Amniotic Membrane as a Carrier for Cultivated Human Corneal Endothelial Cell Transplantation

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Purpose. It would be advantageous if cultivated human corneal endothelial cells (cHCECs) could be transplanted for the treatment of diseases caused by corneal endothelial disorders. To achieve this, a matrix that can serve as a carrier for cHCECs is needed. The present study was conducted to examine the feasibility of using amniotic membrane (AM) as a carrier for this application.

Methods. HCECs obtained from peripheral corneal tissue were cultivated, passaged, and transplanted onto denuded AM. The cell density and morphology of the resultant cHCECs on AM were examined by light, scanning electron, and transmission electron microscopy. To determine whether these cHCEC sheets on AM carrier were functional in vivo, the cHCEC sheets on AM were transplanted onto rabbit corneas whose Descemet's membrane and endothelial cells had been completely removed. After transplantation, the corneal appearance was examined by slit lamp biomicroscopy, and corneal thickness was measured daily by pachymetry. At 7 days after surgery, the grafts were examined by light, scanning electron, and transmission electron microscopy.

Results. The density of the cHCECs on AM was greater than 3000 cells/mm^2. Morphologically, the cHCEC sheets consisted of a fairly continuous layer of flat squamous polygonal endothelial cells that appeared uniform in size with tightly opposed cell junctions in vitro and in vivo after transplantation. The corneas that received transplanted cHCEC sheets had little edema and retained their thinness and transparency.

Conclusions. The cell density and morphology of cHCECs on AM were similar to those of normal corneas, and cHCECs on AM were functional in vivo. These results indicate that AM maintains HCEC morphology and function and could serve as a carrier for cHCEC transplantation. (*Invest Ophthalmol Vis Sci.* 2004;45:800–806) DOI:10.1167/iovs.03-0016

Corneal endothelium is essential for the maintenance of normal corneal hydration, thickness, and transparency.[1] Corneal endothelium of human has essentially no regenerative capacity in vivo.[2,3] Serious corneal endothelial cell loss causes irreversible corneal edema. The type of corneal transplantation performed depends on which corneal layers are damaged. Penetrating keratoplasty is generally the treatment of choice for eyes with a damaged endothelial layer such as in Fuchs' endothelial dystrophy and iatrogenic bullous keratopathy. However, there are several concerns with penetrating keratoplasty: rejection; suture problems, which can cause astigmatism and infection; and denervation, which affects corneal function. In addition, in many countries, including Japan, the supply of donor corneas is insufficient. Therefore, the ideal situation would be if only corneal endothelial cells could be transplanted to treat corneal endothelial diseases. Furthermore, it would be highly desirable if small numbers of corneal endothelial cells could be expanded in culture, and these cultivated human corneal endothelial cells (cHCECs) could be transplanted into eyes of many patients.

For cHCEC transplantation in vivo, some type of carrier is obviously necessary. Up to now, for corneal endothelial cell transplantation, gelatin membranes (Schwartz BD, et al. *IOVS* 1980;21:ARVO Abstract 100; McCully JP, et al. *IOVS* 1981;22: ARVO Abstract 230)[4,5] and coated hydrogel lenses[6] have been used as synthetic carriers for these cells. Human amniotic membrane (AM), widely used as a surgical material,[9] has been used successfully as a carrier for cultivated corneal epithelial cell transplantation.[8–10] In this study, we sought to examine whether AM could also serve as a carrier for cHCECs, both in vitro and in vivo.

Materials and Methods

Human Corneal Tissue

A donor cornea (47 years old, male, endothelial cell density: 3065 cells/mm^2) obtained from Northwest Lions Eye Bank was preserved (Optisol GS; Chiron Vision, Irvine, CA) and transported to our hospital on ice. After using its center for human corneal transplantation the residual limbal tissue was used for this study at day 7 after death.

HCEC Culture

To cultivate HCECs, corneal limbal tissue was placed in a Petri dish containing Dulbecco's modified Eagle's medium (DMEM; Invitrogen Corp., Carlsbad, CA), 50 U/mL penicillin, and 50 μg/mL streptomycin. Under a dissecting microscope, Descemet's membrane with its attached corneal endothelium 1 mm apart from trabecular meshwork was stripped from the stroma and placed in a 35-mm dish containing 1.2 U/mL dispase in phosphate-buffered saline (PBS). The tissue was incubated for 1 hour at 37°C, and the cells were rinsed gently with a sterile pipette. The dispase was then inactivated by suspending the cells in a medium containing DMEM, 50 U/mL penicillin, and 50 μg/mL streptomycin. After gentle centrifugation (3 minutes at 180*g*), the cells were resuspended in culture medium containing DMEM, 50 U/mL penicillin, 50 μg/mL streptomycin, 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, OH), and 2 ng/mL basic fibroblast growth factor (Invitrogen Corp.).

The cells were incubated in wells of a collagen IV–coated 24-well plate at 37°C in 5% carbon dioxide-95% humidified air. The medium was changed every other day. Cells reached confluence in 10 to 20

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days and were then subcultured by treatment with trypsin-EDTA (Invitrogen Corp.) and seeded at a ratio of 1:2 to 1:8.

Preparation of AM

In accordance with the tenets of the Declaration of Helsinki and with proper informed consent, human AMs were obtained at the time of cesarean section. The method of removing the amniotic epithelial cells from the AM has been reported.[11] Briefly, human AM was stored at −80°C in DMEM and glycerol (Nacalai Tesque, Kyoto, Japan) after the AM was washed with PBS containing antibiotics (5 mL of 0.3% ofloxacin). Immediately before use, the thawed AM was deprived of amniotic epithelial cells by incubation with 0.02% EDTA (Wako Pure Chemical Industries, Osaka, Japan) at 37°C for 2 hours, followed by gentle cell scraping with a cell scraper (Nalge Nunc International, Naperville, IL). The tissues were then washed twice with sterile PBS. To confirm whether epithelium was completely removed from AM, light microscopy was used to examine the AM (Fig. 1A).

Seeding cHCECs on Denuded AM

Denuded AMs were spread, basement membrane side up, on the bottom of polyester culture inserts (Corning Corp., Corning, NY), and the inserts were placed in wells of a 12-well plate. Confluent monolayers of cHCECs from passage 5 were trypsinized, centrifuged, and resuspended at a final cell-seeding concentration of 6.0×10^3 cells/mm^2 (1.2×10^6 cells/mL $\times$ 0.5 mL of cell suspension per 100 mm^2 of culture area). Resuspended cells were gently seeded on denuded AM spread on culture inserts in wells of a 12-well plate, centrifuged gently (3 minutes at 180*g*) to increase the cell density of the cHCEC sheets and to increase uniform attachment to the denuded AM, and incubated at 37°C in 5% carbon dioxide-95% humidified air. The culture medium was changed 3 days later and then every other day for 2 weeks.

In some experiments, to investigate the extent of the survival of cHCECs on AM after transplantation in vivo, we labeled the cHCECs with the fluorescent membrane dye DiI (1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate).[12] The stock solution of DiI was made by dissolving 5 mg dye in 5 mL 70% ethanol. The DiI labeling solution was made by diluting the stock solution by 10. Immediately before seeding the HCECs on to the AM, the cells were labeled in vitro by adding DiI solution to the cell suspension. After incubation for 5 minutes at 37°C, the cell suspension was cooled on ice for 15 minutes. Excess dye was then removed by washing the cells twice in PBS. Cells were resuspended at a final cell-seeding concentration of 6.0×10^3 cells/mm^2 and seeded on denuded AM, cultured for 7 days, and transplanted. We conducted this experiment with two cHCEC sheets on AM carriers transplanted into rabbit corneas. At 7 days after transplantation, we killed the animals, removed the grafts, and observed them under the fluorescence microscope. We stained same tissues with alizarin red and examined them under the microscope.

Light and Electron Microscopy

Cultures of cHCECs on denuded AM were examined by light, scanning electron, and transmission electron microscopy.

For light microscopy, tissues were stained with alizarin red and hematoxylin and eosin. For alizarin red staining, day-14 cultures on AM were placed endothelial side up on glass slides. Tissues were briefly rinsed in 0.9% sodium chloride followed by a 1-minute staining with 1% alizarin red in deionized water. Cell density (cells per square millimeter) was calculated by averaging cell density of three cHCEC sheets. For cell density of one sheet, five areas (each area equaled 0.25 mm^2) per one sheet were examined and averaged.

For hematoxylin and eosin stain, day-14 cultures on AM were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Sakura Fine Technical, Tokyo, Japan). After freezing, the resultant blocks were cut into 8-μm-thick sections with a cryostat. The sections were stained with hematoxylin and eosin for examination.

For scanning electron microscopy, day 14 cultures on AM were fixed in 2.5% glutaraldehyde in 0.1 M PBS, washed three times for 15 minutes in PBS, postfixed for 2 hours in 2% osmium tetroxide, and washed three more times in PBS. After dehydration through a graded ethanol series (50%, 70%, 80%, 90%, 95%, and 100%) specimens were transferred to hexamethyldisilazane (Agar Scientific, London, UK) for 2×10 minutes and allowed to air dry. When dry, specimens were mounted on aluminum stubs and sputter coated with gold before examination on a scanning electron microscope (model JSM 5600; Japanese Electron Optical Limited [JEOL], Tokyo, Japan).

For transmission electron microscopy, day-14 cultures on AM were fixed in 2.5% glutaraldehyde in 0.1 M PBS, postfixed in 2% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in epoxy resin (Agar 100; Agar Scientific). Ultrathin (70 nm) sections

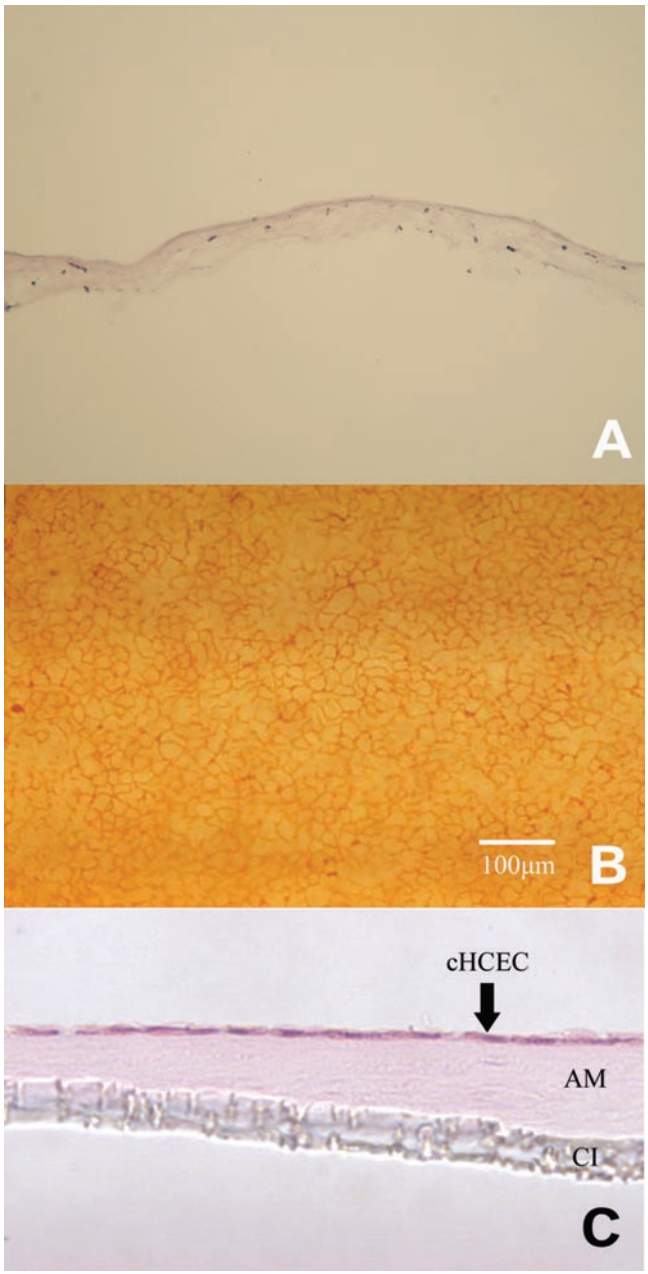

FIGURE 1. (**A**) Denuded AM before cHCECs were seeded. No amniotic epithelial cells were observed. (**B**) Light micrograph of cHCECs cultivated on denuded AM for 2 weeks. Flatmount stained with alizarin red. The cHCECs appeared as a fairly continuous monolayer with a cell density of 3340 cells/mm^2. (**C**) Cross section of the cHCECs on denuded AM on a culture insert (CI). A continuous intact monolayer of cHCECs is evident (*arrow*). Magnification: (**A**) ×100; (**C**) ×200.

TABLE 1. Transplantation Groups

Group	Stripping DM	Transplantation
HCEC	(+)	cHCEC sheet
SD Control	(+)	(−)
AM Control	(+)	Acellular AM only
TO Control	(−)	(−)

were collected on copper grids and stained for 1 hour each with uranyl acetate and 1% phosphotungstic acid and for 20 minutes with Reynolds lead citrate before examination on a transmission electron microscope (JEM 1010; JEOL).

Animals

Male Japanese white rabbits weighing 2 to 3 kg were obtained from Shimizu Laboratory (Kyoto, Japan). All animals were treated in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research. The Committee for Animal Research, Kyoto Prefectural University of Medicine, approved all animal studies.

cHCEC Transplantation

Transplantation was performed on the right eye only. Corneal buttons and graft beds were prepared by excising a 7.00-mm site in the central cornea. Descemet's membranes together with the endothelium were stripped from the corneal buttons. cHCEC sheets, using AM as a carrier, on culture inserts were trephined to a diameter of 6.25 mm, and they were separated gently from inserts with fine forceps. These sheets were placed on the stroma of the corneal buttons, left for a few minutes until dry to secure the sheet to the stroma. The corneal buttons with cHCEC sheets were then placed on the graft bed of the same animal and sutured with eight interrupted sutures and a continuous suture (10-0 nylon). Four groups were prepared in this experiment (Table 1). Each group had three animals. In the first group (the cHCEC group), after Descemet's membrane was stripped, the cHCEC sheet was placed on the corneal button and transplanted as just described. In the second group (the SD control group), Descemet's membrane was stripped and the button transplanted as described. In the third group (the AM control group), after Descemet's membrane was stripped, just the acellular AM was transplanted, as described. In the last group (the TO control group), the host cornea was trephined only and transplanted as described, without stripping Descemet's membrane and transplanting any sheet. All grafted eyes were examined every day after transplantation. Grafts with technical difficulties (e.g., hyphema, infection, or loss of the anterior chamber) were excluded from further consideration. At day 4 after transplantation, the interrupted sutures were removed.

Evaluation of Corneal Appearance, Thickness, and Histology after Transplantation

Each day after transplantation, corneal appearance was examined by slit lamp biomicroscopy, and corneal thickness was determined with an ultrasonic pachymeter (model SP-2000; Tomey, Nagoya, Japan). Corneal thickness is a measure of corneal endothelial function.[13] The mean of 10 measured values was calculated. At 7 days after transplantation, we killed the cHCEC sheet transplant recipients, removed the grafts, and observed them by light and electron microscopes.

RESULTS

Light and Electron Microscopy of the cHCECs Sheet In Vitro

The density of cHCECs seeded on AM was 3285.3 ± 62.0 cells/mm^2, the cHCECs appeared as a fairly continuous monolayer (Figs. 1B, 1C). Scanning electron microscopic images of HCECs cultivated on AM revealed a continuous layer of flat, squamous, polygonal endothelial cells that appeared uniform in size. The interdigitations at the cell boundaries were not as distinct as those in normal endothelium. These cells had a few pits and vacuoles, but appeared to be healthy, well developed, and closely attached to one another, with tightly opposed cell junctions (Fig. 2). Transmission electron microscopic images showed a monolayer of flat endothelial cells, healthy and well formed, with tightly opposed cell junctions. Adjoining cells overlapped each other slightly to maintain maximum contact, as would be expected. At the base of the cHCEC basement membrane, material was clearly being produced (Fig. 3).

Evaluation of Corneal Appearance and Thickness after Transplantation

At 4 days after the operation, the control grafts, consisting of only stripped Descemet's membrane, became highly edematous (Fig. 4A) as did the control grafts with only acellular AM (Fig. 4B). However, grafts with cHCECs on AM had little edema and excellent transparency, and we could see the transplanted cHCEC sheets clearly through the transparent corneal button (Fig. 4C). No signs of rejection or neovascularization were observed. This situation continued for at least 7 days after transplantation (Fig. 5). Corneal grafts with cHCECs were sig-

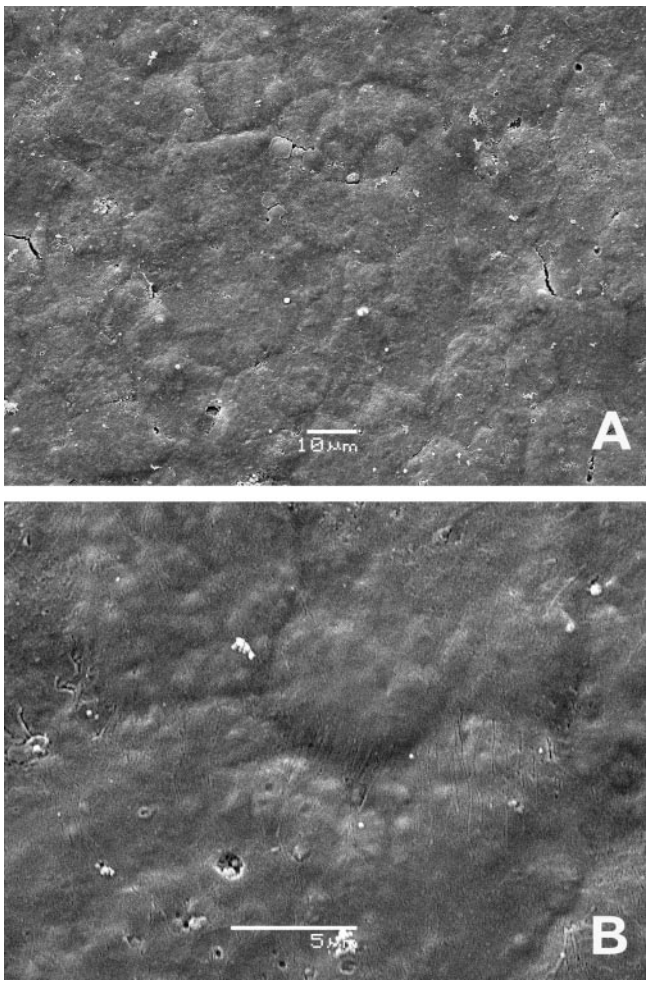

FIGURE 2. Scanning electron micrograph of cultured HCECs. The cells were cultivated on denuded AM for 2 weeks. (**A**) Cells formed a continuous monolayer layer on the AM. (**B**) At higher magnification, it was evident that the cells were polygonal, fairly uniform in size, and in close contact with each other with tightly opposed cell junctions.

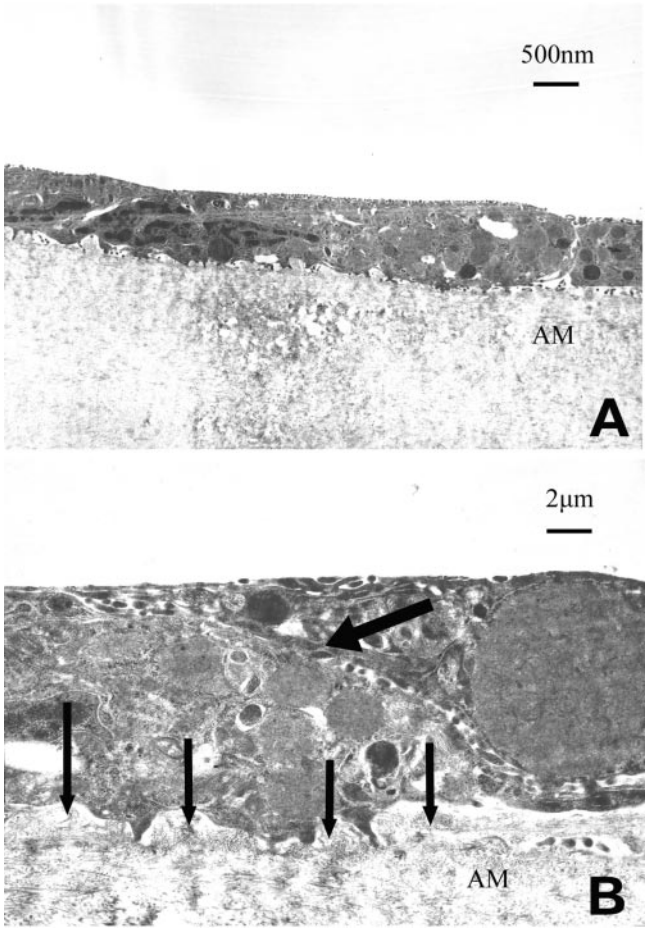

FIGURE 3. Transmission electron micrographs of cultivated HCECs cultivated on denuded AM for 2 weeks. (**A**) A continuous monolayer of these cells covered the surface of denuded AM. These cells had a few pits and vacuoles, but they kept normal endothelial features. (**B**) Adjoining cells either overlapped each other slightly or abutted to maintain good contact. *Thick arrow*: cell–cell junction; *thin arrows*: cells producing basement membrane material.

nificantly thinner when compared with both the stripped Descemet's membrane and AM control groups in daily pachymetry measurements ($P < 0.05$). The average value for rabbit corneal thickness before the operations was 350.7 ± 10.2 μm (mean ± SD). After the operation, average values for corneal thickness were always greater than 800 μm in the acellular AM and stripped Descemet's control groups. However, in the HCEC group, the average values for corneal thickness was consistently below 500 μm from days 5 to 7 after the operation (day 5: 460.7 ± 100.6 μm; day 6: 436.0 ± 100.5 μm; day 7: 460.7 ± 117.7 μm, mean ± SD). The average counts in the HCEC group were not significantly different from those of the TO group (Fig. 6).

Light and electron microscopy of the HCEC sheet 7 days after transplantation showed a continuous monolayer of HCECs on AM. The cells were polygonal, fairly uniform in size, and in close contact with one another (Figs. 7A, 7B). A transmission electron microscopy image of the HCECs shows that a continuous monolayer of cHCECs covered the surface of the denuded AM. Apparent complexity of cell–cell junctions and cell–AM substrates were observed. Many mitochondria and lysosomes were present in the cytoplasm of cHCECs on AM (Fig. 7C).

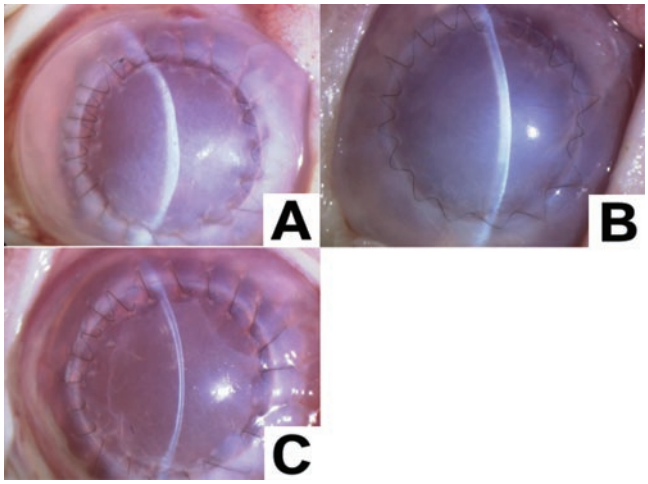

FIGURE 4. At 4 days after the operation, the control corneas consisting of stripped Descemet's membrane (**A**) and acellular AM (**B**) became highly edematous. The grafts consisting of HCECs on AM (**C**) had little edema and excellent transparency.

Assessment of Cultured HCECs Transplanted In Vivo

Before transplantation, cHCECs were present on AM at high density (Fig. 8A). One week after transplantation, the cells were still present on the AM carrier and maintained a high density (2410.0 ± 31.1 cells/mm^2; Fig. 8B). In addition, no proliferation or migration of cHCECs was observed beyond the edge of the AM (Fig. 8C). Alizarin red stain of the cHCEC sheet with corneal button showed that there were no endothelial cells between the cHCEC sheet and the host–graft junction. Therefore, rabbit corneal endothelial cells did not seem to have migrated into the corneal button at the time examined (Fig. 8D).

DISCUSSION

We made cHCEC sheets using denuded acellular AM as a nonsynthetic carrier and transplanted them in vivo in our

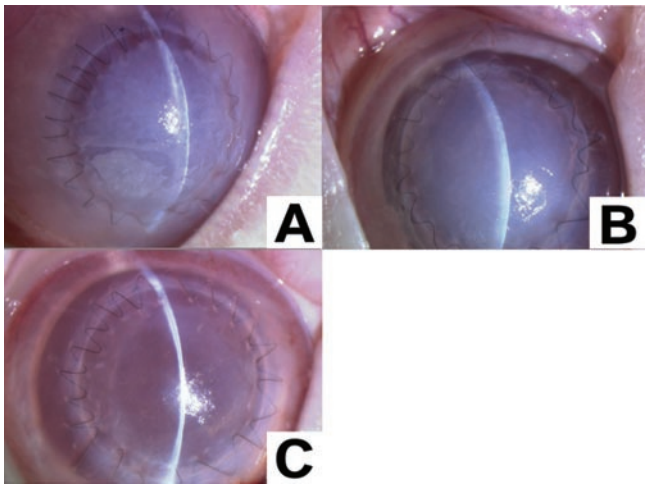

FIGURE 5. At 7 days after the operation, the control corneas consisting of stripped Descemet's membrane (**A**) and acellular AM (**B**) were highly edematous. The grafts consisting of HCECs on AM (**C**) had little edema and corneal transparency.

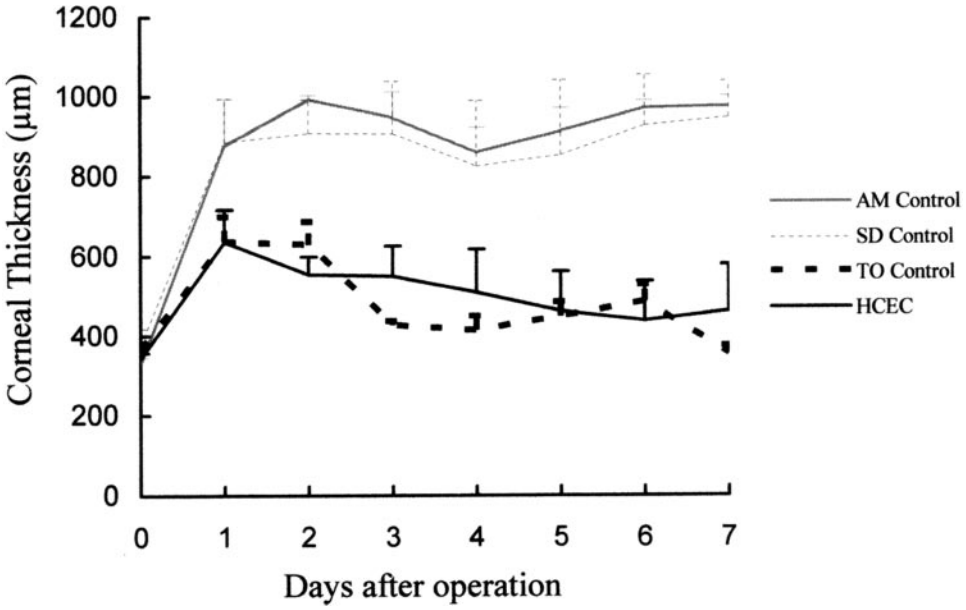

FIGURE 6. Average corneal thickness (mean ± SD) after transplantation. The average corneal thickness was always greater than 800 μm in both the stripped Descemet's membrane and the acellular AM control groups. In the cHCEC transplant recipients, the average corneal thickness was consistently less than that in both the control and AM groups. Between the TO control and cHCEC groups, there was no significant difference in the average corneal thickness.

present study. Cultivated HCEC transplantation on Descemet's membrane in vitro has been reported,[14–16] and the cells were reported to form a stable monolayer and keep their characteristic construction. We think AM is one of the most feasible candidates for cHCEC transplantation, because AM can be obtained more easily than any other nonsynthetic carriers such as Descemet's membrane, and it has been used for cultured corneal epithelial cell transplantation with good clinical results. There have been several studies[4,8,17–19] of corneal endothelial cell transplantation in vivo, but most of these do not involve corneal endothelial cells from humans, but instead cultured corneal endothelial cells from animals: rabbit, bovine, and cat. To our knowledge, in one study,[19] human neonatal corneal endothelial cells were cultivated and seeded onto the Descemet's membrane of the corneal button and then the cells transplanted into African green monkeys. Therefore, this is the first report of cHCECs transplanted in vivo using adult cHCECs.

In our study, the morphology and structure of cHCECs transplanted on denuded AM were evaluated by vital staining, as well as scanning and transmission electron microscopy. We found that the ultrastructure and density of these cells was very similar to that of normal corneal endothelial cells ex vivo. It is also important in cHCEC transplantation to obtain higher cell density. Clinically, it has been proposed that donor corneal tissue with endothelial density of more than 2500 cells/mm^2 are ideal to be transplanted in patients with bullous keratoplasty. Using centrifugation, we obtained cHCEC sheets with a density of more than 3000 cells/mm^2. These cHCEC sheets seem to have high enough cell density to be used in patients for bullous keratopathy.

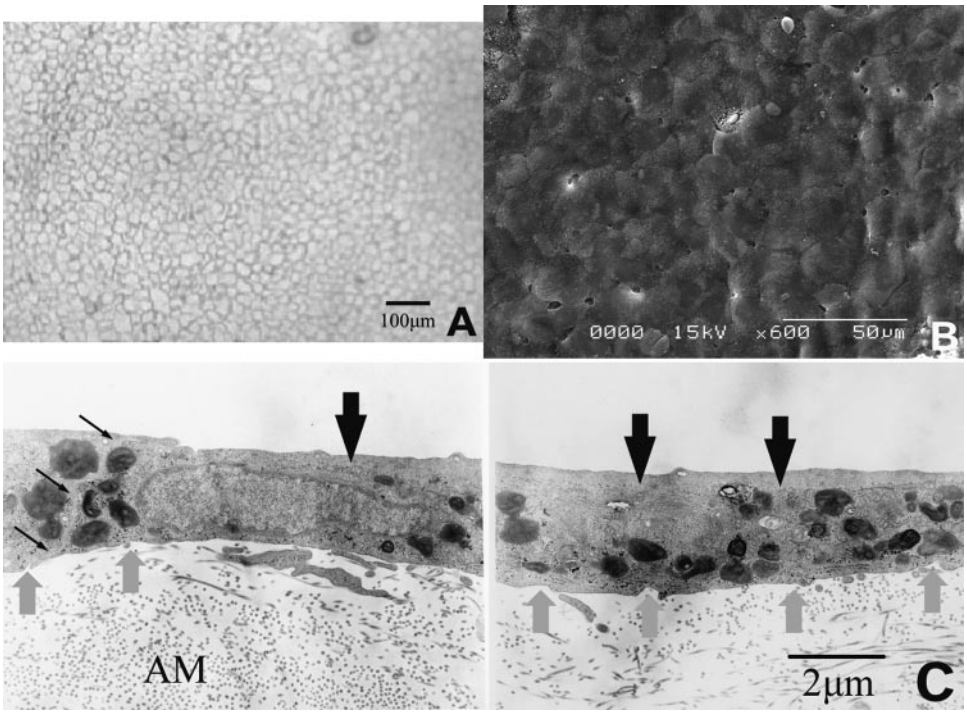

FIGURE 7. Light and electron micrographs of HCECs 7 days after transplantation. (**A**) A fairly continuous monolayer of HCECs. (**B**) Scanning electron micrograph also showing that HCECs formed a continuous monolayer layer on the AM. They were polygonal, fairly uniform in size, and in close contact with one another. (**C**) A transmission electron micrograph of HCECs showing that a continuous monolayer of cHCECs covered the surface of denuded AM. *Thin arrow*: designate cell–cell junction. *Gray arrows*: close association of basal plasma membrane and AM; *thick arrows*: mitochondria in cytoplasm.

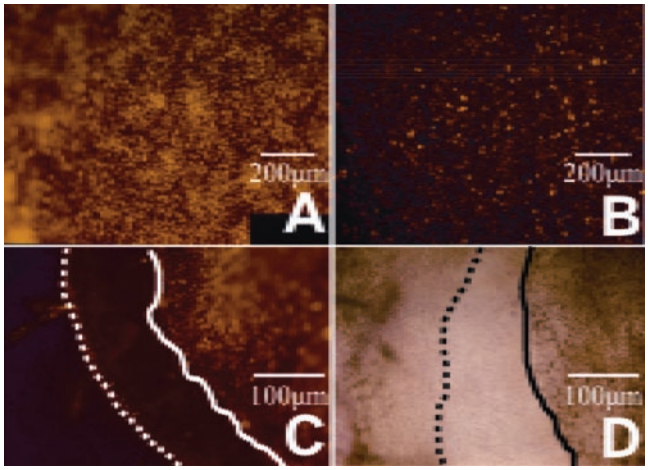

FIGURE 8. DiI-labeled cHCECs on AM. (**A**) At day 0, before transplantation in vivo, the cHCECs were present on the AM at a high density. (**B**) At day 7 after transplantation, the cultured HCECs were present on AM. (**C**) At day 7 after transplantation, the cHCECs showed no evidence of proliferation or migration beyond the edge of the AM. (**D**) Between cHCEC sheet and host–graft junction in the Descemet-stripped area, there were no endothelial cells. Neither donor nor host endothelial cells migrated from the original region. (**C**, **D**) *Solid line*: edge of cHCEC sheet; *dotted line*: host–graft junction. Magnification: (**A**, **B**) ×100; (**C**, **D**) ×40.

In our in vivo study, we also found that the rabbit corneas with transplanted cHCECs on AM retained their thickness and transparency compared with the controls for 1 week, and we also found, but in only one rabbit, that this situation continued for 4 weeks (data not shown). Because rabbit corneal endothelial cells have been known to proliferate in vivo, we wished to investigate the extent of survival of cHCECs transplanted in vivo by using DiI labeling. The results of the DiI labeling showed that the cHCECs remained on the denuded AM transplanted onto the corneal button at least 4 weeks. These results show that transplanted cHCEC sheets remained and were functional for at least 4 weeks. We intend to further investigate the long-term consequences of cHCEC transplantation and the functions of the cHCEC sheet. The HCEC density in the transplants showed 27% reduction 7 days after transplantation, either because of the tissue damage at the time of surgery or the short life of some cultivated endothelial cells. To investigate the long-term consequences of cHCEC transplantation, it is not suitable to use rabbits as recipients, because their corneal endothelial cells proliferate in vivo. It may be better to use cats or monkeys as recipients, because their corneal endothelial cells more closely mimic HCECs in having little or no mitotic activity and a limited regenerative capacity.

The transplanted cHCEC corneas using AM as a carrier are clearer and thinner than either corneas transplanted with AM only or corneas with removed Descemet's membranes and endothelial cells. Furthermore, the transplanted cHCEC corneas are as thin as corneas with trephination only. These results indicate that HCECs on AM function as well as that of normal endothelium at least until 7 days after transplantation. The transplanted cHCEC corneas with AM regained partial transparency after transplantation. Normal corneal stromal clarity depends on the regular arrangement of collagen fibers, AM does not have such a characteristic structure. However, we think thinning of AM after transplantation increased its transparency, as seen in the eyes with cultured corneal epithelial cells transplanted for corneal epithelial diseases.[10] There was a gap of 0.1 to 0.2 mm between donor human endothelial cells and host rabbit endothelial cells, but the transplanted corneas retained their corneal thickness and transparency. We think that the gap had some influence on the pump–leak balance. The water permeating into the corneal stroma through the nonendothelial area may pump out through the adjacent human and rabbit endothelial area. Because an area of central cornea more than 6 mm in diameter is covered by transplanted HCECs, it is reasonable to speculate that endothelial cells pump water from corneal stroma, although other possible factors such as evaporation may contribute to this to some extent.

In our present study, we transplanted cHCEC sheets by trephining the central corneas, removing Descemet's membranes with corneal endothelial cells, placing cHCEC sheets on the stroma of corneal buttons, and suturing them. A technique termed posterior lamellar keratoplasty, an operation for the treatment of bullous keratopathy, has been reported by Melles et al.[20] In this method the full-thickness cornea is not transplanted, just the posterior lamella of the cornea, and the method could be adapted for cHCEC transplantation. We have now investigated a cHCEC transplantation technique to remove corneal endothelial cells with Descemet's membrane and transplant a cHCEC sheet through a corneoscleral incision, similar to posterior lamellar keratoplasty. cHCEC sheet transplantation by this technique would be expected to have the same advantages as a posterior lamellar keratoplasty: fewer problems with sutures after they are in place, lower astigmatism, and more efficient use of donor tissue. In addition cHCEC transplantation may well have the advantage that scheduled operations could be performed, because we would not be dependent on the availability of corneoscleral discs. This possibility therefore has many advantages for both patients and health professionals.

cHCEC transplantation has the potential to be performed, not only as an allogeneic transplantation procedure but also as an autotransplantation procedure, if a small number of corneal endothelial cells from a healthy eye were cultivated, expanded, and transplanted to the contralateral endothelial damaged eye of the same patient. Moreover, in regenerative medicine, the potential of some pluripotent stem cells[21–23] for use in clinical treatments has been noted. Therefore, if pluripotent stem cells (e.g., hematopoietic stem cells and mesenchymal stem cells obtained from bone marrow) could be obtained from patients who undergo bullous keratoplasty and these stem cells could be induced to differentiate into corneal endothelial cells, it would be possible to transplant autologous corneal endothelial sheets without any risk of rejection.

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E R R A T U M

Erratum in: In "Quantitative Analysis of Retinal Ganglion Cell (RGC) Loss in Aging DBA/2NNia Glaucomatous Mice: Comparison with RGC Loss in Aging C57/BL6 Mice" by Danias et al. (*Invest Ophthalmol Vis Sci.* 2003;44:5151–5162), the authors discovered an error in Table 1 of the above manuscript. The numbers in the fourth column are incorrect. The ratio of columns 3 and 4 should result in the fifth column numbers, but does not. Below is the corrected table.

TABLE 1. Mean RGC Counts, Retinal Area and Average RGC Density for Each Age Group in Both Strains

Strain	Age (mo)	RGC Count (*n*)	Retinal Area (mm^2)	RGC Density per Retina (RGC/mm^2)
DBA2/NNia	3	89,492 ± 5,746.0	16.59 ± 0.17	5,396 ± 362
	6	73,834 ± 4,698.8	15.33 ± 0.31	4,805 ± 243
	9	86,363 ± 9,995.3	19.46 ± 0.82	4,403 ± 417
	12	72,322 ± 5,192.7	18.69 ± 0.50	3,882 ± 295
	15	30,769 ± 8,877.0	19.32 ± 0.32	1,583 ± 440
	18	25,106 ± 13,087.3	19.22 ± 0.65	1,248 ± 605
C57/BL6	3	84,027 ± 2,171.6	15.97 ± 0.37	5,268 ± 136
	6	83,925 ± 5,549.8	16.82 ± 0.26	4,980 ± 279
	9	85,168 ± 6,118.7	17.14 ± 0.44	4,954 ± 280
	12	69,824 ± 7,943.6	16.43 ± 0.85	4,212 ± 323
	15	69,002 ± 7,419.0	17.61 ± 0.59	3,916 ± 389
	18	45,890 ± 3,549.8	16.90 ± 0.17	2,724 ± 232

Density was calculated as total RGC count divided by total retinal area. Data are expressed as the mean ± SEM.

The online version of this article was corrected on January 28, 2004, in departure from print.