Development of a Bioengineered Corneal Endothelial Cell Sheet to Fit the Corneal Curvature

Miwa Kimoto,1 Nobuyuki Shima,2 Masahiro Yamaguchi,3 Yosuke Hiraoka,4 Shiro Amano,2 and Satoru Yamagami1,5

1Corneal Regeneration Research Team, Foundation for Biomedical Research and Innovation, Kobe, Japan
2Department of Ophthalmology, University of Tokyo Hospital, Tokyo, Japan
3Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan
4Nitta Gelatin, Inc., Osaka, Japan
5Corneal Transplantation Section, University of Tokyo Graduate School of Medicine, Tokyo, Japan

Correspondence: Nobuyuki Shima, Department of Ophthalmology, University of Tokyo Hospital, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan; noshima.tky@umin.ac.jp.
Satoru Yamagami, Corneal Transplantation Section, University of Tokyo Graduate School of Medicine, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan; syamagami.tky@umin.ac.jp.
Submitted: August 29, 2013
Accepted: March 3, 2014
Citation: Kimoto M, Shima N, Yamaguchi M, Hiraoka Y, Amano S, Yamagami S. Development of a bioengineered corneal endothelial cell sheet to fit the corneal curvature. Invest Ophthalmol Vis Sci. 2014;55:2337–2343. DOI:10.1167/iovs.13-13167

PURPOSE. To evaluate a novel bioengineered corneal endothelial cell sheet that fits the curvature of the posterior corneal surface.

METHODS. A spherically curved gelatin hydrogel sheet (SCGS) was prepared by the dehydrothermal cross-linking method, and its permeability to water and protein was tested. Monkey corneal endothelial cells (MCECs) were seeded onto these hydrogel sheets, and the cells were examined by immunohistochemistry. Then MCEC-SCGS constructs were transplanted in monkeys with bullous keratopathy to assess the efficacy of the hydrogel sheets as a scaffold.

RESULTS. The hydrogel sheets showed similar permeability to water and protein as that of atelocollagen and vitrigel sheets. After transplantation, the SCGS did not show wrinkling and adhered tightly to the posterior corneal surface, whereas the flat sheets developed wrinkles that inhibited tight adhesion. Monkey corneal endothelial cells grown on hydrogel sheets expressed anti–zonula occludens-1 (ZO-1), N-cadherin, and sodium, potassium, and adenosine triphosphatase (Na,K-ATPase) along the plasma membrane. In a monkey model of bullous keratopathy, transplanted MCEC-SCGS constructs showed good adhesion to the posterior corneal surface, with subsequent improvement of corneal edema and transparency.

CONCLUSIONS. A novel MCEC-SCGS construct was effective in a monkey model of bullous keratopathy. The SCGS achieves close adhesion to the posterior corneal surface without wrinkling and may contribute to clinical transplantation of corneal endothelial cell sheets.

Keywords: corneal endothelium, curved gelatin hydrogel sheet, transplantation

Corneal endothelial cells (CECs) form a monolayer of hexagonal cells on the posterior surface of the cornea and play a critical role in maintaining corneal transparency by regulating stromal hydration. Because human CECs have no proliferative capacity in vivo, these cells decrease in number with aging, disease, or trauma, leading to corneal stromal edema known as bullous keratopathy. Corneal transplantation is the only available therapy for bullous keratopathy associated with CEC deficiency. However, there is a worldwide shortage of transplant-grade donor corneas. On the other hand, CECs have been found to proliferate under certain conditions in vitro,1–5 raising the possibility that CEC grafts could be developed by tissue engineering techniques.6–12

Several approaches have been tried for the transplantation of cultured CECs, such as injection of a cell suspension,8,13 or cell sheet transplantation.6,7,9,12 We previously reported on the in vivo efficacy of injecting a cell suspension.8 However, this technique could be difficult to apply clinically because it raises safety issues such as the problem of achieving selective attachment of the cells to Descemet’s membrane without distribution to other organs. Yet, cell sheet transplantation is a more reasonable technique for clinical application because this method is similar to some techniques that are already used for corneal transplantation (e.g., Descemet’s stripping automated endothelial keratoplasty [DSEAEEK] and Descemet’s membrane endothelial keratoplasty [DMEK]), in which only the inner endothelial cell layer is transplanted.14-16

We and other groups have reported on CEC sheet transplantation in animal models of bullous keratopathy using various scaffolds, such as a collagen sheet,8 amniotic membrane,7 vitrigel sheet,17 or corneal stromal disc.12 Corneal endothelial cell sheet transplantation without a scaffold has also been reported,9,11 but unsupported CEC sheets are too fragile to be practical for clinical use. So far, all of the scaffolds used for CEC transplantation have been flat sheets of carrier material. However, a flat sheet sometimes becomes wrinkled at the periphery after transplantation to the posterior surface of the cornea, which has a curved surface. This wrinkling becomes more obvious when we use a thinner and larger sheet as the scaffold. If no wrinkles are observed, the sheet can attach to the posterior surface of the cornea, however, if the wrinkles are large or numerous, aqueous humor can flow under the transplanted sheet and cause complete or partial detachment from the posterior surface of the cornea. Such problems led us to develop a spherically curved cell scaffold that fits the corneal curvature. Gelatin is easy to form into any desired shape and is
used in a wide variety of fields, including the food, pharmaceutical, and medical industries. Watanabe et al.\textsuperscript{10} reported that CECs cultured on a gelatin hydrogel sheet show a normal phenotype and that the gelatin sheet displays good transparency and permeability, suggesting that such sheets have appropriate characteristics as a scaffold for CEC sheet transplantation.

Although many studies of cultured CEC transplantation have used rabbit bullous keratopathy models, these rabbit models have the serious disadvantage of having a very short observation period because residual peripheral host CECs show a high proliferative capacity in rabbits. This means that corneas with bullous keratopathy recover to normal after approximately 1 to 2 weeks even in the control group.\textsuperscript{7,9} In contrast to rabbits, the in vivo proliferative capacity of monkey CECs (MCECs) has been reported to be comparatively low.\textsuperscript{19,20} Therefore, we have used a monkey bullous keratopathy model with allogenic transplantation to obtain in vivo data as a prelude to clinical application.

In the present study, we evaluated the feasibility of using our new spherically curved gelatin hydrogel sheet (SCGS) as a scaffold for cultured CEC. We found that the curved sheet is more suitable than conventional flat sheets as a scaffold for transplantation of CEC sheets.

**Materials and Methods**

**Materials**

Low-glucose Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), PBS, anti–zonula occludens-1 (ZO-1) antibody, Hoechst, and AlexaFluor 488-labeled anti-mouse, anti-goat, and anti-rabbit antibodies were all obtained from Life Technologies (Carlsbad, CA, USA), while mouse or rabbit normal IgG, BSA, and trypsin/EDTA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human BFGF, polyoxyethylene (10) octyphenyl ether (Triton X-100), and 4% paraformaldehyde phosphate buffer solution were from Wako (Osaka, Japan). Lascorbic acid 2-phosphate was purchased from Showa Denko (Tokyo, Japan). Atelocollagen implants came from Koken (Tokyo, Japan), anti–sodium, potassium, and adenosine triphosphatase (Na,K-ATPase) antibody was obtained from Merck Millipore (Billerica, MA, USA), and anti-N-cadherin antibody was sourced from Abcam (Cambridge, UK).

**Preparation of a Spherically Curved Gelatin Sheet (SCGS) Fitting the Posterior Corneal Curvature**

Low endotoxin gelatin (beMatrix Gelatin LS-H) isolated from porcine skin by an alkaline process was obtained from Nitta Gelatin, Inc. (Osaka, Japan) to prepare curved gelatin sheets. A 2\% wt/vol aqueous beMatrix Gelatin LS-H solution was poured into a Teflon mold (Fig. 1A) with an 8-mm radius of curvature and was cooled at 4°C for more than 17 hours. Then, the gel was dried at room temperature, after which cross-linking was done by vacuum drying (0.75 torr) in an oven (Yamato Scientific Co., Ltd., Tokyo, Japan) for 72 hours at 140°C. The sheets thus prepared were stored at −30°C until use. For comparison with the curved sheets, flat gelatin sheets were also prepared as described above, except that a flat mold with the same depth was used.

**Sodium Fluorescein and Protein Permeability Assay**

Evaluation of the permeability of the curved sheets was done by using sodium fluorescein (NaFl) and BSA. Sheets (SCGS, atelocollagen, and vitrigel) were soaked in sterile distilled water and then attached to the bottom of the inserts of a multi-well insert culture plate (Cat No. 353182; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with nail varnish. The sheet-attached insert was set to each well of the multi-well plate, and a mixture of 20 μg/mL NaFl and 10 μg/mL BSA in PBS (400 μL) was added to the insert. Then, 400 μL PBS was added to each well of the multi-well plate. The plates were incubated at room temperature for 1.5 hours, and the fluorescence in each well of the multi-well plate was measured by using a microplate reader (Powerscan HT Multi-Detection Microplate Reader; DS Pharma Biomedical, Osaka, Japan) with excitation at 485 nm and emission at 525 nm. Absorbance of BSA was measured at 280 nm.

**Cell Culture**

Monkey corneal endothelial cells\textsuperscript{17} were kindly provided by Professor Koizumi (Doshisha University, Kyoto, Japan) and were cultured on atelocollagen-coated dishes in basal medium (DMEM with 15% FBS and antibiotics) containing 2 ng/mL BFGF and L-ascorbic acid 2-phosphate (Asc-2P; 0.3 mM) as described previously.\textsuperscript{5} Curved sheets were soaked in sterile distilled water and then were attached to the frame of a membrane-denuded Transwell insert (Corning, Tewksbury, MA, USA) with concave side up with an O-ring. Then, each sheet was coated with 50 μg/mL atelocollagen for 2 hours at 37°C. After washing with PBS, the sheet was soaked in culture medium and seeded with MCECs at 4000 cells/mm\textsuperscript{2}. Incubation was done for 1 week, and the culture medium was changed every other day. In some experiments, to distinguish them from host cells, transplanted MCECs were labeled with PKH26 (Sigma-Aldrich) according to the manufacturer’s protocol before seeding onto the curved sheets.

**Immunocytochemistry**

For staining with ZO-1/N-cadherin and Na,K-ATPase, the MCECs on curved sheets were fixed in 4\% paraformaldehyde for 10 minutes at room temperature and in ice-cold methanol
for 10 minutes, respectively. After washing with PBS containing 0.15% Triton X-100, the specimens were blocked for 30 minutes in blocking buffer containing 3% BSA and 0.5% Triton X-100 in PBS. Primary and secondary antibodies were diluted with the blocking buffer. Incubation was done for 2 hours with the primary antibody (anti-ZO-1 at 1:50, anti–Na,K-ATPase at 1:200, or anti–N-cadherin at 1:200). After washing, incubation was then performed with the secondary antibody (1:200) for 1 hour. Subsequently, the specimens were washed and mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA) containing Hoechst. Negative controls were prepared by using irrelevant IgG of the same species, subtype, and concentration. Specimens were observed under an inverted fluorescence microscope equipped with an epifluorescence attachment (Eclipse TS100, Nikon, Japan).

Transplantation of MCEC-SCGS Constructs Into Monkeys

Nine male cynomolgus monkeys (3.5–5.0 kg; Narita Animal Science Laboratory Co., Ltd., Chiba, Japan) were used to assess the in vivo efficacy of the curved sheets as a cell scaffold. The animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experiments were performed at Narita Animal Science Laboratory Co., Ltd., according to the protocol approved by the company’s Animal Care and Use Committee (Approval No. 120620-1). One day before the operation, each animal received an intravenous injection of methylprednisolone sodium succinate (20 mg/kg). For anesthesia, animals were given an intramuscular injection containing a mixture of ketamine hydrochloride (5 mg/kg; Sankyo, Tokyo, Japan) and xylazine (1 mg/kg; Bayer, Munich, Germany), followed by inhalation of isoflurane. Sodium heparin solution (Ajinomoto Pharmaceuticals, Tokyo, Japan) was injected intravenously at 2000 IU. The surgical procedure was similar to DSAEK as performed in humans. After an anterior chamber maintainer (Alcon Japan Ltd., Tokyo, Japan) was inserted into peripheral cornea, a 3.2-mm scleralcorneal incision was made with a slit knife (Mani, Tochigi, Japan). Then, a viscoelastic agent (HealonV; Abbott Medical Optics, Inc., Santa Ana, CA, USA) was infused into the anterior chamber, and the corneal endothelium was scraped off the central regional curvature (8 mm in diameter) with a 20-gauge silicone needle (soft tapered needle; Inami, Tokyo, Japan). The scraped area was then confirmed by 0.08% trypan blue staining. Descemet’s membrane was stripped off the central regional curvature (4 mm in diameter) with a 25-gauge needle, because monkeys with bullous keratopathy often show spontaneous recovery of corneal transparency by regeneration of their own CECs if Descemet’s membrane is intact and because removing the Descemet’s membrane promoted adhesion of the grafts to the posterior corneal surface. Because our preliminary experiments demonstrated that Descemet’s stripping enhanced migration of inflammatory cells around the transplanted sheet, we stripped only a 4-mm diameter circle size as a minimal surgical procedure. Monkey corneal endothelial cell–SCGS constructs and MCEC-denuded curved sheets were stained with 0.2% trypan blue, cut with a 6-mm biopsy punch, and then the concave side of each sheet was coated with Viscoat (Alcon Japan Ltd.). Each sheet was set into a transplantation injector (ETERNITY Navi, XJ-70; Santen, Osaka, Japan), and then the head of the injector was inserted into the anterior chamber through the corneal incision. The injected sheet was moved to the center of the anterior chamber with the cell side facing the anterior chamber using DSAEK forceps (Asico, Westmont, IL) through the side-port on the opposite side of the 3.2-mm incision using a 25-gauge V-lance (Mani). Then, the sheet was attached to the posterior cornea by air tamponade. Transplantation was only done in one eye of each animal. After surgery, animals received intramuscular administration of tacrolimus hydrate (Astellas Pharma, Inc., Tokyo, Japan) once daily at 0.6 mg/kg for 4 weeks and intravenous injection of levofloxacin hydrate (Daiichi Sankyo Co., Tokyo, Japan) once daily at 50 mg/kg for 1 week. In addition, subconjunctival injection of 10 mg triamcinolone acetonide (Bristol-Myers Squibb, New York, NY, USA) and instillation of 0.1% betamethasone sodium phosphate (Shionogi, Osaka, Japan) and 0.5% levofloxacin hydrate were done once a week for 4 weeks. Blepharorrhaphy was done once a week after surgery to protect against rubbing the eyes. After transplantation, the anterior segment of the eye was examined under a surgical microscope on the next day, then once a week for 4 weeks.

Statistical Analysis

Statistical comparisons between two groups were performed with the unpaired Student’s t-test, while comparisons among multiple groups were done with ANOVA and the Tukey-Kramer test.

Results

Characteristics of the SCGS

We developed the SCGS (Fig. 1A) by using a mold with an 8-mm radius of curvature (Fig. 1B). After hydration, the sheets became transparent (Fig. 1C). Frozen section examination showed that the thickness of the sheets was approximately 20 μm (Fig. 1D).

The permeability of the curved sheets to water and protein was evaluated by using NaFI and BSA and was compared with that of atelocollagen sheets and vitrigel sheets. A diagram of permeability assay is shown in Figure 2A. As a result, permeability of the SCGS for water was similar to that of both atelocollagen sheets and vitrigel sheets (Fig. 2B). Similar results were obtained with respect to permeability for BSA (Fig. 2C).

Preparation of MCEC-SCGS Constructs

Monkey corneal endothelial cells were seeded onto sheets that were attached to the frame of membrane-denuded Transwell inserts with an O-ring (Fig. 3A). A diagram of MCECs culture on sheet is shown in Figure 3B. When we seeded MCECs on the curved sheets, we attached each curved sheet to a well of a culture dish in order to flatten its curvature. This made it possible to seed cells uniformly over the SCGS without being concentrated in the center location. We performed a cross-section analysis and confirmed that MCEC-SCGS constructs consisted of a monolayer of cells but not multi-layer (Fig. 3C).

After 1 week of culture, MCECs were stained for ZO-1 (a tight junction–associated protein), Na,K-ATPase (an integral membrane protein complex responsible for regulating pump functions). The MCECs attached to the sheets grew as hexagonal cells (Fig. 3D). ZO-1 and N-cadherin were localized at the cell borders (Fig. 3E), and Na,K-ATPase was also localized to the intercellular regions (Fig. 3E). Localization of these markers to the plasma membrane was similar to the findings for CEC in vivo, suggesting that MCEC-SCGS constructs could maintain barrier and ion pump functions.

Findings After Surgery

After transplantation, the curved sheets adhered tightly to the posterior surface of the cornea (Fig. 4A), while the flat sheets
**FIGURE 2.** Evaluation of the permeability of spherically curved gelatin sheets. (A) Diagram of permeability assay. (B) Water permeability was assessed using NaFl. (C) Permeability for protein was assessed using BSA. Water and protein permeability of the SCGS was compared with that of atelocollagen sheets and vitrigel sheets. The SCGS showed similar permeability properties to the other sheets.

**FIGURE 3.** Preparation of a cultured MCEC-SCGS construct. (A) Monkey corneal endothelial cells were seeded onto an SCGS attached to the frame of a membrane-denuded Transwell insert with an O-ring. (B) Diagram of MCEC culture on sheet. (C) Frozen cross-section of MCEC culture on SCGS. The sheet was a monolayer of cells. (D) Representative phase-contrast images of MCECs after culture for 1 week on an SCGS. The cells show a regular hexagonal morphology. (E) Immunohistochemistry of MCECs cultured on an SCGS for ZO-1 (left), Na,K-ATPase (middle), and N-cadherin (right). As is the case for normal corneal endothelium, these markers are localized on the plasma membrane of cells with a hexagonal shape. Scale bar: 10 μm.
showed wrinkling at the periphery (Fig. 4B). If aqueous humor flows under the sheets via the wrinkles, there is a possibility that the transplanted sheets will be detached from the cornea. Therefore, the curved sheets have a crucial advantage as a scaffold for CEC transplantation.

The MCEC-SCGS constructs were closely adherent to the posterior cornea, and no detachment was observed after 4 weeks (Fig. 5). Corneal thickness was increased at 7 days but returned to normal on day 21 in the MCEC-SCGS group (Fig. 5), and corneal transparency also started to improve from day 14. In contrast, corneal thickness was >1000 μm throughout the 28-day observation period in the untransplanted and MCEC-denuded SCGS–transplanted control groups, and corneal transparency also did not improve (Fig. 5). Figure 6A shows an MCEC-SCGS construct attached to the posterior corneal stroma and the recovery of corneal transparency on day 28. PKH-positive MCECs are seen uniformly covering the SCGS. At 28 days, MCEC-SCGS constructs were histologically examined. Hematoxylin and eosin (HE) staining showed that the constructs were tightly attached to the posterior corneal stroma by collagenous tissue containing fibroblast-like cells (Fig. 6B). Monkey corneal endothelial cells were abundant on the SCGS (Fig. 7A), and actin ring formation suggested that these cells were functional (Fig. 7B). The density of MCECs on the SCGS scaffolds before transplantation was 2944 ± 350 cells/mm², but it declined to 2300 ± 100 cells/mm² by 28 days after surgery. A number of CD68-positive cells were observed at the periphery of the transplanted constructs, and PKH-labeled cells were decreased in the peripheral region (Figs. 7C, 7D), presumably owing to destruction by the infiltrating CD68-positive cells.

**DISCUSSION**

The present study demonstrated that an SCGS did not show wrinkling after transplantation to the posterior surface of the cornea, while a flat sheet showed wrinkling that could possibly lead to detachment. After transplantation of MCEC-SCGS constructs, no detachment of sheets was observed for up to 4 weeks. These results suggest that the SCGS is more suitable than a conventional flat sheet as a scaffold for CEC sheet transplantation. We used a gelatin sheet with an 8-mm radius of curvature, while the radius of curvature of the posterior corneal surface of the monkeys used in this study was approximately 5 mm (Shima S, Yamagami S, unpublished observation, 2013). Thus, our finding that the transplanted MCEC-SCGS constructs did not show wrinkling indicates that complete conformity of curvature between the scaffold and the posterior cornea is not necessary, probably because of the flexibility of gelatin hydrogel sheets. Histologic examination revealed that the transplanted sheets were tightly attached to the corneal stroma by fibroblast-like cells that had been generated from the corneal stroma. When MCEC-SCGS constructs were transplanted without peeling Descemet’s membrane, approximately 60% became detached within 4 weeks, although the transplanted corneas still showed recovery of transparency (Shima S, Yamagami S, unpublished observation, 2012). Koizumi and coworkers also reported...
detachment of all sheets within 2 weeks when they transplanted MCECs in monkeys without Descemet’s membrane peeling, using a flat vitrigel sheet as the scaffold. A new clinical transplantation technique called “Descemet’s membrane endothelial keratoplasty” (DMEK) has been reported, in which only Descemet’s membrane is replaced with CECs, and this also involves Descemet’s membrane peeling.

In the present monkey model of bullous keratopathy, the MCEC-SCGS group showed recovery of corneal transparency, whereas both control groups (MCEC-denuded SCGS transplantation or no transplantation) had severe persistent bullous keratopathy throughout the follow-up period. Microscopic observation demonstrated that the transplanted MCECs on SCGS scaffolds were uniformly PKH26-positive and morphologically similar to CECs in vivo. These findings indicate that allogenic transplantation of MCEC-SCGS constructs can be done to regulate corneal hydration in vivo. A previous study using a flat vitrigel-MCEC construct in a monkey model of bullous keratopathy showed the efficacy of transplantation even though detachment of the transplanted sheets occurred, and the authors suggested that this was a result of MCEC migration from the vitrigel sheet onto the host Descemet’s membrane. Therefore, the present study is the first to directly demonstrate the feasibility of MCEC sheet transplantation in a monkey bullous keratopathy model.

At 4 weeks after transplantation, a number of CD68-positive cells were detected on the grafts, despite the local administration of steroids (betamethasone and triamcinolone acetonide) and systemic immunosuppression (tacrolimus). We could not suppress the migration of such inflammatory cells with higher doses of these medications and/or use of a minimally invasive surgical procedure without Descemet’s membrane stripping (Kimoto M, unpublished observation, 2012). When monkeys with bullous keratopathy received transplantation with human CEC-SCGS constructs instead of MCEC-SCGS constructs, most of the transplanted CECs disappeared and more inflammatory cells infiltrated the grafts, so human constructs were inferior to monkey constructs with regard to improving corneal transparency (Kimoto M, unpublished observation, 2012). These results surprised us, because we had previously succeeded with human-to-rabbit xenotransplantation and had not detect-
ed leukocyte infiltration in a rabbit bullous keratopathy model.\textsuperscript{6,8,12} We observed infiltration of CD68-positive cells in both the MCEC-SCGS group and the MCEC-denuded SCGS group. Therefore, we initially suspected that infiltration was owing to immunogenicity of the SCGS itself, although biosafety of gelatin has been proven through a long history of use.\textsuperscript{25} Therefore, we transplanted these sheets onto the posterior surface of the cornea in rabbits and confirmed that there was no inflammatory response after 3 months of observation even without steroids and immunosuppressive therapy (Kimoto M, unpublished observation, 2013). Moreover, we have transplanted human CEC-SCGS constructs in a rabbit bullous keratopathy model, and our preliminary results showed no infiltration of CD68-positive cells. We also determined the residual level of BSA in the transplanted MCEC-SCGS constructs and confirmed that it was too low to elicit an immune response, suggesting that a reaction to residual BSA or porcine gelatin did not trigger postoperative ocular inflammation in the monkeys and that the infiltration of CD68-positive cells was due to nonspecific inflammation evoked by surgical stress. Although the exact mechanism leading to severe postoperative inflammation in monkeys is unknown, in addition to the species-specific immunologic environment of the monkey anterior chamber, use of young animals (4 years old) in the present study may have been a reason for such postoperative inflammation, because the success rate of human pediatric corneal transplantation is very low owing to a strong inflammatory response.\textsuperscript{23} Moreover, the monkeys frequently rubbed their eyes after surgery, and we could not prevent this, even though we performed blepharorrhaphy. Such behavior may also have led to the enhancement of nonspecific inflammation. Accordingly, CD68-positive cell infiltration may be caused by factors that are specific to monkeys. Thus, this monkey bullous keratopathy model is not useful for long-term in vivo observation.

In summary, SCGS constructs did not show wrinkling after transplantation onto the posterior corneal stroma, while flat constructs showed wrinkling. Transplanted MCECs expressed ZO-1, N-cadherin, and Na,K-ATPase at the plasma membrane in vivo observation.

Acknowledgments

The authors thank Kazusa Izaki and Motoko Tsukazaki for excellent technical assistance. Disclosure: M. Kimoto, None; N. Shima, None; M. Yamaguchi, None; Y. Hiraoka, None; S. Amano, None; S. Yamagami, None

Supported by knowledge cluster initiative grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) and adaptable and seamless technology transfer program through target-driven research and development, Japan Science and Technology Agency (A-STEP).

References