A Novel Murine Model for Keratoprosthesis

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PURPOSE. To establish a murine model for keratoprosthesis.

METHODS. A miniature keratoprosthesis (m-KPro) device was created consisting of a poly[methyl methacrylate] front part and a titanium back plate, designed after the Boston KPro, which is in widespread clinical use. BALB/c mice were used and a 2 mm in diameter donor cornea was punched out. After 2-mm trepanation of the syngeneic recipient cornea, extracapsular crystalline lens extraction was performed. The m-KPro was assembled onto the cornea button in a similar manner to human KPro implantation. The cornea-device complex was secured to the recipient bed with eight interrupted 11-0 sutures. All mice ($n = 10$) were followed up for 8 weeks postoperatively.

RESULTS. All m-KPros were successfully implanted and retained in all 10 animals. There were no critical complications such as endophthalmitis, corneal melting, device extrusions, leakage, extensive inflammation, or weight loss in the animals. We observed mild to moderate donor and host corneal neovascularization in all cases throughout the follow-up period.

CONCLUSIONS. We have established a novel murine model of KPro implantation that we anticipate will serve as a good experimental system for evaluating host responses after KPro surgery.

Keywords: keratoprosthesis, murine model

In severe corneal disease, a corneal allograft has been the mainstay of therapy for the last century. However, when a standard penetrating keratoplasty has failed or is unlikely to succeed, an artificial cornea is an alternative given that it is not susceptible to an immune rejection. Currently, the Boston Keratoprosthesis (B-KPro) is the most frequently used such device, often resulting in substantial visual restoration.^{1–8} However, long-term safety has been an issue. Both tissue melt around the device, resulting in leak or extrusion, or devastating endophthalmitis, were common problems in the past but their incidence has decreased substantially in more recent years.^{9–11} The development of retroprosthetic membranes, a more benign complication, has also been reduced to approximately 20% through some recent design modifications such as the titanium back plate.¹² Today, postoperative glaucoma has emerged as the greatest threat to good long-term visual acuity outcomes,^{12–15} particularly after chemical burns, but also in most other patient categories. In addition, other optic neuropathies without glaucomatous cupping can occur. The mechanisms remain obscure, but may be related to chronic postoperative inflammation, difficult to detect clinically in these often very diseased eyes. Postoperative complications, such as epiretinal membrane, macular edema, and retinal detachment,^{16–18} may likewise be caused by chronic inflammation. However, systematic studies of the pathophysiologic mechanisms that lead to these complications have been hampered owing to the difficulties encountered in clinical studies, where numerous confounding factors and comorbidities make it difficult to study

mechanisms. The development of a standard and validated animal model would address these shortcomings.

To date, most animal B-KPro research regarding design and retention has for convenience been performed on eyes of rabbits^{19–21} because of similar size and dimensions compared to human eyes. However, the modern study of immunoinflammatory responses is best done in the mouse where access to inbred mice with well-defined immune and genetic variability, as well as extensive access to immunologic reagents, makes it an optimal species for immunologic research. The purpose of the present study was to develop a novel murine model of the B-KPro.

METHODS

Animals

A total of 15 BALB/c male mice (8–10 weeks old; Jackson Laboratory, Bar Harbor, ME, USA), 10 used as recipient and five as donor, were used in this study. They were housed in a climate-controlled animal facility at the Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, United States, and kept under cyclic light conditions (12 hours on/off). All animal experiments were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

FIGURE 1. Design and dimensions of the m-KPro. (A) The m-KPro consists of a poly[methyl methacrylate] front plate (2×) and a titanium back plate. (B) m-KPro size in relation to a pencil tip. Dimensions of the front plate (C) and the back plate (D) are shown.

Device

A miniature keratoprosthesis (m-KPro) (JG Machine, Wilmington, MA, USA) was designed, minimized, but otherwise identical to the B-KPro device used in humans.²² It consisted of a poly[methyl methacrylate] (PMMA) front plate with central stem and a titanium back plate with eight peripheral holes (Figs. 1A, 1B). The dimensions are given in Figures 1C and 1D.

Procedure

The surgery was performed under a surgical microscope (Zeiss, Jena, Germany). All procedures were performed by a single investigator (MO), with a surgical time of 25 to 30 minutes per procedure. After euthanasia of the donor mice, corneas were marked with a 2-mm-diameter biopsy punch (Miltex, Plainsboro, NJ, USA). To maintain a deep anterior

chamber, the viscoelastic (Viscoat; Alcone, Irvine, CA, USA) was injected through the groove with a 30-gauge cannula (Rumex, Clearwater, FL, USA). The cornea was excised with Vannas scissors (Storz Instruments Company, San Dimas, CA, USA). To prepare for the assembly of the m-KPro device, donor corneas were trephined centrally with a 0.5-mm-diameter punch (Harris UniCore punch; Ted Pella, Inc., Redding, CA, USA), and then slid over the stem of the PMMA front plate. The titanium back plate was positioned and locked on the stem such that the donor cornea became securely sandwiched between the front and back plates (Fig. 2A). The donor-device complex was then placed in phosphate-buffered saline (PBS) until the host corneal bed had been prepared.

Recipient mice were anesthetized by intraperitoneal injection of ketamine (120 mg/kg) and xylazine (20 mg/kg). A drop of tropicamide 1% (Bausch & Lomb, New York, NY, USA) and a drop of 0.5% phenylephrine hydrochloride (Altaire Pharmaceu-

FIGURE 2. Surgical procedure of the m-KPro implantation. (A) The titanium back plate was positioned and locked on the stem such that the donor cornea became securely sandwiched between the front and back plates. The donor-device complex was then placed in PBS until the host corneal bed had been prepared. (B) The host cornea was marked with a 2-mm-diameter biopsy punch. (C) The groove was performed with a 30-gauge needle and the viscoelastic was injected through the groove with 30-gauge cannula. (D) The cornea was excised with Vannas scissors. (E) A curvilinear continuous capsulorhexis was performed with a jeweler's forceps. (F) A hydrotomy was performed with a 30-gauge cannula and PBS to deliver the lens en bloc. (G, H) The donor cornea-m-KPro complex was placed in the recipient bed and secured with eight interrupted 11-0 nylon sutures in a standard penetrating keratoplasty fashion. (I) A mouse with implanted m-KPro.

FIGURE 3. Clinical observations after implantation of the m-KPro, using slit-lamp microscopy. (A) Moderate host and donor corneal neovascularization (arrowhead) was observed (week 8). (B) To assess the surface for leakage, fluorescein was applied and no leakage of the aqueous humor was detected (week 2). (C) Anterior chambers remained deep after m-KPro implantation (arrow, week 2).

tics, New York, NY, USA) were applied to the right eye to dilate the pupil 15 minutes before the procedure. The cornea was marked with a 2-mm-diameter biopsy punch (Fig. 2B). Before opening the eye, viscoelastic was injected via a 30-gauge cannula (Fig. 2C). The cornea was excised with Vannas scissors (Fig. 2D). Throughout the procedure viscoelastic was used to maintain the depth of the anterior chamber. The host lens was removed as previously described.²³ Briefly, a curvilinear continuous capsulorrhexis was performed with a jewelers' forceps (Katena, Denville, NJ, USA) (Fig. 2E) and a hydro-dissection was performed with a 30-gauge cannula and PBS to deliver the lens en bloc (Fig. 2F). Visible cortex remnants were carefully aspirated through the cannula. The donor cornea-m-KPro complex was then placed in the recipient bed and secured with eight interrupted 11-0 nylon sutures (Sharpoint; Angiotech Pharmaceuticals, Vancouver, BC, Canada) in a standard penetrating keratoplasty fashion (Figs. 2G-I).

One drop of 1% corticosteroid (Pred Forte; Allergan, Irvine, CA, USA) and antibiotic ointment (AK-POLYBAC; Akorn, Lake Forest, IL, USA) were applied and a tarsorrhaphy was performed by using 8-0 nylon sutures (Sharpoint). The tarsorrhaphy was removed 48 hours after the procedure, and the same antibiotic and steroid drops were administered once a day for 7 consecutive days following tarsorrhaphy removal. One week following m-KPro implantation, corneal sutures were removed. As per standard institutional animal care and use protocols, 0.1 mg/kg buprenorphine (Reckitt Benckiser Healthcare [UK] Ltd., Hull, England) was applied subcutaneously after the procedure and every 12 hours for 48 hours postoperatively.

The mice were examined for the first time 48 hours after the implantation (following tarsorrhaphy removal), then every day for the first week and once a week thereafter. Animal behavior, weight, and signs of inflammation of the conjunctiva and the anterior chamber (AC), such as redness, vascular injection, discharge, and AC reaction, were evaluated. In addition, fluorescein (Fluorets, Chauvin Pharmaceuticals Ltd., Surrey, UK) was applied to assess the surface for leakage and epithelial defects right after tarsorrhaphy removal (48 hours post operation) and at weekly intervals thereafter.

Images of the eyes were taken by using a slit-lamp with a camera (Nikon D100, Tokyo, Japan). Images of the anterior segment were taken by using anterior segment-optical coherence tomography (AS-OCT; Bioptigen, Durham, NC, USA). The mice were euthanized at week 8 postoperatively.

Histology

After euthanasia, the eyes were removed by dissecting the conjunctiva, cutting the extraocular muscles and the nerve. Then the donor cornea, together with the rim of the host cornea, was removed and cut radially on one side with a straight ophthalmic knife (Mani, Utsonomiya, Japan); m-KPros were removed through the cut without disassembling and the

host eyes were embedded in OCT compound (Sakura Finetek, Torrance, CA, USA) and frozen at -80°C . Five-micrometer-thick serial sections of the eyes were cut in the sagittal plane on a microkeratome (TBS, Durham, NC, USA) and prepared for staining on slides. To analyze ocular tissue morphology, we performed hematoxylin-eosin staining and observed it under the microscope (Olympus BX41, Tokyo, Japan).

RESULTS

Clinical Observations

The m-KPros were successfully implanted and retained in all animals ($n = 10$). No critical complications, such as endophthalmitis, corneal melting, device extrusion, epithelial defects, or weight loss, were observed in any of the animals throughout the follow-up period (from 48 hours through 8 weeks post implantation). We observed moderate host and donor corneal neovascularization in all cases throughout the follow-up period (Fig. 3A). No leakage of aqueous humor was detected after application of fluorescein (Fig. 3B), and anterior chambers were deep upon slit-lamp examination (Fig. 3C).

Anterior Segment-OCT

Anterior segment-OCT imaging revealed that m-KPros were retained in the appropriate anatomic location, the host cornea maintained normal structure (Fig. 4A), and the anterior chamber remained deep (Fig. 4B). Membranes originating at the graft-m-KPro junction were observed pulling the iris anteriorly in three eyes (Fig. 4C). Elschnig pearl-like formations were observed in all eyes (Fig. 4D).

Histology

To analyze structural ocular tissue changes, we performed hematoxylin-eosin staining 8 weeks after implantation. The host eyes showed normal morphology and structure. No epithelial defects or corneal thinning was observed. The iris maintained a normal structure and was partially fused with the anterior capsule. The posterior capsule was intact and a homogenous structure likely consisting of regenerating lens fibers was visible filling the space between anterior and posterior capsule. The vitreous cavity was clear and the retina appeared normal, with no retinal detachment.

DISCUSSION

In this study, we successfully implanted m-KPros into 10 murine eyes and observed retention of the device in all implanted eyes. The surgical procedures performed in mice were identical to the procedure in humans, and materials used for the device were comparable to those in clinical usage²²

FIGURE 4. AS-OCT. (A) AS-OCT imaging at week 7 confirmed that m-KPros were retained (*asterisk*), the host cornea maintained normal structure, and (B) the anterior chamber remained deep. (C) Membranes originating at the graft-m-KPro junction pulling the iris anteriorly were visible (*arrowhead*). (D) Elschnig pearl-like formations (*arrow*) were observed in all eyes.

(Fig. 2). We thus established a novel murine model of KPro implantation that we anticipate will serve as a good experimental system for evaluating host responses after KPro surgery.

To our knowledge, this procedure, which is virtually identical to human KPro surgery, is the first study to report KPro implantation in small rodents. Our study demonstrated the feasibility of a murine model of KPro implantation and may allow for future investigations of the host response to this device.

One aspect of our study was to assess the impact of the implanted device on the host eye. We observed moderate neovascularization of the host and donor cornea in the m-KPro-implanted eyes, but no other complications such as epithelial defects, corneal melting, leaking, device extrusion, extensive inflammation, or endophthalmitis (from 48 hours through 8 weeks post implantation). Using OCT, we observed membranes in the AC, pulling the iris anteriorly, but the AC remained deep. These membranes appeared to originate at either the graft-m-KPro junction or graft-host junction, and may be the analog to fibrin membranes or possibly retroprosthetic membranes in humans. Homogenous Elschnig pearl-like structures were observed in the space between the lens capsules, most likely originating from residual equatorial lens epithelium cells that migrated and proliferated in the space between the anterior and posterior lens capsule as described before.^{24,25} Lens epithelial cells often remain in the capsular bag after lens removal, and are known to regenerate rapidly.²⁵ Future studies in this model may need to consider the potential inflammatory role of these cells. Importantly, the posterior capsule remained intact, maintaining the barrier between the anterior and posterior segments of the eye, equal to what surgeons aim for in humans.

Since most of the long-term, vision-threatening complications following KPro implantation affect retinal cells (glaucoma, optic nerve atrophy, epiretinal membrane, macular edema, etc), we were primarily interested in assessing the host retina following m-KPro implantation. Histologic examination confirmed that the retina remained grossly unaffected up to 8 weeks post KPro implantation.

However, more detailed analysis, including later time points, are required to further study the effects of KPro surgery on the retina and optic nerve. Our results indicate the stability and utility of our model as an experimental tool for KPro implantation.

This novel murine model of KPro implantation described in the present study will hopefully allow for immunopathologic investigations of the host response to KPro implantation, in particular in the chronic stage, well after the acute wound-healing response has completed.

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