Development of a Newly Designed Double-Fixed Seoul-Type Keratoprosthesis

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Objective: To develop a newly designed double-fixed keratoprosthesis (Seoul-type keratoprosthesis [S-KPro]) and to assess its mechanical stability and biocompatibility.

Methods: Twenty-five rabbits were divided into 4 groups by fixation technique, amniotic membrane (AM) implantation, and skirt material. The eyes were studied with the use of slitlamp, light, and electron microscopy. Stress testing was performed. In addition, 2 human subjects underwent S-KPro implantation. Best-corrected visual acuity was checked, and ophthalmic examination was performed.

Results: The average retention period of the group receiving double-fixated polyurethane–S-KPro with AM was longer (>24 weeks) than that of the others. Fibroblast invasions were found in polyurethane pores but not in polytetrafluoroethylene (Gore-Tex) pores on light microscopy. The minimal pressure that induced aqueous leakage was greater than 250 mm Hg in all of the tested eyes. Two human subjects have maintained a good postoperative condition for 18 and 8 months.

Conclusions: The double-fixation technique of applied S-KPro and AM appears to be helpful in improving the stability of the keratoprosthesis. Polyurethane with relatively large pore size (40 µm) may be used successfully as a material for the keratoprosthesis skirt.

Clinical Relevance: Our results may be important for improving the clinical outcome of keratoprosthesis.

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PENETRATING keratoplasty has been used successfully to treat many corneal diseases. However, several diseases, including ocular cicatricial pemphigoid, chemical burns, Stevens-Johnson syndrome, ectodermal dysplasia, and mucolipidosis IV, are still untreatable using the procedure. In such cases, the only alternative for visual rehabilitation is the implantation of a keratoprosthesis. The first human implantation was attempted in 1859. Although keratoprostheses have improved significantly since, the failure incidence remains high. The main problem is one of bioincompatibility between the implant and the surrounding tissues, which results in vitritis, retroprosthetic membrane, endophthalmitis, and extrusion. Microporous, biocompatible materials have been shown to improve the long-term stability of keratoprostheses. Porous polytetrafluoroethylene (PTFE), for example, has been reported to be suitable material for the skirt of keratoprosthesis. A new fluorcarbon polymer also showed excellent tolerance and a high degree of tissue adhesion and colonization.

We herein report the development of the newly designed Seoul-type keratoprosthesis (S-KPro), which consists of an optic portion made of polymethyl methacrylate (PMMA), a skirt of expanded PTFE (e-PTFE) or polyurethane, and haptics of monofilament-polypropylene (Prolene; Ethicon Ltd, Edinburgh, Scotland). The main difference between the conventional keratoprosthesis and S-KPro is the method of fixation to the eyeball. In the case of S-KPro, the skirt is anchored to the cornea, and the polypropylene haptics are anchored to the sclera to improve its biostability. Kim and Tseng reported that the transplantation of preserved human amniotic membrane (AM) was useful for surface reconstruction in severely damaged rabbit corneas. In our study, the effects of double fixation and AM transplantation on the biostability of S-KPro were investigated.

RESULTS

CLINICAL OBSERVATIONS OF ANIMAL EXPERIMENTS

Group 1

Of the 10 eyes undergoing implantation, 8 eyes were followed up for a minimum of 2 months. One rabbit died 3 weeks post-
MATERIALS AND METHODS

SEOUL-TYPE KERATOPROSTHESIS

The S-KPro consists of 3 parts (Figure 1 and Figure 2): a long cylindrical optic surrounded by a mushroom-shaped anterior flange, a skirt for corneal fixation, and haptics for scleral fixation. The optic is made of PMMA, 4 mm in diameter and 4 mm in length; the anterior flange consists of a fluorinated silicone about 0.2 mm thick and 6 mm in diameter; the skirt, e-PTFE (Gore-Tex; W. L. Gore and Associates, Inc, Flagstaff, Ariz) or polyurethane 0.4 mm thick. Pore sizes were 20 µm in the e-PTFE and 40 µm in the polyurethane. Apart from the different material in the skirt, polyurethane–S-KPro differs from e-PTFE–S-KPro in that the tear stabilization is improved by a surface modification with ion-grafting polymerization, and that dark brown color was chosen for the anterior flange portion to mimic the oriental iris color. The skirt, with a diameter of 10 mm and a width of 3 mm, was attached to the rear surface of the anterior flange using n-butyl-2-cyanoacrylate (Histoacryl; B. Braun, Melsungen, Germany). The polypropylene haptics, which are used for internal scleral fixation, are a pair of U-shaped haptics that are passed through the periphery of the PMMA cylinder using ultrasonic energy.

PREPARATION OF HUMAN AM

Human placentas were obtained shortly after cesarean deliveries and prepared as previously described. Results of serological examinations for human immunodeficiency virus, hepatitis B surface antigen, and VDRL test proved negative. Discs 1.5 cm in diameter were cut and frozen at −70°C in the mixture of glycerol and minimal essential media.

METHODS OF FABRICATING POLYURETHANE MEMBRANE

We adopted the solvent-casting particulate-leaching method, which was cheaper than other techniques to fabricate polyurethane and allowed control of polyurethane membrane pore size. The principle steps were as follows. (1) Polyurethane pellets (Pellethane® 2363-80AE; The Dow Corporation, Midland, Mich) were extracted with methanol for 3 days to remove low-molecular-weight components. (2) The methanol-extracted polyurethane pellets were dissolved in N,N-dimethylacetamide (Junsei Chemical Co Ltd, Tokyo, Japan) to produce 14% (weight/volume) solution. (3) An analytical mill (model A-10; Tekmer, Cincinnati, Ohio) was used to grind granular sodium chloride and to segregate the following sizes: diameter of less than 53 µm, between 53 and less than 106 µm, and between 106 and 150 µm. (4) Sieved salt particles were added to the solution and then whirled to achieve dispersion. Three different polymer-salt compositions were used in our study: 1 g of solution per 1.5 g of salt (salt weight fraction of 60%), 0.75 g of solution per 1.75 g of salt (salt weight fraction of 70%), and 0.25 g of solution per 2.25 g of salt (salt weight fraction of 80%). (5) The vortexed dispersion was cast in a 5-cm Petri dish, and the membrane thickness was determined by means of solution volume. (6) The composite membranes were dried using a vacuum dryer. (7) Salt leaching was performed using tertiary distilled water.

We adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research throughout this study. The right eyes of 20 New Zealand white rabbits weighing 2.0 to 3.0 kg were implanted with the keratoprostheses. The rabbits were anesthetized using ketamine hydrochloride (Ketalar) at a dose of 30 to 45 mg/kg and xylazine hydrochloride (Rompun) at a dose of 5 to 10 mg/kg. Proparacaine hydrochloride (Alcaine) was used for topical anesthesia.

The rabbits were then divided into the following 4 groups (Figure 3): (1) e-PTFE–S-KPro was implanted with AM transplantation in 10 eyes (group 1); (2) e-PTFE–keratoprosthesis without haptics was implanted with AM transplantation in 5 eyes (group 2); (3) e-PTFE–S-KPro was implanted without AM transplantation in 5 eyes (group 3); and (4) polyurethane–S-KPro was implanted with AM transplantation in 5 eyes (group 4).

The surgical technique for S-KPro implantation was as follows. After dilation of pupil, half-thickness corneal trephination 6 mm in diameter was performed, and 360° operatively, but the implanted S-KPro was well positioned, without retinal detachment. Another S-KPro was partially extruded suddenly at 9 weeks; the probable cause of the extrusion was incidental trauma, probably caused by scratching.

All S-KPros were well tolerated, and transplanted AMs were translucent at 1 week. Few inflammatory cells were shown in a chamber behind all of the S-KPros. At 2 or 3 weeks, the most prominent change was the appearance of neovascularization, which started from the limbus and grew toward the center. At this time, the AM was partially dissolved and the fundus red reflex was normal. Retinoscopic refraction showed hyperopia of about 10 diopters (D). At 5 to 7 weeks, peripheral retinal detachment was observed in most cases, but the S-KPros were well retained. After 8 weeks, the retinas were found to be totally detached.

One eye was enucleated for histological examination at 8 weeks. The interface between the e-PTFE skirt and the cornea looked smooth on gross examination; however, a closed, funnel-shaped total detachment of the retina was observed posterior to the S-KPro. No retroprosthetic membrane formation was observed. Another rabbit was killed humanely for stress testing at 8 weeks.

In 2 rabbits, the e-PTFE skirts were slowly protruding from the surrounding cornea with partial melting of the anterior flap of the corneal pocket at 10 weeks; however, no leakage was observed. The S-KPros were extruded at 12 weeks and 13 weeks, respectively.
intrallamellar dissection was performed with a disposable crescent blade to make an intrastromal pocket 2 mm in length. After excision of trephinated cornea, capsulorhexis of the lens capsule was then performed, and a nucleus was removed. The residual cortex and a part of the anterior vitreous were removed with the use of a vitreous cutter, and a sector iridectomy was then performed. The S-KPro was put into an eyeball, and haptics were fixed to each side of the sclera with 10-0 polypropylene suture from the inside out (ab interno technique). The skirt was inserted into the previously prepared corneal pocket, and eight 10-0 nylon interrupted sutures were placed between the anterior lamellar part of the cornea and the skirt. Cryopreserved AM covered the surface of the keratoprosthesis. At the end of operation, gentamicin sulfate, 20 mg, and dexamethasone sodium phosphate, 20 mg, were injected subconjunctivally. Tarsoorrhaphy was performed to prevent the AM from drying out. Postoperatively, topical dexamethasone with polymyxin B sulfate and neomycin sulfate (Maxitrol; Alcon-Courvreur, Puurs, Belgium) and oxytetracyclin hydrochloride with polymyxin B sulfate (Teramycin; Pfizer Inc, New York, NY) ointments were administered twice a day for 1 month.

**HUMAN CASES**

We confirm that the research followed the tenets of the Declaration of Helsinki and that informed consent was obtained from the patients after being given an explanation of the nature and possible consequences of the study. The first patient who received an e-PTFE–S-KPro implantation had a diagnosis of Stevens-Johnson syndrome; she had had a corneal perforation. The second patient, who had a diagnosis of chemical burn, underwent polyurethane–S-KPro implantation.

**EVALUATION OF BIOSTABILITY OF KERATOPROSTHESIS IN IMPLANTED EYES**

**Clinical Observation**

The implanted eyes were examined on a weekly basis for evidence of complications such as melting, aqueous leakage, retroprosthetic membrane formation, retinal detachment, or proliferative vireo-retinopathy by means of slit-lamp microscopy and indirect ophthalmoscopy. The maximal retention periods of the keratoprosthesis in the implanted eye were recorded. Rabbits that were killed humanely at 8 weeks for histological examination and stress testing and the rabbit that died during follow-up were excluded from the calculation of retention time.

**Stress Testing**

At postoperative 8 weeks, the stress test was undertaken in 1 eye of each group with the use of a specially designed device. An infusion cannula was inserted into the sclera 4 mm posterior to the limbus. It was connected to a bottle of isotonic sodium chloride solution by an intravenous line. A pump was connected to the air portion of the bottle with another intravenous line, and a manometer was connected using a 3-way stopcock in the middle of the line. Intracocular pressure was increased in stages by 50 mm Hg every 5 minutes, and the intraocular pressure that first induced aqueous leakage around the keratoprosthesis was recorded.

**Histological Examination**

The S-KPro–inserted eyes were enucleated at 2 months and fixed in 10% buffered formaldehyde. Two-micrometer sections were stained with hematoxylin-eosin and with Masson trichrome. Sections were also prepared for immunohistochemical experiments, dehydrated in upgraded ethanol (70%, 95%, and 100%), cleared in xylene, and embedded in paraffin. Each cornea was sectioned at 4 to 6 µm, mounted on poly-l-lysine–coated glass slides, and air-dried overnight at room temperature. The sections were dewaxed with alcohol, and then immersed in methanol with 0.5% hydrogen peroxide to block endogenous peroxidase activity. They were then immersed in water and immunostained (ABC method; DAKO, Copenhagen, Denmark) with primary vimentin incubations for 1 or 18 hours (ie, overnight) at a range of dilutions. 3,3'-Diaminobenzidine–hydrogen peroxide was used as a chromogen, and methyl green as a counterstain. In group 4, the cornea was fixed for 2 hours in 2.5% phosphate-buffered glutaraldehyde, rinsed with sodium cacodylate buffer, 0.1 mol/L (pH, 7.4), and postfixed in 1% sodium cacodylate-buffered osmium tetroxide. The specimen was dehydrated in ethanol and propylene oxide and embedded in epoxy resin (Epon). Sections of 50 nm were cut, anchored with 1% uranyl acetate and lead citrate, and examined with a transmission electron microscope (H-7100; Hitachi, Tokyo, Japan).

Four rabbits with intact S-KPro were killed humanely at 16 weeks. At that time, a smooth connection between the prosthesis and the cornea was found. A transparent thin fibrovascular membrane covering the peripheral anterior flange of the prosthesis was observed.

**Group 2**

Clinical results were almost the same as those of group 1 for up to 6 weeks. However, at 6 weeks, the e-PTFE skirts began to protrude from the surrounding cornea. Two eyes were enucleated for histological examination and stress testing. In the remaining eyes, the keratoprostheses were extruded spontaneously at 8, 9, and 10 weeks. At that time, they showed retinal detachment similar to that of group 1.

**Group 3**

This group showed similar findings to the clinical observations in group 1 for up to 4 weeks, but S-KPros were extruded at 7 or 8 weeks.

**Group 4**

Clinical results were almost the same as in those of group 1 during the initial postoperative period. The neovascularized cornea over the polyurethane skirt was well preserved for 4 months without melting or retinal detachment, in contrast to the results of the previous groups. Three of 5 eyes showed a good postoperative state for up to 6 months after the implantation without any complications.
The average retention period of S-KPros in group 1 (n=7) was 13.1 weeks. However, the average retention time would be longer than 13.1 weeks, if we had not intentionally terminated 4 rabbits with eyes that had well-placed S-KPros for 16 weeks. In groups 2 (n=3) and 3 (n=3), the average retention periods were 9.0 and 7.7 weeks, respectively. In group 4, the average retention period was up to 24 weeks (n=3). The average retention periods in groups 1 and 4 were longer than those of groups 2 and 3; however, the statistical analysis could not be performed because of the small sample sizes.

STRESS TESTING

The minimum pressure that induced aqueous leakage was 300 mm Hg in single eyes from groups 1, 2, and 4, and 250 mm Hg in a single eye from group 3.

HISTOPATHOLOGIC EXAMINATION

In groups 1 through 3, light microscopy revealed a mild to moderate inflammation, which consisted of mononuclear and polymorphonuclear leukocytes in and around the e-PTFE skirts and in the corneal stroma. No fibrovascular ingrowth into the pores of e-PTFE was observed, although a few fibroblasts were in evidence. No collagen accumulation in the e-PTFE skirts was found with Masson trichrome staining.

Figure 1. Schematic diagram of Seoul-type keratoprosthesis made of polyurethane skirt. A, Front view; B, side view. PMMA indicates polymethyl methacrylate.

However, fibroblast invasion into the pores of the polyurethane skirt was detected at 2 months after operation (Figure 4). Transmission electron microscopy demonstrated many activated fibroblasts with abundant endoplasmic reticula and mitochondria in pores of the polyurethane skirt. Newly formed collagen fibrils were noted adjacent to these fibroblasts (Figure 5).

HUMAN SUBJECTS

Patient 1

A 55-year-old woman visited Seoul National University Hospital, Seoul, South Korea, complaining of discharge and ocular pain in her right eye in 1997. The lens and vitreous prolapse through the corneal defect were noted, with visual acuity of hand motions. In her left eye, cornea covered with conjunctiva and symblepharon were observed, again with visual acuity of hand motions. She had had Stevens-Johnson syndrome for 14 years and had undergone unsuccessful penetrating keratoplasty in both eyes. The e-PTFE–S-KPro implantation was conducted as described above.

On the latest examination, 18 months after surgery, the S-KPro was found well placed in the eye, the retina was flat, and the corrected visual acuity was 20/100 (Figure 6).

Patient 2

A 28-year-old man had acid burn to both eyes 14 months before surgery. The scarred corneas were completely covered with highly vascularized conjunctiva after the failure of repeated AM applications (Figure 7A). Light perception was preserved in both eyes. Polyurethane–S-KPro was implanted in his left eye in 1998. On the latest follow-up, 8 months after the operation, S-KPro was well preserved to the eye (Figure 7B), and the visual acuity improved to 20/50 with a myopic correction of −7.5 D sphere.
Since keratoprosthesis is exposed to external environment, it is difficult to unite keratoprosthesis and cornea completely. Although the concept of keratoprosthesis has existed for 2 centuries, limited progress has been made because of the problem of implant bioincompatibility. In view of recent experimental and clinical results, microporous biocompatible polymers seem to offer satisfactory alternatives for keratoprosthetic support. These polymers are easy to implant and decrease mechanical tissue damage. Expanded PTFE appears to have the characteristics of an ideal prosthesis. It is inert, maintains its strength for a long period, and has a porosity that encourages tissue colonization.

Legeais et al used e-PTFE with a pore size of either 20- or 50-µm and reported that cellular ingrowth was significantly greater in the 50-µm material, which resulted in collagen deposition within the pores. In this experiment, we used e-PTFE with a pore size of 20 µm because it was the only available pore size at that time. However, our study revealed that this pore size was too small to allow fibroblast invasion. Polyurethane membranes, however, may be produced in a range of pore sizes in the laboratory; moreover, polyurethane is used for vascular prostheses. Therefore, the skirt material used during our work was composed of polyurethane rather than e-PTFE. Moreover, we demonstrated that rabbit fibroblasts invaded the 40-µm polyurethane more easily than they did the 20-µm e-PTFE.

The potential advantage of S-KPro is that it is double-fixed to healthy and stable sclera internally and to the cornea externally. Since most prostheses are placed in diseased corneas, many complications result not only from inadequate corneal healing capacity, but also from a deficiency in the compatibility of the keratoprosthesis and the surrounding cornea. Scleral fixation provides strong...
support for keratoprosthesis, even in the absence of corneal support.

Ion-implanted PMMA has been introduced to improve tear stability and reduce retroprosthetic membrane formation. Work on this material is ongoing in our laboratory.

In addition, AM apparently contributed to the stabilization of the surgical wound during the initial postoperative period and might provide an alternative to conjunctiva or mucous membrane.

The formation of a retroprosthetic membrane is a common postoperative complication of keratoprosthesis. To reduce retroprosthetic membrane formation, we introduced the long cylinder-shaped optic, mixed the heparin with the infusion fluid, and included a hydrophilic surface modification of the PMMA. In our study, no retroprosthetic membrane was observed, although the follow-up was relatively short.

The most common complication in the implanted eyes of rabbits was total retinal detachment with severe anterior proliferative vitreoretinopathy. Possible explanations are an injury caused by the scleral-fixated haptic itself or a vitritis induced by the surgery. However, our work demonstrated the possibility of long-term success of S-KPro in human eyes compared with the results in rabbit eyes.

The causes of the extrusion of the keratoprostheses in rabbit eyes are unclear. Possible explanations are melting of the cornea; incomplete integration between the cornea and the skirt, which is a result of poor fibroblast invasion; and increased intraocular pressure. However, the difficulty in measuring the intraocular pressure in implanted eyes prevented our being able to clarify the relationship between glaucoma and extrusion.

In conclusion, the double support design of S-KPro and AM implantation seemed to improve the stability of keratoprosthesis. Further experiments to improve the surgical outcomes of keratoprosthesis are pending.

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