Articles
Transplant of Corneal Epithelium to Rabbit Corneal Wounds In Vivo
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Sheets of corneal epithelium removed from 9-mm buttons of adult rabbit corneas using Dispase II were placed on abraded (basement membrane intact) or keratectomized corneas of anesthetized rabbits. Both types of wounds extended from limbus to limbus. The host animals were maintained under deep anesthesia for 3 hr, during which time culture medium was dripped onto the surface of the transplant. A soft contact lens then was placed over the cornea and the eye bandaged shut. Short-term experiments indicated that after 24 hr the transplanted epithelium was adherent to both abraded and keratectomized corneas (n = 4). Hemidesmosomes had formed between basal cells of donor epithelium and denuded host basement membrane, and cytoplasmic blebs had extended from donor epithelium into host keratectomized stroma. Seven transplants to abraded corneas and 17 transplants to keratectomized corneas were followed for longer time periods. Six of the seven transplants to abraded corneas were maintained until termination of the experiment (four at 4 weeks, one at 2 weeks, one at 1 week). Three of the 17 transplants to keratectomized corneas were maintained until termination (one at 4 weeks, one at 2 weeks, and one at 6 days). The remaining 14 sloughed between days 2 and 6. These data indicate that it is feasible to transplant corneal epithelial sheets and that they can be maintained most successfully if the host basement membrane is present. Invest Ophthalmol Vis Sci 26:425-433, 1985

Ocular surface diseases and injuries, such as ocular cicatricial pemphigoid, Stevens-Johnson syndrome, and chemical injuries, result in serious damage to the surface of the eye, including superficial scarring and vascularization of the cornea, corneal epithelial defect formation and persistence, and conjunctival scarring. Since most of the damage is superficial, replacement of the superficial tissues might be expected to be beneficial. Indeed, conjunctival transplantation1 and keratopithelioplasty2 have been used successfully to treat ocular surface disorders by replacing the surface layers. However, because these techniques are limited, in the first case to autograft situations and in the second to availability of donor material, methods to replace the ocular surface are still being sought.3

Recently, a technique was developed to remove intact sheets of corneal epithelium using the bacterial neutral protease, Dispase II.4 The intact epithelium is viable and rapidly forms hemidesmosomes when placed on freshly denuded corneal basement membranes in vitro.5 Furthermore, it maintains normal stratified epithelial morphology and desmosomes, and surface microplacae are intact.

We report here results of studies designed to determine whether freed epithelial sheets could be transplanted to and maintained on corneal wounds in vivo. To do so, sheets were placed on rabbit corneas from which the host epithelium was removed either by scraping or by keratectomy, and the survival of the sheet in vivo was followed clinically and morphologically.

Materials and Methods
All investigations involving animals reported in this study conform to the ARVO Resolution on the Use of Animals in Research.

Epithelial sheets 9 mm in diameter were removed from corneal buttons of New Zealand white rabbits, as previously described.4 Briefly, 9-mm corneal buttons were placed in culture medium containing 1.2 U/ml Dispase II (Boehringer, Mannheim, Indianapolis, IN). The posterior half of the stroma was removed, and the anterior half was incubated at 35°C for 1 hr. Epithelial sheets were removed and washed in three changes of fresh medium to remove Dispase II.
Fig. 1. Appearance of epithelial sheet on abraded cornea 24 hr after transplant. a, Whole excised cornea; darkly stained area outlines remaining epithelial defect. The donor epithelial sheet was placed slightly off center and has joined the host epithelium in the region in the upper right (×4). b, Light micrograph of section through central area of transplant. The transplanted sheet has maintained normal stratification (×300). c, Electron micrograph demonstrating tight adhesion between donor epithelium and host basement membrane. Hemidesmosomes have formed between the two (×25,000).

**Placement of Epithelial Sheets on Rabbit Wounds**

Rabbits were anesthetized with intramuscular ketamine (200 mg). Fifteen minutes prior to administration of anesthesia, intramuscular thorazine (30 mg) was injected. After rabbits were deeply anesthetized, topical proparacaine drops were applied. One of two types of wound was made; either a limbal-to-limbal scrape to remove all epithelium, leaving the basal laminae intact, or a limbal-to-limbal keratectomy removing both epithelium and basal lamina. This was confirmed by histology. After wounding, rabbits were placed under a dissecting microscope and culture medium was dripped on the surface of the cornea to keep the wound moist. A stainless steel weighing spoon was used to transfer the epithelial sheet to the wound. The spoon held culture medium, upon which the epithelial sheet floated. With the wound surface in focus at low magnification, the sheet was transferred to the wound by the addition of drops of culture medium, which caused the sheet to float onto the wound surface. Drops of medium were placed directly on top of the sheet to “straighten” and flatten it on the wound. The rabbit was kept under anesthesia with ketamine (100 mg every half hour for 1.5 hr, then 100 mg hourly) for 3 hr after placement of the sheet. An inverted bottle of culture medium with capillary tubing provided a constant drip of medium to the surface of the transplanted sheet. At the end of the 3-hr period, a soft contact lens was placed over the eye and the eye was taped shut. After 24 hr, the lens (if still in place) was removed and fluorescein instilled in the eye to determine presence or absence of the transplant and extent of epithelial wound healing. Eyes were photographed daily, posttransplant, for the duration of the experiment. At the end of the experiment, eyes were fixed in half-strength Karnovsky’s fixative, stained with Richardson’s stain to delineate any remaining epithelial defect, and photographed under a dissecting microscope. Corneas then were processed for light and electron microscopy and hemidesmosomes counted, where appropriate, as previously described.5

**Results**

Epithelial sheets were transplanted to 11 abraded corneas (basement membrane intact) and 21 keratec-
Fig. 2. Epithelial sheet on keratectomized stroma 24 hr after transplant. a, Whole excised cornea stained with Richardson's stain to delineate remaining epithelial defect. Donor epithelial sheet was placed centrally and has joined host epithelium at upper left (X4). b, Light micrograph of section taken from central region of transplant. Donor epithelium has maintained stratification and has surrounded bits of stromal lamellae (arrows), which resulted from keratectomy procedure (X300). c, Electron micrograph demonstrates extension of cytoplasmic blebs from basal cells of donor epithelium into host stroma (X25,000).

tomized corneas. Four rabbits from each group were killed 24 hr after transplant; these experiments are described as short-term experiments. The remaining transplants, seven to abraded corneas and 17 to keratectomized corneas, were followed for longer periods and are designated as long-term experiments.

Short-Term Experiments

At 24 hr, transplanted epithelial sheets were adherent to both abraded and keratectomized corneas in all animals (Figs. 1, 2). Light and electron micrographs of sections of the central "island" of transplanted epithelium in Figures 1a and 2a are shown in Figures 1b, c, and 2b, c, respectively. The transplanted epithelia maintained their normal stratification (Figs. 1b, 2b) and were tightly adherent to their substrates (Figs. 1c, 2c). In the four transplants onto abraded corneas with denuded basement membranes, hemidesmosomes had formed between the basal cells of the donor epithelium and the host basement membrane (Fig. 1c). In the four transplants onto keratectomized corneas, cytoplasmic extensions or blebs extended from basal cells into the stroma (Fig. 2c). The region of the cornea where the transplant was in place in situ was remarkably clear at 24 hr, on both abraded and keratectomized corneas (Figs. 3a, 4a). Because of the initial success with short-term experiments, additional transplants were done and followed for longer time periods.

Long-Term Experiments

The results of experiments are summarized in Table 1. Of the seven transplants on abraded corneas, six remained adherent for the duration of the experiment. One transplant sloughed between 24 and 48 hr. Eyes 1, 2, and 4 days posttransplant are shown in Figure 3. At 24 hr the cornea is clear in the area of the transplant; by 3–4 days epithelial wound closure...
occurred and all corneas remained clear until the animals were killed. Of the six transplants followed long term, four were harvested at 4 weeks, one at 1 week, and one at 2 weeks. We detected no graft rejection in these allograft transplants. Sections of corneas from the center of the transplant region taken 1 week and 4 weeks posttransplant (Fig. 5) demonstrate normal stratification and tight adhesion between transplanted epithelia and host basal lamina. There were 2.3 hemidesmosomes present per \( \mu m \) membrane after 4 weeks, which did not differ significantly from the 2.26/\( \mu m \) membrane for controls. In no instance did we see goblet cells on corneas of these transplant regions. If host epithelium had replaced graft epithelium at 4 weeks, one would see goblet cells in central regions of the cornea.67

Eyes with epithelial transplants on the keratectomized stroma are shown in Figure 4. Of the 17 transplants to keratectomized corneas, only three remained adherent until the animal was killed. Of these three, one was taken at 6 days, one at 2 weeks, and one at 4 weeks. The remaining 14 sloughed between days 2 and 6. It is not clear whether those that sloughed began to slough centrally, and then slowly (over a 24-hr period) lost all cells, or whether some transplants sloughed as an entire sheet. In seven transplants a central defect was noted, and in the other seven, the transplant was entirely gone 24 hr after the sheet was found to be intact.

Comparison of a 6-day fully adherent transplant on a keratectomized surface with a 6-day transplant that was beginning to slough can be made in Figures 6 and 7. The major difference we noted between the adherent and sloughing epithelium was the presence of extracellular debris between the basal cells and the stroma in the sloughing cornea (compare Figs. 6c–7c). We also found inflammatory cells in and below the epithelial transplants.

The major difference we noted between 24-hr and 6-day transplants on keratectomized surfaces was that
cytoplasmic blebs from basal cells no longer extended into the stroma at 6 days (compare Fig. 2c with Figs. 6c and 7c).

Electron micrographs of the 2- and 4-week transplants on keratectomized surfaces that remained adherent demonstrate partial (2-week, Fig. 8a, b) and complete (4-week, Fig. 8c) basement membrane synthesis and assembly. Numerous keratocytes populated the stroma beneath the 2-week transplant, and the stratification of the epithelium was not normal. In the 4-week transplant, epithelial stratification was normal and keratocyte populations below the transplant were no longer dense.

Discussion

Our data indicate that it is feasible to transplant sheets of corneal epithelium to wounds in vivo and to maintain such transplants. Successful maintenance of the transplants increases if the basement membrane is present on the host cornea. Maintenance of transplants on keratectomies is not as successful. The sloughing of the initially adherent sheet (days 2–6) present on the keratectomized cornea may be due to subsequent retraction of the cytoplasmic blebs that penetrate into the stroma as early as 24 hr. Bleb

Table 1. Results of epithelial transplants followed long term

<table>
<thead>
<tr>
<th>Wound type</th>
<th>No.</th>
<th>Wound closure</th>
<th>No. sloughed</th>
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<tr>
<td>Abraded</td>
<td>7</td>
<td>3–4 days</td>
<td>6</td>
</tr>
<tr>
<td>Keratectomy</td>
<td>17</td>
<td>2–3 days</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Didn’t close</td>
<td>(10)</td>
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Fig. 5. Micrographs of sections through transplants on abraded corneas a, 1 week and b, 4 weeks posttransplant. Light micrograph insets (X300) demonstrate that at both time points the epithelium and stroma appear relatively normal. The nuclei of some of the basal cells are irregularly shaped. Electron micrographs demonstrate the presence of hemidesmosome complexes between donor and host. The number of hemidesmosomes per μm membrane at 4 weeks did not differ from that of normal corneas (X25,000).

Fig. 6. Keratectomized cornea with intact epithelium 6 days after transplant. Epithelial wound healing had been complete since day 3. a, Whole excised cornea demonstrating intact epithelium (X4). b, Light micrograph of section through center of transplant region. Uneven stromal surface from keratectomy is evident and basal cells appear cuboidal (X300). c, Electron micrograph of junction area between basal cell membrane and stroma. Note absence of blebs into stroma (X25,000).
retraction may occur as the cells begin to synthesize and lay down new basement membrane components. New segments of basal lamina have been observed 7 days after 8.5-mm keratectomy wounds in vivo. In fact, Sugrue and Hay have demonstrated that cytoplasmic blebs are retracted by basal cells of freed embryonic chick corneal epithelium in the presence of soluble laminin, a basement membrane component.

Recurrent corneal erosions may be due to the same process. If segments of the basal lamina are lost due to wounding, epithelial cells that cover the wound may initially send blebs down into the stroma. These blebs may be responsible for adhesion of the epithelium for a period of time, but as the cells begin their synthesis and deposition of basal lamina, the blebs may be withdrawn. At this time, when there is a lack of interdigitating blebs and a lack of complete basement membrane with hemidesmosomes, the epithelium may be weakly adherent and subject to erosion.

The time required for complete redeposition of basement membrane after keratectomy in rabbits is 6–8 weeks. In our two transplants that were maintained on keratectomized corneas until animals were killed (one at 2 weeks, one at 4 weeks), basal lamina synthesis appeared to be ahead of schedule. Indeed, in the 4-week transplant, basal lamina deposition was complete. Perhaps this is due to the earlier closing of the epithelial wound that occurred in 3–4 days with transplant, as compared with 10–13 days without transplant.

One of our problems in interpreting our data is that we lack definitive proof that the transplant has not been replaced slowly by host epithelium. During the first 24 hr after placement of the epithelial sheet,
Fig. 8. Micrographs of sections of keratectomized corneas with adherent donor epithelium two weeks after transplant, a, b, upper inset. Light micrograph inset demonstrates many keratocytes below transplant. These actively synthesizing cells, i.e., cell at bottom of a, are laying down new stroma. The epithelium, while normally stratified, has cuboidal basal cells (×300). a and b, Electron micrographs of two regions along juncture between epithelium and stroma. The region in a has no basement membrane and no blebs extending into the stroma. Segments of newly synthesized basement membrane are obvious in b. Where segments are present, hemidesmosomes are present (arrows) (×25,000). Micrographs of sections of keratectomized cornea with adherent epithelium 4 weeks after transplant, c, lower inset. Epithelium from the transplant region appears normally stratified and the stroma has been replaced (×300). Cellularity within the stroma appears normal. The electron micrograph demonstrates that the basal lamina has been replaced and is continuous at 4 weeks. This is approximately 2–4 weeks earlier than in studies in which keratectomies have been done without transplant of epithelium (×25,000).
it is unclear whether the remaining area of epithelial defect surrounding the sheet is covered by host or graft tissue. There is no question that the central area is covered by graft epithelium and the peripheral area by host epithelium initially. Since no epithelial rejection was seen in these eyes, it is also apparent that no obvious takeover of graft epithelium occurred during the 4 weeks the eyes were observed. Whereas it is likely that peripheral host epithelium would eventually take over the donor epithelium, it has been demonstrated that the time for this gradual takeover is 3–6 months or longer following penetrating keratoplasty in rabbits. Therefore, such a takeover would not be seen in the experiments we described, which were only followed up for 1 month. Furthermore, histology showed corneal-appearing epithelium on the corneas at all times postoperatively. Since the original defects were limbal to limbal, healing from the host conjunctival epithelium would have resulted in the histologic stages described previously for healing of conjunctival epithelium over the cornea. In that case, conjunctival-appearing epithelium and goblet cells would have been seen. Therefore, we feel that donor epithelium was retained for at least 1 month in these experiments.

There are several technical difficulties with the procedure reported here. The corneal epithelial sheet is fragile, difficult to handle, and difficult to place precisely, since it must be immersed in fluid to be moved. One can, however, always discern apical from basal surface since the sheet’s edges curl inward toward the apical surface. In summary, our data indicate that it is technically feasible to transplant corneal epithelial sheets to wounds in vivo. Further refinement of the procedure could potentially lead to clinical ocular surface replacement therapy.

**Key words:** corneal epithelial transplantation in vivo, corneal wound healing, ocular surface replacement, rabbit cornea

**References**