Biological Response to a SupraDescemetic Synthetic Cornea in Rabbits

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Objective: To evaluate the biocompatibility of a novel nonpenetrating keratoprosthesis (supraDescemetic synthetic cornea) in a rabbit model.

Methods: Seven rabbits received a supraDescemetic synthetic cornea (7-mm diameter, 350-µm-thick optical zone, 100-µm-thick peripheral flange) in their healthy right eyes. A surgical technique was developed that allowed implantation of the device on top of the bare Descemet membrane. Three rabbits received a supraDescemetic synthetic cornea made of hydroxyethyl methacrylate–methyl methacrylate26, 1 received a hydroxyethyl methacrylate–N-vinyl pyrrolidone75 mesoplant, and 3 were implanted with devices made of polymethyl methacrylate. All rabbits were euthanized after 8 weeks; the eyes were enucleated and examined by conventional histological and immunohistochemical evaluations.

Results: All eyes became quiet within several days. The Descemet membrane remained transparent during the observation period. Indirect ophthalmoscopy performed through the prosthesis allowed accurate examination of the posterior pole. Histological evaluation of the implanted corneas displayed no signs of an acute or chronic inflammatory reaction to the supraDescemetic synthetic cornea in 5 eyes; a few inflammatory cells were detected in the corneas of 2 rabbits. The interface between the Descemet membrane and the mesoplan displayed ingrowth of very thin (<10-µm) tissues colonized by kerocytes in 3 of the 7 corneas.

Conclusions: This study validates the biocompatibility of this new type of nonpenetrating keratoprosthesis. Because opening of the anterior chamber is not required with the supraDescemetic synthetic cornea, the risk for intraocular infection is minimal, and the implantation procedure is less traumatic compared with a penetrating device.

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METHODS

SYNTHETIC CORNEA

Three different materials were used for the synthetic corneas tested: polymethyl methacrylate.
(PMMA; n=3), hydroxyethyl methacrylate–methyl methacrylate (HEMA-MMA; n=3), and hydroxyethyl methacrylate–N-vinyl pyrrolidone (HEMA-NVP; n=1), the latter 2 with a water content of 26% and 75%, respectively. All implants were 7 mm in diameter and had the same design, adjusted to the dimensions of the rabbit’s cornea (Figure 1): a thicker central optical zone was surrounded by a thinner outer flange with perforations, which would allow nutrition transfer and tissue ingrowth for improved fixation of the implant. The central part had a diameter of 4.5 mm and a thickness of 350 µm. Both anterior and posterior curvatures measured 8 mm. The outer flange showed a thickness of 100 µm. The transition zone between the central optical part and the peripheral part was made conical. All implants were produced and provided by Cornéal Laboratoires (Paris, France).

SURGICAL PROCEDURE

The study was conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and the study protocol was approved by the University of Miami School of Medicine Animal Care and Use Review Board.

Implantation of an artificial cornea was performed in the right eyes of 7 female New Zealand White rabbits with 3.0 to 4.7 kg in body weight. The animals were anesthetized with an intramuscular injection of a ketamine-xylazine-acepromazine mixture (35 mg/kg, 5 mg/kg, and 0.75 mg/kg, respectively).

Ultrasonic pachymetry was performed in the outermost corneal periphery next to the limbus. A 7-mm curved incision along the limbus was performed from 9 to 11 o’clock in the clear cornea, at a calculated 85% of the corneal depth using a diamond knife with double footplate and adjustable blade. An intralamellar dissection was made using a disposable knife (SatinCrescent Knife, bevel down; Alcon Laboratories Inc, Fort Worth, Tex) and a blunt curved spatula (K3-3000; Katena Products Inc, Denville, NJ). A custom-made, round-shaped metal plate with a diameter of 6.5 mm was inserted in the created pocket, followed by trephination of the corneal tissue above the plate using a handheld custom-made 3.7-mm vacuum trephine (Figure 2A). The remaining stromal layers were removed by gentle tearing and cutting. Once a small patch of the DM was exposed, a fine spatula shaped like blunt wire (Straight spatula, 0.25 mm; Rumex International Co, Miami, Fla) was carefully slid along the plane between the DM and the remaining stroma. The stroma was then lifted and cut away until the DM was completely exposed to the edges of the trephination (Figure 2B). The synthetic cornea was inserted via the incision; the optical part was positioned on top of the DM and the peripheral skirt placed in deep corneal stroma. Three PMMA, 3 HEMA-MMA26, and 1 HEMA-NVP75 devices were implanted in this series. The incision was closed using 3 interrupted 10-0 nylon sutures. Surgical procedures were performed by 2 of the authors (J.S., V.F.). Dexamethasone with neomycin and polymyxin B ointment (Maxitrol; Alcon Laboratories Inc) was topically applied twice daily for 3 days, starting at the end of surgery. No local or systemic treatment was administered after the third postoperative day. Slitlamp exami-
nation was performed on the first 3 postoperative days and on a weekly basis thereafter. Slitlamp photography was done during each examination. All sutures were removed 1 week after surgery. Prior to euthanasia of the rabbits, the clarity of the remaining tissue was judged, and indirect ophthalmoscopy of the posterior segment was performed, using a 90-diopter lens.

Eight weeks after surgery, all animals were euthanized with a lethal dose of pentobarbital and phenytoin (Eutasol; Diamond Animal Health Inc, Des Moines, Iowa) administered intravenously through the marginal ear vein.

**HISTOLOGICAL EVALUATION**

The operated eye of each rabbit was immediately enucleated and placed in 10% buffered formaldehyde for at least 24 hours. Corneas, including the mesoplant, were removed and further processed for histological evaluation. Sections were stained with hematoxylin-eosin, periodic acid-Schiff, and Masson trichrome techniques.

To determine the actual extent of epithelial downgrowth into the cornea-polymer interface in each animal, immunohistochemical evaluation was performed using a pan-specific cocktail of antibodies displaying primary reactivity with cytokeratins: AE1/AE3, 34BE12 (Dako Corp, Carpinteria, Calif), and CAM5.2 (BD Biosciences, San Jose, Calif).

**CONTROL**

To determine if the DM plane was actually reached using the technique previously described, surgery was performed also on the contralateral left eyes of 4 rabbits immediately before planned euthanasia, the fragility of the bare DM not permitting prolonged follow-up unless protected by the synthetic cornea. Three of those corneas were processed for light microscopic analysis. The surface characteristics of the exposed membrane were studied on the fourth eye using scanning electron microscopy.

**RESULTS**

**CLINICAL OBSERVATION**

All rabbits showed an uneventful initial postoperative phase. All eyes became quiet on the first or second postoperative day. The DM was found to be firmly attached to the posterior surface of the implant and was detectable by careful slitlamp examination. The corneal tissue overlying the outer flange showed slight edema postoperatively that resolved within 2 weeks, resulting in a more continuous transition between the cornea and the implant's optic. Neovascularization of the cornea did not occur in any of the operated eyes within the observation period. The implants maintained their optical transparency and displayed no alterations in their outer surface (Figure 3). The last layer of the corneal stroma could not be removed completely in the area of the trephination edge at the time of surgery in each of the cases. Those remnants were detectable postoperatively between the implant and the DM, appearing as a narrow circular band next to the edge of the trephination. With time, opacification of these remnants slightly increased, displaying a very slow progression toward the optical center. The central part of the denuded DM remained transparent in all rabbits that received PMMA or HEMA-MMA26 implants. When we inserted the HEMA-NVP75 implant, a small bundle of loose stromal tissue was accidentally implanted between the synthetic cornea and the DM, which resulted in minor opacification in its surrounding areas with time.

The cornea above the sDSC flange showed continuous retraction with time, leaving the inner part of the flange uncovered. This was the case for both PMMA and the softer hydrophilic materials tested, occurring mostly at the 6- and 12-o’clock positions. Tissue retraction did not reach the flange openings in any of the cases. Indirect ophthalmoscopy performed prior to euthanasia allowed very accurate examination of the posterior pole with vascular details discernible through the artificial cornea (Figure 4), even in those few rabbits that displayed minor opacification of the DM-sDSC interface.

**HISTOPATHOLOGICAL EXAMINATION**

All mesoplants appeared to be well tolerated by the corneal tissue. A few inflammatory cells (neutrophils) could...
be detected at the trephination edge of 1 cornea that had received a mesoplant; 1 cornea with a HEMA-NVP75 mesoplant showed some clusters of inflammatory cells underneath. None of the other corneas displayed any noticeable signs of acute or chronic inflammatory reaction. Epithelial thinning up to 2 layers was found on top of areas above the sDSC flange. Trephination edges, on the other hand, showed marked thickening of the epithelial layer; however, epithelial coverage could not be detected on the anterior surface of the optic itself. Immunohistochemical examination displayed a tendency of the corneal epithelium to grow backwards onto the upper surface of the flange (Figure 5A). In 1 of the eyes, epithelial ingrowth reached the posterior surface of the flange (Figure 5B) but did not extend into the interface between the DM and the sDSC optic. The flange openings were filled with newly synthesized collagen tissue, lined partially by epithelial cells (Figure 6A). The interface between the DM and the mesoplant displayed ingrowth of very thin (<10-μm) keratocyte-containing tissue in 3 of the 7 corneas (1 × PMMA, 1 × HEMA-MMA26, and 1 × HEMA-NVP75). In all others, the DM was still found to be in direct contact with the polymer (Figure 6B, C). The endothelium was found to be healthy and normal in all of the rabbits.

CONTROL

Histological examination showed a completely exposed DM, without any residual stromal layer remaining on top. With scanning electron microscopy, the outer surface of the DM appears as an extremely smooth and even structure (Figure 7); even with high magnification (×10000), no structural roughness could be discovered.

COMMENT

The concept of implanting a lamellar KPro was described by Stone five decades ago.22-23 His devices were made of PMMA and were implanted in rabbit eyes using a 2-stage procedure. The KPro’s were inserted in a corneal pocket, trephining the central upper part of the cornea after a sufficient time interval when firm fibrosis had developed at the implant’s perforated periphery. In this series, a 1-stage procedure was used; no mesoplant was lost spontaneously in the early postoperative phase as fibrous ingrowth in the flange openings occurred within a few weeks, providing excellent fixation of the sDSC within the host cornea. However, ongoing retraction of the corneal tissue above the peripheral flange was noticed, jeopardizing the stability of the mesoplant with time. With the KPro periphery acting as a barrier, a disrupted or restricted nutrient flow to keratocytes anterior to the flange could eventually compromise their homeostasis. In addition, enzymatic degradation of the collagen24 could finally lead to the observed tissue reduction. This phenomenon was found less frequently and was delayed in cases where the trephination edge became vascularized (J.S., P.D.L., J-M.P., and E.A., unpublished data, 2002). This suggests a potential benefit of neovascularization for the long-term survival of corneal tissue located above a material with restricted permeability. Vascularized corneas were found to be much less likely to melt after KPro implantation compared with avascular corneas,25 probably because vessels provide proteolytic enzyme inhibitors and nutrients essential for tissue preservation. However, mesoplant presence has not stimulated neovascularization within the observation period in this series. Because vascularization of the fixation material seems to be desirable, coverage with a conjunctival flap might prevent melting in cases of nonvascularized host corneas. A central opening above the KPro optics could then be performed following integration of the conjunctiva, similar to the procedure proposed for the AlphaCor.28 The material itself was tolerated very well by the host tissue, and a toxic reaction, as previously reported for other polymers,29 could not be detected. Biocompatibility of the soft polymers used in this study was tested in an earlier study.27 Small disks of the materials implanted in intrastromal pockets demonstrated some cellular reactions at the sites of mechanical stress, but no major cellular reaction against the implant material itself could be detected. Although PMMA has good optical property, it has disadvantages when used as material for synthetic corneas because of its rigid nature. Both HEMA-MMA26 and HEMA-NVP75 are found to be soft and flexible. Biocompatibility has been defined as the ability of a material to perform with an appropriate response in a specific application. Although the International Organization for Standardization recommends longer test periods, up to 78 weeks for long-term biocompatibility testing of novel biomaterials, our results strongly indicate that those hydrophilic polymers are well tolerated by the corneal stroma and are suitable as material for sDSC KPro.

Ingrowth of a thin membrane of fibrous tissue underneath the optics occurred in several rabbits of our series even within the relatively short observation period of 8 weeks. One might also speculate on the incidence and intensity of fibrous ingrowth after a longer period. However, because the regenerative capacity of the rabbit’s eye tissue is found to be greater compared with that of human eye tissue,28-29 scarring of the DM-polymer interface might actually be less frequent when the sDSC is implanted in patients. Following opacification underneath a synthetic polymer, the actual visual acuity that could be achieved with such a lamellar KPro might there-
fore be lower compared with those with penetrating devices. But on the other hand, the increase in safety in the long run—as there is no need to enter the anterior chamber of the eye—might compensate for a potentially lower but still useful visual acuity. Retinal detachments could not be found in any of our rabbits at the end of the observation period, in contrast to animal studies on perforating KPro’s, which reported retinal detachments in a high number of rabbits following implantation.30 In cases of severe interface scarring, removal of this membrane with both the DM and the endothelial layer might be an option for regaining visual acuity, using instruments similar to those already described for deep lamellar endothelial keratoplasty.31 Another surgical approach for sDSC implantation was tested in an earlier study, inserting the device in such a way that its complete base (base and flange) was located on the bare DM.32 This technique could theoretically postpone fibroblast ingrowth into the DM-polymer interface from the side. However, those mesoplants showed a postoperative tendency for decentration and instability, most likely because tissue ingrowth into flange openings could
just occur for one (the stromal) side and might therefore not be as strong as with the implantation technique previously described.

Although the results of this study are promising, one has to consider that all mesoplates have so far been implanted in healthy and clear corneas. Because the biological response might differ in diseased and vascularized corneas (eg, after corneal burns), it seems advisable to further study sDSC reliability and long-term stability in an appropriate animal model for these conditions before proceeding to human trials.

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