Long-term Observation After Transplantation of Cultured Human Corneal Endothelial Cells for Corneal Endothelial Dysfunction

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Research Article

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Abstract

Background

At present, corneal transplantation is still the only way to treat serious corneal diseases caused by corneal endothelial dysfunction. However, the shortage of donor cornea tissues and human corneal endothelial cells (HCECs) remains a worldwide challenge. We cultivated HCECs by the use of a conditioned medium from orbital adipose-derived stem cells (OASC-CM) \textit{in vitro}. Then the HCECs were used to treat animal corneal endothelial dysfunction models via cell transplantation. The initial effect was gratifying. The purpose of this study was to conduct a long-term observation and evaluation after cell transplantation.

Methods

First, orbital adipose-derived stem cells (OASCs) were isolated to prepare conditioned medium (CM). Then HCECs were cultivated and expanded by the usage of CM (CM-HCECs). Related CEC markers were analyzed by immunofluorescence. Cells proliferation ability was also tested. CM-HCECs were then transplanted into monkey corneal endothelial dysfunction models by cell injection. We carried out a 24-month postoperative preclinical observation and verified the long-term effect by histological examination and transcriptome sequencing.

Results

CM-HCECs expressed HCEC related markers and maintained polygonal cell morphology after several passages. During 24 months of cell transplantation into the monkey's anterior chamber, the cornea thickness and transparency kept healthy status, and the corneal endothelial cell density remained in the normal range. Gene sequencing showed that the gene expression pattern of CM-HCECs was similar to that of transplanted cells and HCECs.

Conclusions

The proliferation and repair ability of HCECs were significantly improved due to the effect of OASC-CM. The result of this study confirmed long-term therapeutic efficacy of CM-HCECs \textit{in vivo}. Our research provided an extensive cell source and a promising prospect for regenerative medicine and cell-based therapy.

Background

Human corneal endothelial cells (HCECs) have very limited proliferative capacity \textit{in vivo} \cite{1,2}. Multiple factors such as Fuchs endothelial corneal dystrophies (FECD), Peter's anomaly, iridocorneal endothelial
syndrome (ICE), intraocular surgery, hypoxia, infection or trauma could damage HCECS, which will lead to corneal endothelial dysfunction and even irreversible corneal blindness [3, 4]. Researchers have been committed to the treatment of corneal endothelial dysfunction. Besides traditional corneal transplantation, new procedures such as Descemet's stripping endothelial keratoplasty (DSEK), Descemet's stripping automated endothelial keratoplasty (DSAEK) and Descemet's membrane endothelial keratoplasty (DMEK) were applied to clinical treatment [5–8]. At present about 185,000 corneal transplants were performed worldwide per year, it has been far from inadequate in addressing the need of patients yet [9].

Researchers have studied novel therapeutic modalities such as cell-based therapy, regenerative medicine, bio-engineered or tissue-engineering cornea construct and gene therapy in recent years [10–15]. These studies provide commendable ideas and methods. But whatever the method used, the resource of seed cells remains the critical issue. HCECs as the most important seed cell have very limited proliferative capacity [2]. When cultured in vitro with classic methods HCECs began a fibroblastic change and showed endothelial-to-mesenchymal transition (EMT) after several passages [16, 17]. Therefore, it becomes particularly important to obtain sufficient corneal endothelial cells with therapeutic function. We innovatively cultivated HCECs with conditioned medium obtained from human orbital adipose-derived stem cells (OASCs) in our previous research. The HCECs cultured with conditioned medium (CM-HCECs) could maintain good proliferative and cell-based therapeutic capacity even after 10 passages. Then we produced animal corneal endothelial dysfunction models and treated them via cell transplantation. The results show that opaque and edematous cornea recovered and kept normal thickness and transparence rapidly during the observation of 10 months after cell transplantation [18].

In this study, we expanded HCECs with OASC-CM in vitro. Then CM-HCECs were transplanted into monkey endothelial dysfunction models and a 24-month preclinical observation was carried out. Further transcriptome sequencing was also carried out on HCECs, CM-HCECs and transplanted cells. Results showed that the treated corneas remained transparent and moderate thickness. CM-HCECs possessed therapeutic ability. The transplanted cells could adapt well to the microenvironment. The studies indicated that more useful cells would be available for the research of HCECs and clinical cell-based therapy for corneal endothelial dysfunction in the future.

## Methods

### Cell culture and evaluation of HCECs

OASC-CM and HCECs were prepared as previously described [19–21]. Orbital adipose tissues were collected from 15 patients aged between 23 and 65 (45.3 ± 9.8) years following blepharoplastic surgeries. Briefly, OASCs were isolated and cultured in DMEM-LG supplemented with 10% FBS (Gibco) and 10% penicillin-streptomycin (Sigma), and incubated at 37°C in 5% CO2. Cells were washed three times with phosphate buffered saline (PBS) when they were at 60–80% confluence and the medium was
replaced with basal growth medium. The OASCs were kept for an additional 12–24 hours. The medium was then collected and filtered (0.22 µm) and stored at −80°C.

HCECs were obtained from discarded corneal-scleral rings after penetrating keratoplasty (PK) and from the Eye Tissue Bank of Shandong Province, China. The age of donors ranged from 15 to 78 years (n = 10). The Descemet’s membranes (DM) containing HCECs were stripped and incubated in basal culture medium (BM) for stabilization, followed by digestion with 1 mg/mL collagenase type I (Sigma). The BM was composed of Opti-MEM-I (Gibco), 8% FBS, 5 ng/mL human epidermal growth factor (hEGF; PeproTech), 20µg/mL ascorbic acid (Sigma), 200 mg/L calcium chloride, 0.08% chondroitin sulfate, and 50µg/mL penicillin-streptomycin [22]. After digestion the HCECs were cultured in BM containing 10% OASC-CM as CM-HCECs. CEC related functional proteins as Na⁺/K⁺ ATPase and tight junction protein zona occludens 1 (ZO-1) were evaluated by Immunofluorescence according to the previous protocol [18]. Cell proliferation, migration and repair capacity were detected with a Cell Counting Kit-8 (CCK-8) Assay and Wound Healing Assay. Passage 9 (P9) and passage11 (P11) of CM-HCECs were used for cell transplantation in the study.

Animals

Four rhesus monkeys weighing 3.0–4.0 kg (3 to 5 years of age; HongLi Medical Animal Experimental Research Center, Jinan, Shandong Province, China) were used for animal experiments.

Transplantation of CM-HCECs into the monkey corneal endothelial dysfunction models

Monkeys were randomly divided into the experimental group (n=3) and the control group (n=1). The nonsurgical eyes of monkeys were used as the normal group. Monkey corneal endothelial dysfunction models and CM-HCECs transplantation were performed according to our previous method. Briefly, monkeys were under general anesthesia of ketamine hydrochloride. The CECs of monkeys were mechanically scraped with a modified irrigator needle (Shandong Weigao) from the DM of four monkeys (Fig. 1A1, B1). Aqueous humor (50 µl) was first extracted from the anterior chamber. CM-HCECs (2.7×10⁵ cells for each eye) were suspended in 50ul culture medium and injected into the anterior chamber of three monkeys as the experimental group (Fig. 1A2, B2). The other one monkey only had 50ul culture medium injected without any cells as the control group. A peribulbar injection of triamcinolone and a subconjunctival injection of dexamethasone were given at the end of the surgery. All the monkeys were then immediately kept in a face-down position for 5 hours under general anesthesia (Fig. 1A3, B3). Tobramycin and Dexamethasone drops were given 4 times a day. The corneas were examined by a slit-lamp microscopy, AccuPen Handheld Tonometer (Accutome), OCT (Carl Zeiss), non-contact specular microscopy (Topcon), gonioscope (Volk), B-ultrasonography (Suoer) and fundus camera (Carl Zeiss) at certain times after surgery. Three monkeys in the experimental group were euthanized at 24 months after
the transplantation which were labeled as the TR2Y group. The monkey of the control group was euthanized 24 months after surgery. Postoperative eyes were removed. The cornea is divided into several parts, one for RNA-Sequencing, and one for immunofluorescent staining in the frozen section, and the other part was subjected to Hematoxylin and eosin (H&E) staining.

**Immunofluorescence And Histological Examination**

Postoperative eyes were removed and part of the cornea was embedded in Tissue-Tek optimum cutting temperature compound (Sakura) and sectioned into 5µm slices. The frozen slices were subjected to standard immunofluorescent staining [18]. Primary antibodies were anti-Na$^+/K^+$ ATPase α-1 (1:200), anti-zonula occludens-1 (ZO-1, 1:100). Part of the cornea was fixed in 4% formaldehyde and subjected to standard H&E staining.

**RNA-Sequencing**

The total RNA of HCECs, CM-HCECs and corneal endothelial cells of TR2Y groups were isolated using TRIzol reagent (Life Technologies) according to the manufacturer’s protocol. RNA-seq transcriptome library was prepared following the TruSeqTM RNA sample preparation Kit from Illumina (San Diego, CA) using 1mg of total RNA. Sequencing was performed by Majorbio Biotech (Shanghai, China) using the Illumina HiSeq 4000 150 bp Paired-End Platform. The raw paired end reads were trimmed and quality controlled by SeqPrepand Sickle with default parameters. Then clean reads were separately aligned to human reference genome with orientation mode using HIASAT software. The mapped reads of each sample were assembled by StringTie.

To identify differential expression genes (DEGs) between two different samples, the expression level was calculated according to the TPM method. RSEM was used to quantify gene abundances. R statistical package software EdgeR was utilized for differential expression analysis. Genes with FDR<0.05 and $|\log2FC|\geq1$ were considered as significant. Venn analysis (TPM>1) was used to demonstrate common and uniquely expressed genes transcripts between samples. The correlation analysis provides basic reference for the analysis of differential genes. GO functional enrichment analysis were performed by Goatools to identify which DEGs were significantly enriched at Bonferroni-corrected P-value $\leq0.05$ compared with the whole-transcriptome background.

**Statistical analysis**

Data are presented as the means±SEM. The Student’s t test was used to examine differences between the two groups. All data analyses were calculated by GraphPad Prism version 7 (GraphPad Software, Inc.).

**Results**
Cell culture and characterization of OASCs and CM-HCECs

In the study human OASCs were plastic adherent, spindle-shaped, fibroblast-like cells. They were highly expressed CD29 and CD105 by flow cytometry (data not shown). Cultured OASCs demonstrated similar morphology and phenotype, indicating their stem cell origin.

HCECs displayed typical hexagonal morphology on the DM in vivo (Fig. 2A-B). When cultured in OASC-CM, HCECs maintained polygonal morphology and contact-inhibited monolayer even after 10 passages (Fig. 2C). Meanwhile CM-HCECs expressed CEC related functional proteins such as Na⁺/K⁺ ATPase and ZO-1 (Fig. 2D). In contrast, HCECs began a fibroblastic change, became larger with vacuoles, and showed EMT after 4-5 passages cultured in BM (data not shown).

Long-term observation after Transplantation of Cultivated CM-HCECs into the primate models

Slit-lamp microscopy and OCT showed that the cornea recovered transparent at about 7 days after cell transplantation in the TR2Y group. Slight keratic precipitates (KP) and anterior chamber exudation could be noticed at 10 to 14 day after the transplantation. The depth of the anterior chamber was normal, the iris texture was clear, the pupil was round with normal direct light reflection and the crystalline lens was transparent. The corneas of the TR2Y group remained transparent until 24 months after surgery (Fig. 3A). While the cornea in control group had obvious corneal opacity and stroma edema. The iris, pupil and crystalline lens could not be seen at 3 months after surgery. Meanwhile obvious corneal neovascularization also appeared by slit lamp (Fig. 3B).

The central corneal thickness (CCT) of the TR2Y group was about 720µm at 1 month and between 600µm-700µm from 1-3 months after surgery. Then it was stable at about 500µm from 6-24 months after surgery the corneal thickness. Yet the central corneal thickness of the control group maintained above 1000µm at the end of 24 months after surgery (Fig. 4A, F). The CECs in the TR2Y group showed multilateral morphology under the examination of non-contact specular microscopy. The average endothelial cell density was about 1800 cells/mm² at the first month after surgery. And it decreased to about 1682 cells/mm² at the third month. After that, the number of CECs increased continuously. The average cell density reached 2530 cells/mm² at the end of 24 months (Fig. 4B, G). The cell density of CECs in control group could not be detected with specular microscopy or confocal microscope because of the obvious corneal opacity and edema. In addition, a gonioscope, fundus photography and B-mode ultrasound showed no pathological changes of eyes in the TR2Y group (Fig. 4C-E). There was no difference in intraocular pressure (IOP) between the TR2Y group and the normal group (Fig. 4H).

Histological Examination and Immunofluorescence

HE staining showed that the corneal thickness of the TR2Y group was similar to that of the normal group. Cells created a closely arranged monolayer on DM. The cornea of the control group was significantly
thickened, and almost no cells were detected on the endothelial surface. There were more inflammatory cells in corneal stroma, irregular arrangement and fracture of collagen fibers, and vacuolar changes in corneal epithelium (Fig. 5A).

Immunofluorescence staining of frozen sections showed that Na\(^{+}/K^{+}\) ATPase and ZO-1 were strongly expressed in the corneal endothelium of the TR2Y group and the normal group, suggesting the pump function and tight junction function of CECs. On the contrary, there was almost no nuclear staining on DM in the control group. The staining results of Na\(^{+}/K^{+}\) ATPase and ZO-1 were negative (Fig. 5B).

**The differential expression gene and correlation in HCECs, CM-HCECs and TR2Y cells**

RNA-seq was carried out to research the gene expression of HCECs, CM-HCECs and cells after transplantation (TR2Y cells). The result of Venn analysis displayed the co-expressed genes and specially expressed genes between samples. The Venn analysis of HCECs and CM-HCECs showed that 12472 genes were co-expressed, accounting for 77.48%. The number of specific genes of HCECs and CM-HCECs was 1914 and 1712, accounting for 11.89% and 10.63% separately (Fig. 6A). The Venn analysis of HCECs and TR2Y cells showed that 12738 genes were co-expressed, accounting for 77.53%. The number of specific genes of HCECs and TR2Y cells was 1648 and 2044, accounting for 10.03% and 12.44% separately (Fig. 6B). The Venn analysis of CM-HCECs and TR2Y cells showed that 13611 genes were co-expressed, accounting for 88.64%. The number of specific genes of CM-HCECs and TR2Y cells was 573 and 1171, accounting for 3.73% and 7.63% separately (Fig. 6C).

Correlation analysis was carried out to detect the relativity between samples. The r value between HCECs and CM-HCECs was both about 0.712. The r value between HCECs and TR2Y cells was about 0.809. The r value between CM-HCECs and TR2Y cells was 0.911. (Fig. 6D).

**Differential expression gene analysis of CM-HCECs and TR2Y cells**

Expression variance analysis was carried out to detect differential expression genes (DEGs) between CM-HCECs and TR2Y cells. 129 significant DEGs were detected between CM-HCECs and TR2Y cells, among which 104 genes (80.6%) were up-regulated and 25 genes (19.4%) were downregulated (Fig. 7A, B).

The GO enrichment analysis of CM-HCECs and TR2Y cells showed the top 20 GO sets of abundance, including classification of biological process, cellular component, and molecular function. Results showed that the DEGs mainly enriched in function about extracellular region, different binding region and cell adhesion (Fig. 7C). The KEGG enrichment analysis of CM-HCECs and TR2Y cells showed the top 20 KEGG sets of abundance. ECM-receptor interaction, Nitrogen metabolism, Protein digestion and absorption, PI3K-Akt signaling pathway and Focal adhesion were the top 5 sets of abundance (Fig. 7D).
The up-regulated DEGs were mainly enriched in extracellular matrix (ECM) of these pathways such as collagen, laminin and fibronectin.

**Discussion**

DSEK, DSAEK and DEMK had brought new technologies for corneal transplantation in the treatment of corneal endothelial dysfunction in recent years [6, 23]. But these methods also have disadvantages such as technical difficulty, cell loss, lamellae dislocation, graft rejections and graft failure after surgery [24–27]. However, all types of corneal transplantation depend on donor cornea and healthy HCECs. Corneal substitutes are expected to completely solve the worldwide shortage of corneal donors with the rapid development of tissue engineering and regenerative medicine[15]. Among the three elements of seed cells, scaffold materials and three-dimensional culture, seed cells are the most critical factor. Recently researchers had studied the effect of pharmacological agents on CEC proliferation, such as rho-associated protein kinase inhibitor Y-27632 and inhibitors of the p38 mitogen-activated protein kinase (MAPK) [10, 28]. The former researchers used cell injection with Y-27632 to treat human bullous keratopathy and the experimental results were gratifying. However, excessive cell proliferation usually leads to EMT with loss of function [29]. Therefore, how to obtain a sufficient number of HCECs with therapeutic ability remains a knotty problem.

OASCs and CECs are both derived from neural crest cells of the neuroectoderm [30, 31]. OASCs possess a powerful proliferative capacity and multi-lineage differentiation potential [20]. In the previous study, we prepared a conditioned medium obtained from OASCs (OASC-CM) and cultivated HCECs with OASC-CM (CM-HCECs). The results of *in vitro* experiments showed that CM-HCECs could highly express CEC related markers (N-Cadherin, Na+/K+ ATPase and ZO-1) even after 10 passages. Meanwhile, the proliferation and repair ability of HCECs were significantly enhanced. In order to test the therapeutic ability of CM-HCECs *in vivo*, we carried out animal experiments of corneal endothelial dysfunction in rabbit and monkey models. The results of 10 months observation after surgery showed that the cornea could recover and remain transparent in a short time which preliminarily proved the therapeutic effect of CM-HCECs [18].

In this study, we conducted monkey experiments and observed 24 months after cell transplantation. Through the experimental results we discovered that the corneas of the TR2Y group recovered rapidly and remained transparent. The central corneal thickness (CCT) maintained at about 500µm 1-24 months after surgery. The average endothelial cell density was the lowest in the third month, which was considered to be related to postoperative immune reaction, microenvironment changes and apoptosis caused by inflammatory factors [32, 33]. After 3 months, the cell density gradually increased, indicating that the transplanted cells had adapted to the new microenvironment and remained stable. At 24 months after cell transplantation, the cell density in the TR2Y group remained at about 2500 cells/mm², which was slightly lower than that of over 3000 cells/mm² in normal monkeys before the operation. In addition, there were no pathological changes in eyes of the TR2Y group by a gonioscope, fundus photography and B-mode ultrasound. The result indicated that transplanted cells coexisted harmoniously with the host.
Histological examination and Immunofluorescence also showed that the transplanted cells formed single cell layer on DM and played the function of tight junction and pump function.

RNA-sequencing was used to study the gene expression pattern of HCEC, CM-HCECs and TR2Y cells after cell transplantation. Venn analysis and correlation analysis showed that the proportion of co-expressed genes and the correlation coefficient in HCECs, CM-HCECs and TR2Y cells were quite high. The results of GO annotation analyses also showed that the three cells were similar in gene expression pattern and belonged to the same type of cell.

Venn analysis showed that the CM-HCECs and TR2Y cells had high proportion of co-expressed genes. Correlation analysis showed that there was a high correlation between CM-HCECs and TR2Y cells and the r value was 0.911. It showed that most cells maintained the original expression pattern and remained in stable status after the transplantation. Venn analysis showed that the proportion of co-expressed genes of HCECs and tr2y was 77.53% which was higher than that of HCECs and CM-HCECs (77.48%). And the correlation analysis showed that the r value was 0.809 in HCECs vs TR2Y cells and 0.712 in HCECs vs CM-HCECs. The result indicated the transplanted cells were closer to HCECs. It could be seen from the analysis of the expression difference between CM-HCECs and TR2Y groups that transplanted cells had obvious changes in extracellular matrix, cell adhesion and other related functions. The result indicates that the adhesion of TR2Y cells is enhanced after cell transplantation. We consider that the microenvironment in monkey anterior chamber is more conducive to cell survival and growth than that \textit{in vitro} [34].

In this study, keratic precipitates (KP) and anterior chamber exudation occurred after the transplantation. This may due to the immune reaction caused by some transplanted cells falling onto the lens or into the anterior chamber. In addition, heterologous grafts and usage of serum containing medium could also cause immune or rejection reactions [18]. But the reactions were moderate and could be controlled by conventional therapy. This may benefit from that the cultured HCECs are endowed with the function of immunomodulatory by CM [35, 36]. On the other hand, transplantation through cell-injection also has limitations such as inaccurate location of cells, cells lost and the requirement for special body position after surgery. We provide long-term observation and analysis results on advanced primates although the number of experimental animals is limited.

In this preclinical research the general condition of the monkeys was good during 24 months of postoperative observation. There were no indications of abnormal intraocular pressure, lens opacity and changes of fundus. In the future research, we will improve experiment method and procedure and conduct clinical trials.

\textbf{Conclusions}

In conclusion, CM-HCECs can successfully treat the monkey corneal endothelial dysfunction model, survive for a long time and remain stable in the host. The corneas of the experimental group remain transparent during the long-term observation after cell transplantation. The gene expression pattern of
transplanted cells is closer to that of HCECs. Our research provides more cell resources and ideas for regenerative medicine and tissue engineering.

**Abbreviations**

BM: basal culture medium; CCK-8: Cell Counting Kit-8; CCT: central corneal thickness; CECs: corneal endothelial cells; CM: conditioned medium; CM-HCECs: cultivated HCECs with conditioned medium; DEGs: differential expression genes; DM: Descemet's membranes; DMEK: Descemet's membrane endothelial keratoplasty; DMEM-LG: Dulbecco's modified Eagle's medium low glucose; DSAEK: Descemet's stripping automated endothelial keratoplasty; DSEK: Descemet's stripping endothelial keratoplasty; ECM: extracellular matrix; EMT: endothelial-to-mesenchymal transition; FECD: fuchs endothelial corneal dystrophies; HCECs: human corneal endothelial cells; H&E: Hematoxylin and eosin; ICE: iridocorneal endothelial syndrome; IOP: intraocular pressure; KP: keratic precipitates; MAPK: mitogen-activated protein kinase; OASCs: orbital adipose-derived stem cells; OASC-CM: conditioned medium obtained from orbital adipose-derived stem cells; PBS: phosphate buffered saline; PK: penetrating keratoplasty; ZO-1: zonula occludens-1

**Declarations**

**Ethics approval and consent to participate**

All animals were treated in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experiments were approved by the Medical Ethics Committee of Qilu Hospital of Shandong University, Yantai Yuhuangding Hospital and the Association for Laboratory Animal Care of HongLi Medical Animal Experimental Research Center, China. Samples were collected following written informed consents, and the study was performed in adherence to the Declaration of Helsinki. All the experimental protocols were approved by the Ethical Committee of Qilu Hospital of Shandong University and Yantai Yuhuangding Hospital and were conducted following the institutional guidelines.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
Competing interests

The authors declare no competing interests.

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Authors’ contributions

P. S and L. S contributed equally to this work. P. S and X. W were responsible for conception and design. P. S and L. S conducted the cell experiments, analyzed the data and wrote the manuscript. P. S, L. S and L. D performed the animal experiment. Y. L and X. W were responsible for provision of study material and revision of the manuscript.

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Figures
Figure 1

Schema of CM-HCECs transplantation into the Monkey corneal endothelial dysfunction model. A1 Scrape of CECs (the blue dots) on DM (A1). A2 Injection of CM-HCECs (the green dots) into the anterior chamber. A3 The eyes were kept in a face-down position for the CM-HCECs (the green dots) sinking onto the DM. B1 The CECs were completely scraped from the DM of the monkey model with a modified irrigator needle. B2 CM-HCECs suspended in MEM were injected into the anterior chamber with an insulin needle. B3 The monkeys were kept in the face-down position immediately after cell transplantation.
Figure 2

Isolation, culture and characteristics of human corneal endothelial cells (HCECs) by OASC-CM. A, B Peeled DM layer that contained endothelial cells. C The morphology of different passages of CM-HCECs. D Expression of CEC relative markers by Immunofluorescence. Scale bar: 100 μm.
Figure 3

Observation of TR2Y group and control group during 24 months after transplantation of CM-HCECs. A Corneal transparency, thickness and anterior chamber condition were examined by slit-lamp in the TR2Y group. B Corneal transparency, thickness and anterior chamber condition were examined by slit-lamp in the control group.
Figure 4

Ophthalmologic examination results of TR2Y group and control group after cell transplantation. A OCT showed differences of central corneal thickness (CCT) in the TR2Y group and the control group. B CECs were detected by noncontact specular microscopy in the TR2Y group. C-E Images of anterior chamber angle, fundus and ocular B-mode ultrasound in the TR2Y group. F Mean CCT was measured in the TR2Y
group and the control group before and after surgery. G Changes of cell density in the TR2Y group. H IOP was measured in the TR2Y group and the normal group during the 24 months observation.

Figure 5

Histological examination and Immunofluorescence of normal group, TR2Y group and control group. A H&E staining of cornea in different groups. B Immunofluorescent staining of Na⁺/K⁺ ATPase and Zo-1 (blue: DAPI, red: Na⁺/K⁺ ATPase, Zo-1). Scale bar: 100 μm.
Figure 6

Differential expression gene and correlation in HCECs, CM-HCECs and TR2Y cells. A Venn analysis of HCECs and CM-HCECs. B Venn analysis of HCECs and TR2Y cells. C Venn analysis of CM-HCECs and TR2Y cells. D The correlation analysis of HCECs, CM-HCECs and TR2Y cells.

Figure 7

Differential expression gene analysis of CM-HCECs and TR2Y cells. A Heatmap of CM-HCECs versus TR2Y cells. B Volcano of CM-HCECs versus TR2Y cells. C GO enrichment analysis of CM-HCECs versus TR2Y cells. D KEGG enrichment analysis of CM-HCECs versus TR2Y cells.