudy around the edges of the graft until the eighth postoperative month, but later cleared.

Postoperative refractive data of the transplants showed progressive development of myopia (-5.50 to -8.50 D) and steep corneas, compared with the unoperated eyes (+1.00 to +1.50 D) in each case. While the surface topography of the unoperated corneas indicated curvatures of 47 to 48 D, the cell/carrier transplant produced very steep corneas (54.6 to 56.4. D, respectively). The steepening of the grafts had progressed with time and reached the 63 D, but did not increase after the eighth postoperative month. Progressive steepening of all grafts occurred independently of the

TABLE I.

| | LENSE TYPE: B&L or Chiron | LENGTH OF FOLLOW-UP Days | CLEAR GRAFT | IOP | RETINOSCOPY | | HISTOLOGY | ULCER |
|---|---|---|---|---|---|---|---|---|
| **EYE** | | | | | | | | |
| **1** #316 | Chiron #243-28-04 | 239 | Yes | NA | -5 | -8 | Damaged | Yes |
| **2** #551 | Chiron #243-28-01 | 267 | Yes | NL | -7 | -10 | NL endo | Yes |
| **3** #555 | Chiron #243-28-05 | 340 | Yes | NL | -10 | -15 | Damaged | Yes |
| **4** #118 | Chiron #243-28-04 | 370 | Yes | NL | -6 | -9 | NL endo | Yes |
| **5** #36 | Chiron col 3-16-92-10 | 370 | Yes | NA | -7 | -10 | NL endo | Yes |
| **6** #602 | Chiron col 3-16-92-3 | 189 | Yes | NL | -17 | -16 | NL endo | Yes |
| **7** #14 | Chiron #183-74-3 | 437 | Yes | NA | -6 | -7 | Damaged | No |
| **8** #12 | Chiron #243-28-02 | 728 | Yes | NL | -5 | -8 | NL endo | No |
| **9** #412 | Chiron #243-28-02 | 246 | Yes | NL | -4 | -8 | NL endo | Yes |
| **10** #801 | B & L | 411 | Yes | NL | NA | NA | NL endo | Yes |
| **11** #731 | B & L | 371 | Yes | NL | NA | NA | NL endo | No |
| **12** #721 | B & L | 287 | Yes | NA | NA | NA | Damaged | Yes |
| **13** #791 | B & L | 120 | Yes | NA | NA | NA | NL endo | No |
| **14** #728 | B & L | 347 | Yes | NA | NA | NA | NL endo | Yes |
| **15** #767 | B & L | 274 | Yes | NA | NA | NA | Damaged | Yes |
| **16** #742 | B & L | 363 | Yes | NA | NA | NA | NL endo | Yes |
| **17** #702 | B & L | 325 | Yes | NA | NA | NA | Damaged | Yes |

NA, ; NL, normal.

*Average length of survival for Chiron lens was 361-5 days and for B & L lens 279-22 days.

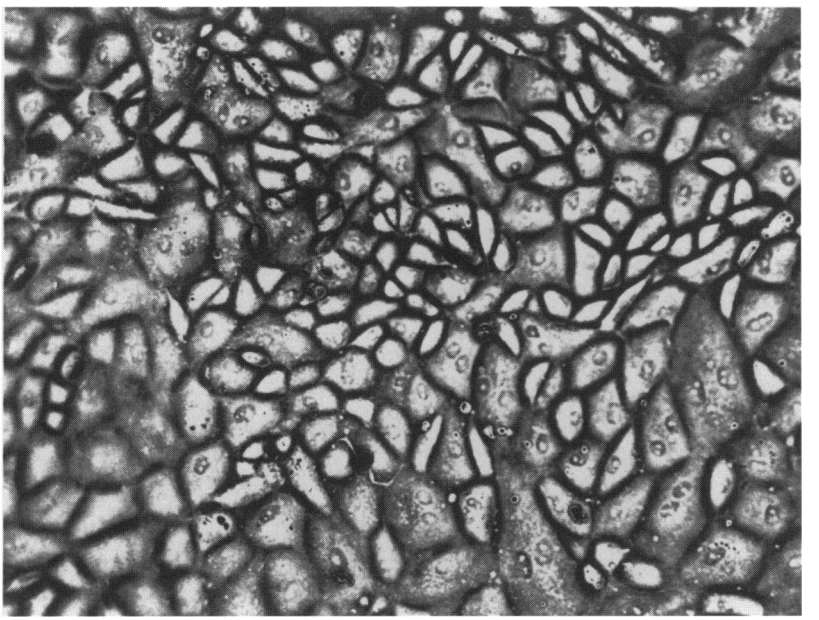

FIGURE 1
Light microscopy of rabbit corneal cells growing on carrier before transplantation (cell density 3,000 cell/mm2). Bar = 50 $\mu$m.

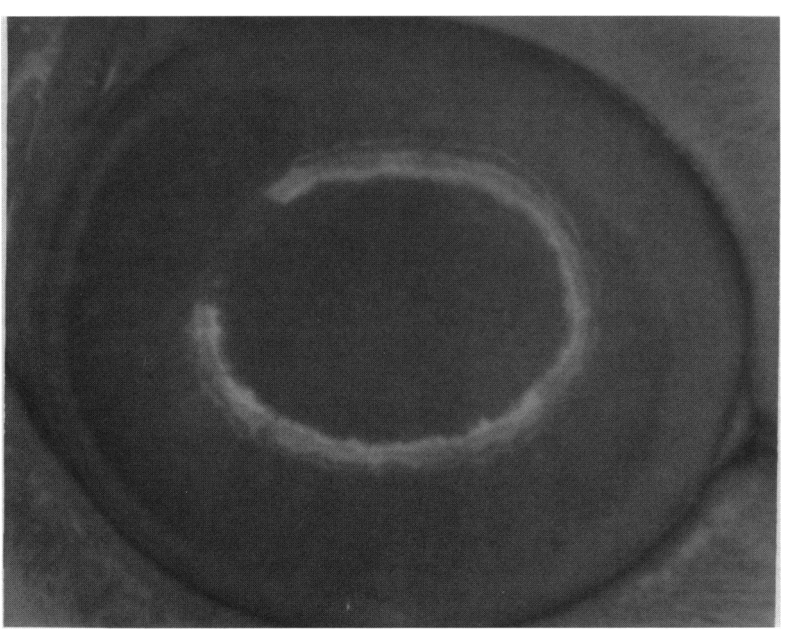

FIGURE 2A
Rabbit cornea before corneal endothelial transplantation.

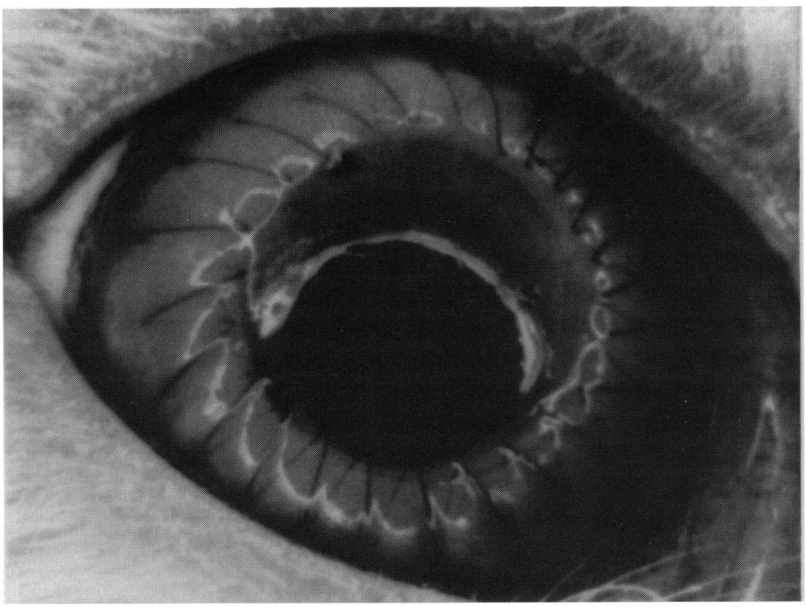

FIGURE 2B
Rabbit cornea 3 days after transplantation of cell/carrier.

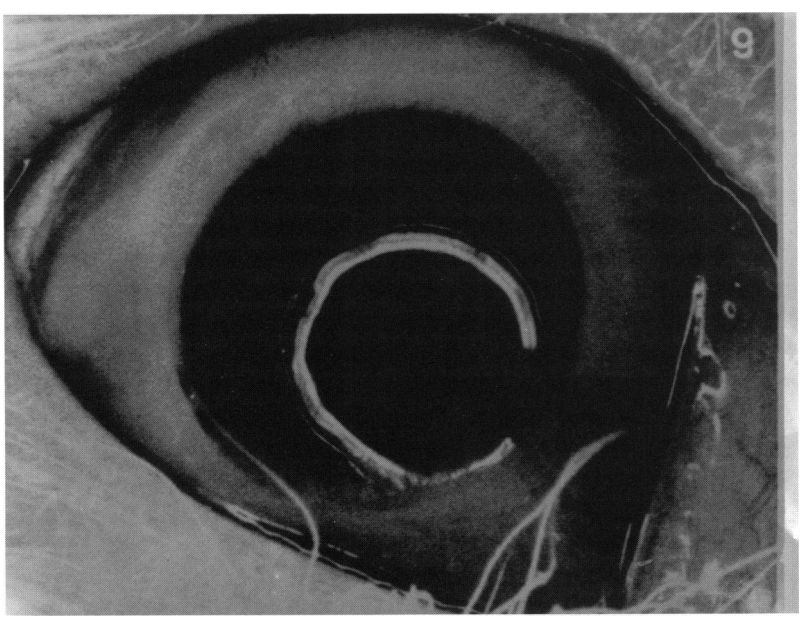


FIGURE 2C
Rabbit cornea 1 year after corneal endothelial cell transplantation.

carrier lens type, thickness, or other parameters (Fig 3, A and B). The only complication commonly observed was a sterile ulceration of the graft at the edge of the original trephine cut, which occurred in 16 cases out of the 19 at the end of the survival period (Fig 4).

**CONFOCAL MICROSCOPY**

Confocal microscopy of the 1- and 2-year grafts demonstrated a regular hexagonal pattern of the transplanted endothelial cells without polymegathism (Fig 5), and a thickened Descemet's membrane (18 to 36 $\mu$m). The endothelial cell density was 1,675 cells/mm$^2$ in the 1-year graft and 1,728 cells/mm$^2$ in the 2-year graft. Confocal microscopy of the unoperated eyes from both animals demonstrated a normal endothelial monolayer with an endothelial cell density of 2,317 cells/mm$^2$ in the 1-year survival and 3,066 cells/mm$^2$ in the 2-year survival.

**LIGHT AND TRANSMISSION ELECTRON MICROSCOPY**

Light microscopy of the flat mount preparation from the 2-year graft showed a regular, hexagonal pattern of the endothelial cells without polymegathism in the central area of the graft and some degree of polymegathism at the periphery (Fig 6A). A distinct pattern of actin filaments was detectable in the endothelial monolayer of the graft with a 2-year survival, resembling the intact cytoskeleton of the normal corneal endotheli-

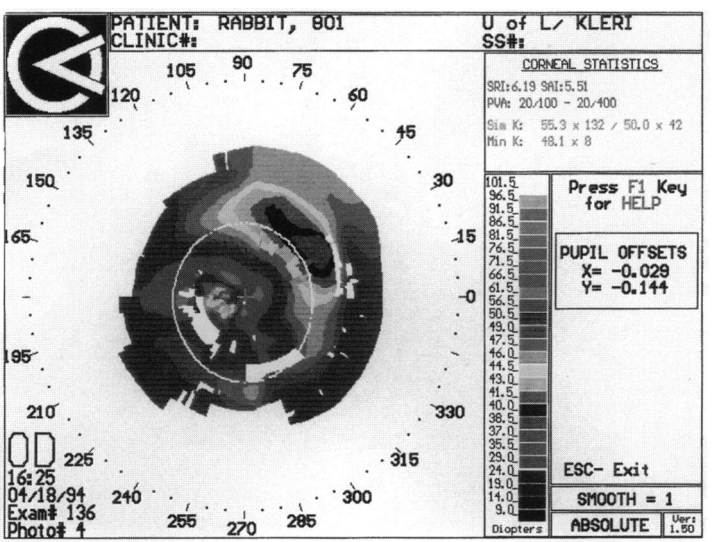


FIGURE 3A

Corneal topography of grafted cornea 1 week after transplantation.

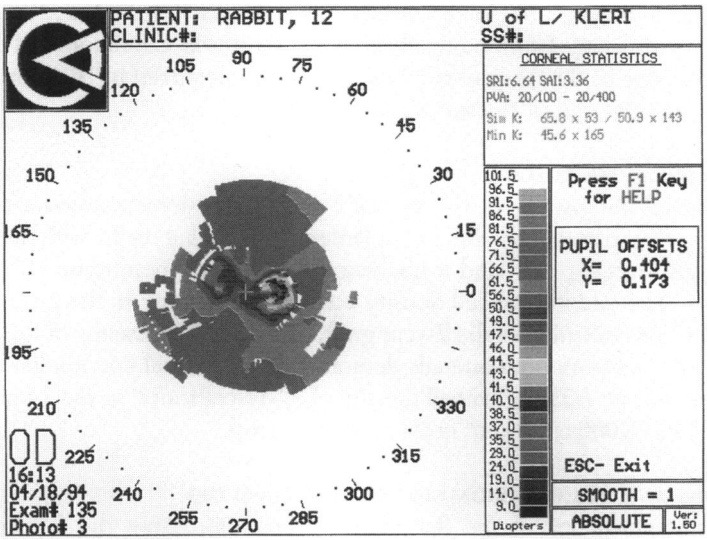


FIGURE 3B
Corneal topography of grafted cornea 6 months after transplantation.

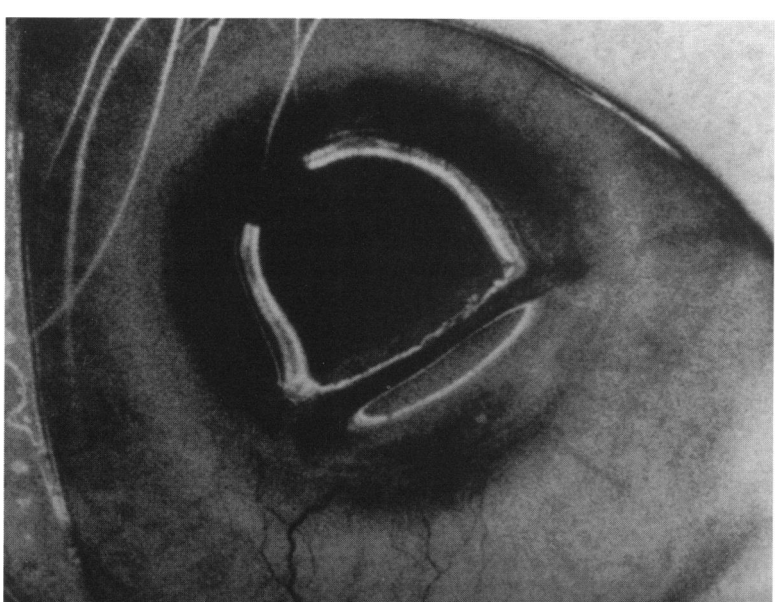

FIGURE 4
Ulcerated cornea in a 1-year graft at time of sacrifice.

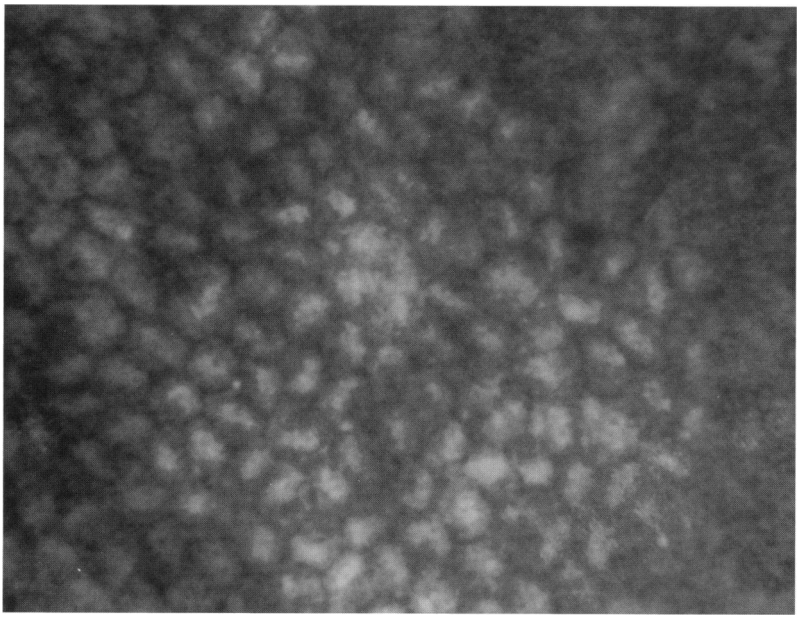

FIGURE 5
In vivo confocal microscopy of endothelium of 2-year graft.

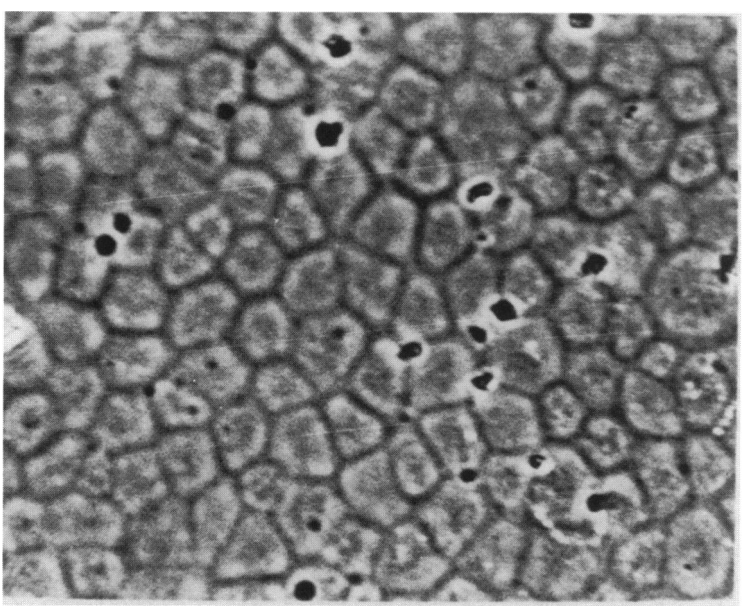

FIGURE 6A
Phase-contrast microscopy of flat mount corneal preparation from 2-year graft. Endothelial cells have regular, hexagonal shape with little polymegathism.

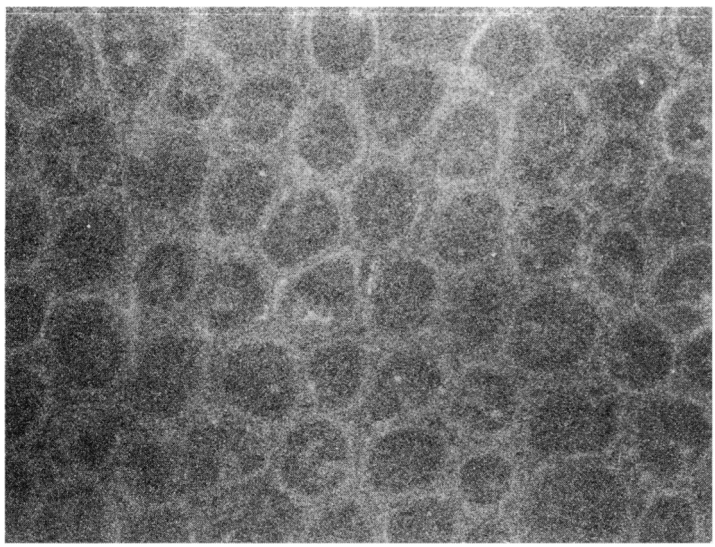

FIGURE 6B
F-actin staining of corneal endothelium in a 2-year graft. Note normal cytoskeletal architecture.

um (Fig 6B). A well-positioned carrier with an intact endothelial cell monolayer and its basement membrane was present in all 17 cases (Fig 7). The basement membrane stained light blue with Masson trichrome and was PAS-positive, indicating that a normal endothelial basement membrane material was deposited by the donor cells (data not shown). There was no evidence of stromal infiltration by cells, epithelial or stromal edema, retrocorneal membrane, or neovascularization.

Transmission electron microscopy of the endothelium in the 1- and 2-year grafts showed identical ultrastructure with the native corneal endothelium (Fig 8, A and B). The newly formed Descemet's membrane was significantly thicker (18 to 36 $\mu$m) than the native Descemet's of the unoperated eye (10 $\mu$m). Enlarged interlamellar spaces and keratocytes were detected in the stroma of the graft, along with irregularities in the periodicity of the lamellae (Fig 8, C and D). The ultrastructure of the epithelium was normal, but the cells and nuclei were significantly flattened in all areas of the graft compared with the unoperated epithelium (Fig 8, E and F).

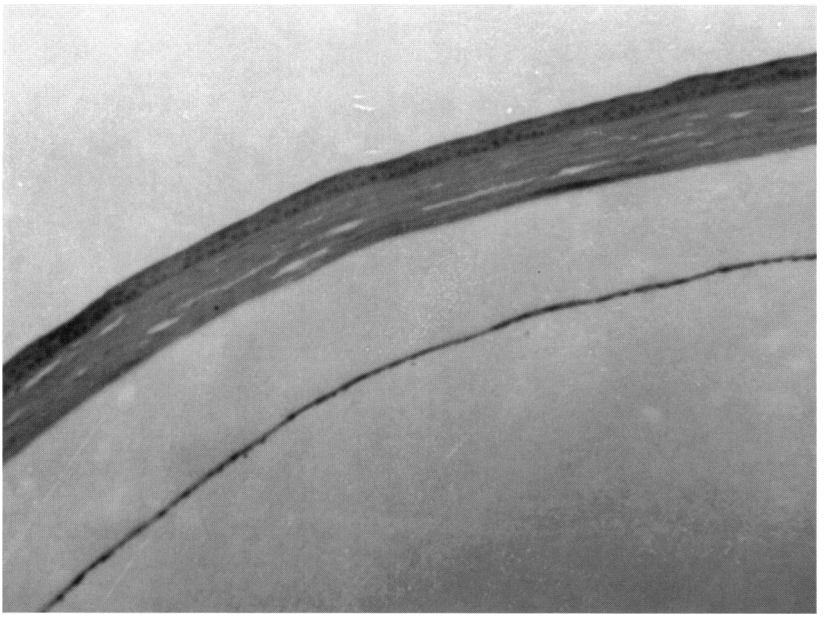

FIGURE 7
Light microscopy of corneal endothelial transplant. Epi = epithelium; S = stroma; C = Hydrogel carrier; Endo = endothelium; Bar = 100 $\mu$m.

**BRDU LABELING AND F-ACTIN STAINING**

Homogenous BrdU immunolabeling was present in the nuclei of the transplanted endothelial cells in all areas of the graft after 6 months survival (Fig 9).

## DISCUSSION

Our results demonstrated with a high level of consistency that grafted corneal endothelial cells on a hydrogel carrier remained functional for a long period of time without rejection and without using immunosuppression. Similar long-term survival rates have not been well documented in the literature. Gospodarowicz[2,4] reported survival times of 8 months, whereas other investigators[1,2,3,5-8] demonstrated 3 to 6 months survival of grafts with endothelial cell replacement. Although different species, techniques, and carriers were used in these studies, a high rejection rate was usually present. A high rejection rate was seen in our initial study,[3] but with slight modification of the cell culture and surgical techniques and early removal of the sutures, we significantly improved the outcome of the replacement procedure.

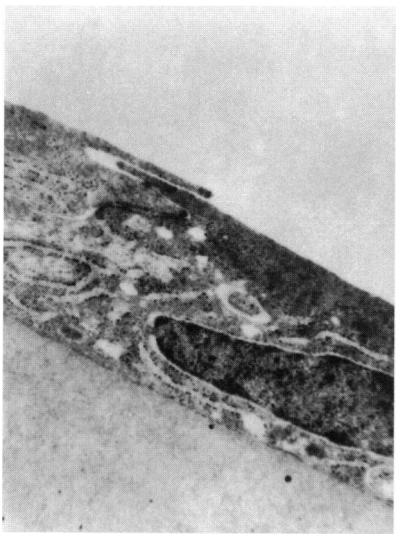

FIGURE 8A
TEM of transplanted endothelium (2-year graft).

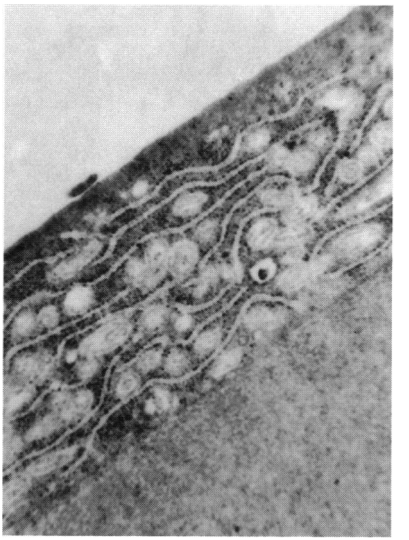

FIGURE 8B
TEM of native endothelium.

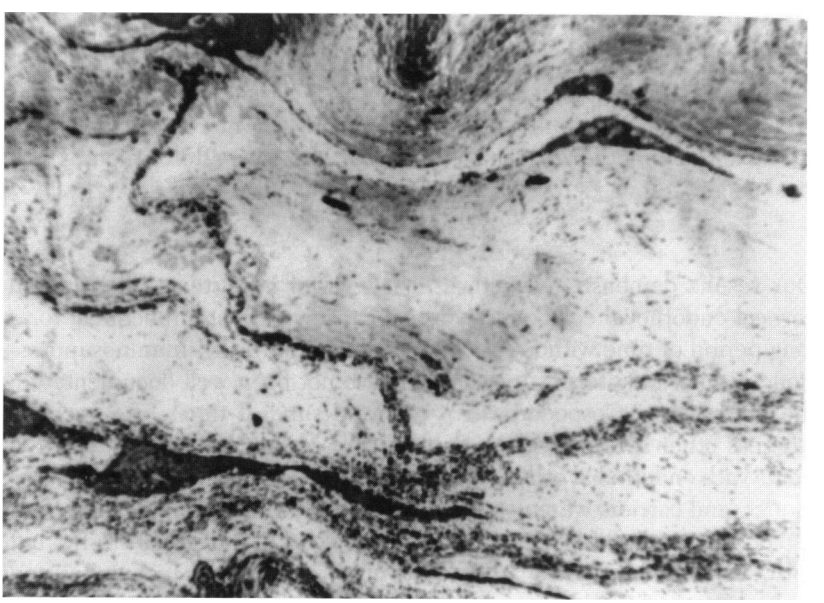

FIGURE 8C
TEM of stroma of 2-year graft with irregularities in periodicity of lamellae.

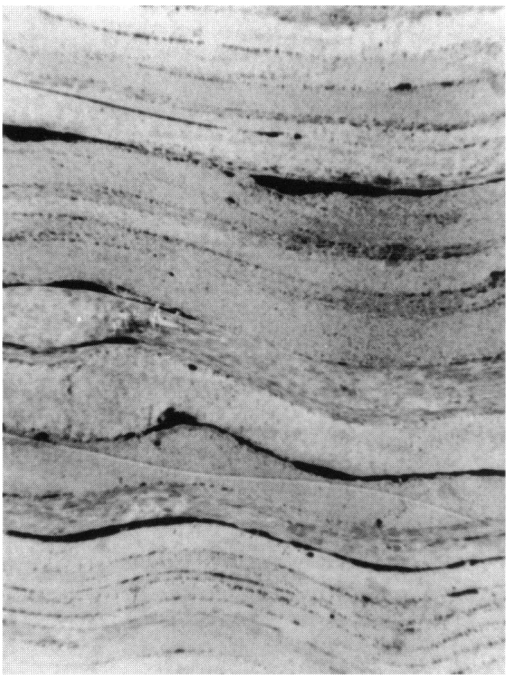

FIGURE 8D
TEM of normal corneal stroma.

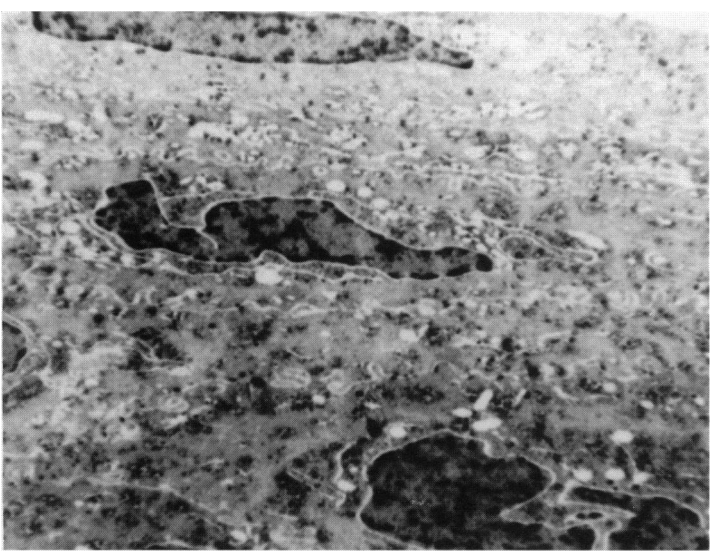

FIGURE 8E
TEM of epithelium of 2-year graft. Note flattened epithelial cells with normal ultrastructure.

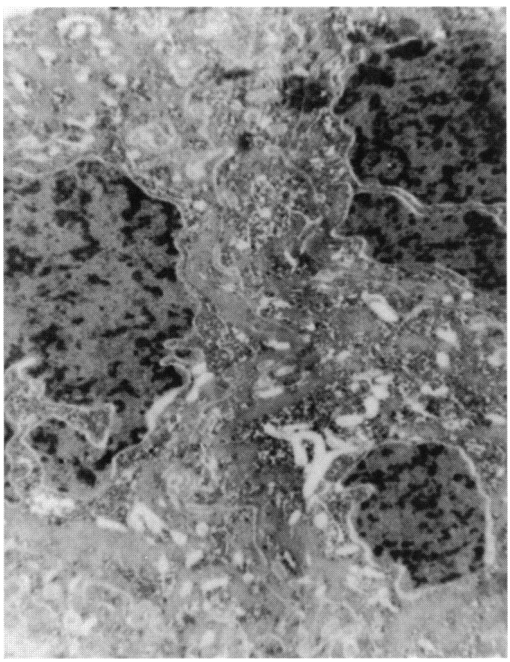

FIGURE 8F
TEM of epithelium in unoperated eye.

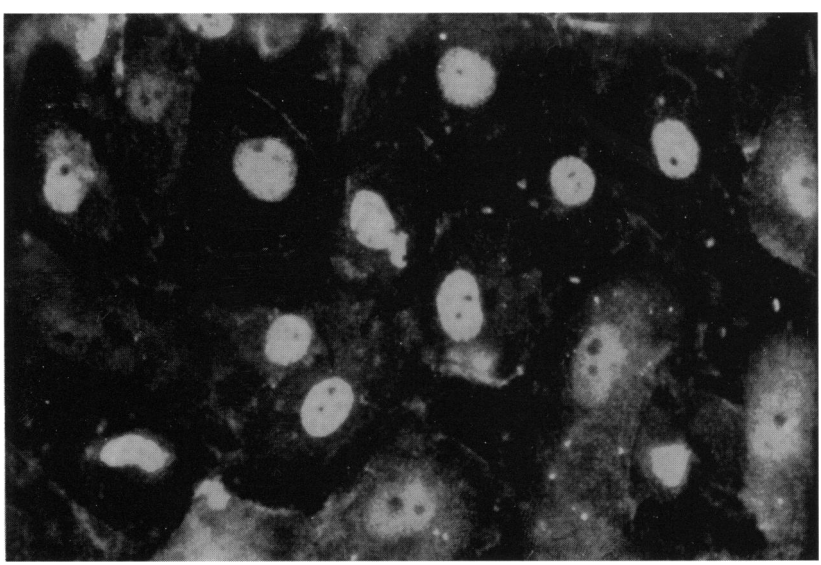

FIGURE 9
BrdU immunolabeling of cell nuclei was present in all areas of graft after 6-month survival.

By using different microscopic techniques for the evaluation of the long-term transplants, we had the opportunity to correlate the postoperative observations and the in vivo confocal microscopic findings with the ex vivo histologic findings. A regular hexagonal pattern of the transplanted endothelial cells was a consistent finding with both confocal and light microscopy. The absence of major morphologic abnormalities or polymegathism in the transplanted corneal endothelial monolayers correlated well with the observation of long-term transparency in these grafts.

Histochemical staining of the grafted corneas showed that the histologic characteristics of the newly formed Descemet's membrane by donor cells on the lens carriers were identical with the histologic properties of the native Descemet's membrane. The increased thickening was progressive with time, and the velocity of extracellular matrix deposition by the transplanted endothelial cells in our study was exactly the same (1 $\mu$m per month) as predicted earlier by Schwartz and McCulley[9] for native corneal endothelium. It is possible that the cells were initially programmed by the culture conditions (serum and growth factors) to produce this high amount of extracellular matrix, or the hydrogel biomaterial itself stimulated matrix production. Regeneration from the host cornea into the grafted area was a real concern, considering the high mitotic activity of the rabbit corneal endothelium. In our earlier study,[3] we demonstrated that implantation of a hydrogel without cultured corneal endothelial cells did not result in regeneration from the host, and the transplant remained cloudy. In the present study, the BrdU-labeled, cultured corneal endothelial cells showed a homogenous labeling of cell nuclei 6 months after transplantation, which demonstrated that the original donor endothelial cells had remained on the carrier. This finding is consistent with the study of Gospodarowicz and associates,[4] who used karyotype analysis to distinguish between the host and grafted corneal endothelial cells and demonstrated that there was no invasion of the transplanted corneal button by the recipient endothelium.

The present investigation supports the fact that hydrogels are biocompatible, stable, and excellent candidates[10-15] for use in "tissue engineering" in the cornea. Tissue engineering is a new trend in biotechnology in which tissue-cultured cells and biomaterials are used to replace damaged tissue.[16] This study demonstrates that corneal endothelial monolayers grown on a biomaterial substrate can be placed in vivo and remain functional with few side effects, and suggests that tissue engineering may be a feasible alternative to current corneal transplantation procedures. The technique presented here has a far better prospect in clinical use than any of the other techniques investigated in recent years (epithelial replacement, intracorneal transplants, keratoprosthesis) for the following reasons: (1) the endothelial monolayer is less immunogenic and not as complex a

structure as the epithelium or full-thickness cornea, (2) there is no tear film or eyelid interaction, (3) there is no innervation, (4) the anterior architecture of the cornea can be preserved, and (5) this is a surgical technique that can be easily learned using available microsurgical instrumentation.

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**DISCUSSION**

JAMES P. MCCULLEY, MD. I would like to congratulate Drs Mohay, Wood, and McLaughlin on an excellent and provocative paper. This work builds on the pioneering work of David M. Maurice and D. Gospodarowicz. David Maurice began evaluating the feasibility of transplantation of tissue-cultured rabbit corneal endothelium in the early 1970s. I worked with him in this area in the mid to late 1970s. We were able to transplant successfully tissue-cultured rabbit and human corneal endothelium into the rabbit and have return of normal function. Dr Wood has similarly achieved this capability with rabbit endothelium transplanted into a rabbit host and demonstrated return of function in vivo. One of the advantages of transplanting tissue-cultured endothelium is that it might indeed be less immunogenic. We found no difficulties with rejection when transplanting rabbit into rabbit, but did have problems with xenograft rejections when transplanting human endothelium into a rabbit host. We were able to demonstrate return of normal function and clearing of the transplanted corneas prior to the onset of xenograft rejection. The authors imply that previous investigators typically had difficulty with rejection, a point that I would like to clarify, in that we did not experience difficulties when performing allografts.

Our interest in this field somewhat decreased because of two persistent problems: (1) maintenance of an adequate endothelial cell density after transplantation and (2) availability of an adequate carrier for the tissue-cultured endothelium into the host. The authors achieved a good cell density in vivo with 3,000 cells/mm$^2$ on the hydrogel carriers. Unfortunately, specular microscopy was not possible in their model, and therefore they did not report on cell density postoperatively. It is therefore not possible to assess whether they have overcome this limiting factor with the present model. It would have been helpful if they would have done flat-mount preparations after the recipient animals were sacrificed, since in vivo specular microscopy was not possible. The confocal microscopy that they did allowed evaluation of the endothelium in vivo, but unfortunately they do not report on cell density determinations, which according to Drs Jester and Petroll were done. (Their recollection is that the density was decreased and they presently are searching their archives for the exact count.)

We previously developed a gelatin membrane that served as a carrier for tissue-cultured cells, but unfortunately it required a cyanoacrylate glue to hold the membrane in place. We were able to demonstrate the utility of this approach in rabbits but could not justify its use in humans. The authors' approach has been a very clever one, in which they mechanically secure a hydrogel carrier in a two-phase penetrating keratoplasty

wound. This provides adequate mechanical stability, which is necessary because the tissue-cultured endothelial cells require several days in vivo before they recover from the shock of tissue culture and have return of normal function. I do have some concerns about the permeability of the hydrogel carrier. The authors report 16 of 19 rabbits with sterile ulceration at the graft edge, which might be related to bunching of the hydrogel in the periphery, with resultant decrease in nutrient flow anteriorly. They were placing an 8-mm hydrogel carrier into a 7.5-mm bed, which might very well have resulted in excessive hydrogel tissue in the periphery. If this is not the explanation for the graft edge ulceration, I would like to know what other mechanisms they might propose for this ulceration. I further question a problem with hydrogel permeability based on the fact that they reported an increase in interlamellar spaces on transmission electron microscopy, ie, a suggestion that the anterior stroma might be edematous. I question whether such anterior stromal edema might account for the increasing anterior corneal curvature that they report. Rabbits do not have a Bowman's membrane, and therefore can swell both anteriorly and posteriorly. Unfortunately, ultrasonic pachymetry was not possible in the animals with the hydrogel in place. It would have been useful to have performed optical pachymetry to evaluate whether or not the anterior corneal stroma was swelling. Additional concerns arise about adequate carrier permeability based on the present report of thinned epithelium. Also, Dr Woods' 1995 ARVO abstract reported a decrease in the keratocyte population.

The authors utilized confocal microscopy to evaluate in vivo the status of the corneal endothelium, whereas in vivo specular microscopy had not worked. In vivo confocal microscopy is an excellent, but expensive, tool and is not yet widely available. I note that Drs Jester, Petroll, and Cavanagh from our department in Dallas provided this capability to the authors. It allows one, in effect, to obtain an optical biopsy of living tissue in vivo, without actually removing tissue. A more in-depth report of the confocal microscopic findings would have given us more insights into the adequacy of this model.

As the authors point out, as more keratorefractive surgery is done, we may, indeed, face a problem with eye banks. Not only may donor tissue availability be compromised, but we will have increasing problems in determining whether corneas are suitable for transplantation when there is no knowledgeable family member available to provide a past ocular history. What role "tissue engineering" or transplantation of tissue-cultured corneal endothelium will play in the future remains to be seen. I still am very hopeful that we will be able to develop a suitable mechanism to transplant tissue-cultured corneal endothelial cells as an alternative to full-thickness penetrating keratoplasty. I remain concerned about the postop-

erative cell density that we can achieve and the identification of an optimal carrier for the cells.

Once again, I would like to compliment the authors on his excellent paper, and I would like to ask three specific questions:

1. Do you have any laboratory information or do you recall the cell density determined by confocal microscopy that would allow us to evaluate the achieved cell density of the tissue-cultured endothelium after transplantation?

2. Would you comment about the potential problems of hydrogel permeability and its possible role in the peripheral ulceration, keratocyte dropout, increased interlamellar spaces, thinned epithelium, and their possible role in the increasing anterior corneal curvature noted postoperatively?

3. Did the transplanted tissue-cultured cells spread onto the previously denuded host posterior corneal surface (and explain the delayed clearing of the peripheral host), or did the animal repopulate the peripheral cornea with residual host endothelium?

STEVEN KRAMER, MD. Our group at UCSF has been interested in this for many years. Dr David Huang, the director of our corneal service, is most enthusiastic at the moment about genetically altering endothelial cells in the cultured state, cells that might, for example, elaborate factors to suppress neovascularization or to decrease rejection, and using the altered cells for replacement. We eliminate the carrier problem by not using one. The recipient cornea is removed, coated with the altered cells that are in suspension, and then replaced. Our direction is therefore to alter the endothelium by genetic engineering.

THOMAS WOOD, MD. Thank you. The cell density was approximately 1,600 at one year and 1,700 at 2 years, just slightly less than the density of approximately 2,000 and 3,000 in the opposite, untreated eye. The implant was actually thinner in situ than in the laboratory. I deleted some of the confocal microscopy (2 specimens) because the findings could not be confirmed with the 17 specimens undergoing electron microscopy. Our objective was to get a clear transplant for an extended period of time with transplanted endothelium. I do not think the permeability is a problem. The soft lens tends to mold the cornea to its shape; we need a material that will remain the shape of the cornea. There is evidence that the endothelium spreads to the periphery; we saw that with the confocal microscope, and the average rabbit peripheral cornea cleared 8 months after the transplant.