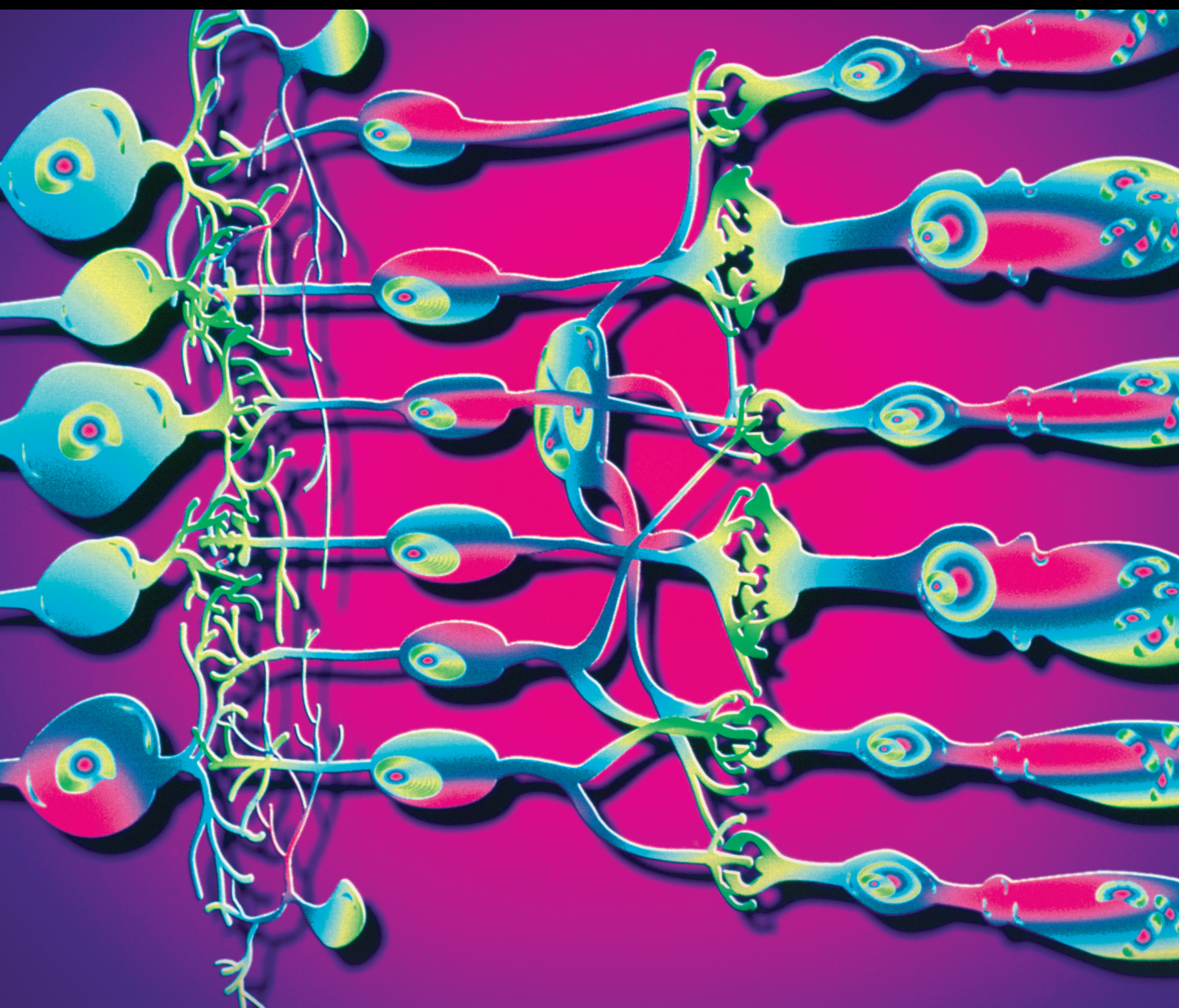
Journal of Ophthalmology

Corneal Tissue Engineering: From Bench to Clinic

Lead Guest Editor: Sang Beom Han
Guest Editors: Jodhbir S. Mehta, Yu-Chi Liu, and Karim Mohamed-Noriega



Corneal Tissue Engineering: From Bench to Clinic

Journal of Ophthalmology

Corneal Tissue Engineering: From Bench to Clinic

Lead Guest Editor: Sang Beom Han
Guest Editors: Jodhbir S. Mehta, Yu-Chi Liu,
and Karim Mohamed-Noriega

Editorial Board

Contents

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 6091389, 1 page
https://doi.org/10.1155/2018/6091389

Editorial

Corneal Tissue Engineering: From Bench to Clinic

Sang Beom Han,[1] **Jodhbir S. Mehta**,[2,3,4] **Yu-Chi Liu**,[2,3,4] **and Karim Mohamed-Noriega**[5]

[1]*Department of Ophthalmology, Kangwon National University Hospital, Kangwon National University School of Medicine, Chuncheon, Republic of Korea*
[2]*Singapore National Eye Centre, Singapore*
[3]*Singapore Eye Research Institute, Singapore*
[4]*Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[5]*Department of Ophthalmology, Autonomous University of Nuevo Leon, Monterrey, Mexico*

Correspondence should be addressed to Sang Beom Han; m.sangbeom.han@gmail.com

Received 26 September 2018; Accepted 26 September 2018; Published 23 December 2018

Corneal blindness is one of the major causes of blindness worldwide, for which corneal transplantation has long been the only treatment method. In addition to penetrating keratoplasty, development of partial thickness corneal transplantation techniques including deep anterior lamellar keratoplasty (DALK), Descemet's stripping endothelial keratoplasty (DSEK), and Descemet's membrane endothelial keratoplasty (DMEK) allowed improved visual anatomical outcome. Keratoprosthesis including Boston keratoprosthesis (KPro) and osteo-odonto keratoprosthesis (OOKP) also enabled visual recovery in patients with serious ocular surface destruction. However, the demands for corneal transplant can never be met because of global shortage of donor corneal tissue.

We have introduced in the call for papers for this special issue that various methods of corneal tissue engineering such as cultivation and expansion of corneal tissues using novel drugs and biomaterials have been studied. These novel methods are expected to allow the production of large amount of synthetic corneal tissues for transplantation without the risk of disease transmission. Further researches on corneal tissue engineering, i.e., cell therapy for corneal tissues including the epithelium, stroma, and endothelium; development of biomaterials, drugs, and imaging techniques that can be applied to corneal tissue engineering; application of artificial corneal tissue; and anterior segment reconstruction would provide even more improved visual and anatomical results.

In this special issue, the authors contributed 4 original articles and 2 review papers regarding researches of corneal tissue engineering and its clinical application.

The authors reported the results of their original researches on various topics in corneal tissue engineering: (1) outcomes of Descemet membrane endothelial keratoplasty for vitrectomized eyes with sutured posterior chamber intraocular lens; (2) generation of femtosecond laser-cut decellularized corneal lenticule using hypotonic trypsin-EDTA solution for corneal tissue engineering; (3) modern corneal eye-banking using a software-based IT management solution; and (4) preliminary investigation of the mechanical anisotropy of the normal human corneal stroma.

This special issue also includes review articles on the following topics: (1) application of novel drugs for corneal cell regeneration and (2) from DMEK to corneal endothelial cell therapy: technical and biological aspects.

We believe these articles will provide readers with valuable information on corneal tissue engineering and novel ideas for researches on related topics.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by 2017 Research Grant from Kangwon National University (No. 520170034).

Sang Beom Han
Jodhbir S. Mehta
Yu-Chi Liu
Karim Mohamed-Noriega

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 5392041, 7 pages
https://doi.org/10.1155/2018/5392041

Research Article

Preliminary Investigation of the Mechanical Anisotropy of the Normal Human Corneal Stroma

Chao Xue,[1] Yaoqi Xiang,[2] Min Shen,[2] Di Wu,[1] and Yan Wang[1]

[1]*Clinical College of Ophthalmology, Tianjin Medical University, Tianjin Eye Hospital, Tianjin Key Laboratory of Ophthalmology and Visual Science, Tianjin Eye Institute, Tianjin, China*
[2]*School of Mechanical Engineering, Tianjin University, Tianjin, China*

Correspondence should be addressed to Yan Wang; wangyan7143@vip.sina.com

Received 5 April 2018; Revised 20 July 2018; Accepted 16 September 2018; Published 17 October 2018

Guest Editor: Karim Mohamed-Noriega

Purpose. To investigate the anisotropic characteristics of the normal human corneal stroma using fresh corneal tissue. *Methods*. Sixty-four corneal specimens extracted from stromal lenticules were included in this study. The specimens were cut in the temporal-nasal (horizontal) or superior-inferior (vertical) direction. Strip specimens were subjected to uniaxial tensile testing. The tensile properties of the specimens were measured and compared in the two directions. *Results*. The low-strain tangent modulus was statistically significantly greater in the vertical direction than in the horizontal direction (1.32 ± 0.50 MPa vs 1.17 ± 0.43 MPa; $P = 0.035$), as was the high-strain tangent modulus (51.26 ± 8.23 MPa vs 43.59 ± 7.96 MPa; $P \leq 0.001$). The elastic modulus in the vertical direction was also higher than that in horizontal direction at stresses of 0.01, 0.02, and 0.03 MPa, but not statistically significant; so, $P = 0.338$, 0.373, and 0.417, respectively. *Conclusions*. The biomechanical behavior in normal human corneal stroma tissue is slightly stiffer in the vertical direction than in the horizontal direction. This information may aid our understanding of the biomechanical properties of the cornea and related diseases.

1. Introduction

The transparent cornea is a significant component of the outer ocular tunic. In addition to serving as the primary refractive component of the eye, the cornea has unique mechanical characteristics [1–3]. Mechanical anisotropy is an important property of the cornea and may provide valuable information in terms of determining the onset and severity of many diseases [4]. Meek and Boote reported that the orientation of collagen fibrils in keratoconus was different from that in a healthy cornea [5].

Previous microstructural studies found that the majority of collagen fibrils in the central region of the human cornea had a preferential orientation in the inferior-superior (vertical) or nasal-temporal (horizontal) direction [2]. Given that collagen fibrils are the main load-carrying elements of the stroma, their preferential orientation may determine the mechanical anisotropy of the cornea [6–8].

Uniaxial tensile testing has been used to compare the mechanical behavior of porcine and cadaveric human cornea strips from different directions and showed that the elastic response differs according to the cutting direction [9, 10]. However, this test cannot describe the mechanical anisotropy of the normal human cornea accurately. Fortunately, with the advent of small incision lenticule extraction (SMILE) surgery since 2011 [11], it is now possible to obtain fresh human corneal tissue, allowing direct study of mechanical anisotropy in the fresh human corneal stroma.

While the material parameters of the corneal stromal lenticules have be measured through uniaxial tensile test in the previous study [12], the purpose of this study was to investigate the mechanical anisotropy of the normal human corneal stroma using stromal lenticules extracted during SMILE as part of an effort to elucidate the biomechanical behavior of the human cornea.

2. Materials and Methods

2.1. Surgical Procedures and Preparation of Specimens. All patients underwent a systematic ophthalmic examination to confirm a healthy cornea, including but not limited to slit-lamp microscopy, corneal topography, and measurement of intraocular pressure. The study exclusion criteria were keratoconus or suspected keratoconus, active ocular or systemic disease, a clinical history of ocular surgery or trauma, and any other condition that could affect the health of the cornea.

The SMILE procedures were performed by the same experienced surgeon using the VisuMax femtosecond laser system (Carl Zeiss Meditec AG, Jena, Germany). The details of this procedure have been described elsewhere [11, 13, 14]. After creation of a refractive lenticule and a small incision, the surgeon dissected the lenticule from the surrounding tissue and extracted it through the incision.

After extraction, the 12 o'clock position of the corneal lenticule was marked with gentian violet. Each specimen was preserved in storage medium (Eusol-C; Alchima, Padova, Italy) below 4° in a refrigerator for no more than 24 hours, after which the lenticule was prepared for the experiment.

The lenticule was gently placed on a rubber base and cut into 1.0 mm-wide strip specimens from the central region in the required direction using a customized double-blade knife. The lengths of the corneal specimens were different according to the diameter of the corneal lenticule and were approximately 6.5 mm. Specimens from left eyes were cut in the temporal-nasal (horizontal) direction, and those from right eyes were cut in the superior-inferior (vertical) direction (Figure 1).

2.2. Uniaxial Tensile Testing. The specimens were then clamped between the rough-surfaced jaws to prevent slippage. Next, they were subjected to uniaxial tensile testing, starting with three loading/unloading cycles to precondition the specimen, followed by loading to failure.

The tests were performed using an IBTC-50 (in situ bidirectional tension and compression) testing system (Tianjin Care Measure and Control Co., Ltd., Tianjin, China) in a laboratory water bath filled with normal saline at room temperature. The distance between the two clamps was 1.5 mm. The rate of deformation was 0.01 mm/s. The central thickness of the corneal specimen was obtained from the SMILE surgery data. The specimen width was 1 mm for this experiment.

The study was approved by the Ethics Committee at Tianjin Eye Hospital, Tianjin Medical University, and adhered to the tenets of the Declaration of Helsinki. Informed consent to use clinical data for analysis and publication was obtained from all patients.

2.3. Statistical Analysis. The statistical analysis was performed using IBM SPSS software version 20 (IBM Corp., Armonk, NY). Eye pairs were compared using the paired t-test. A P-value <0.05 was considered statistically significant.

3. Results

Sixty-four corneal specimens obtained from the left and right eyes of 32 patients (11 men; 21 women) of mean age 21.11 ± 3.24 (range 16–31) years were included in the study. The mean preoperative sphere was −4.18 ± 1.59 D in the left eyes and −4.51 ± 1.64 D in the right eyes, and there was no significant statistical significance between these two groups ($P = 0.065$). Twenty-eight of 32 left eyes and twenty-seven of 32 right eyes have both spherical and cylindrical errors. All the astigmatism was with the rule. The mean preoperative cylinder was −0.95 ± 0.91 D in the left eyes and −0.93 ± 0.90 D in the right eyes, and there was no significant statistical significance between these two groups ($P = 0.810$). The mean central lenticule thickness was 103.78 ± 25.85 μm.

A typical stress-strain curve is shown in Figure 2. The curve can be divided into four sections. The first section is the linear elastic OA segment, in which the load changes are very small and deformation increases rapidly. The second section is the AB segment, in which the load increases exponentially with an increase in deformation, thus reflecting a nonlinear relationship. The third section is the BC segment, which is an approximately straight line, where the maximum stress is reached at point C. The fourth section is D, which is the fracture point. The elastic modulus (E) of the OA segment is defined as the low-strain tangent modulus (LSTM) and that of the BC segment is defined as the high-strain tangent modulus (HSTM) [15].

Figure 3 shows the stress-strain curves for the 64 specimens in the different directions, most of which are concentrated in a relatively small range.

Average stress-strain curves for eye pairs were compared in the vertical direction and the horizontal direction (Figure 4). The stress-strain behavior of each corneal specimen was described using the following equation:

$$\sigma = A\left(e^{B\varepsilon} - 1\right), \tag{1}$$

where A and B are constants [10]. The values of A and B that provided the best fit are shown in Table 1. The difference between the two curves increased with increasing strain/stress (Figure 4). Under the physiological intraocular pressure, the corneal stress is about 0.02 MPa. We calculated Young's modulus with a stress growth from 0.01 MPa to 0.03 MPa.

The uniaxial tensile test data of corneal stroma were selected. By plotting the values of σ for the early part of the tests (corresponding to the strain of less than 5%), we can determine Young's modulus E by linear regression analysis (Figure 5). On the early part of the tests, the data fluctuated greatly, and their increasing trend can be reflected by linear fitting. We used the area of strain from 0 to 0.5 as a low-strain zone, and the high-strain zones can easily be identified through the stress-strain curves. We can roughly estimate Young's modulus in low-strain zones and high-strain zones through this method.

The LSTM and HSTM values in the vertical direction were significantly higher than those in the horizontal direction (Table 2).

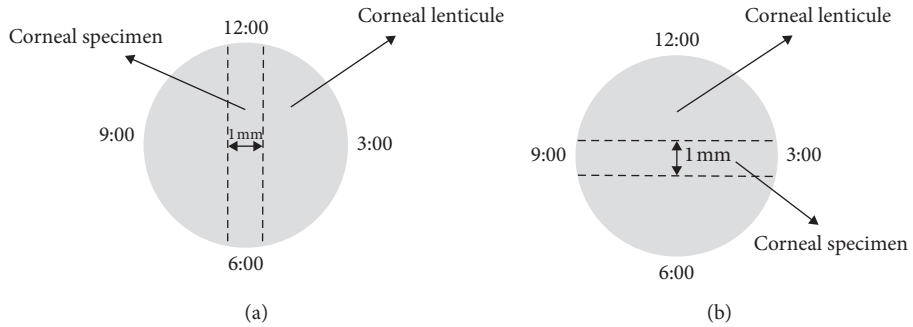


Figure 1: Sketch map of corneal specimen in different directions: (a) vertical; (b) horizontal.

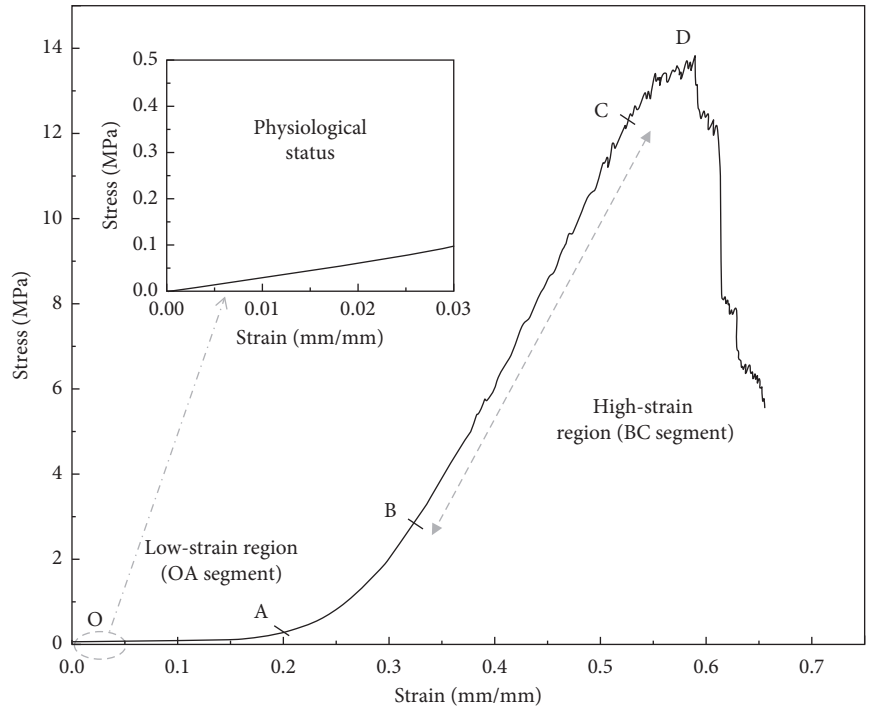


Figure 2: The stress-strain curve of a corneal strip.

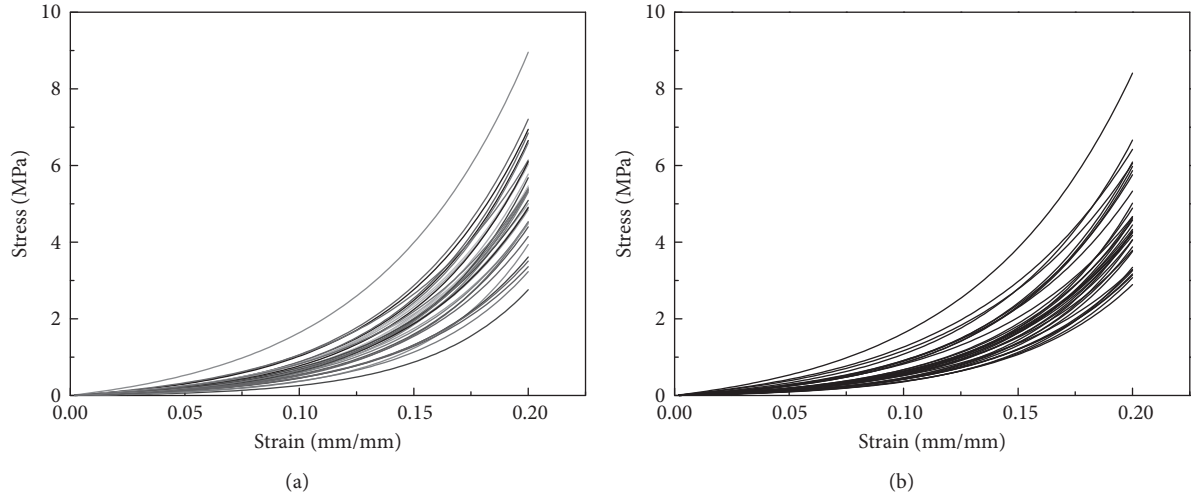


Figure 3: Stress-strain curves of 32 specimens in different directions: (a) vertical; (b) horizontal.

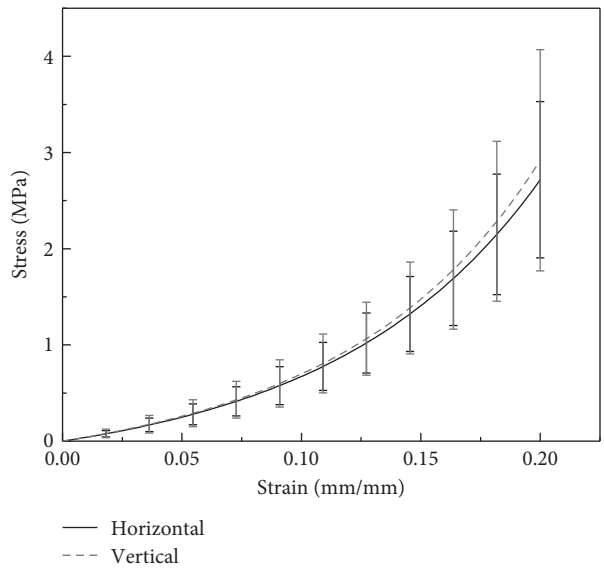


Figure 4: Average stress-strain curves of specimens.

Table 1: Values of constants A and B.

No.	A		B	
	Vertical (MPa)	Horizontal (MPa)	Vertical	Horizontal
1	0.15	0.184	18.958	17.644
2	0.140	0.063	19.333	20.549
3	0.030	0.258	22.632	16.445
4	0.042	0.0720	22.771	20.021
5	0.132	0.063	18.23	21.219
6	0.075	0.075	19.502	18.987
7	0.114	0.139	19.331	17.604
8	0.222	0.127	17.548	17.683
9	0.215	0.369	17.533	14.288
10	0.276	0.238	15.732	15.774
11	0.122	0.135	19.084	17.432
12	0.119	0.119	19.516	17.446
13	0.086	0.0773	18.463	18.689
14	0.156	0.168	18.388	18.002
15	0.090	0.113	20.488	18.708
16	0.171	0.100	17.972	19.108
17	0.152	0.088	19.142	20.281
18	0.211	0.222	16.048	15.459
19	0.056	0.119	20.381	17.939
20	0.076	0.085	20.536	19.668
21	0.126	0.082	17.993	18.369
22	0.132	0.058	18.746	19.956
23	0.083	0.083	18.812	20.179
24	0.131	0.060	19.302	21.107
25	0.103	0.049	19.023	21.189
26	0.144	0.128	17.959	18.332
27	0.472	0.172	14.969	17.799
28	0.129	0.101	18.256	18.586
29	0.094	0.211	19.039	16.713
30	0.126	0.133	18.438	17.57
31	0.183	0.511	17.078	14.302
32	0.108	0.064	19.634	19.178
Mean ± SD	0.140 ± 0.08	0.140 ± 0.10	18.78 ± 1.63	18.32 ± 1.83

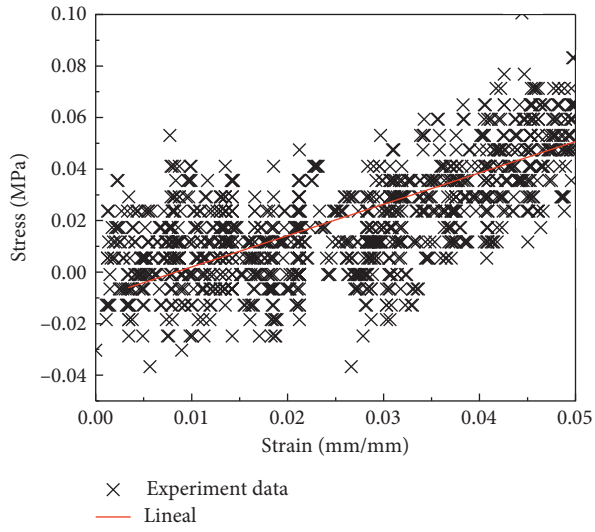


Figure 5: Curve fitting to obtain Young's modulus.

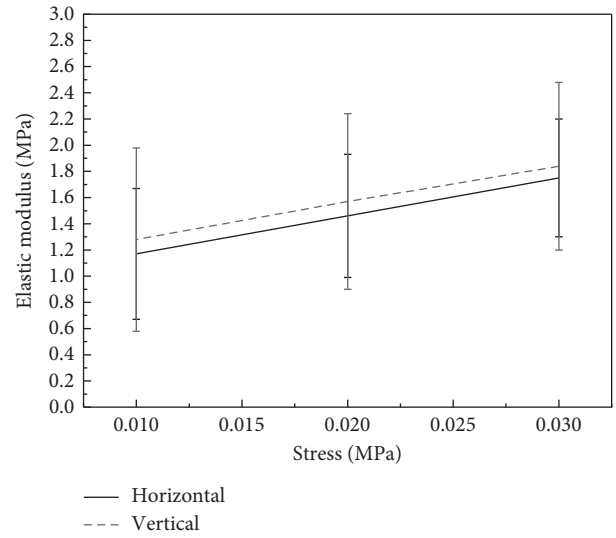


Figure 6: Relationships between elastic modulus and stress.

Table 2: Comparison of LSTM and HSTM in eye pairs.

Parameters	Horizontal	Vertical	P value
LSTM(MPa)	1.17 ± 0.43	1.32 ± 0.50	0.035
HSTM(MPa)	43.59 ± 7.96	51.26 ± 8.23	≤0.001

Table 3: Comparison of elastic modulus at different stress levels.

Stress (MPa)	Vertical (MPa)	Horizontal (MPa)	P
0.01	1.28 ± 0.70	1.17 ± 0.50	0.338
0.02	1.57 ± 0.67	1.46 ± 0.47	0.373
0.03	1.84 ± 0.64	1.75 ± 0.45	0.417

Figure 6 shows the relationship between the elastic modulus (E) and stress (σ) according to the physiologic state of the cornea. Very small scatter could be seen between the two lines, but the difference was not statistically significant ($P > 0.05$; Table 3). Figure 6 shows that the relationship between the elastic modulus and stress was linear.

4. Discussion

Many groups have recognized the importance of the anisotropic characteristics of the cornea, and much research effort has been focused on the biomechanical anisotropy of this structure. In previous research, the biomechanical properties of strip specimens from porcine or cadaveric human cornea tissue cut in different directions were compared using uniaxial tensile tests, and the results showed that the biomechanical behavior varied depending on the direction in which the specimen was cut [9, 10]. In one study, inflation tests were performed to investigate the global characteristics of the entire bovine cornea, and an inverse finite element method was developed to determine its anisotropic properties [16]. In another study, a computational model was used to determine the biomechanical deformation of corneal tissue that had been cut and removed and the effect of mechanical anisotropy resulting from the fibrillar architecture [17]. All these approaches provided valuable information on corneal mechanical anisotropy but could not describe the mechanical anisotropy of the normal human cornea accurately because data could only be obtained from animal or cadaveric human corneas, which may not be representative of the normal fresh human cornea. With the advent of SMILE surgery, it is now possible to obtain fresh normal corneal tissue for research purposes. Although corneal lenticules have been used in previous studies to observe changes in cell morphology [18–20], there have been no reports of biomechanical tests performed using fresh human corneal tissue.

The corneal stroma contains several hundred lamellae, each of which is composed of parallel collagen fibrils embedded in an extracellular matrix [21, 22]. The stroma comprises about 90% of the corneal thickness and determines the mechanical behavior of the cornea. Knowledge of the biomechanical properties of the corneal stroma would aid our understanding of the biomechanical properties of the cornea itself.

Eusol-C was used to preserve the corneal lenticule immediately after it was surgically extracted. The effectiveness of this storage medium in maintaining stromal hydration after 7 days of storage has been confirmed in previous studies [10, 23]. In the present study, the corneal lenticule was preserved in the storage medium, Eusol-C, for no more than 24 hours so that the specimens would maintain their good quality for the tests. Although the tensile tests were conducted in saline solution in the present study, the experiment was accomplished in a very short time; therefore, it is unlikely that significant swelling occurred during the experiments.

In this study, scatter was seen on comparison of the stress-strain curves for eye pairs in the vertical and horizontal directions. Under the same level of strain, the stress in the vertical direction was greater than that in the horizontal

direction, and the difference increased with increasing strain. Both the LSTM and HSTM were significantly higher in the vertical direction than in the horizontal direction. This finding confirms that the biomechanical behavior of the cornea differs in different directions.

In this study, we analyzed the biomechanical behavior of the cornea in the physiological level of stress. Under the same level of stress, the elastic modulus of each specimen was greater in the vertical direction than in the horizontal direction at stress levels of 0.01, 0.02, and 0.03 MPa, but the difference was not statistically significant. These three stress levels were chosen because they are within the normal physiologic state of the cornea [24] and were applied immediately after the start of uniaxial tensile testing, that is, in a different phase from that in which the LSTM and HSTM were calculated. This could explain why there was a statistically significant difference in the LSTM and HSTM between the vertical direction and the horizontal direction and no statistically significant difference in the value of the elastic modulus between the two directions at stress levels of 0.01, 0.02, and 0.03 MPa. The implications of this finding require further study. In addition, there was a linear relationship between the elastic modulus and stress in this study. The cornea is a soft tissue with elastic nonlinearity properties, so the elastic modulus increases with increasing stress [9].

Previous works showed that subjects with high myopia had lower normalized corneal tangent moduli than subjects with low myopia had [25–27]. In addition, the cut pattern of the lenticule is different between pure spherical correction and those with sphere and cylinder correction; it could impact the results. In the present study, both preoperative sphere and cylinder had no significant statistical significance between eyes from horizontal and vertical directions. Based on this result, we are able to compare the tensile properties of the specimens in different directions.

Elsheikh et al. [10] reported that vertical strips of cornea from cadaveric human eyes were slightly stiffer and stronger than horizontal strips, by 10%–25% on average at a rate of deformation of 1% per minute and 16%–18% at a rate of deformation of 500% per minute. In the present study, the vertical specimens were also slightly stiffer and stronger than the horizontal specimens. We found that the vertical specimens were stronger on average than the horizontal specimens by 13% for LSTM and 18% for HSTM at a rate of deformation of 40% per minute, which is consistent with the findings of Elsheikh et al. [10]. However, in the physiological state, the difference was much smaller at stress levels of 0.01, 0.02, and 0.03 MPa, with corresponding rates of 1%, 4%, and 5%, respectively. Figures 4 and 5 show that the difference in the elastic modulus between the vertical and horizontal direction increases with increasing stress. This difference is much smaller in the very early portion of the stress-strain curve than in the later portions. The same trend was present in the study reported by Elsheikh et al. [10]. This phenomenon may also explain the much smaller difference found in the physiologic state.

In conclusion, the results of this study show that the biomechanical behavior of normal human corneal stroma tissue is slightly stiffer in the vertical direction than in the horizontal direction. To the best of our knowledge, the present study is the first attempt to investigate mechanical corneal anisotropy using fresh human corneal stroma tissue. Different elongation rates will be necessary in future studies to assess the possible effect of corneal viscoelasticity on mechanical anisotropy. Also, obtaining overall corneal properties through local property measurement will be the focus of further work.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant no. 81670884).

References

[1] D. P. Pinero and N. Alcon, "Corneal biomechanics: a review," *Clinical and Experimental Optometry*, vol. 98, no. 2, pp. 107–116, 2015.

[2] C. R. Ethier, M. Johnson, and J. Ruberti, "Ocular biomechanics and biotransport," *Annual Review of Biomedical Engineering*, vol. 6, no. 1, pp. 249–273, 2004.

[3] C. J. Roberts, "Concepts and misconceptions in corneal biomechanics," *Journal of Cataract and Refractive Surgery*, vol. 40, no. 6, pp. 862–869, 2014.

[4] M. Singh, J. Li, Z. Han et al., "Assessing the effects of riboflavin/UV-A crosslinking on porcine corneal mechanical anisotropy with optical coherence elastography," *Biomedical Optics Express*, vol. 8, no. 1, pp. 349–366, 2017.

[5] K. M. Meek and C. Boote, "The use of X-ray scattering techniques to quantify the orientation and distribution of collagen in the corneal stroma," *Progress in Retinal and Eye Research*, vol. 28, no. 5, pp. 369–392, 2009.

[6] K. L. Goh, J. R. Meakin, R. M. Aspden, and D. W. Hukins, "Influence of fibril taper on the function of collagen to reinforce extracellular matrix," *Proceedings of the Royal Society B: Biological Sciences*, vol. 272, no. 1575, pp. 1979–1983, 2005.

[7] K. L. Goh, J. R. Meakin, R. M. Aspden, and D. W. Hukins, "Stress transfer in collagen fibrils reinforcing connective tissues: effects of collagen fibril slenderness and relative stiffness," *Journal of Theoretical Biology*, vol. 245, no. 2, pp. 305–311, 2007.

[8] K. M. Meek and R. H. Newton, "Organization of collagen fibrils in the corneal stroma in relation to mechanical properties and surgical practice," *Journal of Refractive Surgery*, vol. 15, no. 6, pp. 695–699, 1999.

[9] A. Elsheikh and D. Alhasso, "Mechanical anisotropy of porcine cornea and correlation with stromal microstructure," *Experimental Eye Research*, vol. 88, no. 6, pp. 1084–1091, 2009.

[10] A. Elsheikh, M. Brown, D. Alhasso, P. Rama, M. Campanelli, and D. Garway-Heath, "Experimental assessment of corneal anisotropy," *Journal of Refractive Surgery*, vol. 24, no. 2, pp. 178–187, 2008.

[11] W. Sekundo, K. S. Kunert, and M. Blum, "Small incision corneal refractive surgery using the small incision lenticule extraction (SMILE) procedure for the correction of myopia and myopic astigmatism: results of a 6 month prospective study," *British Journal of Ophthalmology*, vol. 95, no. 3, pp. 335–339, 2011.

[12] Y. Xiang, M. Shen, C. Xue, D. Wu, and Y. Wang, "Tensile biomechanical properties and constitutive parameters of human corneal stroma extracted by SMILE procedure," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 85, pp. 102–108, 2018.

[13] Y. Wang, J. Ma, J. Zhang et al., "Incidence and management of intraoperative complications during small-incision lenticule extraction in 3004 cases," *Journal of Cataract and Refractive Surgery*, vol. 43, no. 6, pp. 796–802, 2017.

[14] D. Wu, Y. Wang, L. Zhang, S. Wei, and X. Tang, "Corneal biomechanical effects: small-incision lenticule extraction versus femtosecond laser-assisted laser in situ keratomileusis," *Journal of Cataract and Refractive Surgery*, vol. 40, no. 6, pp. 954–962, 2014.

[15] L. A. Davis, S. E. Stewart, C. G. Carsten, B. A. Snyder, M. A. Sutton, and S. M. Lessner, "Characterization of fracture behavior of human atherosclerotic fibrous caps using a miniature single edge notched tensile test," *Acta Biomaterialia*, vol. 43, pp. 101–111, 2016.

[16] T. D. Nguyen and B. L. Boyce, "An inverse finite element method for determining the anisotropic properties of the cornea," *Biomechanics and Modeling in Mechanobiology*, vol. 10, no. 3, pp. 323–337, 2011.

[17] P. M. Pinsky, D. van der Heide, and D. Chernyak, "Computational modeling of mechanical anisotropy in the cornea and sclera," *Journal of Cataract and Refractive Surgery*, vol. 31, no. 1, pp. 136–145, 2005.

[18] Y. W. Ji, M. Kim, D. S. Y. Kang et al., "Effect of lowering laser energy on the surface roughness of human corneal lenticules in SMILE," *Journal of Refractive Surgery*, vol. 33, no. 9, pp. 617–624, 2017.

[19] M. Lombardo, M. P. De Santo, G. Lombardo et al., "Surface quality of femtosecond dissected posterior human corneal stroma investigated with atomic force microscopy," *Cornea*, vol. 31, no. 12, pp. 1369–1375, 2012.

[20] N. M. Ziebarth, M. A. Lorenzo, J. Chow et al., "Surface quality of human corneal lenticules after SMILE assessed using environmental scanning electron microscopy," *Journal of Refractive Surgery*, vol. 30, no. 6, pp. 388–393, 2014.

[21] L. J. Muller, E. Pels, L. R. Schurmans, and G. F. Vrensen, "A new three-dimensional model of the organization of proteoglycans and collagen fibrils in the human corneal stroma," *Experimental Eye Research*, vol. 78, no. 3, pp. 493–501, 2004.

[22] D. A. Parry and A. S. Craig, "Electron microscope evidence for an 80 a unit in collagen fibrils," *Nature*, vol. 282, no. 5735, pp. 213–215, 1979.

[23] D. Amato, F. Oddone, M. Nubile, R. A. M. C. Gisoldi, C. M. Villani, and A. Pocobelli, "Pre-cut donor tissue for descemet stripping automated keratoplasty: anterior hinged lamella on versus off," *British Journal of Ophthalmology*, vol. 94, no. 4, pp. 519–522, 2010.

[24] X. Wang, X. Li, W. Chen, R. He, Z. Gao, and P. Feng, "Effects of ablation depth and repair time on the corneal elastic modulus after laser in situ keratomileusis," *BioMedical Engineering OnLine*, vol. 16, no. 1, p. 20, 2017.

[25] Y. Hon, G. Z. Chen, S. H. Lu, D. C. Lam, and A. K. Lam, "High myopes have lower normalised corneal tangent moduli (less 'stiff' corneas) than low myopes," *Ophthalmic and Physiological Optics : The Journal of the British College of Ophthalmic Opticians (Optometrists)*, vol. 37, no. 1, pp. 42–50, 2017.

[26] A. Plakitsi, C. O'Donnell, M. A. Miranda, W. N. Charman, and H. Radhakrishnan, "Corneal biomechanical properties measured with the ocular response analyser in a myopic population," *Ophthalmic and Physiological Optics : The Journal of the British College of Ophthalmic Opticians (Optometrists)*, vol. 31, no. 4, pp. 404–412, 2011.

[27] M. Shen, F. Fan, A. Xue, J. Wang, X. Zhou, and F. Lu, "Biomechanical properties of the cornea in high myopia," *Vision Research*, vol. 48, no. 21, pp. 2167–2171, 2008.

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 6482095, 8 pages
https://doi.org/10.1155/2018/6482095

Review Article

From DMEK to Corneal Endothelial Cell Therapy: Technical and Biological Aspects

Raffaele Nuzzi[1,2], **Paola Marolo**[1,2] **and Federico Tridico**[1,2]

[1]*S.C.U. Ophthalmology Unit, "City of Health and Science" University Hospital, Via Cherasco 23, 10126 Turin, Italy*
[2]*Department of Surgical Sciences, University of Turin, Corso Dogliotti 14, 10126 Turin, Italy*

Correspondence should be addressed to Raffaele Nuzzi; prof.nuzzi_raffaele@hotmail.it

Received 1 February 2018; Revised 13 May 2018; Accepted 26 June 2018; Published 1 August 2018

Academic Editor: Karim Mohamed-Noriega

The main treatment available for restoration of the corneal endothelium is keratoplasty and DMEK provides faster visual recovery and better postoperative visual acuity when compared to DSAEK. However, the technical challenges related to this technique and the steep technical learning curve seem to prevent the overcoming of DSAEK in favor of DMEK. Furthermore, the outcome of lamellar keratoplasty techniques is influenced by problems related to corneal grafting tissue availability, management, and quality. On the other hand, improvements in the field of cell engineering have opened the way for the use of stem cells-derived corneal endothelial cells with regenerative intent. In this overview, latest findings in endothelial cell engineering are reported, and perspectives of clinical application of mesenchymal stem cells for corneal endothelial replacement and regeneration are evaluated.

1. Introduction

Currently, keratoplasty is the main solution for the treatment of diseases involving corneal endothelium. Frequent indications to endothelial corneal grafting include Fuchs' endothelial dystrophy, bullous keratopathy following phacoemulsification, and endothelial disfunction after corneal transplant. Descemet's membrane endothelial keratoplasty (DMEK), introduced in 2006 [1], represents the most novel technique for endothelial keratoplasty. It differs from Descemet's stripping-automated endothelial keratoplasty (DSAEK) for the use of a grafted material that includes no corneal stroma but only endothelium and Descemet membrane. The graft can thus be introduced into the anterior chamber and applied to the posterior stroma through the injection of an air bubble. The graft rejection risk is lower in DMEK when compared to DSAEK, and several studies have demonstrated that DMEK provides faster visual recovery and better postoperative visual acuity than DSAEK [2–5]. Furthermore, as of today, the rate of primary graft failure after DMEK seems lower if compared to DSEK [6–8].

Despite the promising outcomes of this new technique, DMEK is affected by several technical difficulties. In first instance, the surgical complexity (e.g., because of the thinner tissue used, graft unfolding can be more challenging) and its steep learning curve discourage many surgeons from leaving DSAEK in favor of this technique [9–11]. In addition, a higher graft detachment rate after DMEK might lead to more frequent rebubbling or graft repositioning [12]. Another problem is primary endothelial cell loss, which seems to be related to surgeon experience [13]. Loss of endothelial cells is higher in the early postoperative time after DMEK and around 7% per year in the following period [14].

Lately, several improvements have been made in the realization of techniques to isolate and administer human corneal cells as an alternative to keratoplasty [15]. Emerging strategies of tissue engineering for corneal endothelial applications focus on transplantable endothelial cells production [16].

Nowadays, cell therapy is focused on culture of corneal endothelial cells retrieved from donors, followed by grafting in the donor's cornea. The current research is focusing on the expansion of human corneal endothelial cells to overcome the shortage of donor tissues [15]; however, bioengineered corneal endothelium could lead to promising perspectives for potential applications with regenerative intent [17].

2. Current Findings in Cell Application for Corneal Endothelial Deficiency

Several studies have been conducted investigating the in vitro expansion of corneal endothelial cells (CECs) derived from humans [18, 19] and animal models [20–24]. Human CECs can be isolated from donor corneas with the application of EDTA, trypsin, or collagenase II. Furthermore, through testing of different culturing factors (such as basal culture media, additives [25, 26], and methods of media modulation [25, 27]), several growth environments have been developed in order to allow the expansion of human CECs (such as human corneal stroma, collagen, amniotic membrane, and biodegradable polymers) [28].

As of today, different applicative approaches have been proposed for human CECs: monolayered cell sheets, cellular injection therapy, and cell-carrying systems [25]. Localization of cultivated human CECs (delivered via intracameral injection) onto the posterior corneal surface has been tested through ferromagnetic induction [29, 30] and gravity due to prone posture [18], or a combination of these methods. Previous studies from Mimura et al. evaluated the treatment of corneal endothelial deficiency in rabbit models with intracameral injection of sphere colonies of corneal endothelial progenitor cells [30]. On the other hand, ultrathin sheets of human corneal endothelial cells have been transplanted with DSAEK devices in animal models. Recently, the function and clinical adaptability of isolated primary human corneal endothelial cells have been evaluated in a preclinical rabbit model of endothelial keratopathy, via a tissue-engineered endothelial keratoplasty approach, with positive outcomes regarding corneal thickness reduction [31]. However, this method may prove to be too challenging to be clinically applicable, since an excessively thin sheet may be difficult to handle. For this reason, cells injections seem more technically feasible. Also, several cell-seeded scaffolds have been evaluated for corneal transplantation (composed of different materials such as porcine Descemet's membrane [32, 33], chitosan [34], hydrogel lens [35], and paramagnetic microspheres [36]) promoting transferring of cultivated cells into target corneas. In accordance with these considerations, a clinical trial was started in 2013 to investigate the application of cultured endothelial cell injections, supplemented with a ROCK inhibitor (which showed to be useful for endothelial wound healing [37]) with improvements in endothelial cell density, corneal thickness reduction, and visual acuity (UMIN000012534) [38, 39].

3. Stem Cells for Corneal Endothelium Diseases

Although derivation of human corneal endothelial cells from embryonic stem cells (with their broad differentiation potential) has been reported [40], several concerns surrounding the use of this type of stem cells, on the ethical level—regarding retrieval/donation of oocytes and extraction from the destruction of embryos—and also in terms of safety with the high risk of teratoma development, seem to limit its clinical application [41]. However, a transcriptome analysis on embryonic-derived endothelial stem cells revealed the expression of several markers shared with CECs (such as ZO1, Col8a, and CRY1), with promising perspectives of future applications of stem cells-derived CECs [42]. Given these considerations, an impressive effort has been dedicated to the identification of alternative sources, more suitable for corneal endothelial cells production.

It has been shown that induced pluripotent stem cells (iPSCs) extracted from monkeys can be modified into corneal endothelial cells (through the use of an endothelium-deriving medium including GSK-3-beta inhibitor, retinoic acid, and a ROCK inhibitor) capable of regulating corneal stromal transparency after transplantation into rabbit eyes [43]. However, relevant matters that currently hinder the use of iPSCs are the extremely low rate at which adult somatic cells can be altered in order to obtain iPSCs and the specific conditions allowing the differentiation of corneal endothelial cells from human iPSCs, which have not been fully discovered yet. Taking into consideration also the potential oncogenic risk related to iPSCs [44], an important work is still required to perfect iPSCs differentiation before their safe and efficient application for corneal tissue engineering can be achieved.

Mesenchymal stem cells (MSCs) can be easily obtained from different human tissues, and their application (similarly to other pluripotent cell types) seems more feasible if compared to primary human corneal endothelial cells, which have limited proliferative capacities [45]. As of today, MSCs have been administered through two different delivery routes: local (through direct injection or cell-seeded scaffolds) and systemic (intravenous or intra-arterial introduction) [46]. As multipotent MSCs and iPSCs are derived from adult tissues, they can be used without the ethical problems surrounding embryonic stem cells. In addition, autologous MSCs avoid the need for immune-suppressive drugs to prevent rejection of allogenic grafts.

The potential of phenotypical alteration of mesenchymal stem cells towards human corneal endothelial-like cells is based on the fact that during eye development in many species, including humans, corneal endothelial cells differentiate from neural crest-derived periocular mesenchymal cells (this embryological development is supported also by several immunohistochemical findings) [47–49]. Moreover, MSCs and human CECs share some mesenchymal features (since CECs are able to alter their shape into a fibroblast-like one and can produce type IV collagen, in presence of FGF and fibroblastic extracellular matrix [50, 51]) and are able to express adhesion proteins such ZO1 and N-cadherin [52], although they are considered as different cell types. However, as of today, directed differentiation of CECs has not been clearly identified and the ability to clearly detect a definitive CEC clone derived from pluripotent or stem cells sources is still insufficient.

4. Potential Applications of MSCs for Endothelial Replacement

MSCs open many perspectives for cell-based clinical applications, due to their regenerating ability and their potential to differentiate into many different cell types [53, 54]. MSCs can be easily retrieved from different sources like bone marrow

(BM-MSC), adipose tissue (AT-MSC), skeletal muscle, dental pulp, umbilical cord (U-MSCs), and blood from umbilical cord (UCB-MSCs) [55–62]. However, the ideal source tissue for endothelial cell differentiation remains to be discovered. In fact, MSCs derived from different sources show similar morphology but different colony generation rate, proliferation, and differentiation capacities. For example, UCB-MSCs possess the highest differentiation capacity, but the lowest colony generation frequency, while AT-MSCs have the highest colony generation frequency and the lowest proliferation capacity goes to BM-MSCs [63]. Moreover, only a limited number of MSCs can be extracted from adult tissues (generally, a 5 ml bone marrow aspirate may contain between 2,500 and 6,000 MSCs) [64]. Even if this amount may be sufficient for repairing corneal endothelial lesions, avoiding excessive manipulation of the cell product, there is not an established number of cells to initiate the treatment, and the expansion of MSCs can still be required for clinical application. However, classical monolayer expansion techniques do not preserve MSCs progenitor potency, but novel alternative methods (such as 3D dynamic cultures, scaffolds, and growth factors applications and hypoxia modulation) can enhance the efficacy of expanded cells for clinical application [65].

It has been demonstrated that human BM-MSCs can differentiate into epithelial corneal cells in vivo and into corneal keratocytes in vitro and AT-MSCs can differentiate into epithelial corneal cells in vitro, while human U-MSCs can differentiate into corneal keratocytes in vivo [66–68]. An appealing aspect of MSCs is their ability to aim mainly to injured areas, where they can differentiate into different cell types in accordance to the surrounding microenvironment [54]. We had also performed preliminary evaluations, highlighting the ability of MSCs to survive, migrate, and integrate at the level of cornea, iris, ciliary body, and lens in eyes of healthy mice after injection in the anterior chamber [69] (Figure 1). Moreover, the application of mesenchymal stem cells, with their high plasticity potential and relative safety, avoids the ethical and biological concerns related to the use of embryonic stem cells and iPSCs [70, 71].

The optimal MSCs delivery strategy for clinical application in corneal diseases is represented by local administration through different methods, since it is also the main technique currently used for hCECs. Several studies have focused on the application of mesenchymal stem cells for corneal diseases treatment. For example, UCB-MSCs have been transplanted into corneas of mice leading to improved corneal transparency and increased stromal thickness [68], and autologous bone marrow MSCs have been used to replace corneal endothelium of rabbits in vivo [72]. These studies provided encouraging results, even if the phenotype of implanted cells was examined using morphological techniques (live confocal imaging and scanning electron microscopy). Joyce et al. reported that the phenotype of UCB-MSCs could be altered toward endothelial cell-like cells and that these modified cells tend to "home" to injured areas of ex-vivo corneal endothelium disease models. Moreover, grafting of these modified MSCs did not occur onto normal areas of corneal endothelium or Descemet's membrane, suggesting a high-specificity action of these cells [73]. Homing of MSCs to wounded endothelium is probably due to chemotaxis induced by tumor necrosis factor-α (TNF-α) and expression of adhesion molecules on the surface of endothelial cells (such as ICAM-1 and ELAM-1), both occurring during inflammation [74, 75] and promoting migration and adhesion of MSCs in injured areas [75, 76]. Also, Zo1 and N-cadherin expressions, used to examine the presence of MSCs, were more distributed at the level of the damaged area [73]. However the presence, even if in a smaller quantity, of the same proteins in untreated cultures raises some doubts regarding the differentiation process of MSCs into endothelial cells. Moreover, the typical hexagonal shape of corneal endothelial cells was not observed in the endothelial-like cells derived from MSCs and the molecular basis for the complete transition from mesenchymal to endothelial type is still not known. Even if further research is needed to better understand the environmental conditions leading to an adequate differentiation, MSC can be considered as a potential candidate for corneal endothelial cells replacement.

MSCs or endothelial-like cells derived from MSCs can be applied with techniques similar to those adopted previously for other cell types, avoiding the limits of human corneal endothelial cells, which can't be expanded indefinitely. Moreover, MSCs can also be cultivated to create an ultrathin sheet for endothelial replacement [77], but with implicit technical difficulties for the surgeon (given the difficult management). In the light of all these findings, we are currently evaluating the application of MSCs through intracameral injection in murine models with two modalities (Figure 2): the first one features total asportation of the diseased endothelium (with Descemet's membrane sparing), followed by the injection of a small quantity of mesenchymal stem cells in the anterior chamber while the other one features the injection of MSCs without previous endothelial asportation, with regenerative and repairing intent, especially at the level of the areas where cell elements are absent.

Both modalities should be carried out with a paralimbal injection, through a dedicated "ad hoc" syringe, on central and/or peripheral corneal areas, after the realization in the anterior chamber of an air-stroma (for the first method) or air-endothelium (for the latter) interface. It will be possible to inject a total amount of 1000/2000/5000 MSCs (dispensed in multiple administrations, three months away from each other, in relation to cell attachment, migration, and survival and depending on a case-by-case evaluation). Integration of MSCs is related to the quantity of injected cells with the possibility of repeated injections.

5. Discussion and Applicative Aspects

Availability of donors for endothelial keratoplasty is an emerging problem that must be addressed. Corneal graft tissue derived from cadavers must meet stringent adequacy criteria that include serological tests (in selected cases) and medical history of the donor [78–80]. However, many potential donor corneas, often from elderly donors, are rejected for transplantation because of their lower endothelial cell count and possible age-related alterations. Moreover, availability of corneas can be also affected by cultural, logistical,

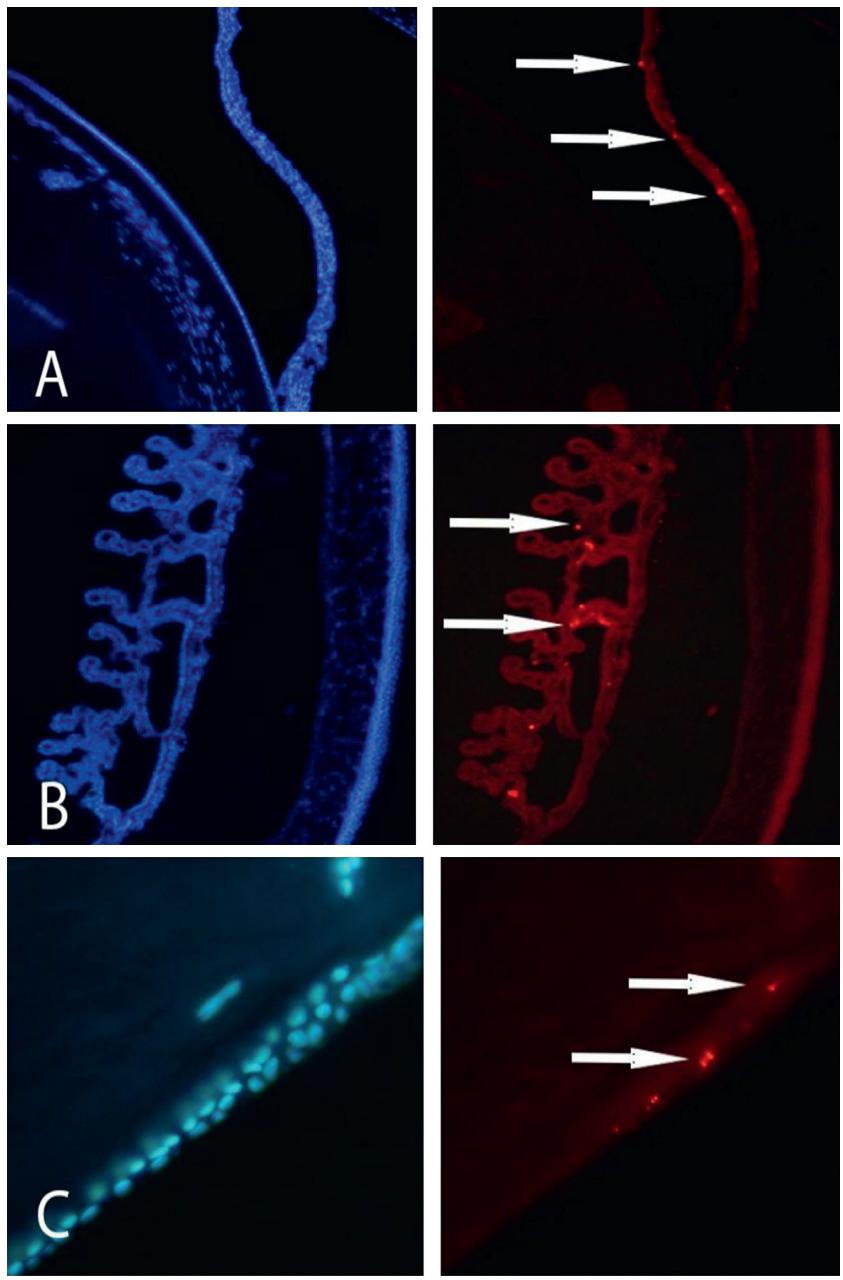


Figure 1: Mesenchymal stem cells introduced in the anterior chamber of murine models can migrate towards the (A) iris, (B) ciliary body, and (C) lens. Migrated cells (indicated by the arrows in the images on the right) could be observed in the anterior segment for 6 weeks. Images on the right are highlighted with a filter specific for bisbenzimide, while images on the right are visualized with a filter specific for Dil Stain.

and technical difficulties [81], and long postmortem time or damage occurred during handling of donor corneas can possibly lead to unsuitable graft material [82]. These are some of the impelling problems that must be taken into account in conjunction with global shortage of suitable corneal tissues. To further alleviate the growing demand for grafting quality tissue, a considerable clinical interest is being amplified in the development of tissue engineering as a suitable alternative for corneal graft [81].

The application of mesenchymal stem cells for corneal endothelial replacement can be considered as a promising perspective, even if its potential still remains to be functionally assessed with additional studies on in vivo animal models. First of all, it reduces the need for donor corneas, saving resources which are more and more limited in time. Moreover, autologous mesenchymal stem cells can be extracted from the same patient, which can act both as donor and receiving subject, further lowering rejection rates and immunological reactions. Since these cells can be injected in the anterior chamber, technical issues related to surgery are reduced (with no corneal flap that needs to be carefully managed). Furthermore, repeated injections can be performed, if needed (such in case of failure or recurrence), after a second cell extraction or after MSCs expansion (that can function as a reservoir sample). MSCs adhesion can also be promoted by coating with antibodies specific for endothelial adhesion

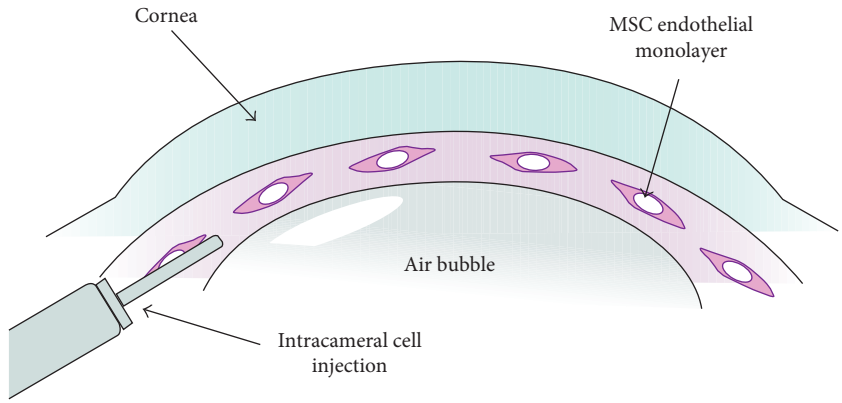


Figure 2: Schematic diagram showing injection of mesenchymal stem cells in the anterior chamber with a cornea-air interface (currently under evaluation in animal models).

proteins, improving targeting of MSCs to wounded areas [76]. In addition, MSC injections can prevent endothelial transplant failure due to cell deficiency which can occur in donor corneas for age-related modifications. For this reason, endothelial cell therapy can be considered also as an adjuvant therapy after DMEK and not only as a first-step approach. Autologous MSCs engraftment can be evaluated in clinical trials on humans with the additional advantage given by the possibility of patients prone positioning, which should facilitate their integration (always preceded by a case-by-case setting). Another problem following DMEK and DSAEK is represented by optical aberrations, due to stromal/donor flap interface. Since the interface can be significantly reduced with the application of mesenchymal stem cells, optical aberration and hyperopic shift should be avoided.

If a translational approach was promoted, and corneal endothelial cell engineering was adopted in a widespread fashion, social and economic costs would be progressively lowered, with saving of donor corneas, surgical material, and management expenses. Currently, specific intracellular signals leading to morphologic changes of MSCs are not known; hence, further investigation of the molecular basis related to mesenchymal-to-epithelial transition of MSCs is needed. Other issues that remain to be addressed before clinical application of mesenchymal stem cells for corneal endothelium replacement are the definition of the best source to obtain MSCs and best conditions for cell expansion, suppression of differentiation to undesired cell types, and homing to damaged corneal areas. Additional studies, including in vivo testing, are now needed to identify the specific conditions that would best support the ability of MSCs to replace corneal endothelial cells lost due to damage or disease as means of restoring corneal transparency. Finally, adverse events related to MSCs application are rare and often related to the microenvironment in which MSCs engraft—leading to unwanted differentiation of transplanted cells—or the presence of preexisting tumors whose growth may be promoted by immunosuppression provided by MSCs [83]. Even if autologous MSCs can be considered safe in terms of immunogenicity and de-novo malignancy development [84], long-term clinical trials are still required to evaluate differentiation concerns and clinical efficacy in human corneas.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] G. R. Melles, T. S. Ong, B. Verves, and J. van der Wees, "Descemet membrane endothelial keratoplasty (DMEK)," *Cornea*, vol. 25, no. 8, pp. 987–990, 2006.

[2] T. Tourtas, K. Laaser, B. O. Bachmann et al., "Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty," *American Journal of Ophthalmology*, vol. 153, no. 6, pp. 1082.e2–1090.e2, 2012.

[3] V. Bhandari, J. K. Reddy, K. Relekar, and V. Prabhu, "Descemet's stripping automated endothelial keratoplasty versus Descemet's membrane endothelial keratoplasty in the fellow eye for fuchs endothelial dystrophy: a retrospective study," *BioMed Research International*, vol. 2015, Article ID 750567, 7 pages, 2015.

[4] F. P. Guerra, A. Anshu, M. O. Price, and F. W. Price, "Endothelial keratoplasty: fellow eyes comparison of Descemet stripping automated endothelial keratoplasty and Descemet membrane endothelial keratoplasty," *Cornea*, vol. 30, no. 12, pp. 1382–1386, 2011.

[5] J. Hori, N. C. Joyce, and J. W. Streilein, "Immune privilege and immunogenicity reside among different layers of the mouse cornea," *Investigative Ophthalmology and Visual Science*, vol. 41, no. 10, pp. 3032–3042, 2000.

[6] S. X. Deng, W. B. Lee, K. M. Hammersmith et al., "Descemet membrane endothelial keratoplasty: safety and outcomes: a report by the American Academy of Ophthalmology," *Ophthalmology*, vol. 125, no. 2, pp. 295–310, 2018.

[7] W. B. Lee, D. S. Jacobs, D. C. Musch et al., "Descemet's stripping endothelial keratoplasty: safety and outcomes: a report by the American Academy of Ophthalmology," *Ophthalmology*, vol. 116, no. 9, pp. 1818–1830, 2009.

[8] P. Maier, T. Reinhard, and C. Cursiefen, "Descemet stripping endothelial keratoplasty—rapid recovery of visual acuity," *Deutsches Aerzteblatt Online*, vol. 110, no. 21, pp. 365–371, 2013.

[9] M. A. Terry, "Endothelial keratoplasty: why aren't we all doing Descemet membrane endothelial keratoplasty?," *Cornea*, vol. 31, no. 5, pp. 469–471, 2012.

[10] M. O. Price and F. W. Price Jr., "Descemet membrane endothelial keratoplasty," *International Ophthalmology Clinics*, vol. 50, no. 3, pp. 137–147, 2010.

[11] F. W. Price and M. O. Price, *DSEK: What You Need to Know about Endothelial Keratoplasty*, SLACK Incorporated, Thorofare, NJ, USA, 2009.

[12] I. Dapena, L. Ham, and G. R. Melles, "Endothelial keratoplasty: DSEK/DSAEK or DMEK-the thinner the better?," *Current Opinion in Ophthalmology*, vol. 20, no. 4, pp. 299–307, 2009.

[13] S. B. Koenig, D. J. Covert, W. J. Dupps Jr., and D. M. Meisler, "Visual acuity, refractive error, and endothelial cell density six months after Descemet stripping and automated endothelial keratoplasty (DSAEK)," *Cornea*, vol. 26, no. 6, pp. 670–674, 2007.

[14] L. Baydoun, C. M. Tong, W. W. Tse et al., "Endothelial cell density after Descemet membrane endothelial keratoplasty: 1 to 5-year follow-up," *American Journal of Ophthalmology*, vol. 154, no. 4, pp. 762-763, 2012.

[15] J. Navaratnam, T. P. Utheim, V. K. Rajasekhar, and A. Shahdadfar, "Substrates for expansion of corneal endothelial cells towards bioengineering of human corneal endothelium," *Journal of Functional Biomaterials*, vol. 6, no. 3, pp. 917–945, 2015.

[16] S. Yuan and G. Fan, "Stem cell-based therapy of corneal epithelial and endothelial diseases," *Regenerative Medicine*, vol. 10, no. 4, pp. 495–504, 2015.

[17] J. Zavala, G. R. López Jaime, C. A. Rodríguez Barrientos, and J. Valdez-Garcia, "Corneal endothelium: developmental strategies for regeneration," *Eye*, vol. 27, no. 5, pp. 579–588, 2013.

[18] G. SL. Peh, K. P. Toh, F. Y. Wu et al., "Cultivation of human corneal endothelial cells isolated from paired donor corneas," *PLoS One*, vol. 6, no. 12, Article ID e28310, 2011.

[19] G. SL. Peh, Z. Chng, H. P. Ang et al., "Propagation of human corneal endothelial cells: a novel dual media approach," *Cell Transplantation*, vol. 24, no. 2, pp. 287–304, 2015.

[20] N. Koizumi, Y. Sakamoto, N. Okumura et al., "Cultivated corneal endothelial cell sheet transplantation in a primate model," *Investigative Opthalmology and Visual Science*, vol. 48, no. 10, pp. 4519–4526, 2007.

[21] N. Okumura, N. Koizumi, M. Ueno et al., "The new therapeutic concept of using a rho kinase inhibitor for the treatment of corneal endothelial dysfunction," *Cornea*, vol. 30, no. 1, pp. S54–S59, 2011.

[22] N. Okumura, N. Koizumi, M. Ueno et al., "Enhancement of corneal endothelium wound healing by Rho-associated kinase (ROCK) inhibitor eye drops," *British Journal of Ophthalmology*, vol. 95, no. 7, pp. 1006–1009, 2011.

[23] N. Okumura, N. Koizumi, M. Ueno et al., "ROCK inhibitor converts corneal endothelial cells into a phenotype capable of regenerating in vivo endothelial tissue," *American Journal of Pathology*, vol. 181, no. 1, pp. 268–277, 2012.

[24] S. Li, C. Wang, Y. Dai et al., "The stimulatory effect of ROCK inhibitor on bovine corneal endothelial cells," *Tissue and Cell*, vol. 45, no. 6, pp. 387–396, 2013.

[25] Y. Q. Soh, G. S. L. Peh, and J. S. Mehta, "Translational issues for human corneal endothelial tissue engineering," *Journal of Tissue Engineering and Regenerative Medicine*, vol. 11, no. 9, pp. 2425–2442, 2017.

[26] L. M. M. Vianna, L. Kallay, T. Toyono et al., "Use of human serum for human corneal endothelial cell culture," *British Journal of Ophthalmology*, vol. 99, no. 2, pp. 267–271, 2015.

[27] X. Sha, Z. Liu, L. Song et al., "Human amniotic epithelial cell niche enhances the functional properties of human corneal endothelial cells via inhibiting P53–survivin–mitochondria axis," *Experimental Eye Research*, vol. 116, pp. 36–46, 2013.

[28] J. S. Choi, E. Y. Kim, M. J. Kim et al., "Factors affecting successful isolation of human corneal endothelial cells for clinical use," *Cell Transplantation*, vol. 23, no. 7, pp. 845–854, 2014.

[29] T. Mimura, N. Shimomura, T. Usui et al., "Magnetic attraction of iron-endocytosed corneal endothelial cells to Descemet's membrane," *Experimental Eye Research*, vol. 76, no. 6, pp. 745–751, 2003.

[30] T. Mimura, S. Yamagami, T. Usui et al., "Long-term outcome of iron-endocytosing cultured corneal endothelial cell transplantation with magnetic attraction," *Experimental Eye Research*, vol. 80, no. 2, pp. 149–157, 2005.

[31] G. SL. Peh, H.-P. Ang, C. N. Lwin et al., "Regulatory compliant tissue-engineered human corneal endothelial grafts restore corneal function of rabbits with bullous keratopathy," *Scientific Reports*, vol. 7, no. 1, article 14149, 2017.

[32] C. Shao, Y. Fu, W. Lu, and X. Fan, "Bone marrow-derived endothelial progenitor cells: a promising therapeutic alternative for corneal endothelial dysfunction," *Cells Tissues Organs*, vol. 193, no. 4, pp. 253–263, 2011.

[33] J. Schwartzkopff, L. Bredow, S. Mahlenbrey, D. Boehringer, and T. Reinhard, "Regeneration of corneal endothelium following complete endothelial cell loss in rat keratoplasty," *Molecular Vision*, vol. 16, pp. 2368–2375, 2010.

[34] T.-J. Wang, I.-J. Wang, J.-N. Lu, and T.-H. Young, "Novel chitosan-polycaprolactone blends as potential scaffold and carrier for corneal endothelial transplantation," *Molecular Vision*, vol. 18, pp. 255–264, 2012.

[35] J. Mohay, T. O. Wood, and B. J. McLaughlin, "Long-term evaluation of corneal endothelial cell transplantation," *Transactions of the American Ophthalmological Society*, vol. 95, pp. 131–148, 1997.

[36] S. V. Patel, L. A. Bachman, C. R. Hann, C. K. Bahler, and M. P. Fautsch, "Human corneal endothelial cell transplantation in a human ex vivo model," *Investigative Opthalmology and Visual Science*, vol. 50, no. 5, pp. 2123–2131, 2009.

[37] N. Okumura, N. Koizumi, E. P. Kay et al., "The ROCK inhibitor eye drop accelerates corneal endothelium wound healing," *Investigative Opthalmology and Visual Science*, vol. 54, no. 4, pp. 2493–2502, 2013.

[38] N. Okumura, S. Kinoshita, and N. Koizumi, "Cell-based approach for treatment of corneal endothelial dysfunction," *Cornea*, vol. 33, no. 11, pp. S37–S41, 2014.

[39] S. Kinoshita, N. Koizumi, M. Ueno et al., "Injection of cultured cells with a ROCK inhibitor for bullous keratopathy," *New England Journal of Medicine*, vol. 378, no. 11, pp. 995–1003, 2018.

[40] K. Pang, K. Zhang, J. Zhu et al., "Differentiation of human embryonic stem cells to corneal epithelium and endothelium like cells for cornea replacement construction," *Investigative Opthalmology and Visual Science*, vol. 56, p. 5831, 2015.

[41] J. Wu and J. C Izpisua Belmonte, "Dynamic pluripotent stem cell states and their applications," *Cell Stem Cell*, vol. 17, no. 5, pp. 509–525, 2015.

[42] Q. Song, S. Yuan, Q. An et al., "Directed differentiation of human embryonic stem cells to corneal endothelial cell-like cells: a transcriptomic analysis," *Experimental Eye Research*, vol. 151, pp. 107–114, 2016.

[43] S. Hatou, S. Yoshida, K. Higa et al., "Corneal endothelial cells derived from monkey iPS cells: a short-term evaluation," *Investigative Opthalmology and Visual Science*, vol. 54, p. 1015, 2013.

[44] I. Gutierrez-Aranda, V. Ramos-Mejia, C. Bueno et al., "Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless of the site of injection," *Stem Cells*, vol. 28, no. 9, pp. 1568–1570, 2010.

[45] N. C. Joyce, "Proliferative capacity of corneal endothelial cells," *Experimental Eye Research*, vol. 95, no. 1, pp. 16–23, 2012.

[46] T. J. Kean, P. Lin, A. I. Caplan, and J. E. Dennis, "MSCs: delivery routes and engraftment, cell-targeting strategies, and, immune modulation," *Stem Cells International*, vol. 2013, Article ID 732742, 13 pages, 2013.

[47] A. Cvekl and E. R. Tamm, "Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases," *Bioessays*, vol. 26, no. 4, pp. 374–386, 2004.

[48] K. Hayashi, K. Sueishi, K. Tanaka, and H. Inomata, "Immunohistochemical evidence of the origin of human corneal endothelial cells and keratocytes," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 224, no. 5, pp. 452–456, 1986.

[49] M. Böhnke, K. Vogelberg, and K. Engelmann, "Detection of neuronespecific enolase in long-term cultures of human corneal endothelium," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 236, no. 7, pp. 522–526, 1998.

[50] A. H. Neufeld, M. M. Jumblatt, E. D. Matkin, and G. M. Raymond, "Maintenance of corneal endothelial cell shape by prostaglandin E2: effects of EGF and indomethacin," *Investigative Opthalmology and Visual Science*, vol. 27, pp. 1437–1442, 1986.

[51] P. Hsieh and J. Baum, "Effects of fibroblastic and endothelial extracellular matrices on corneal endothelial cells," *Investigative Opthalmology and Visual Science*, vol. 26, pp. 457–463, 1985.

[52] W. M. Petroll, J. V. Jester, J. Bean, and H. D. Cavanagh, "The spatial organization of apical junctional complex-associated proteins in feline and human corneal endothelium," *Current Eye Research*, vol. 18, no. 1, pp. 10–19, 1999.

[53] D. Baksh, L. Song, and R. S. Tuan, "Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy," *Journal of Cellular and Molecular Medicine*, vol. 8, no. 3, pp. 301–316, 2004.

[54] G. Chamberlain, J. Fox, B. Ashton, and J. Middleton, "Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing," *Stem Cells*, vol. 25, no. 11, pp. 2739–2749, 2007.

[55] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., "Multilineage potential of adult human mesenchymal stem cells," *Science*, vol. 284, no. 5411, pp. 143–147, 1999.

[56] P. A. Zuk, M. Zhu, P. Ashjian et al., "Human adipose tissue is a source of multipotent stem cells," *Molecular Biology of the Cell*, vol. 13, no. 12, pp. 4279–4295, 2002.

[57] C. De Bari, F. Dell'Accio, P. Tylzanowski, and F. P. Luyten, "Multipotent mesenchymal stem cells from adult human synovial membrane," *Arthritis and Rheumatism*, vol. 44, no. 8, pp. 1928–1942, 2001.

[58] A. Uezumi, K. Ojima, S. Fukada et al., "Functional heterogeneity of side population cells in skeletal muscle," *Biochemical and Biophysical Research Communications*, vol. 341, no. 3, pp. 864–873, 2006.

[59] G. Laino, A. Graziano, R. d'Aquino et al., "An approachable human adult stem cell source for hard-tissue engineering," *Journal of Cellular Physiology*, vol. 206, no. 3, pp. 693–701, 2006.

[60] H. S. Wang, S. C. Hung, S. T. Peng et al., "Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord," *Stem Cells*, vol. 22, no. 7, pp. 1330–1337, 2004.

[61] Y. A. Romanov, V. A. Svintsitskaya, and V. N. Smirnov, "Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord," *Stem Cells*, vol. 21, no. 1, pp. 105–110, 2003.

[62] A. Erices, P. Conget, and J. J. Minguell, "Mesenchymal progenitor cells in human umbilical cord blood," *British Journal of Haematology*, vol. 109, no. 1, pp. 235–242, 2000.

[63] Y. J. Chang, D. T. Shih, C. P. Tseng, T. B. Hsieh, D. C. Lee, and S. M. Hwang, "Disparate mesenchyme-lineage tendencies in mesenchymal stem cells from human bone marrow and umbilical cord blood," *Stem Cells*, vol. 24, no. 3, pp. 679–685, 2006.

[64] R. Cuthbert, S. A. Boxall, H. B. Tan et al., "Single platform quality control assay to quantify multipotential stromal cells in bone marrow aspirates prior to bulk manufacture or direct therapeutic use," *Cytotherapy*, vol. 14, no. 4, pp. 431–440, 2012.

[65] A. I. Hoch and J. K. Leach, "Concise review: optimizing expansion of bone marrow mesenchymal stem/stromal cells for clinical applications," *Stem Cells Translational Medicine*, vol. 3, no. 5, pp. 643–652, 2014.

[66] Y. Ma, Y. Xu, Z. Xiao et al., "Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells," *Stem Cells*, vol. 24, no. 2, pp. 315–321, 2006.

[67] T. Nieto-Miguel, S. Galindo, R. Reinoso et al., "In vitro simulation of corneal epithelium microenvironment induces a corneal epithelial-like cell phenotype from human adipose tissue mesenchymal stem cells," *Current Eye Research*, vol. 38, no. 9, pp. 933–944, 2013.

[68] H. Liu, J. Zhang, C. Y. Liu et al., "Cell therapy of congenital corneal diseases with umbilical mesenchymal stem cells: lumican null mice," *PLoS One*, vol. 5, no. 5, Article ID e10707, 2010.

[69] R. Nuzzi, "Prospettive di terapia cellulare oculare," in *Communication Presented at the XI Meeting of Low Vision Academy*, Rome, Italy, June 2010.

[70] G. Kögler, S. Sensken, J. A. Airey et al., "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential," *Journal of Experimental Medicine*, vol. 200, no. 2, pp. 123–135, 2004.

[71] V. Markov, K. Kusumi, M. G. Tadesse et al., "Identification of cord blood-derived mesenchymal stem/stromal cell populations with distinct growth kinetics, differentiation potentials, and gene expression profiles," *Stem Cells and Development*, vol. 16, no. 1, pp. 53–73, 2007.

[72] X. W. Liu and J. L. Zhao, "Transplantation of autologous bone marrow mesenchymal stem cells for the treatment of corneal endothelium damages in rabbits," *[Zhonghua Yan Ke Za Zhi] Chinese Journal of Ophthalmology*, vol. 43, pp. 540–545, 2007.

[73] N. C. Joyce, D. L. Harris, V. Markov, Z. Zhang, and B. Saitta, "Potential of human umbilical cord blood mesenchymal stem cells to heal damaged corneal endothelium," *Molecular Vision*, vol. 18, pp. 547–564, 2012.

[74] L. Trinh, F. Brignole-Baudouin, A. Labbé, M. Raphaël, J-L Bourges, and C. Baudouin, "The corneal endothelium in an endotoxin-induced uveitis model: correlation between in vivo confocal microscopy and immunohistochemistry," *Molecular Vision*, vol. 14, pp. 1149–1156, 2008.

[75] S. Meirelles Lda, A. M. Fontes, D. T. Covas, and A. I. Caplan, "Mechanisms involved in the therapeutic properties of

mesenchymal stem cells," *Cytokine and Growth Factor Reviews*, vol. 20, no. 5-6, pp. 419–427, 2009.

[76] I. K. Ko, T. J. Kean, and J. E. Dennis, "Targeting mesenchymal stem cells to activated endothelial cells," *Biomaterials*, vol. 30, no. 22, pp. 3702–3710, 2009.

[77] S. Yamagami, T. Mimura, S. Yokoo, T. Takato, and S. Amano, "Isolation of human corneal endothelial cell precursors and construction of cell sheets by precursors," *Cornea*, vol. 25, pp. S90–S92, 2006.

[78] W. J. Armitage, S. J. Moss, D. L. Easty et al., "Supply of corneal tissue in the United Kingdom," *British Journal of Ophthalmology*, vol. 74, no. 11, pp. 685–687, 1990.

[79] K. McColgan, "Corneal transplant surgery," *Journal of Perioperative Practice*, vol. 19, no. 2, pp. 51–54, 2009.

[80] N. Builles, L. Kodjikian, C. Burillon et al., "Major endothelial loss from corneas in organ culture: importance of second endothelial count," *Cornea*, vol. 25, no. 7, pp. 815–820, 2006.

[81] J. W. Ruberti and J. D. Zieske, "Prelude to corneal tissue engineering gaining control of collagen organization," *Progress in Retinal and Eye Research*, vol. 27, no. 5, pp. 549–577, 2008.

[82] K. Engelmann, J. Bednarz, and M. Valtink, "Prospects for endothelial transplantation," *Experimental Eye Research*, vol. 78, no. 3, pp. 573–578, 2004.

[83] V. Volarevic, B. S. Markovic, M. Gazdic et al., "Ethical and Safety Issues of Stem Cell-Based Therapy," *International Journal of Medical Sciences*, vol. 15, no. 1, pp. 36–45, 2018.

[84] M. M. Lalu, L. McIntyre, C. Pugliese et al., "Safety of cell therapy with mesenchymal stromal cells (safecell): a systematic review and meta-analysis of clinical trials," *PLoS One*, vol. 7, no. 10, Article ID e47559, 2012.

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 2645280, 7 pages
https://doi.org/10.1155/2018/2645280

Research Article

Modern Corneal Eye-Banking Using a Software-Based IT Management Solution

C. Kern,[1] **K. Kortuem**,[1,2] **C. Wertheimer**,[1] **O. Nilmayer**,[1] **M. Dirisamer**,[1] **S. Priglinger**,[1] **and W. J. Mayer**[1]

[1]*Department of Ophthalmology, University Hospital LMU, Munich, Germany*
[2]*Moorfields Eye Hospital, London, UK*

Correspondence should be addressed to C. Kern; christoph.kern@med.uni-muenchen.de

Received 12 March 2018; Accepted 26 April 2018; Published 20 May 2018

Academic Editor: Yu-Chi Liu

Background. Increasing government legislation and regulations in manufacturing have led to additional documentation regarding the pharmaceutical product requirements of corneal grafts in the European Union. The aim of this project was to develop a software within a hospital information system (HIS) to support the documentation process, to improve the management of the patient waiting list and to increase informational flow between the clinic and eye bank. *Materials and Methods*. After an analysis of the current documentation process, a new workflow and software were implemented in our electronic health record (EHR) system. *Results*. The software takes over most of the documentation and reduces the time required for record keeping. It guarantees real-time tracing of all steps during human corneal tissue processing from the start of production until allocation during surgery and includes follow-up within the HIS. Moreover, listing of the patient for surgery as well as waiting list management takes place in the same system. *Conclusion*. The new software for corneal eye banking supports the whole process chain by taking over both most of the required documentation and the management of the transplant waiting list. It may provide a standardized IT-based solution for German eye banks working within the same HIS.

1. Introduction

A global survey showed that keratoplasty is the most common tissue transplantation in the world, with 184.576 performed procedures involving 283.530 grafts in 2016 [1]. The regulations for the processing of human corneal grafts, which are regulated as pharmaceutical products, have increased in Germany within the last years because of new European and national regulations. In 2007, new legislation on the Quality and Safety of Human Tissues and Cells (Tissue law) incorporating amended European directives was passed by the German government. This resulted in major changes in medical law, transplant law and drug law. To define and control the quality requirements of donor tissue, corneal grafts were classified as a drug and not a human transplant. Therefore, this tissue is now regulated not only by tissue and transplant law, but also by drug law. The changes in the law generated increased documentation, processing costs and more thorough tests [2]. All regulations were reviewed and summarized by the "Deutsche Ärztekammer" (German medical council) and the "Paul-Ehrlich Institute" and led to new national guidelines [3, 4]. Because of the change in the laws, we adapted quality management and other regulations at our Cornea Bank accordingly to meet the new criteria [5]. As most of the documentation during processing at our tissue bank was paper-based or relied on the manual input of data into software such as Microsoft Excel, we observed an increase in the documentation time required during the processing of corneal grafts.

In 2013, to improve patient-related documentation at the university eye clinic, we started to develop a custom-made electronic health record (EHR) adjusted to the needs of ophthalmology based on the hospital information system (HIS) i.s.h.med (Cerner AG, Erlangen, Germany). This system is now used for the entire clinical documentation at

our hospital [6, 7]. Using a Picture Archiving and Communication System (PACS), we linked diagnostic data from diagnostic devices to the clinical data of patients and built a data-warehouse including clinical and diagnostic data from more than 350,000 patients [8].

The processing of corneal grafts is documented on quality-audited paper forms. Manual repetitive documentation and manual data transfer between the eye hospital and the eye bank risk processing errors and do not guarantee a high safety level or auditability. Efficient, complete and comprehensible record-keeping is listed as one of the key features of eye banks in the literature [9].

Special requirements for electronic documentation were defined by the Bavarian Medical Council: safety of the network against access from outside, daily data and documentation back-up, long-term data storage, assignment of all entries to a responsible employee and, of course, proof of later additional changes in the patient's documentation [10]. These requirements can be achieved and guaranteed by using an established, professionally secured and backed-up HIS [11].

The aim of this project was to develop custom-made documentation algorithm based on our HIS, not only to improve documentation, but also to link waiting list management with transplant allocation. These data could enrich clinical information of importance concerning the number and quality of performed corneal transplantations at our clinic and should additionally simplify data analysis.

2. Material and Methods

2.1. Process Analysis. As a first step, the documentation process during corneal graft processing, together with waiting list management and allocation, was analysed and transferred into a process flow chart to guarantee the proper implementation and functionality of the new custom-made documentation software. Time measurements before and after introduction of the new system were recorded for three different steps: initial documentation on arrival of the donor tissue at the eye bank, documentation of nutritional liquid change of the graft and documentation for final clearance.

2.2. Software Requirements. Many requirements had to be considered before the development process was initiated. First, the software had to meet the criteria for documentation as defined in the "Good manufacturing practice" guideline provided by the Paul-Ehrlich Institute [3]. This involves the legibility and the auditability of the documentation. All changes in the documentation must be clearly visible and linked to the working user. Moreover, according to national guidelines, all documentation must be stored for at least 30 years after the expiry date of the product. These requirements are met by the well-established hospital IT platform run by the university hospital's IT department [12]. Access to the software must be limited for normal users as they should not be aware of links between donor and receiver identities.

Another requirement was the support of the employees of the eye banks during the documentation process, as most of their time was now used for documentation and not processing. In addition to the automatically performed timestamps, user logging, audit trails and lot numbers of the used material, the software must simplify the documentation process itself through pre-allocation.

2.3. Software Implementation. The development of the necessary user interface was performed within our HIS (i.s.h.med, Cerner AG, Erlangen, Germany), which is based on a SAP (SAP SE, Walldorf, Germany) platform by using Advanced Business Application Programming (ABAP) programming language [13]. The development process started in the late summer of 2016, with a first version being available in a HIS testing environment in spring 2017 during which functionality and stability were assessed. After several revisions to the software, the system was launched in December 2017.

3. Results

3.1. Process Analysis. Every single documentation step of the old process was identified and a data input structure was developed to define the requirements of the custom-made information input algorithm.

Several quality-managed Word files (Microsoft Corporation, Redmond, USA) were necessary for the monitoring and documenting of processing in the old workflow (Figure 1). After printing one set of documents per graft, a medical technical assistant (MTA) manually filled in the ongoing process documentation on paper. Once the processing was completed, the paper documents were archived in a folder and stored in an office.

The previous processing documentation and the listing and transplant allocation process were analysed (Figure 1). In the past, the waiting list was managed by using Microsoft Excel Spreadsheets (Microsoft Corporation, Redmond, USA) for which no audit trail was available. The whole process therefore needed to be optimized at every step and was subsequently defined in a detailed development plan of the new software.

3.2. Software Implementation. The software was implemented in the hospital's HIS by creating a new working environment for the eye bank and contained three different views: an overview of the processed transplants, the waiting list and a history of transplanted grafts for both our own and externally provided grafts (Figure 2). Based on assigned access rights, only eye bank employees can access the new working environment. All data are stored in the hospital's professionally managed redundant data centre, which guarantees data protection, high availability and regular data back-ups. Real-time data is stored on six different servers and weekly back-ups of the whole database are stored on servers using redundant array of independent disks technology (RAID-servers). Moreover, the data containing graft

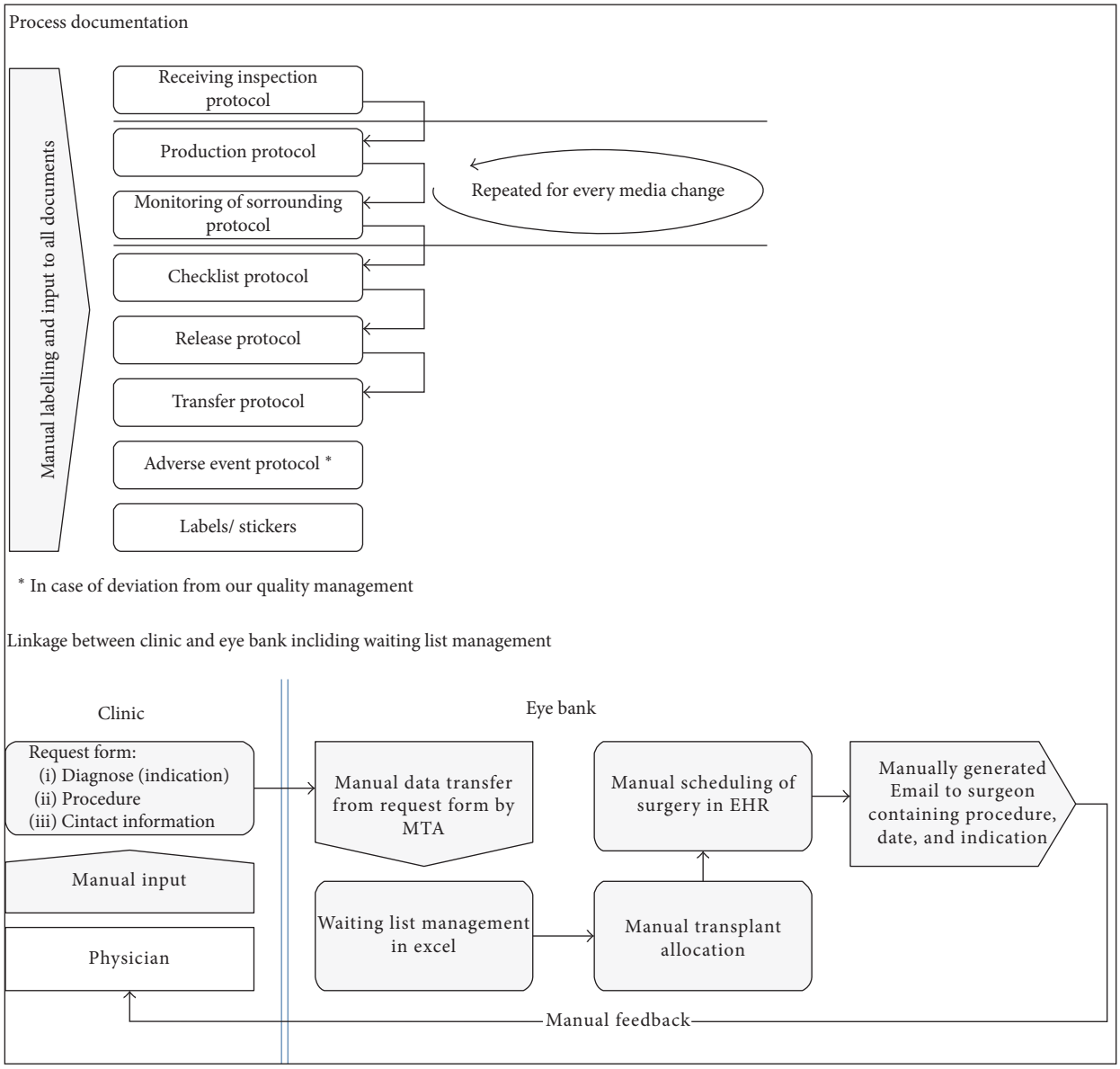


Figure 1: Process analysis before implementation of new software solution.

information are stored in the data-warehouse of the clinic to simplify data access for research and statistics.

3.3. Documentation of the Tissue-Processing Process. Five new user interfaces (HIS-term: parametric medical documents (PMD)) have been developed to allow process documentation. All PMDs are linked to a patient within the HIS patient master index. Therefore, all donors are registered, if they have not been a patient during their lifetime, in the category "cornea donor." This categorization allows data access to be limited to those employees with special access rights. The processing protocol including the graft ID is linked to the recipient's EHR. This allows the linking of graft ID and the donor's identity for eye bank staff (special access rights) in cases of any adverse events. Graft and surgery complications are recorded in the recipient's EHR and can be queried by the eye bank staff at any time from the data warehouse. Figure 3 shows the data structure of the new processing documentation process. In a university setting, final clearance for all grafts is provided by a responsible consultant with the necessary access rights. Follow-up documentation within the recipient's EHR is guaranteed by this data structure, even though, due to information governance issues, backtracking of the graft's origin is not possible for the treating physician. To provide a better overview of multiple processed transplants, all data appear in a view in the working environment (Figure 4). This contains the graft's ID, the donor's information and clearance following various microbiological tests.

3.4. Linkage between Clinic and Eye Bank. Improvements in the listing of patients for corneal transplantation and in the feedback loop were further objectives of this software

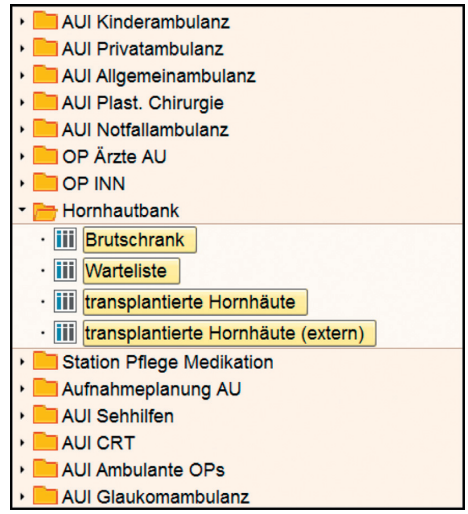


Figure 2: New eye bank working environment within the clinical HIS user interface. Translations related to corneal grafts only: Hornhautbank = Eyebank; Brutschrank = transplants in production; Warteliste = Waiting list; transplantierte Hornhäute = history of transplanted grafts; transplantiert Hornhäute (extern) = history of externally obtained transplanted grafts.

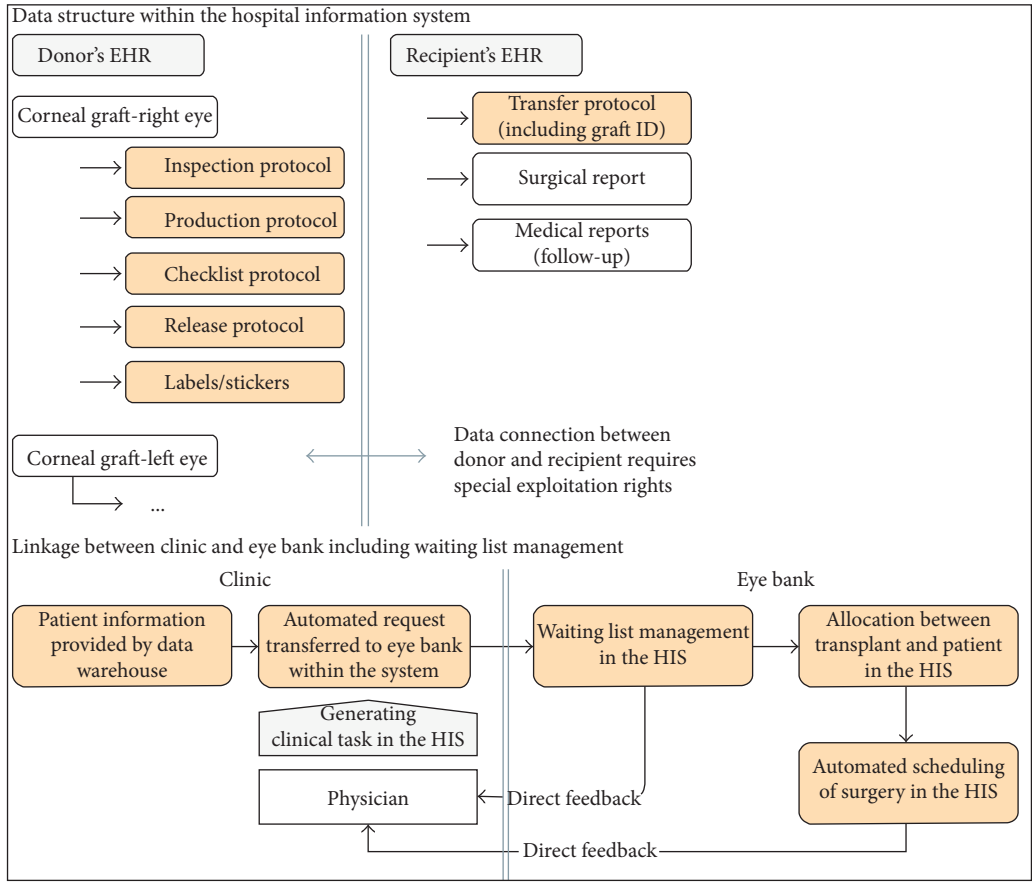


Figure 3: Process analysis after implementation of new software. All documents and steps shaded in peach are supported by the new software.

solution. A new item in the entry management part of the HIS was developed to enable the safe and rapid listing of patients for the physician. The clinical and contact information of the patient is automatically added to data fields before the inclusion of the patient to the waiting list. This step replaces the manually filled-in request form (Figure 3). After the allocation of a transplant to a recipient, a new surgical procedure in the theatre diary will be generated providing the surgeon with direct feedback about the date, time and relevant procedure. Relevant parameters about the

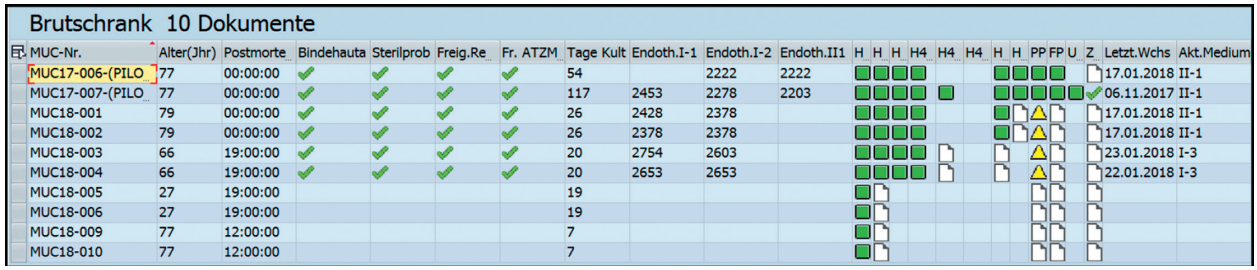


Figure 4: Working environment "Transplants in production." Headings of columns starting on the left side: Graft ID, age donor, time between donor's death and explantation of the corneal graft, microbiological testing of conjunctiva, microbiological testing of first medium, clearance by forensic medicine, clearance by serological testing, number of days cultivated, endothelial count I-1, I-2 and II-1, status of documents (blank sheets means the document can be set up, a yellow triangle symbolizes a document without final clearance (changes still possible) and the green square indicates a finished document), last medium change, actual medium.

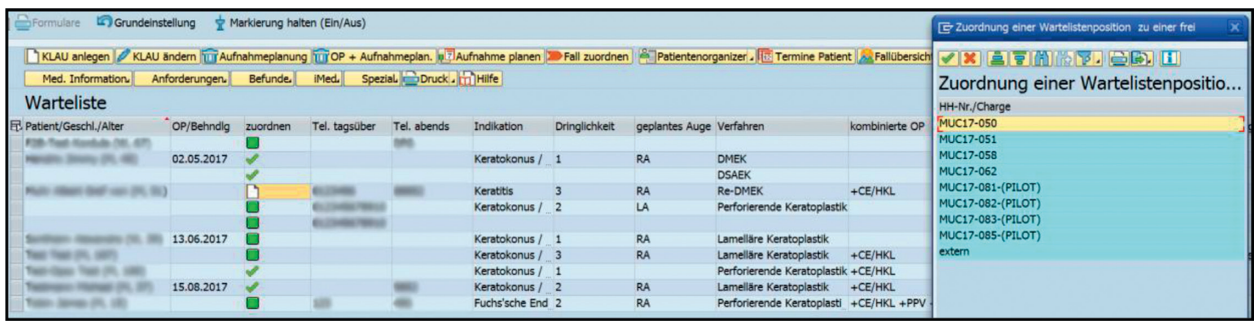


Figure 5: Working environment "waiting list." The process of linking a patient from the waiting list to a graft is shown. Headings of the columns starting on the left: patient/sex/age if available, scheduled surgery, connection, daytime phone number, evening phone number, indication, side, procedure, combined procedure. The column headed connection shows a green tick if the surgery has been performed, a green square if it is scheduled and a blank sheet of paper if no connection between this patient and a graft exists. Clicking on the blank paper, you can see a selection of the available grafts from the working environment of "transplants in production."

graft are provided to the surgeon in the operation theatre on an automatically filled in checklist, which is printed and attached to the graft package. The whole waiting list management takes now place within the user interface of the HIS (Figure 5). To provide a transparent system of allocating tissues in the waiting list, we defined three different urgency groups based on clinical criteria: "elective," "urgent," "emergency." For the first group, grafts are provided depending on the patients' overall waiting time, for the second group within 4 weeks and an emergency listed patient will receive the next available transplant. After a processed transplant has been matched to a patient on the waiting list, all stickers and forms are generated automatically by using previously entered data. After the removal of the patient from a waiting list position, the transplanted cornea is moved to a third subfolder of the eye bank environment, where information about the donor, the processing process and the recipient is visualized. This enables employees with the necessary rights to obtain a rapid overview of donors, corneal graft IDs, recipients, indications and the date and type of surgery. Data can be also filtered by date to simplify annual statistics for internal or official use.

3.5. Time Measurements. Before the introduction of the new system, the time consumed during initial documentation was 30 minutes per graft. Changing the nutritional liquid took 20 minutes and final clearance 60 minutes of documentation time. With the new system, initial documentation time decreased to 10 minutes per graft. Changing the graft liquid only took 5 minutes and the final clearance 20 minutes of documentation per graft. This equals a reduction of 66% for initial documentation, 75% for liquid change and 66% for final clearance.

4. Discussion

After the analysis of the existing process and the definition of certain documentation requirements as legally specified, new software has been implemented to streamline our corneal graft processing. The new software features a high degree of automation and supports the linkage of clinics and our eye bank. Compared with former paper-based and handwritten documentation, many tasks have been automated (e.g., time, date and lot numbers). The implementation within the leading HIS has made a transparent and clear tissue allocation process with the highest amount of information being available to all involved staff to guarantee patient safety. All processing and transplant allocation data are now safely stored on hospital servers according to German/European data safety and storage guidelines [14].

The generation of a waiting list order position via a patient EHR is one of the key features of the new software.

It guarantees easy access from every clinic desk and immediate listing during clinical examination. The automatic addition of each patient's personal and clinical data into the list should eliminate errors during the listing of patients. Moreover, this system simplifies the workflow as the generation of a list position within the EHR replaces the previously used paper-based versions. Subjectively, the data quality within EHR-based listing form has increased through the new system by providing all the necessary clinical information and the correct contact information. After a request for a graft by a physician, the eye bank employee receives immediate feedback through the sending of the clinical task to the worklist as explained by Kortuem et al. [7]. Within the HIS, comments can be added and waiting list positions can be edited and, thus, all information is clearly visible to managing employees.

In 2016, a survey was performed by the "Deutsche Ophthalmologische Gesellschaft" (German ophthalmologic society). It covered questions related to IT infrastructure at eye hospitals. The results have shown that the most commonly used system is i.s.h. med in 13 German eye hospitals [11]. Worldwide, more than 500 hospitals participate in Cerners' HIS solution [15]. Developed as a module of i.s.h. med, our eye bank software can easily be transferred to other clinics by using the same HIS. This will expand the usability spectrum of the systems at other clinics.

Recently, a corneal transplant registry was set up at India's National Eye Bank including the follow-up data of graft recipients. According to the authors, the database simplified data collection for follow-up compared with former paper-based outcome analysis [16]. The connection of the corneal graft documentation process to the EHR simplifies access to data regarding the processing of the tissue and the graft itself [8, 11]. This provides further helpful information about the graft to the surgeon prior surgery. Within the existing data-warehouse, graft-specific data can be easily matched to the clinical data of the follow-up examinations of the recipients. This provides easy access to data for further statistical analysis, quality control, research, recipient follow-up and the correlation of graft data to clinical outcome and possible complications.

Many legal and professional regulations exist concerning the safety assessment of donor and recipient electronic records [10]. In addition to tracking changes in the records made by users and the identification of changes in the existing documentation, network safety must be guaranteed [14]. The used HIS should however provide regular back-up and long-term data storage and, moreover, the servers running the system should receive clearance from a data protection officer [11]. By implementing the software within an established HIS, all mandatory points concerning data protection and auditability have been met. Furthermore, in cases of any required inspections by the responsible authorities, access to the whole documentation can be granted through a digital system.

By the development of a custom-made documentation software into out HIS, we improved the dataflow between the clinic and eye bank, with a saving of 66% of documentation time. Electronic documentation systems can thus reduce the workload for employees and even increase patient safety because of the automatic, less error-prone transfer of data between the clinic and tissue bank.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] P. Gain, R. Jullienne, Z. He et al., "Global survey of corneal transplantation and eye banking," *JAMA Ophthalmology*, vol. 134, no. 2, pp. 167–173, 2016.

[2] C. Hauswald and T. Bredehorn-Mayr, "Practical implications of the law on tissues for cornea banks in Germany," *Developments in Ophthalmology*, vol. 43, pp. 125–130, 2009.

[3] Beschluss der Bundesärztekammer über die Richtlinie zur Gewinnung von Spenderhornhäuten und zum Führen einer Augenhornhautbank," *Deutsches Arzteblatt International*, vol. 115, no. 6, p. 262, 2018.

[4] Richtlinie zur Gewinnung von Spenderhornhäuten und zum Führen einer Augenhornhautbank, Erste Fortbeschreibung," *Deutsches Ärzteblatt*, vol. 115, no. 6, p. 262, 2018.

[5] M. Toniolo, D. Camposampiero, C. Griffoni, and G. L. Jones, "Quality management in European eye banks," *Developments in Ophthalmology*, vol. 43, pp. 70–86, 2009.

[6] K. Kortuem, M. Muller, C. Hirneiss et al., "Smart eye data: development of a foundation for medical research using smart data applications," *Der Ophthalmologe*, vol. 113, no. 6, pp. 469–477, 2016.

[7] K. U. Kortuem, M. Müller, A. Babenko, A. Kampik, and T. C. Kreutzer, "Entwicklung eines augenärztlichen klinischen Informationssystems für bettenführende Augenkliniken," *Der Ophthalmologe*, vol. 112, no. 12, pp. 995–1001, 2015.

[8] K. U. Kortuem, M. Muller, C. Kern et al., "Using electronic health records to build an ophthalmologic data warehouse and visualize patients' data," *American Journal of Ophthalmology*, vol. 178, pp. 84–93, 2017.

[9] R. Tandon, A. Singh, N. Gupta, M. Vanathi, and V. Gupta, "Upgradation and modernization of eye banking services: Integrating tradition with innovative policies and current best practices," *Indian Journal of Ophthalmology*, vol. 65, no. 2, pp. 109–115, 2017.

[10] Berufsordnung für die Ärzte Bayerns–Bekanntmachung vom 09. Januar 2012 i.d.F. der Änderungsbeschlüsse vom 21 Oktober 2017," *Bayerisches Ärzteblatt*, 2017, http://www.blaek.de/docs/epaper/berufsordnung_2015_2016/html5/index.html?&locale=DEU.

[11] K. Kortum, C. Kern, G. Meyer, S. Priglinger, and C. Hirneiss, "Required framework for the collection of real-life data: an example from University Eye Hospital Munich," *Klinische Monatsblätter für Augenheilkunde*, vol. 234, no. 12, pp. 1477–1482, 2017.

[12] *Services Provided by the Departement of "Medizintechnik und IT" (MIT)*, http://www.klinikum.uni-muenchen.de/Medizintechnik-und-IT/de/ueber-uns/index.html.

[13] J. Wendt, *Grundlagen der COBOL-Programmierung. COBOL: Einführung mit PC-Spracherweiterungen und Übungen*, Gabler Verlag, Wiesbaden, Germany, 1994.
[14] Empfehlungen zur ärztlichen Schweigepflicht, Datenschutz und Datenverarbeitung in der Arztpraxis," *Dtsch Arztebl International*, vol. 115, no. 10, p. 453, 2018.
[15] Cerner Health Services, *i.s.h. med–die klinische Welt in SAP for Healthcare*, Cerner Health Services, North Kansas City, MO, USA, 2016.
[16] V. Gogia, S. Gupta, J. S. Titiyal, A. Panda, R. M. Pandey, and R. Tandon, "A preliminary descriptive analysis of Corneal Transplant Registry of National Eye Bank in India," *Contact Lens and Anterior Eye*, vol. 37, no. 2, pp. 111–115, 2014.

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 1215868, 9 pages
https://doi.org/10.1155/2018/1215868

Review Article

Application of Novel Drugs for Corneal Cell Regeneration

Sang Beom Han,[1] **Yu-Chi Liu**,[2,3,4] **Karim Mohamed-Noriega**,[5] **and Jodhbir S. Mehta**[2,3,4]

[1]*Department of Ophthalmology, Kangwon National University Hospital, Kangwon National University, Chuncheon, Republic of Korea*
[2]*Singapore National Eye Centre, Singapore*
[3]*Singapore Eye Research Institute, Singapore*
[4]*Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[5]*Department of Ophthalmology, Faculty of Medicine, University Hospital "Jose E. Gonzalez", Autonomous University of Nuevo Leon, Monterrey, NL, Mexico*

Correspondence should be addressed to Jodhbir S. Mehta; jodmehta@gmail.com

Received 27 December 2017; Revised 26 February 2018; Accepted 27 March 2018; Published 2 May 2018

Academic Editor: Enrique Mencía-Gutiérrez

Corneal transplantation has been the only treatment method for corneal blindness, which is the major cause of reversible blindness. However, despite the advancement of surgical techniques for corneal transplantation, demand for the surgery can never be met due to a global shortage of donor cornea. The development of bioengineering and pharmaceutical technology provided us with novel drugs and biomaterials that can be used for innovative treatment methods for corneal diseases. In this review, the authors will discuss the efficacy and safety of pharmacologic therapies, such as Rho-kinase (ROCK) inhibitors, blood-derived products, growth factors, and regenerating agent on corneal cell regeneration. The promising results of these agents suggest that these can be viable options for corneal reconstruction and visual rehabilitation.

1. Introduction

Corneal blindness is one of the leading causes of reversible blindness worldwide [1]. Corneal transplantation has been the only method for the treatment of corneal blindness, of which penetrating keratoplasty has long been the major surgical procedure. More recently, partial thickness corneal transplantation, such as Descemet's stripping endothelial keratoplasty (DSEK), Descemet's membrane endothelial keratoplasty (DMEK), and deep anterior lamellar keratoplasty (DALK), has been gaining popularity due to better visual prognosis and reduced risk of rejection. However, a global shortage of donor corneal tissue makes it impossible to meet the demands for corneal transplantation with current cornea donation programs. In addition, despite the advancement of surgical techniques and devices, corneal transplantation is still associated with limitations, such as graft failure or rejection, difficulty of the surgical procedure, and complications including secondary glaucoma [2, 3].

Remarkable developments of novel biomaterials and stem cell-based tissue cultivation and expansion techniques during the past few decades might enable the mass production of synthetic corneal tissue and are expected to provide innovative treatment methods for corneal disease. Medical treatment using drugs, such as Rho-kinase (ROCK) inhibitors, blood-derived products, growth factors, and regenerating agent eye drops (RGTA), is also anticipated to have therapeutic potential. In this review, we aimed to provide information on the pharmacologic therapies for corneal cell regeneration using these drugs.

2. Rock Inhibitors

ROCK is a serine/threonine protein kinase that is activated by Rho and forms Rho/ROCK complex that regulates a variety of cellular functions, such as cell proliferation, differentiation, migration, contraction, and apoptosis [4, 5]. Therefore, the ROCK signaling pathway has drawn interest as a potential

target for the treatment of diseases of multiple organs [4, 5]. Recent studies showed that the ROCK inhibitor might be an innovative therapeutic agent for various ocular diseases, particular for corneal endothelial decompensation [4]. Although corneal transplantation has been the only therapy for corneal endothelial dysfunction, studies indicate the potential of ROCK inhibitors as a less-invasive alternative to graft surgery [3].

2.1. ROCK Inhibitor for Corneal Endothelial Cell (CEC) Generation. Okumura et al. [6] demonstrated that a selective ROCK inhibitor, Y-27632, promoted CEC proliferation and adhesion and suppressed its apoptosis, indicating that the topical ROCK inhibitor has been therapeutic for CEC regeneration [6]. An experimental study showed that a ROCK inhibitor facilitated the proliferation of CECs by the modulation of cyclin and p27; both are regulators of the G1/S transition [7]. Peh et al. [8] also revealed that Y-27632 increased cell proliferation. In their study, the effect of the ROCK inhibitor on the proliferation of CEC was significant only in the corneas from younger donors, suggesting that CECs from older corneas might lose the proliferation potential that could be activated by the ROCK inhibitor [8]. The findings of another study that Y-27632 had no effect on the proliferation of human CECs, although it facilitated corneal endothelial wound healing ex vivo and *in vitro*, support the assumption [9].

ROCK inhibitor eye drops enhanced corneal endothelial wound healing in a rabbit CEC injury model [6, 7, 10]. Topical ROCK inhibitor instillation also led to the facilitation of corneal endothelial wound healing and the recovery of corneal transparency in a primate CEC damage model [11]. Y-27632 was also shown to enhance the proliferation and adhesion and suppress the apoptosis of primate CECs cultured *in vitro* [6, 12]. Considering that primate CECs also have limited proliferative capacity, these results suggest that the ROCK inhibitor may have a therapeutic effect on human corneal endothelial dysfunction.

Okumura et al. [13] postulated that topical application of the ROCK inhibitor could facilitate the proliferation and migration of the residual CECs after acute corneal endothelial damage, thereby decreasing the risk of corneal endothelial decompensation [13]. A preliminary study showed that topical administration of the ROCK inhibitor resulted in the recovery of corneal transparency in 1-2 months in all 3 patients with postoperative acute corneal endothelial decompensation [3].

The ROCK inhibitor could be an alternative to corneal transplantation for Fuchs' endothelial corneal dystrophy (FECD). A human pilot study demonstrated that treatment with Y-27632 eye drops for patients with FECD resulted in the decrease in central corneal thickness and the recovery of corneal transparency in patients with corneal edema confined to the center, whereas the effect was not evident in those with diffuse corneal edema [3, 11, 14]. A recent case series study also revealed that descemetorhexis without graft surgery followed by topical ROCK inhibitor administration resulted in the restoration of corneal clarity and visual rehabilitation in patients with FECD [15].

2.2. ROCK Inhibitor for Tissue Engineering. For the treatment of corneal endothelial decompensation, the following two strategies for tissue engineering appears to be promising [1]: transplantation of bioengineered corneal endothelial cell sheet and [2] direct injection of cultivated CEC suspension to the anterior chamber [3].

Experimental studies showed that the transplantation of cultivated CECs on a collagen sheet can result in the restoration of corneal transparency and reduction of corneal edema [16, 17]. However, manipulation of the fragile monolayer sheet in the anterior chamber is technically challenging and is associated with the risk of CEC loss [3].

Theoretically, direct injection of CECs into the anterior chamber might be simple and less invasive compared to CEC sheet transplantation. In addition, the preparation of cells for transplantation may be easier, without the need for an artificial substrate [3]. The risk of CEC damage during the procedure might also be reduced. As the CECs injected into the anterior chamber may not spontaneously attach to Descemet's membrane, methods for improving the adhesion of the injected CECs have been attempted, such as magnetic guidance of iron powder or superparamagnetic microspheres incorporated in the CECs [18–20].

Considering that the ROCK inhibitor was proven to promote CEC adhesion onto a substrate [12], it may be postulated that the ROCK inhibitor can be helpful for promoting the attachment of CECs injected into the anterior chamber. An experimental study using a rabbit corneal endothelial dysfunction model demonstrated that intracameral injection of rabbit CECs combined with Y-27632, followed by keeping each rabbit in the facedown position for 3 hr, achieved enhanced attachment of donor cells onto host Descemet's membrane and the restoration of corneal transparency [21], whereas CEC injection without the inclusion of Y-27632 led to persistent corneal edema [21].

Y-27632 also promoted the adhesion of intracamerally injected CECs onto Descemet's membrane in both rabbit and primate corneal endothelial decompensation models and upregulated the expression of functional proteins including Na+/K+ ATPase and ZO-1, thereby leading to the resolution of corneal edema [22]. Another study revealed that intracameral injection of either human or monkey CECs in combination with Y-27632 led to the regeneration of monkey corneal endothelium, suggesting that intracameral injection of cultivated human CECs combined with the ROCK inhibitor may be a plausible therapeutic option for corneal endothelial diseases [2]. Preliminary data from a human clinical study showed that cultured human CECs injected in conjunction with ROCK achieved improvement of corneal edema and visual acuity without any serious adverse effect [3]. Although the results appear to be promising, further prospective randomized studies with a long-term follow-up are necessary to evaluate the efficacy and safety of ROCK inhibitors for the treatment of corneal endothelial diseases [3].

2.3. Other Effects of ROCK Inhibitor on Corneal Regeneration. The ROCK inhibitor was suggested to have additional effects that can be potentially applicable for corneal regeneration. Y-27632 was shown to promote both ex vivo and *in vitro*

proliferation of limbal epithelial cell proliferation, suggesting it can be useful for the treatment of limbal stem cell deficiency [23]. Zhou et al. [24] also demonstrated that Y-27632 enhanced the cloning efficiency of limbal stem/progenitor cells by promoting their adhesion and capacity of reactive oxygen species scavenging in a rabbit model [24]. Y-27632 inhibited the transition of rabbit keratocyte to myofibroblast and modulated a wound healing process after a superficial lamellar keratectomy in a rabbit cornea [25]. Animal experimental studies revealed that ROCK inhibitors, fasudil and AMA0526, inhibited corneal neovascularization and opacity and facilitated corneal epithelial regeneration after corneal alkali burn [26, 27].

3. Blood-Derived Products

Application of blood-derived products for ocular surface diseases was first introduced in 1975 for 6 patients with chemical burns [28]. Since Tsubota et al. [29, 30] proved the efficacy and safety of autologous serum eye drops (ASE) for the treatment of dry eye disease (DED) and persistent epithelial defect (PED) [29, 30], studies have proven that blood-derived products are innovative therapeutic agents for various ocular surface disorders, such as DED [31], PED [32], neurotrophic keratitis [33], recurrent corneal erosion [34], and chemical burns [35].

3.1. Autologous Serum Eye Drops (ASE) [36]. ASE has a similar biochemical composition as human tears [37] and includes growth factors including epidermal growth factor (EGF) and transforming growth factor- (TGF-) β [38], chemokines, fibronectin, and various nutrients [38].

Consensus for the preparation method has never been established yet [37]. An *in vitro* experimental study indicated that an increased clotting time of 120 min or longer, a sharp centrifugation (3000g for 15 min), and dilution with balanced salt solution at 12.5–25% were optimal for corneal epithelial healing [39]. Clinically, 20% ASE is most frequently used to match the TGF-β concentration, which is 5 times higher in serum than in tears, to prevent delayed wound healing and promotion of corneal haze caused by TGF-β [30]. However, higher concentrations of ASE (50 to 100%) were also suggested to be effective and safe [40, 41]. A randomized prospective study showed that 100% ASE was more effective for the treatment of PED, Sjögren's syndrome (SS), and non-Sjögren DED than 50% ASE [42].

Randomized clinical studies revealed that ASE was more effective than conventional treatment for the improvement of both symptoms and signs of DED [43–45]. Kojima et al. [46] demonstrated that ASE suppressed apoptosis in the ocular surface epithelium and albumin contained in ASE recovered ocular surface damage [46]. ASE was superior to artificial tear in the improvement of dry eye signs after refractive surgery [47]. In addition, ASE can be a therapeutic option for PED refractory to conventional treatment [40, 48]. Schrader et al. [49] suggested that ASE combined with silicone hydrogel contact lenses might be helpful in recalcitrant PED [49]. Moreover, ASE was shown to be effective in facilitating reepithelialization of corneal graft after penetrating keratoplasty [50]. It also promoted corneal epithelial healing in patients with neurotrophic keratitis and aniridic keratopathy [51, 52]. A prospective study with a long-term follow-up revealed that ASE was effective for the prevention of recurrence in patients with recurrent corneal erosion [53]. However, a review of five randomized clinical trials that compared AS versus artificial tears or saline in DED patients revealed that no evidence of a benefit was found after two weeks of treatment, although there might be some short-term effect on symptoms with AS compared with artificial tears [54]. Therefore, we also believe further well-designed, large-scale randomized controlled studies are warranted to evaluate the efficacy of AS [54]. The absence of preservatives and high nutrient level in ASE increases the risk of sample contamination [55, 56]. Therefore, attention should be paid for the signs of infection in patients using ASE [36]. Lagnado et al. [55] recommended that sample vials should be stored frozen at −20°C for up to 6 months, and each vial should be thawed and used for only 24 hours. A prospective randomized human study showed that containers equipped with a sterilizing filter can be used for up to 4 weeks without any increased risk of contamination [56]. The concentrations of growth factors in 20% ASE remained stable for up to 9 months when kept frozen at −20°C and up to 4 weeks when defrosted [57].

3.2. Allogeneic Serum Eye Drops (SE). Allogeneic SE from healthy donors can be advantageous in patients with fear or difficulty of blood sampling or coexisting blood disorders including anemia [58]. Moreover, it can also be a therapeutic option for patients with graft-versus-host disease (GVHD) or (SS), in which a considerable amount of proinflammatory cytokines could be included in their autologous serum [59]. Allogeneic SE can be prepared using the same protocol of ASE [58]. Because allogeneic SE includes anti-A and anti-B antibodies, it can theoretically cause an immune reaction against ABO antigens expressed on corneal and conjunctival epithelium [58]. Therefore, preparation of allogenic SE from ABO-identical donors or blood type AB donors is recommended [60, 61]. Studies demonstrated the efficacy of allogenic SE in DED, PED, neurotrophic keratitis, GVHD, and exposure keratopathy [60, 62, 63], indicating its potential as a viable alternative to ASE [36].

3.3. Umbilical Cord Serum Eye Drops (UCSE). UCSE samples can be prepared using umbilical cord blood collected during delivery [36]. Rigorous screening for blood-borne infections is mandatory prior to donation [36, 58]. UCSE can also be a therapeutic option for patients with blood disorders or systemic inflammatory diseases, in which ASE is contraindicated [58]. Although allogeneic serum does have the same advantage, UCS contains a higher level of growth factors, neurotrophic factors, and essential tear components compared to allogeneic serum [31, 33, 64]. Moreover, compared to allogeneic serum, a substantially larger amount of UCSE can be obtained with a single sampling from one donor and can be distributed to multiple patients [58]. As UCSE contains high levels of neurotrophic factors such as substance P (SP), insulin-like growth factor- (IGF-) 1, and nerve growth

factor (NGF) [32, 33], as well as growth factors including EGF and TGF-β [64], it is conceivably helpful for corneal nerve regeneration and epithelial healing. Studies have revealed that UCSE accelerated the recovery of PED and neurotrophic keratitis recalcitrant to conventional treatment [32, 33]. UCSE was shown to be more effective than ASE for the improvement of symptoms and signs of DED, particularly in severe cases associated with GVHD and SS [64, 65]. UCSE was superior to artificial tear in treating recurrent corneal erosions and reducing its recurrence [34]. In ocular chemical burn, UCSE resulted in faster corneal epithelial healing and milder corneal opacity compared to ASE or artificial tears [35]. UCSE is also shown to decrease early corneal haze and improve ocular surface parameters after laser epithelial keratomileusis (LASEK) [66].

3.4. Platelet-Derived Plasma Preparations. Platelet-derived plasma preparations contain a large amount of growth factors and cytokines [36, 58] and have been successfully used in maxillofacial and orthopedic surgery as well as in regenerative medicine for the promotion of tissue healing [67]. Various preparations have been developed, such as plasma rich in growth factors (PRGF), platelet-rich plasma (PRP), and platelet lysate [58]. PRGF is obtained by the filtration of plasma supernatants after centrifugation of the whole blood [68]. PRP is a plasma with increased concentrations of platelets obtained with an additional centrifugation of the whole blood [69]. Platelet lysate is collected by inducing platelet lysis and release of growth factors including platelet-derived growth factor (PDGF) using PRP [70].

Kim et al. [71] demonstrated that PRP was superior to ASE in the treatment of PED. PRGF was also suggested to be useful for the healing of PED [72]. PRP was shown to be effective for the improvement of both symptoms and signs of DED [73]. Plasma lysate was suggested to be helpful for the treatment of DED associated with GVHD or SS [74, 75]. PRP was also superior to conventional treatment for the recovery of visual acuity and corneal transparency in patients with ocular chemical injury [76]. In addition, PRP is potentially available for a biomaterial for ocular surface reconstruction [77, 78].

4. Growth Factors for Corneal Diseases

4.1. Nerve Growth Factor (NGF). NGF facilitates corneal epithelial healing, which is mediated by the cleavage of β4 integrin and the upregulation of matrix metalloproteinase-9 [79]. Topical administration of NGF was shown to be effective in neurotrophic keratitis refractory to conventional treatment [80–82]. NGF is also expected to be effective for the treatment of diabetic keratopathy, as it could alleviate inflammation and apoptosis of corneal cells that can occur in diabetes mellitus [83].

Topical NGF was also shown to have a beneficial effect for postoperative corneal wound healing [80]. Cellini et al. [84] demonstrated that topical NGF was superior to artificial tear for corneal reconstruction after cataract. Animal experimental studies revealed that topical application of NGF accelerated restoration of corneal sensitivity and promoted cornea epithelial proliferation and nerve regeneration after laser in situ keratomileusis (LASIK) or photorefractive keratectomy (PRK) [85–87].

4.2. Substance P (SP) and Insulin-Like Growth Factor- (IGF-) 1. A randomized prospective study revealed that topical SP and IGF-1 combination therapy was useful for the prevention of superficial punctate keratopathy after cataract surgery in diabetic patients [88]. SP was shown to promote an epithelial healing process in diabetic cornea and attenuate hyperosmotic stress-induced apoptosis of corneal epithelial cells through the neurokinin-1 receptor signaling pathway [89, 90]. IGF-1 also facilitated the regeneration of corneal surface ultrastructure and nerves after LASIK in rabbit eyes [91].

4.3. Vascular Endothelial Growth Factor (VEGF). VEGF can facilitate the functional and anatomical recovery after peripheral nerve damage [92]. Guaiquil et al. [93] demonstrated that VEGF-B treatment selectively promoted nerve regeneration and restored sensory and trophic functions of injured corneal nerves, suggesting that it might have a therapeutic potential for peripheral corneal nerve injury [93].

An experimental study revealed that the expression of endogenous VEGF-B was attenuated in regenerated corneal epithelium in a diabetic mouse model, whereas supplementation of exogenous VEGF-B accelerated corneal nerve regeneration [94].

4.4. Other Grow Factors. An animal study showed that pigment epithelial-derived factor, in conjunction with docosahexaenoic acid, might be effective for the treatment of DED caused by corneal nerve damage and neurotrophic keratitis [95]. Topical administration of neuroprotectin D1 was also shown to attenuate inflammation and facilitate nerve regeneration after corneal damage in a rabbit model [96]. Ciliary neurotrophic factor was shown to be able to activate corneal epithelial stem/progenitor cells and promote the corneal nerve regeneration and epithelial recovery, suggesting its therapeutic potential for diabetic keratopathy and limbal stem cell deficiency [97]. A randomized clinical study demonstrated that basic fibroblast growth factor promoted corneal epithelial healing after PRK, indicating it could be a therapeutic option for delayed healing [98].

4.5. Regenerating Agent Eye Drops (RGTAs). RGTA (OTR4120 Cacicol20®; Théa, Clermont-Ferrand, France) is a carboxymethyl dextran sulfate polymer bioengineered to replace heparan sulfate, which is an important factor both for matrix proteins and for growth factors [99, 100]. Thus, RGTA is conceivably helpful for restoring equilibrium in cellular microenvironment [99, 101]. It is also expected to be useful for corneal wound healing, and several studies have shown promising results [99–107].

Experimental studies using a rabbit corneal burn model and a clinical case series study suggested that RGTA might be an innovative agent for promoting corneal regeneration and attenuating ocular surface inflammation by reducing oxidative, proteolytic, and nitrosative corneal damage [104, 108, 109]. A clinical pilot study also revealed the efficacy of RGTA for corneal ulcers and dystrophies refractory

Table 1: Effect and possible application of the novel drugs.

Drug	Effect	Possible application
ROCK inhibitor	Corneal endothelial cell regeneration	Topical eye drops for recovery of corneal clarity in corneal endothelial dysfunction
	Promotion of corneal endothelial cell adhesion	Adjuvant therapy for the corneal endothelial cell injection
Blood-derived products	Promotion of healing of ocular surface epithelium	Recovery of persistent epithelial defect or neurotrophic keratitis
		Promoting ocular surface regeneration and prevention of corneal haze after ocular chemical injury or keratorefractive surgery
Growth factors	Facilitation of corneal epithelial healing and nerve regeneration	Treatment of persistent epithelial defect or neurotrophic keratitis
		Topical eye drops for diabetic keratopathy
		Recovery of cornea epithelium and nerve after keratorefractive surgery
RGTA*	Promotion of corneal regeneration and attenuation of ocular surface inflammation	Treatment of persistent epithelial defect or neurotrophic keratitis
		Recovery of corneal epithelium after corneal cross-linking
		Corneal recovery after keratorefractive surgery

*RGTA: regenerating agent eye drops.

to conventional treatment [105]. A prospective clinical study demonstrated that RGTA might be effective and safe for the treatment of neurotrophic keratitis [106]. Chappelet et al. [101] recently showed that RGTA could be useful for the treatment of a PED after bacterial keratitis [101].

RGTA ophthalmic solution was also reported to facilitate corneal epithelial healing after corneal cross-linking (CXL) by reconstruction of the extracellular matrix in the corneal wound area [99, 100]. A randomized clinical trial demonstrated that RGTA might be superior to topical hyaluronic acid for corneal wound recovery after CXL in patients with keratoconus [103].

In animal excimer laser models, topical RGTA reduced corneal haze and promoted nerve regeneration, suggesting that it could be a useful option for the restoration of corneal microarchitecture after keratorefractive surgery [102, 107].

5. Conclusion

In this review paper, we have introduced a number of research papers that have demonstrated the efficacy of pharmacologic therapies, such as ROCK inhibitors, blood-derived products, growth factors, and RGTA on corneal cell regeneration. The promising results of these studies suggest that these agents can be viable options to aid corneal cell regeneration.

In summary, the ROCK inhibitor can promote the regeneration of CECs and its adhesion to Descemet's membrane. Thus, it can be used as a topical eye drops for the treatment of corneal endothelial dysfunction. It can also be used as an adjuvant therapy for CEC injection to AC. Blood-derived products can promote healing of ocular surface epithelium. Hence, it can be used for PED or neurotrophic keratitis. It can also be used for promoting ocular surface reconstruction and prevention of corneal opacity after ocular chemical injury. Growth factors promote the recovery of cornea epithelium and nerve; thus, these drugs can especially be helpful for PED, neurotrophic keratitis, and diabetic keratopathy. As RGTA facilitates corneal regeneration and attenuates ocular surface inflammation, it can be indicated for corneal regeneration after corneal CXL. It can also be helpful for the treatment of PDE or neurotrophic keratitis. The recovery of corneal epithelium and nerve after keratorefractive surgery can be facilitated by blood-derived products, growth factors, and RGTA (Table 1).

Although these therapeutic agents are innovative, further prospective randomized studies are needed for the verification of the efficacy and safety of the drugs [3]. Further studies are also required for the development of novel therapeutic agents for corneal cell regeneration.

Conflicts of Interest

None of the authors have a proprietary interest in the study or financial interests to disclose.

Acknowledgments

This study was supported by 2017 Research Grant from Kangwon National University.

References

[1] J. P. Whitcher, M. Srinivasan, and M. P. Upadhyay, "Corneal blindness: a global perspective," *Bulletin of the World Health Organization*, vol. 79, no. 3, pp. 214–221, 2001.

[2] N. Okumura, Y. Sakamoto, K. Fujii et al., "Rho kinase inhibitor enables cell-based therapy for corneal endothelial dysfunction," *Scientific Reports*, vol. 6, no. 1, article 26113, 2016.

[3] N. Okumura, S. Kinoshita, and N. Koizumi, "Application of Rho kinase inhibitors for the treatment of corneal endothelial diseases," *Journal of Ophthalmology*, vol. 2017, Article ID 2646904, 8 pages, 2017.

[4] R. Nourinia, S. Nakao, S. Zandi, S. Safi, A. Hafezi-Moghadam, and H. Ahmadieh, "ROCK inhibitors for the treatment of ocular diseases," *British Journal of Ophthalmology*, vol. 102, no. 1, 2017.

[5] N. Koizumi, N. Okumura, M. Ueno, and S. Kinoshita, "New therapeutic modality for corneal endothelial disease using Rho-associated kinase inhibitor eye drops," *Cornea*, vol. 33, Supplement 11, pp. S25–S31, 2014.

[6] N. Okumura, N. Koizumi, M. Ueno et al., "Enhancement of corneal endothelium wound healing by Rho-associated kinase (ROCK) inhibitor eye drops," *British Journal of Ophthalmology*, vol. 95, no. 7, pp. 1006–1009, 2011.

[7] N. Okumura, S. Nakano, E. D. P. Kay et al., "Involvement of cyclin D and p27 in cell proliferation mediated by ROCK inhibitors Y-27632 and Y-39983 during corneal endothelium wound healing," *Investigative Ophthalmology & Visual Science*, vol. 55, no. 1, pp. 318–329, 2014.

[8] G. S. L. Peh, K. Adnan, B. L. George et al., "The effects of Rho-associated kinase inhibitor Y-27632 on primary human corneal endothelial cells propagated using a dual media approach," *Scientific Reports*, vol. 5, no. 1, p. 9167, 2015.

[9] A. Pipparelli, Y. Arsenijevic, G. Thuret, P. Gain, M. Nicolas, and F. Majo, "ROCK inhibitor enhances adhesion and wound healing of human corneal endothelial cells," *PLoS One*, vol. 8, no. 4, article e62095, 2013.

[10] N. Okumura, N. Koizumi, M. Ueno et al., "The new therapeutic concept of using a rho kinase inhibitor for the treatment of corneal endothelial dysfunction," *Cornea*, vol. 30, Supplement 1, pp. S54–S59, 2011.

[11] N. Okumura, N. Koizumi, E. D. P. Kay et al., "The ROCK inhibitor eye drop accelerates corneal endothelium wound healing," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 4, pp. 2493–2502, 2013.

[12] N. Okumura, M. Ueno, N. Koizumi et al., "Enhancement on primate corneal endothelial cell survival in vitro by a ROCK inhibitor," *Investigative Ophthalmology & Visual Science*, vol. 50, no. 8, pp. 3680–3687, 2009.

[13] N. Okumura, S. Kinoshita, and N. Koizumi, "The role of rho kinase inhibitors in corneal endothelial dysfunction," *Current Pharmaceutical Design*, vol. 23, no. 4, pp. 660–666, 2017.

[14] N. Koizumi, N. Okumura, M. Ueno, H. Nakagawa, J. Hamuro, and S. Kinoshita, "Rho-associated kinase inhibitor eye drop treatment as a possible medical treatment for Fuchs corneal dystrophy," *Cornea*, vol. 32, no. 8, pp. 1167–1170, 2013.

[15] G. Moloney, C. Petsoglou, M. Ball et al., "Descemetorhexis without grafting for Fuchs endothelial dystrophy-supplementation with topical ripasudil," *Cornea*, vol. 36, no. 6, pp. 642–648, 2017.

[16] N. Koizumi, Y. Sakamoto, N. Okumura et al., "Cultivated corneal endothelial cell sheet transplantation in a primate model," *Investigative Ophthalmology & Visual Science*, vol. 48, no. 10, pp. 4519–4526, 2007.

[17] T. Mimura, S. Yamagami, S. Yokoo et al., "Cultured human corneal endothelial cell transplantation with a collagen sheet in a rabbit model," *Investigative Ophthalmology & Visual Science*, vol. 45, no. 9, pp. 2992–2997, 2004.

[18] T. Mimura, N. Shimomura, T. Usui et al., "Magnetic attraction of iron-endocytosed corneal endothelial cells to Descemet's membrane," *Experimental Eye Research*, vol. 76, no. 6, pp. 745–751, 2003.

[19] T. Mimura, S. Yamagami, T. Usui et al., "Long-term outcome of iron-endocytosing cultured corneal endothelial cell transplantation with magnetic attraction," *Experimental Eye Research*, vol. 80, no. 2, pp. 149–157, 2005.

[20] I. H. Ma, L. W. Chen, W. H. Tu, C. J. Lu, C. J. Huang, and W. L. Chen, "Serum components and clinical efficacies of autologous serum eye drops in dry eye patients with active and inactive Sjogren syndrome," *Taiwan Journal of Ophthalmology*, vol. 7, no. 4, pp. 213–220, 2017.

[21] T. Mimura, S. Yamagami, S. Yokoo et al., "Sphere therapy for corneal endothelium deficiency in a rabbit model," *Investigative Ophthalmology & Visual Science*, vol. 46, no. 9, pp. 3128–3135, 2005.

[22] N. Okumura, N. Koizumi, M. Ueno et al., "ROCK inhibitor converts corneal endothelial cells into a phenotype capable of regenerating *in vivo* endothelial tissue," *The American Journal of Pathology*, vol. 181, no. 1, pp. 268–277, 2012.

[23] C. C. Sun, H. T. Chiu, Y. F. Lin, K. Y. Lee, and J. H. S. Pang, "Y-27632, a ROCK inhibitor, promoted limbal epithelial cell proliferation and corneal wound healing," *PLoS One*, vol. 10, no. 12, article e0144571, 2015.

[24] Q. Zhou, H. Duan, Y. Wang, M. Qu, L. Yang, and L. Xie, "ROCK inhibitor Y-27632 increases the cloning efficiency of limbal stem/progenitor cells by improving their adherence and ROS-scavenging capacity," *Tissue Engineering. Part C: Methods*, vol. 19, no. 7, pp. 531–537, 2013.

[25] M. Yamamoto, A. J. Quantock, R. D. Young et al., "A selective inhibitor of the Rho kinase pathway, Y-27632, and its influence on wound healing in the corneal stroma," *Molecular Vision*, vol. 18, pp. 1727–1739, 2012.

[26] P. Zeng, R. B. Pi, P. Li et al., "Fasudil hydrochloride, a potent ROCK inhibitor, inhibits corneal neovascularization after alkali burns in mice," *Molecular Vision*, vol. 21, pp. 688–698, 2015.

[27] D. Sijnave, T. Van Bergen, K. Castermans et al., "Inhibition of Rho-associated kinase prevents pathological wound healing and neovascularization after corneal trauma," *Cornea*, vol. 34, no. 9, pp. 1120–1129, 2015.

[28] R. A. Ralph, M. G. Doane, and C. H. Dohlman, "Clinical experience with a mobile ocular perfusion pump," *Archives of Ophthalmology*, vol. 93, no. 10, pp. 1039–1043, 1975.

[29] K. Tsubota, E. Goto, S. Shimmura, and J. Shimazaki, "Treatment of persistent corneal epithelial defect by autologous serum application," *Ophthalmology*, vol. 106, no. 10, pp. 1984–1989, 1999.

[30] K. Tsubota, E. Goto, H. Fujita et al., "Treatment of dry eye by autologous serum application in Sjogren's syndrome," *British Journal of Ophthalmology*, vol. 83, no. 4, pp. 390–395, 1999.

[31] K. C. Yoon, S. K. Im, Y. G. Park, Y. D. Jung, S. Y. Yang, and J. Choi, "Application of umbilical cord serum eyedrops for the treatment of dry eye syndrome," *Cornea*, vol. 25, no. 3, pp. 268–272, 2006.

[32] K. C. Yoon, H. Heo, I. Y. Jeong, and Y. G. Park, "Therapeutic effect of umbilical cord serum eyedrops for persistent corneal epithelial defect," *Korean Journal of Ophthalmology*, vol. 19, no. 3, pp. 174–178, 2005.

[33] K. C. Yoon, I. C. You, S. K. Im, T. S. Jeong, Y. G. Park, and J. Choi, "Application of umbilical cord serum eyedrops for the treatment of neurotrophic keratitis," *Ophthalmology*, vol. 114, no. 9, pp. 1637–1642.e2, 2007.

[34] K. C. Yoon, W. Choi, I. C. You, and J. Choi, "Application of umbilical cord serum eyedrops for recurrent corneal erosions," *Cornea*, vol. 30, no. 7, pp. 744–748, 2011.

[35] N. Sharma, M. Goel, T. Velpandian, J. S. Titiyal, R. Tandon, and R. B. Vajpayee, "Evaluation of umbilical cord serum therapy in acute ocular chemical burns," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 2, pp. 1087–1092, 2011.

[36] N. G. Soni and B. H. Jeng, "Blood-derived topical therapy for ocular surface diseases," *British Journal of Ophthalmology*, vol. 100, no. 1, pp. 22–27, 2015.

[37] G. Geerling, S. Maclennan, and D. Hartwig, "Autologous serum eye drops for ocular surface disorders," *British Journal of Ophthalmology*, vol. 88, no. 11, pp. 1467–1474, 2004.

[38] C. Yamada, K. E. King, and P. M. Ness, "Autologous serum eyedrops: literature review and implications for transfusion medicine specialists," *Transfusion*, vol. 48, no. 6, pp. 1245–1255, 2008.

[39] L. Liu, D. Hartwig, S. Harloff, P. Herminghaus, T. Wedel, and G. Geerling, "An optimised protocol for the production of autologous serum eyedrops," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 243, no. 7, pp. 706–714, 2005.

[40] B. H. Jeng and W. J. Dupps Jr, "Autologous serum 50% eyedrops in the treatment of persistent corneal epithelial defects," *Cornea*, vol. 28, no. 10, pp. 1104–1108, 2009.

[41] K. Lekhanont, P. Jongkhajornpong, L. Choubtum, and V. Chuckpaiwong, "Topical 100% serum eye drops for treating corneal epithelial defect after ocular surgery," *BioMed Research International*, vol. 2013, Article ID 521315, 7 pages, 2013.

[42] Y. K. Cho, W. Huang, G. Y. Kim, and B. S. Lim, "Comparison of autologous serum eye drops with different diluents," *Current Eye Research*, vol. 38, no. 1, pp. 9–17, 2013.

[43] B. A. Noble, R. S. Loh, S. MacLennan et al., "Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease," *British Journal of Ophthalmology*, vol. 88, no. 5, pp. 647–652, 2004.

[44] T. Kojima, R. Ishida, M. Dogru et al., "The effect of autologous serum eyedrops in the treatment of severe dry eye disease: a prospective randomized case-control study," *American Journal of Ophthalmology*, vol. 139, no. 2, pp. 242–246, 2005.

[45] A. R. C. Celebi, C. Ulusoy, and G. E. Mirza, "The efficacy of autologous serum eye drops for severe dry eye syndrome: a randomized double-blind crossover study," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 252, no. 4, pp. 619–626, 2014.

[46] T. Kojima, A. Higuchi, E. Goto, Y. Matsumoto, M. Dogru, and K. Tsubota, "Autologous serum eye drops for the treatment of dry eye diseases," *Cornea*, vol. 27, Supplement 1, pp. S25–S30, 2008.

[47] T. Noda-Tsuruya, N. Asano-Kato, I. Toda, and K. Tsubota, "Autologous serum eye drops for dry eye after LASIK," *Journal of Refractive Surgery*, vol. 22, no. 1, pp. 61–66, 2006.

[48] A. C. Poon, G. Geerling, J. K. Dart, G. E. Fraenkel, and J. T. Daniels, "Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies," *British Journal of Ophthalmology*, vol. 85, no. 10, pp. 1188–1197, 2001.

[49] S. Schrader, T. Wedel, R. Moll, and G. Geerling, "Combination of serum eye drops with hydrogel bandage contact lenses in the treatment of persistent epithelial defects," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 244, no. 10, pp. 1345–1349, 2006.

[50] Y. M. Chen, F. R. Hu, J. Y. Huang, E. P. Shen, T. Y. Tsai, and W. L. Chen, "The effect of topical autologous serum on graft re-epithelialization after penetrating keratoplasty," *American Journal of Ophthalmology*, vol. 150, no. 3, pp. 352–359.e2, 2010.

[51] Y. Matsumoto, M. Dogru, E. Goto et al., "Autologous serum application in the treatment of neurotrophic keratopathy," *Ophthalmology*, vol. 111, no. 6, pp. 1115–1120, 2004.

[52] J. S. Lopez-Garcia, L. Rivas, I. Garcia-Lozano, and J. Murube, "Autologous serum eyedrops in the treatment of aniridic keratopathy," *Ophthalmology*, vol. 115, no. 2, pp. 262–267, 2008.

[53] N. G. Ziakas, K. G. Boboridis, C. Terzidou et al., "Long-term follow up of autologous serum treatment for recurrent corneal erosions," *Clinical & Experimental Ophthalmology*, vol. 38, no. 7, pp. 683–687, 2010.

[54] Q. Pan, A. Angelina, M. Marrone et al., "Autologous serum eye drops for dry eye," *Cochrane Database of Systematic Reviews*, vol. 2, article CD009327, 2017.

[55] R. Lagnado, A. J. King, F. Donald, and H. S. Dua, "A protocol for low contamination risk of autologous serum drops in the management of ocular surface disorders," *British Journal of Ophthalmology*, vol. 88, no. 4, pp. 464-465, 2004.

[56] J. S. Lopez-Garcia and I. Garcia-Lozano, "Use of containers with sterilizing filter in autologous serum eyedrops," *Ophthalmology*, vol. 119, no. 11, pp. 2225–2230, 2012.

[57] J. S. Lopez-Garcia, I. Garcia-Lozano, L. Rivas, N. Ramírez, M. T. Méndez, and R. Raposo, "Stability of growth factors in autologous serum eyedrops after long-term storage," *Current Eye Research*, vol. 41, no. 3, pp. 1–7, 2016.

[58] G. Giannaccare, P. Versura, M. Buzzi, L. Primavera, M. Pellegrini, and E. C. Campos, "Blood derived eye drops for the treatment of cornea and ocular surface diseases," *Transfusion and Apheresis Science*, vol. 56, no. 4, pp. 595–604, 2017.

[59] P. A. Stenwall, M. Bergstrom, P. Seiron et al., "Improving the anti-inflammatory effect of serum eye drops using allogeneic serum permissive for regulatory T cell induction," *Acta Ophthalmologica*, vol. 93, no. 7, pp. 654–657, 2015.

[60] L. H. Harritshoj, C. Nielsen, H. Ullum, M. B. Hansen, and H. O. Julian, "Ready-made allogeneic ABO-specific serum eye drops: production from regular male blood donors, clinical routine, safety and efficacy," *Acta Ophthalmologica*, vol. 92, no. 8, pp. 783–786, 2014.

[61] K. G. Badami and M. McKellar, "Allogeneic serum eye drops: time these became the norm?," *British Journal of Ophthalmology*, vol. 96, no. 8, pp. 1151-1152, 2012.

[62] C. C. Chiang, W. L. Chen, J. M. Lin, and Y. Y. Tsai, "Allogeneic serum eye drops for the treatment of persistent corneal epithelial defect," *Eye*, vol. 23, no. 2, pp. 290–293, 2009.

[63] K. S. Na and M. S. Kim, "Allogeneic serum eye drops for the treatment of dry eye patients with chronic graft-versus-host disease," *Journal of Ocular Pharmacology and Therapeutics*, vol. 28, no. 5, pp. 479–483, 2012.

[64] K. C. Yoon, H. Heo, S. K. Im, I. C. You, Y. H. Kim, and Y. G. Park, "Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome," *American Journal of Ophthalmology*, vol. 144, no. 1, pp. 86–92.e2, 2007.

[65] K. C. Yoon, I. Y. Jeong, S. K. Im, Y. G. Park, H. J. Kim, and J. Choi, "Therapeutic effect of umbilical cord serum eyedrops for the treatment of dry eye associated with graft-versus-host disease," *Bone Marrow Transplantation*, vol. 39, no. 4, pp. 231–235, 2007.

[66] K. C. Yoon, H. J. Oh, J. W. Park, and J. Choi, "Application of umbilical cord serum eyedrops after laser epithelial

keratomileusis," *Acta Ophthalmologica*, vol. 91, no. 1, pp. e22–e28, 2013.

[67] B. H. Choi, C. J. Im, J. Y. Huh, J. J. Suh, and S. H. Lee, "Effect of platelet-rich plasma on bone regeneration in autogenous bone graft," *International Journal of Oral and Maxillofacial Surgery*, vol. 33, no. 1, pp. 56–59, 2004.

[68] E. Anitua, M. de la Fuente, F. Muruzabal, A. Riestra, J. Merayo-Lloves, and G. Orive, "Plasma rich in growth factors (PRGF) eye drops stimulates scarless regeneration compared to autologous serum in the ocular surface stromal fibroblasts," *Experimental Eye Research*, vol. 135, pp. 118–126, 2015.

[69] S. T. Tanidir, N. Yuksel, O. Altintas, D. K. Yildiz, E. Sener, and Y. Caglar, "The effect of subconjunctival platelet-rich plasma on corneal epithelial wound healing," *Cornea*, vol. 29, no. 6, pp. 664–669, 2010.

[70] L. Liu, D. Hartwig, S. Harloff et al., "Corneal epitheliotrophic capacity of three different blood-derived preparations," *Investigative Ophthalmology & Visual Science*, vol. 47, no. 6, pp. 2438–2444, 2006.

[71] K. M. Kim, Y. T. Shin, and H. K. Kim, "Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis," *Japanese Journal of Ophthalmology*, vol. 56, no. 6, pp. 544–550, 2012.

[72] S. Lopez-Plandolit, M. C. Morales, V. Freire, J. Etxebarría, and J. A. Durán, "Plasma rich in growth factors as a therapeutic agent for persistent corneal epithelial defects," *Cornea*, vol. 29, no. 8, pp. 843–848, 2010.

[73] J. L. Alio, A. E. Rodriguez, R. Ferreira-Oliveira, D. Wróbel-Dudzińska, and A. A. Abdelghany, "Treatment of dry eye disease with autologous platelet-rich plasma: a prospective, interventional, non-randomized study," *Ophthalmology and Therapy*, vol. 6, no. 2, pp. 285–293, 2017.

[74] S. Pezzotta, C. Del Fante, L. Scudeller, M. Cervio, E. R. Antoniazzi, and C. Perotti, "Autologous platelet lysate for treatment of refractory ocular GVHD," *Bone Marrow Transplantation*, vol. 47, no. 12, pp. 1558–1563, 2012.

[75] A. M. Fea, V. Aragno, V. Testa et al., "The effect of autologous platelet lysate eye drops: an in vivo confocal microscopy study," *BioMed Research International*, vol. 2016, Article ID 8406832, 10 pages, 2016.

[76] A. Panda, M. Jain, M. Vanathi, T. Velpandian, S. Khokhar, and T. Dada, "Topical autologous platelet-rich plasma eyedrops for acute corneal chemical injury," *Cornea*, vol. 31, no. 9, pp. 989–993, 2012.

[77] J. L. Alio, A. E. Rodriguez, and L. M. Martinez, "Bovine pericardium membrane (tutopatch) combined with solid platelet-rich plasma for the management of perforated corneal ulcers," *Cornea*, vol. 32, no. 5, pp. 619–624, 2013.

[78] J. L. Alio, A. E. Rodriguez, L. M. Martinez, and A. L. Rio, "Autologous fibrin membrane combined with solid platelet-rich plasma in the management of perforated corneal ulcers: a pilot study," *JAMA Ophthalmology*, vol. 131, no. 6, pp. 745–751, 2013.

[79] T. Blanco-Mezquita, C. Martinez-Garcia, R. Proenca et al., "Nerve growth factor promotes corneal epithelial migration by enhancing expression of matrix metalloprotease-9," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 6, pp. 3880–3890, 2013.

[80] A. Lambiase, M. Sacchetti, and S. Bonini, "Nerve growth factor therapy for corneal disease," *Current Opinion in Ophthalmology*, vol. 23, no. 4, pp. 296–302, 2012.

[81] F. R. Ghosheh, F. Cremona, B. D. Ayres et al., "Indications for penetrating keratoplasty and associated procedures, 2001-2005," *Eye & Contact Lens*, vol. 34, no. 4, pp. 211–214, 2008.

[82] S. Bonini, A. Lambiase, P. Rama, G. Caprioglio, and L. Aloe, "Topical treatment with nerve growth factor for neurotrophic keratitis," *Ophthalmology*, vol. 107, no. 7, pp. 1347–1351, 2000.

[83] J. H. Park, S. S. Kang, J. Y. Kim, and H. Tchah, "Nerve growth factor attenuates apoptosis and inflammation in the diabetic cornea," *Investigative Ophthalmology & Visual Science*, vol. 57, no. 15, pp. 6767–6775, 2016.

[84] M. Cellini, E. Bendo, G. O. Bravetti, and E. C. Campos, "The use of nerve growth factor in surgical wound healing of the cornea," *Ophthalmic Research*, vol. 38, no. 4, pp. 177–181, 2006.

[85] M. J. Joo, K. R. Yuhan, J. Y. Hyon et al., "The effect of nerve growth factor on corneal sensitivity after laser in situ keratomileusis," *Archives of Ophthalmology*, vol. 122, no. 9, pp. 1338–1341, 2004.

[86] S. Esquenazi, H. E. P. Bazan, V. Bui, J. He, D. B. Kim, and N. G. Bazan, "Topical combination of NGF and DHA increases rabbit corneal nerve regeneration after photorefractive keratectomy," *Investigative Ophthalmology & Visual Science*, vol. 46, no. 9, pp. 3121–3127, 2005.

[87] K. Ma, N. Yan, Y. Huang, G. Cao, J. Deng, and Y. Deng, "Effects of nerve growth factor on nerve regeneration after corneal nerve damage," *International Journal of Clinical and Experimental Medicine*, vol. 7, no. 11, pp. 4584–4589, 2014.

[88] N. Chikamoto, T. Chikama, N. Yamada, T. Nishida, T. Ishimitsu, and A. Kamiya, "Efficacy of substance P and insulin-like growth factor-1 peptides for preventing postsurgical superficial punctate keratopathy in diabetic patients," *Japanese Journal of Ophthalmology*, vol. 53, no. 5, pp. 464–469, 2009.

[89] L. Yang, G. Di, X. Qi et al., "Substance P promotes diabetic corneal epithelial wound healing through molecular mechanisms mediated via the neurokinin-1 receptor," *Diabetes*, vol. 63, no. 12, pp. 4262–4274, 2014.

[90] L. Yang, W. Sui, Y. Li et al., "Substance P inhibits hyperosmotic stress-induced apoptosis in corneal epithelial cells through the mechanism of Akt activation and reactive oxygen species scavenging via the neurokinin-1 receptor," *PLoS One*, vol. 11, no. 2, article e0149865, 2016.

[91] C. Wang, Y. Peng, S. Pan, and L. Li, "Effect of insulin-like growth factor-1 on corneal surface ultrastructure and nerve regeneration of rabbit eyes after laser in situ keratomileusis," *Neuroscience Letters*, vol. 558, pp. 169–174, 2014.

[92] Z. Pan, S. Fukuoka, N. Karagianni, V. H. Guaiquil, and M. I. Rosenblatt, "Vascular endothelial growth factor promotes anatomical and functional recovery of injured peripheral nerves in the avascular cornea," *The FASEB Journal*, vol. 27, no. 7, pp. 2756–2767, 2013.

[93] V. H. Guaiquil, Z. Pan, N. Karagianni, S. Fukuoka, G. Alegre, and M. I. Rosenblatt, "VEGF-B selectively regenerates injured peripheral neurons and restores sensory and trophic functions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 48, pp. 17272–17277, 2014.

[94] G. Di, X. Zhao, X. Qi et al., "VEGF-B promotes recovery of corneal innervations and trophic functions in diabetic mice," *Scientific Reports*, vol. 7, article 40582, 2017.

[95] J. He, M. S. Cortina, A. Kakazu, and H. E. P. Bazan, "The PEDF neuroprotective domain plus DHA induces corneal nerve regeneration after experimental surgery," *Investigative Ophthalmology & Visual Science*, vol. 56, no. 6, pp. 3505–3513, 2015.

[96] M. S. Cortina, J. He, T. Russ, N. G. Bazan, and H. E. P. Bazan, "Neuroprotectin D1 restores corneal nerve integrity and function after damage from experimental surgery," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 6, pp. 4109–4116, 2013.

[97] Q. Zhou, P. Chen, G. Di et al., "Ciliary neurotrophic factor promotes the activation of corneal epithelial stem/progenitor cells and accelerates corneal epithelial wound healing," *Stem Cells*, vol. 33, no. 5, pp. 1566–1576, 2015.

[98] A. Meduri, P. Aragona, P. L. Grenga, and A. M. Roszkowska, "Effect of basic fibroblast growth factor on corneal epithelial healing after photorefractive keratectomy," *Journal of Refractive Surgery*, vol. 28, no. 3, pp. 220–223, 2012.

[99] K. Gumus, M. G. Guerra, S. H. de Melo Marques, S. Karaküçük, and D. Barritault, "A new matrix therapy agent for faster corneal healing and less ocular discomfort following epi-off accelerated corneal cross-linking in progressive keratoconus," *Journal of Refractive Surgery*, vol. 33, no. 3, pp. 163–170, 2017.

[100] G. D. Kymionis, D. A. Liakopoulos, M. A. Grentzelos et al., "Effect of the regenerative agent poly(carboxymethylglucose sulfate) on corneal wound healing after corneal cross-linking for keratoconus," *Cornea*, vol. 34, no. 8, pp. 928–931, 2015.

[101] M. A. Chappelet, D. Bernheim, C. Chiquet, and F. Aptel, "Effect of a new matrix therapy agent in persistent epithelial defects after bacterial keratitis treated with topical fortified antibiotics," *Cornea*, vol. 36, no. 9, pp. 1061–1068, 2017.

[102] I. Alcalde, A. Inigo-Portugues, N. Carreño, A. C. Riestra, and J. M. Merayo-Lloves, "Effects of new biomimetic regenerating agents on corneal wound healing in an experimental model of post-surgical corneal ulcers," *Archivos de la Sociedad Española de Oftalmología*, vol. 90, no. 10, pp. 467–474, 2015.

[103] A. M. Bata, K. J. Witkowska, P. A. Wozniak et al., "Effect of a matrix therapy agent on corneal epithelial healing after standard collagen cross-linking in patients with keratoconus: a randomized clinical trial," *JAMA Ophthalmology*, vol. 134, no. 10, pp. 1169–1176, 2016.

[104] F. Brignole-Baudouin, J. M. Warnet, D. Barritault, and C. Baudouin, "RGTA-based matrix therapy in severe experimental corneal lesions: safety and efficacy studies," *Journal Français d'Ophtalmologie*, vol. 36, no. 9, pp. 740–747, 2013.

[105] C. K. Chebbi, K. Kichenin, N. Amar et al., "Pilot study of a new matrix therapy agent (RGTA OTR4120) in treatment-resistant corneal ulcers and corneal dystrophy," *Journal Français d'Ophtalmologie*, vol. 31, no. 5, pp. 465–471, 2008.

[106] M. Guerra, S. Marques, J. Q. Gil et al., "Neurotrophic keratopathy: therapeutic approach using a novel matrix regenerating agent," *Journal of Ocular Pharmacology and Therapeutics*, vol. 33, no. 9, pp. 662–669, 2017.

[107] M. Xeroudaki, B. Peebo, J. Germundsson, P. Fagerholm, and N. Lagali, "RGTA in corneal wound healing after transepithelial laser ablation in a rabbit model: a randomized, blinded, placebo-controlled study," *Acta Ophthalmologica*, vol. 94, no. 7, pp. 685–691, 2016.

[108] R. P. J. Arvola, A. Robciuc, and J. M. Holopainen, "Matrix regeneration therapy: a case series of corneal neurotrophic ulcers," *Cornea*, vol. 35, no. 4, pp. 451–455, 2016.

[109] J. Cejkova, C. Olmiere, C. Cejka, P. Trosan, and V. Holan, "The healing of alkali-injured cornea is stimulated by a novel matrix regenerating agent (RGTA, CACICOL20): a biopolymer mimicking heparan sulfates reducing proteolytic, oxidative and nitrosative damage," *Histology and Histopathology*, vol. 29, no. 4, pp. 457–478, 2014.

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 3127126, 7 pages
https://doi.org/10.1155/2018/3127126

Research Article

Outcomes of Descemet Membrane Endothelial Keratoplasty for Vitrectomized Eyes with Sutured Posterior Chamber Intraocular Lens

Norihiro Yamada,[1] Takahiko Hayashi,[1,2,3,4,5] Kentaro Yuda,[2,3,4] Toshiki Shimizu,[2,3] Itaru Oyakawa,[6] Hidenori Takahashi,[5] and Naoko Kato[1]

[1]*Department of Ophthalmology, Saitama Medical University Hospital, Saitama, Japan*
[2]*Department of Ophthalmology, Yokohama City University Hospital, Yokohama, Japan*
[3]*Department of Ophthalmology, Yokohama Minami Kyosai Hospital, Yokohama, Japan*
[4]*Kikuna Yuda Eye Clinic, Yokohama, Japan*
[5]*Department of Ophthalmology, Jichi Medical University, Tochigi, Japan*
[6]*Department of Ophthalmology, Heart Life Hospital, Okinawa, Japan*

Correspondence should be addressed to Takahiko Hayashi; takamed@gmail.com

Received 31 December 2017; Accepted 22 March 2018; Published 11 April 2018

Academic Editor: Karim Mohamed-Noriega

Purpose. To evaluate the clinical outcomes of Descemet membrane endothelial keratoplasty (DMEK) for vitrectomized eyes that underwent pars plana vitrectomy (PPV) and transscleral-sutured intraocular lens (IOL) implantation. *Methods*. In this retrospective study, DMEK cases were reviewed from medical records and divided into two groups: the eyes after PPV and transscleral-sutured IOL implantation (vitrectomized group) and the eyes with in-the-bag IOL implantation (control group) prior to DMEK. The main outcome measures included time of graft unfolding during surgery and best spectacle-corrected visual acuity (BSCVA), central corneal thickness (CCT), and endothelial cell density (ECD) at 1, 3, and 6 months after the DMEK. *Results*. Twenty-three eyes (vitrectomized group, $n = 8$; control group, $n = 15$) in 23 patients were included in this study. The unfolding time was significantly longer in the vitrectomized group than in the control group ($P < 0.001$). Postoperative BSCVA was worse in the vitrectomized group (0.16 ± 0.15) than in the control group (-0.06 ± 0.06; $P = 0.017$). The improvement in BSCVA was negatively correlated with the patients' age and frequency of previous surgeries. *Conclusions*. Despite the longer graft unfolding time and limited visual recovery, DMEK should be applicable to vitrectomized eyes with transscleral-sutured IOL implantation.

1. Introduction

Descemet membrane endothelial keratoplasty (DMEK) is a new method of corneal endothelial keratoplasty introduced by Melles et al. [1], which allows rapid recovery of visual acuity and minimizes immunological rejection [1–5]. The surgical steps in DMEK include careful graft preparation, safe graft insertion into the anterior chamber, recognition of graft orientation, smooth graft unfolding, and successful graft attachment in the anterior chamber by air or gas tamponade [6–13].

The eyes that are most suitable for DMEK are thought to be pseudophakic bicameral eyes with normal anterior chamber depth. When DMEK is performed on an eye containing a sutured intraocular lens (IOL), the IOL should be properly centered within the lens capsule, providing an intact iris-IOL diaphragm. Although the indications of DMEK have been widely expanded to many endothelial disorders, the eyes with iris abnormalities or sutured IOL are thought to be unsuitable for DMEK [14–18], because the graft might be lost through the peripheral iris defect or the interspace between the iris and fixed IOL [18].

Vitrectomized eyes are further challenging because the absence of vitreous pressure during surgery requires unfolding and attaching the graft using air, which is difficult. Previous reports indicated that higher rates of postoperative graft dislocation were observed following DMEK for vitrectomized eyes and required rebubbling, resulting in a higher incidence of primary graft failure [15, 18]. However, some eyes develop bullous keratopathy that requires suturing of IOLs as well as pars plana vitrectomy (PPV) prior to endothelial keratoplasty.

The purpose of the present study was to determine the clinical outcomes, postoperative complications, and features of DMEK in the eyes that underwent previous PPV and transscleral-sutured IOLs.

2. Material and Methods

2.1. Participants. This was a retrospective study, and the study protocol was approved by the Institutional Review Board of Yokohama Minami Kyosai Hospital (approval number 29_03_05) and Saitama Medical University (approval number 17-032). The research followed the tenets of the Declaration of Helsinki. Patients with bullous keratopathy who underwent DMEK at the Yokohama Minami Kyosai Hospital and Saitama Medical University Hospital from January 2016 to December 2016 and who were followed up for more than 6 months were retrospectively analyzed. In this analysis, the eyes were classified into two groups based on the status of the IOL. The "vitrectomized" group consisted of patients with eyes that underwent PPV and transscleral-sutured IOL implantation before DMEK. The control group consisted of patients who had routine IOL implantation before DMEK. The eyes that underwent previous trabeculectomy or penetrating keratoplasty or had a history of birth injury or endotheliitis were excluded. Patients who did not agree with this study or could not be followed up were not included.

2.2. Surgical Procedure

2.2.1. Donor Preparation. BBG 250® (BBG; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in balanced saline solution (BSS® or BSS-plus®; Alcon, Osaka, Japan) to 0.1% (*w*/*v*). All grafts were peeled as described previously. BBG (0.1%, *w*/*v*) was used to stain the graft edges during peeling. A punch was gently placed on the endothelial surface to indent a circle 7.75, 8.0, or 8.25 mm in diameter. Subsequently, 1.0 and 1.5 mm- diameter dermatological biopsy punches (Kai Industries, Seki, Japan) were used to place asymmetric marks on the edges of the identified circles [19]. Donor grafts thus marked were stained with 0.1% (*w*/*v*) BBG (1.0 mg/mL) for 1 min and stored in BSS prior to insertion, 30 min later [20]. Because unfolding time depends on donor age, we usually selected donors over 60 years old.

2.2.2. Surgical Techniques. All surgeries were performed under local anesthesia. After establishing retrobulbar anesthesia and Nadbath facial nerve block, two paracenteses and a 2.8 mm upper corneal or corneoscleral incision were made for the recipient cornea. Peripheral iridotomy was performed at the 6 o'clock position to prevent a postoperative pupillary block. The donor membrane graft, stained with 0.1% (*w*/*v*) BBG (1.0 mg/mL), was placed into an IOL injector (model WJ-60M; Santen Pharmaceuticals, Osaka, Japan) and inserted into the anterior chamber.

The inserted graft was unfolded using a noncontact technique by shallowing the anterior chamber [13]. In the vitrectomized group, if shallowing the anterior chamber was difficult, a small amount of air was also injected between the host cornea and donor graft, and the rolled up donor graft was subsequently unfolded. After the graft was unfolded, additional air was added slowly underneath the graft from the center, and the rolled graft was gradually attached to the host cornea. The folded edges of the graft were additionally stretched using "bubble-bumping maneuver" [13]. In cases of severe bullous keratopathy, we used the chandelier illumination technique during DMEK surgery via the pars plana approach [21]. After the correct orientation was confirmed, the anterior chamber was filled with air to adhere the graft to the host cornea. Fifteen minutes later, the air was partially replaced with BSS. Finally, 0.4 mg of betamethasone (Rinderon®; Shionogi, Osaka, Japan) was subconjunctivally administered in 1.5% (*w*/*v*) levofloxacin eye drops (Cravit®; Santen Pharmaceuticals).

Postoperative medications included 1.5% (*w*/*v*) levofloxacin (Cravit) and 0.1% (*w*/*v*) betamethasone sodium phosphate (Sanbetasone®; Santen Pharmaceuticals) commencing at four times daily for 3 months and tapering thereafter.

2.2.3. Postoperative Follow-Up and Examinations. In addition to the standard ophthalmic examination, the best spectacle-corrected visual acuity (BSCVA), corneal endothelial cell density (ECD), central corneal thickness (CCT), and graft adaptation were evaluated both preoperatively and for up to 6 months postoperatively in all eyes. BSCVA was measured as decimal visual acuity and was converted to a logarithm of the minimum angle of resolution (LogMAR) values. Graft adaptation was assessed with both slit-lamp microscopy and an anterior segment OCT (SS1000; Tomey, Nagoya, Japan). Corneal thickness was measured by corneal tomography (SS1000; Tomey). When progressive graft detachment occurred near the central area, a rebubbling procedure was performed as described previously [22]. Preoperative ECD values were retrieved from donor eye bank records, and postoperative ECD values were measured with the aid of a specular microscope (FA3509; Konan Medical, Nishinomiya, Japan). Spectral-domain optical coherence tomography (RS 3000; Nidek, Japan) was performed 1, 3, and 6 months after DMEK. When CME was diagnosed postoperatively, topical bromfenac (Bronuck®, Senju, Pharmaceutical Co., Osaka, Japan) and sub-Tenon injection of triamcinolone acetonide (MaQaid®; Wakamoto Pharmaceutical Co., Tokyo, Japan) were immediately applied.

2.2.4. Graft Unfolding Time. The graft unfolding time was evaluated using surgical videos and compared between the two groups. The time from the first tap used to unfold the tissue to the start of air injection underneath the graft was measured and defined as the unfolding time.

Table 1: Patient characteristics before surgery.

	Vitrectomized group	Control group	P^*
Number of eyes	8	15	
Sex (male/female)	5/3	3/12	0.051*
Age	72.8 ± 10.5	74.1 ± 5.1	0.906†
Eye (R/L)	3/5	7/8	0.632*
BSCVA (LogMAR)	1.15 ± 0.60	0.98 ± 0.52	0.795†
CCT (μm)	765 ± 63	722 ± 88	0.194†
Frequency of previous surgeries	3.11 ± 0.78	1	**<0.001**

BSCVA: best spectacle-corrected visual acuity; CCT: central corneal thickness; L: left; LogMAR: logarithm of the minimal angle of resolution; R: right. *χ^2 test (comparison between two groups); †unpaired *t*-test.

2.2.5. Statistical Analysis. The Wilcoxon test or paired *t*-test was used to compare values preoperatively and postoperatively, as appropriate. The Mann–Whitney *U* test or unpaired t-test was used to compare values between the two groups, as appropriate. Due to the distribution of unfolding times, they were compared using the two-sided Student's *t*-test after logarithmic transformation. The male/female and right/left ratios were compared using the chi-square test. To explore related factors, multiple regression analysis with stepwise variable selection (minimum Bayesian information criterion, increasing number of variables) was performed. All analyses were performed using JMP Pro Software version 11.2.0 (SAS Institute, Cary, NC, USA). A P value < 0.05 was considered statistically significant.

3. Results

3.1. Patients. Twenty-three eyes in 23 patients (8 men and 15 women) were considered eligible for the study. Eight eyes came from the vitrectomized group, and the other fifteen eyes came from the control group. Ages ranged from 52 to 82 years (mean, 73.8 years). Preoperative patient profiles of the two groups are summarized in Table 1.

In the present study, the frequency of previous surgeries prior to DMEK was 3.11 ± 0.78 in the vitrectomized group. In contrast, only phacoemulsification and simultaneous IOL implantation were performed in the control group. The comparison between the two groups was statistically significant ($P < 0.001$).

3.1.1. Vitrectomized Group. Eight eyes had previously undergone PPV and transscleral-sutured IOL implantation (vitrectomized group). Five of the eight eyes showed an aphakic state derived from complicated cataract surgeries, one eye underwent phacoemulsification and aspiration for pseudoexfoliation syndrome, and two eyes underwent intracapsular cataract extraction. These latter three eyes revealed pseudoexfoliation corneal endotheliopathy. One of the three eyes underwent aspiration for congenital cataract during childhood, after which the patient used a hard contact lens for a long time. Another eye was implanted with an IOL that was subluxated, which required the extraction of the lens and the secondary implantation of a new lens.

In three eyes, bullous keratopathy was caused by vitreoretinal surgeries; two had undergone pars plana vitrectomy for rhegmatogenous retinal detachment with silicone oil injection, while one had pars plana vitrectomy with silicone oil injection for endophthalmitis after cataract surgery. Detailed patient profiles of the vitrectomized group are summarized in Table 2.

3.1.2. Control Group. Fifteen eyes underwent routine cataract surgery with in-the-bag IOL implantation prior to DMEK (control group). Six eyes had Fuchs' corneal endothelial dystrophy, nine had iatrogenic bullous keratopathy, six underwent argon laser iridotomy, and three were subjected to phacoemulsification and IOL implantation.

3.2. Visual Acuity. In the vitrectomized group, BSCVA improved from 1.15 ± 0.60 preoperatively to 0.37 ± 0.19 at 1 month, 0.28 ± 0.15 at 3 months, and 0.16 ± 0.15 at 6 months. In the control group, BSCVA improved from 0.98 ± 0.52 preoperatively to 0.20 ± 0.23 at 1 month, 0.07 ± 0.12 at 3 months, and −0.06 ± 0.06 at 6 months. A statistically significant improvement in BSCVA was obtained in the vitrectomized group at all observation points ($P = 0.011$ at 1 month, 0.005 at 3 months, and 0.003 at 6 months). A statistically significant improvement of BSCVA was also obtained in the control group at all observation points ($P = 0.002$ at 1 month, 0.001 at 3 months, and 0.001 at 6 months). The BSCVA in the control group was significantly better than that in the vitrectomized group at all the examination points ($P = 0.795$ preoperatively, 0.032 at 1 month, 0.007 at 3 months, and 0.017 at 6 months; Figure 1).

3.3. Central Corneal Thickness. In the vitrectomized group, the CCT decreased from 764.5 ± 62.7 μm preoperatively to 529.5 ± 56.6 μm at 1 month, 520.6 ± 51.6 at 3 months, and 513.3 ± 43.3 at 6 months. In the control group, the CCT decreased from 722.0 ± 88.8 μm preoperatively to 555.9 ± 64.8 μm at 1 month, 507.3 ± 53.7 at 3 months, and 513.8 ± 52.9 at 6 months. A statistically significant improvement in CCT was observed in each group at all examination points ($P < 0.001$, Wilcoxon rank sum test in both groups). There was no significant difference in CCT between the two groups at any examination point. The P values were 0.194 preoperatively, 0.136 at 1 month, 1.0 at 3 months, and 0.810 at 6 months.

3.4. Corneal Endothelial Cell Density. In the vitrectomized group, the donor corneal ECD decreased from 2629 ± 303 cells/mm^2 preoperatively to 1728 ± 429 cells/mm^2 at 1 month, 1620 ± 414 cells/mm^2 at 3 months, and 1548 ± 401 cells/mm^2 at 6 months postoperatively (40.7 ± 11.2% less than the preoperative value of the donor graft). In the control group, the donor corneal ECD decreased from 2707 ± 238 cells/mm^2 preoperatively to 2021 ± 466 cells/mm^2 at 1 month, 1837 ± 440 cells/mm^2 at 3 months, and 1679 ± 419 cells/mm^2 at 6 months postoperatively (38.2 ± 18.6% less than the preoperative value of the donor graft). There was no significant difference in ECD between the two groups at

Table 2: Profiles of the enrolled patients (vitrectomized group).

Case	Sex	Age	OD/OS	Etiology for PPV	Previous surgeries	Preop BSCVA	Preop CCT (μm)	Treatment before DMEK
1	F	79	OD	PEX	PEA, PPV + IOLs	20/2000	793	
2	M	79	OS	PEX	ICCE, PPV + IOLs	20/1000	724	
3	F	74	OS	PEX	ICCE + PPV + IOLs, DSAEK	20/200	836	ASR
4	F	52	OS	Extended CL wearing	Cataract aspiration, PPV + IOLs	20/50	672	
5	F	64	OS	Dropped IOL	PPV + IOLs	20/2000	734	ASR
6	F	56	OS	RRD	PPV + SOi, SOr + IOLs	20/50	757	
7	M	79	OD	RRD	PPV + SOi, SOr + IOLs	20/500	939	ASR
8	M	74	OD	Endophthalmitis	PPV + IOLr + SOi, SOr + IOLs	20/100	658	ASR

ASR: anterior segment reconstruction; BSCVA: best spectacle-corrected visual acuity; CCT: central corneal thickness; DMEK: Descemet membrane endothelial keratoplasty; ICCE: intracapsular cataract extraction; IOLr: removal of intraocular lens; IOLs: transscleral-sutured posterior chamber intraocular lens implantation; OD: right eye; OS: left eye; PEA: phacoemulsification and aspiration; PEX: pseudoexfoliation syndrome; PPV: pars plana vitrectomy; Preop: preoperative; RRD: rhegmatogenous retinal detachment; SOi: silicone oil injection; SOr: silicon oil extraction.

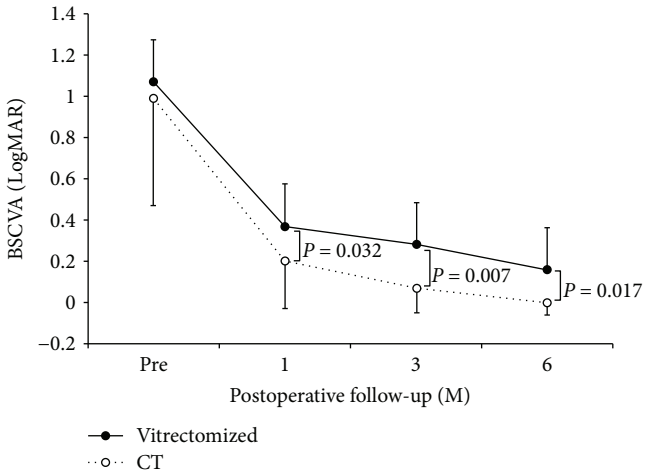


Figure 1: Changes in best spectacle-corrected visual acuity (BSCVA). Statistically significant improvement in BSCVA was seen in the vitrectomized group ($P = 0.011$ at 1 month, 0.005 at 3 months, and 0.003 at 6 months; Wilcoxon rank sum test). In the control group, a statistically significant improvement of BSCVA was seen at all observation points ($P = 0.002$ at 1 month, 0.001 at 3 months, and 0.001 at 6 months; Wilcoxon rank sum test). There was also a statistically significant difference in BSCVA between the two groups at all postoperative examinations ($P = 0.795$ preoperatively, 0.032 at 1 month, 0.007 at 3 months, and 0.017 at 6 months; Mann–Whitney U test). Vitrectomized: vitrectomized group; CI: confidence interval; CT: control group.

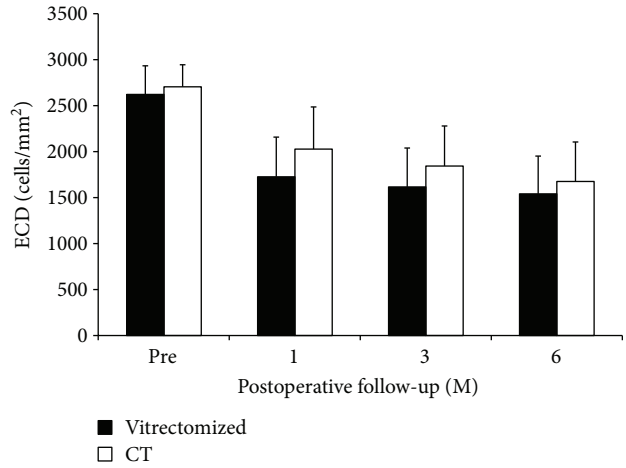


Figure 2: Changes in endothelial cell density (ECD). In the vitrectomized group, the donor corneal ECD decreased from 2629 ± 303 cells/mm^2 preoperatively to 1548 ± 401 cells/mm^2 at 6 months postoperatively ($40.7 \pm 11.2\%$ less than the preoperative value of the donor graft). In the control group, the donor corneal ECD decreased from 2707 ± 238 cells/mm^2 at preoperative point to 1679 ± 419 cells/mm^2 at 6 months postoperatively ($38.2 \pm 18.6\%$ less than the preoperative value of the donor graft). There was no significant difference in ECD between the two groups at any pre- and postoperative points (P value = 0.832 preoperatively, 0.136 at 1 month, 0.259 at 3 months, and 0.526 at 6 months; Mann–Whitney U test). Vitrectomized: vitrectomized group; CI; confidence interval; CT; control group.

any pre- and postoperative points (Figure 2). The P values were 0.832 preoperatively, 0.136 at 1 month, 0.259 at 3 months, and 0.526 at 6 months.

3.5. Graft Unfolding Time. The geometric mean of the graft unfolding time was 19.0 min in the vitrectomized group (95% confidence interval (CI), 13.4–24.7) and 7.1 min in the control group (95% CI, 3.2–10.9). The graft unfolding time was significantly longer in the vitrectomized group than the control group ($P < 0.001$; Figure 3).

3.6. Complications after DMEK. None of the eyes showed intraoperative complications. Rebubbling for partial detachment was required in two eyes (25.0%) of the vitrectomized group and in four eyes (26.7%) of the control group; no significant difference between the two groups was observed ($P = 0.554$). CME was present in four eyes (50.0%) in the vitrectomized group and two eyes (13.3%) in the control group ($P = 0.081$). In all affected eyes, the CME resolved with topical bromfenac and sub-Tenon injection of

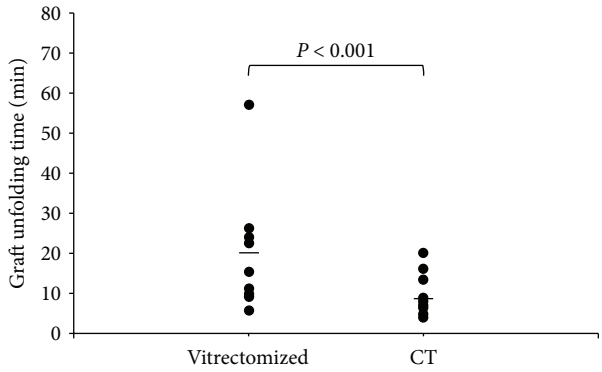


Figure 3: Comparison of graft unfolding time. Graft unfolding time was significantly longer in the vitrectomized group than in the control group (*$P < 0.001$). The geometric mean graft unfolding time (indicated with bars) was 19.0 min in the vitrectomized group and 7.1 min in the control group. Vitrectomized: vitrectomized group; CI; confidence interval; CT; control group.

triamcinolone acetonide. The mean BSCVA at 6 months after DMEK was 0.11 ± 0.12 in the eyes without CME and 0.037 ± 0.14 in the eyes with CME ($P = 0.30$).

3.7. BSCVA Prognosis-Related Factors. For BSCVA prognosis, we performed multiple regression analysis after stepwise variable selection. We used BSCVA at 6 months after DMEK as the response variable, and age, anterior chamber depth before DMEK, axial length, frequency of previous surgeries, graft unfolding time, and baseline BSCVA were examined as explanatory variables. The results are summarized in Table 3. After stepwise selection, age (estimated value = 0.006, $P = 0.007$) and frequency of previous surgeries (estimated value = 0.015, $P < 0.001$) were obtained as significant factors.

4. Discussion

The present investigation indicated that DMEK could be successfully performed in vitrectomized eyes with transscleral-sutured IOL, even though the graft unfolding times were significantly longer in the vitrectomized eyes. BSCVA was significantly improved in both groups, and postoperative CCT, ECD, and complication rates were comparable between the two groups.

In vitrectomized eyes, unfolding the graft can be challenging because the anterior chamber becomes shallow. Indeed, the geometric mean of the graft unfolding time was 19.0 min in the vitrectomized group, significantly longer than that of the control group (7.1 min; $P < 0.001$). This result is consistent with previous findings [23]. Yoeruek et al. reported 55% rebubbling rates after DMEK for postvitreous surgery, while 10% showed iatrogenic primary graft failure in the immediate postoperative period [15]. Weller et al. reported that graft detachments were observed in 45.8% of cases [14]. These previous findings indicate that longer manipulation to unfold the graft may cause more endothelial damage to the transplanted grafts. Fortunately, there was no primary graft failure, and the rebubbling rates were 22.2% in the vitrectomized group and 26.7% in the controls, with no group difference. Similarly, the ratio of ECD decrease at 6 months after DMEK was comparable between the two groups, with $40.7 \pm 11.2\%$ in the vitrectomized group and $38.2 \pm 18.6\%$ in the control group. These results indicate that careful preparation and manipulation during surgery might have contributed to successful DMEK even in the vitrectomized eyes.

Table 3: Multiple regression analysis for correlates of postoperative best spectacle-corrected visual acuity (BSCVA).

Predictor	Estimated value	SE	P value
Age	0.00061	0.0021	**0.009**
AXL	Unselected		0.95
ACD	Unselected		0.26
Frequency of previous surgeries	0.11	0.016	**<0.001**
Unfolding time	Unselected		0.95
CME	Unselected		0.45
Preoperative BSCVA	Unselected		0.68

SE: standard error; AXL: axial length; ACD: anterior chamber depth; CME: cystoid macular edema. Multivariate analysis was constructed after stepwise variable selection (BIC, forward method).

The results suggest some important points for performing DMEK on the eyes that have undergone transscleral-sutured IOL implantation combined with PPV. First, an intact iris-IOL diaphragm is necessary. In such eyes, anterior segment reconstruction by suturing the iris could be necessary before DMEK. In the present investigation, we performed anterior segment reconstruction in four of the eight eyes in the vitrectomized group. Second, the position of the transscleral-sutured IOL is important. If the intraocular lens was sutured far posterior from the iris, the graft could be lost into the interspace between the iris and IOL or fall into the vitreous cavity [24]. Careful preoperative selection of the eyes and appropriate anterior segment reconstruction are thus necessary for successful DMEK.

Although the postoperative BSCVA significantly improved in both the vitrectomized and the control groups, it was also significantly lower in the vitrectomized group than in the control group. To clarify the underlying cause for this difference, we performed multiple regression analysis after stepwise variable selection for BSCVA prognosis, and the results showed that the patients' age and the frequency of the previous surgeries were highly related to the postoperative BSCVA. Similar findings that the visual acuity of younger patients tended to improve have also been reported after other ocular surgeries, such as cataract surgery and vitrectomy for macular hole [25–27]. Several factors, including unrecognized or subclinical comorbidity, age-related changes in macular function, and the tendency to perceive functional impairment irrespective of vision in elderly people, could have contributed to these differences [28].

Another important factor affecting postoperative BSCVA was the frequency of previous surgeries. Postoperative BSCVA has been reported to be worse for previous repeated intraocular surgeries including penetrating keratoplasty,

Boston keratoplasty, and PPV [29–31]. Repeated surgeries may cause persistent inflammation, elevated intraocular pressure, and/or insufficient ocular circulation, resulting in deteriorated retinal function. Moreover, precisely centering the IOL can be difficult for the eyes with transscleral-sutured IOL, also contributing to BSCVA impairment. However, this aspect was not evaluated in the present study. The worse BSCVA in the vitrectomized group compared with the control group may thus be caused by various factors.

Flanary et al. reported that the incidence of CME was 8.0% (7 among 88 eyes) after staged DMEK that was performed within 6 months after cataract surgery and 7.1% (6 among 85 eyes) in solitary DMEK performed more than 6 months after cataract surgery [32]. According to a cohort study by Heinzelmann et al., 13% of the eyes developed a single episode of CME during the follow-up time after DMEK [33]. In the current study, the incidence of development of CME was similar to that of previous reports in the control group (13.3%), but much higher in the vitrectomized group (50.0%), although there was no significant difference between the two groups. Furthermore, CME occurrence was not significantly correlated with postoperative BSCVA (0.110 versus 0.037, resp.; $P = 0.300$). We speculate that this is probably because we applied topical bromfenac and sub-Tenon injection of triamcinolone acetonide immediately upon the detection of CME postoperatively. However, further studies are still needed to ascertain the influence of CME on the final visual outcome after DMEK.

Our findings indicate that we could obtain comparable outcomes with respect to the mean ECD and complication rates using DMEK for typical pseudophakic nonvitrectomized eyes. Moreover, DMEK could produce excellent visual outcomes and low rejection rates. Even in complex cases such as vitrectomized eyes, we observed impressive visual recovery after DMEK. In fact, the BSCVA in one case improved from 20/200 to 20/50, despite the fact that the patient had been limited to 20/100 after previous DSAEK. Caution should be exercised in the selection of the candidates, including presurgical preparation such as creating an intact iris-IOL diaphragm. Careful postoperative evaluation of the occurrence of CME and its immediate treatment may also contribute to improvement of the surgical outcomes.

5. Conclusion

In conclusion, DMEK can improve visual function in the eyes that underwent previous PPV and transscleral-sutured IOL implantation.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

Norihiro Yamada and Takahiko Hayashi contributed equally to this work.

References

[1] G. R. J. Melles, T. S. Ong, B. Ververs, and J. van der Wees, "Descemet membrane endothelial keratoplasty (DMEK)," *Cornea*, vol. 25, no. 8, pp. 987–990, 2006.

[2] T. Tourtas, K. Laaser, B. O. Bachmann, C. Cursiefen, and F. E. Kruse, "Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty," *American Journal of Ophthalmology*, vol. 153, no. 6, pp. 1082–1090.e2, 2012.

[3] A. Anshu, M. O. Price, and F. W. Price Jr., "Risk of corneal transplant rejection significantly reduced with Descemet's membrane endothelial keratoplasty," *Ophthalmology*, vol. 119, no. 3, pp. 536–540, 2012.

[4] T. Hayashi, S. Yamagami, K. Tanaka et al., "Immunologic mechanisms of corneal allografts reconstituted from cultured allogeneic endothelial cells in an immune-privileged site," *Investigative Ophthalmology & Visual Science*, vol. 50, no. 7, pp. 3151–3158, 2009.

[5] M. Ang, M. R. Wilkins, J. S. Mehta, and D. Tan, "Descemet membrane endothelial keratoplasty," *British Journal of Ophthalmology*, vol. 100, no. 1, pp. 15–21, 2016.

[6] I. Dapena, L. Ham, K. Droutsas, K. van Dijk, K. Moutsouris, and G. R. J. Melles, "Learning curve in Descemet's membrane endothelial keratoplasty: first series of 135 consecutive cases," *Ophthalmology*, vol. 118, no. 11, pp. 2147–2154, 2011.

[7] E. C. Hamzaoglu, M. D. Straiko, Z. M. Mayko, C. S. Sales, and M. A. Terry, "The first 100 eyes of standardized Descemet stripping automated endothelial keratoplasty versus standardized Descemet membrane endothelial keratoplasty," *Ophthalmology*, vol. 122, no. 11, pp. 2193–2199, 2015.

[8] J. R. Nussbaumer, S. Alloju, and W. Chamberlain, "Clinical outcomes of Descemet membrane endothelial keratoplasty during the surgeon learning curve versus Descemet stripping endothelial keratoplasty performed at the same time," *Journal of Clinical & Experimental Ophthalmology*, vol. 7, no. 5, 2016.

[9] C. Monnereau, R. Quilendrino, I. Dapena et al., "Multicenter study of Descemet membrane endothelial keratoplasty: first case series of 18 surgeons," *JAMA Ophthalmology*, vol. 132, no. 10, pp. 1192–1198, 2014.

[10] F. Arnalich-Montiel, A. Pérez-Sarriegui, and A. Casado, "Impact of introducing 2 simple technique modifications on the Descemet membrane endothelial keratoplasty learning curve," *European Journal of Ophthalmology*, vol. 27, no. 1, pp. 16–20, 2016.

[11] M. Ang, J. S. Mehta, S. D. Newman, S. B. Han, J. Chai, and D. Tan, "Descemet membrane endothelial keratoplasty: preliminary results of a donor insertion pull-through technique using a donor mat device," *American Journal of Ophthalmology*, vol. 171, pp. 27–34, 2016.

[12] T. Hayashi, I. Oyakawa, and N. Kato, "Techniques for learning Descemet membrane endothelial keratoplasty for eyes of Asian patients with shallow anterior chamber," *Cornea*, vol. 36, no. 3, pp. 390–393, 2017.

[13] I. Dapena, K. Moutsouris, K. Droutsas, L. Ham, K. van Dijk, and G. R. Melles, "Standardized "no-touch" technique for Descemet membrane endothelial keratoplasty," *Archives of Ophthalmology*, vol. 129, no. 1, pp. 88–94, 2011.

[14] J. M. Weller, T. Tourtas, and F. E. Kruse, "Feasibility and outcome of Descemet membrane endothelial keratoplasty in complex anterior segment and vitreous disease," *Cornea*, vol. 34, no. 11, pp. 1351–1357, 2015.

[15] E. Yoeruek, G. Rubino, T. Bayyoud, and K. U. Bartz-Schmidt, "Descemet membrane endothelial keratoplasty in vitrectomized eyes: clinical results," *Cornea*, vol. 34, no. 1, pp. 1–5, 2015.

[16] N. Sorkin, A. Einan-Lifshitz, Z. Ashkenazy et al., "Enhancing Descemet membrane endothelial keratoplasty in postvitrectomy eyes with the use of pars plana infusion," *Cornea*, vol. 36, no. 3, pp. 280–283, 2017.

[17] H. Yazu, T. Yamaguchi, M. Dogru, N. Ishii, Y. Satake, and J. Shimazaki, "Descemet-stripping automated endothelial keratoplasty in eyes with transscleral-sutured intraocular lenses," *Journal of Cataract & Refractive Surgery*, vol. 42, no. 6, pp. 846–854, 2016.

[18] V. S. Liarakos, L. Ham, I. Dapena et al., "Endothelial keratoplasty for bullous keratopathy in eyes with an anterior chamber intraocular lens," *Journal of Cataract & Refractive Surgery*, vol. 39, no. 12, pp. 1835–1845, 2013.

[19] A. Matsuzawa, T. Hayashi, I. Oyakawa et al., "Use of four asymmetric marks to orient the donor graft during Descemet's membrane endothelial keratoplasty," *BMJ Open Ophthalmology*, vol. 1, no. 1, article e000080, 2017.

[20] T. Hayashi, K. Yuda, I. Oyakawa, and N. Kato, "Use of brilliant blue G in Descemet's membrane endothelial keratoplasty," *BioMed Research International*, vol. 2017, Article ID 9720389, 5 pages, 2017.

[21] T. Shimizu, T. Hayashi, K. Yuda et al., "Chandelier illumination for Descemet membrane endothelial keratoplasty," *Cornea*, vol. 36, no. 9, pp. 1155–1157, 2017.

[22] M. Dirisamer, K. van Dijk, I. Dapena et al., "Prevention and management of graft detachment in Descemet membrane endothelial keratoplasty," *Archives of Ophthalmology*, vol. 130, no. 3, pp. 280–291, 2012.

[23] E. Yoeruek, T. Bayyoud, J. Hofmann, and K.-U. Bartz-Schmidt, "Novel maneuver facilitating Descemet membrane unfolding in the anterior chamber," *Cornea*, vol. 32, no. 3, pp. 370–373, 2013.

[24] N. A. Afshari, M. S. Gorovoy, S. H. Yoo et al., "Dislocation of the donor graft to the posterior segment in Descemet stripping automated endothelial keratoplasty," *American Journal of Ophthalmology*, vol. 153, no. 4, pp. 638–642.e2, 2012.

[25] C. M. Mangione, R. S. Phillips, M. G. Lawrence, J. M. Seddon, E. J. Orav, and L. Goldman, "Improved visual function and attenuation of declines in health-related quality of life after cataract extraction," *Archives of Ophthalmology*, vol. 112, no. 11, pp. 1419–1425, 1994.

[26] P. Gogate, V. Vakil, R. Khandekar, M. Deshpande, and H. Limburg, "Monitoring and modernization to improve visual outcomes of cataract surgery in a community eyecare center in western India," *Journal of Cataract and Refractive Surgery*, vol. 37, no. 2, pp. 328–334, 2011.

[27] Y. Kim, E. S. Kim, S. Y. Yu, and H. W. Kwak, "Age-related clinical outcome after macular hole surgery," *Retina*, vol. 37, no. 1, pp. 80–87, 2017.

[28] T. Y. Wong, "Effect of increasing age on cataract surgery outcomes in very elderly patients," *BMJ*, vol. 322, no. 7294, pp. 1104–1106, 2001.

[29] V. Patel, M. R. Moster, L. Kishfy et al., "Sequential versus concomitant surgery of glaucoma drainage implant and Boston keratoprosthesis type 1," *European Journal of Ophthalmology*, vol. 26, no. 6, pp. 556–563, 2016.

[30] M. Vanathi, N. Sharma, R. Sinha, R. Tandon, J. S. Titiyal, and R. B. Vajpayee, "Indications and outcome of repeat penetrating keratoplasty in India," *BMC Ophthalmology*, vol. 5, no. 1, p. 26, 2005.

[31] E. Moisseiev, I. D. Fabian, J. Moisseiev, and A. Barak, "Outcomes of repeated pars plana vitrectomy for persistent macular holes," *Retina*, vol. 33, no. 6, pp. 1137–1143, 2013.

[32] W. E. Flanary, J. M. Vislisel, M. D. Wagoner et al., "Incidence of cystoid macular edema after Descemet membrane endothelial keratoplasty as a staged and solitary procedure," *Cornea*, vol. 35, no. 8, pp. 1040–1044, 2016.

[33] S. Heinzelmann, P. Maier, D. Böhringer, S. Hüther, P. Eberwein, and T. Reinhard, "Cystoid macular oedema following Descemet membrane endothelial keratoplasty," *British Journal of Ophthalmology*, vol. 99, no. 1, pp. 98–102, 2015.

Hindawi
Journal of Ophthalmology
Volume 2018, Article ID 2590536, 12 pages
https://doi.org/10.1155/2018/2590536

Research Article

Generation of Femtosecond Laser-Cut Decellularized Corneal Lenticule Using Hypotonic Trypsin-EDTA Solution for Corneal Tissue Engineering

Man-Il Huh,[1,2] Kyoung-Pil Lee,[1,2] Jeongho Kim,[2] Soojin Yi,[2] Byeong-Ung Park,[2] and Hong Kyun Kim[1,2]

[1]*Bio-Medical Institute, Kyungpook National University Hospital, Daegu, Republic of Korea*
[2]*Department of Ophthalmology, School of Medicine, Kyungpook National University, Daegu, Republic of Korea*

Correspondence should be addressed to Hong Kyun Kim; okeye@knu.ac.kr

Received 2 February 2018; Accepted 10 March 2018; Published 1 April 2018

Academic Editor: Sang Beom Han

Purpose. To establish an optimized and standardized protocol for the development of optimal scaffold for bioengineering corneal substitutes, we used femtosecond laser to process human corneal tissue into stromal lenticules and studied to find the most efficient decellularization method among various reagents with different tonicities. *Methods*. The decellularization efficacy of several agents (0.1%, 0.25%, and 0.5% of Triton X-100, SDS, and trypsin-EDTA (TE), resp.) with different tonicities was evaluated. Of all protocols, the decellularization methods, which efficiently removed nuclear materials examined as detected by immunofluorescent staining, were quantitatively tested for sample DNA and glycosaminoglycan (GAG) contents, recellularization efficacy, and biocompatibilities. *Results*. 0.5% SDS in hypertonic and isotonic buffer, 0.25% TE in hypotonic buffer, and 0.5% TE in all tonicities completely decellularized the corneal lenticules. Of the protocols, decellularization with hypotonic 0.25 and 0.5% TE showed the lowest DNA contents, while the GAG content was the highest. Furthermore, the recellularization efficacy of the hypotonic TE method was better than that of the SDS-based method. Hypotonic TE-treated decellularized corneal lenticules (DCLs) were sufficiently transparent and biocompatible. *Conclusion*. We generated an ideal protocol for DCLs using a novel method. Furthermore, it is possible to create a scaffold using a bioengineered corneal substitute.

1. Introduction

The cornea is the front and outermost part of the eyeball. It serves not only as a mechanical barrier but also as a visual gateway because of its transparency. Transparency is a unique characteristic of the corneal tissue and enables proper visual function [1], but the cornea can be damaged by numerous diseases and injuries that affect its structure. Some causes are irreversible and can be treated by corneal transplantation using human donor tissue [2]. Although corneal transplantation has been successfully performed since the first human corneal transplant in 1905, there are three major disadvantages to this procedure: immunologic graft rejection, possible graft failure, and the lack of donors. Thus, from a clinical perspective, it would be very useful to generate a corneal substitute for the human cornea [3–5].

Currently, tissue engineering methods have been developed as a promising solution for tissue replacement and regeneration. Tissue-engineered corneal equivalents are based on the principle of tissue engineering, which is seeding and proliferating cells within a scaffold. Several scaffolds for corneal equivalents using biological materials have been developed using collagen [6–8] or in combination with glycosaminoglycan (GAG) [9, 10] and fibrin agarose gel [11–13]. Although these approaches have shown some success, there are several limitations compared to the use of human tissue. Decellularized extracellular matrix scaffolds derived from tissues and organs have been successfully used in both preclinical animal studies and human clinical applications. Decellularization methods have unique advantages, such as the use of intact extracellular matrix, no immunologic response, and suitable mechanical strength [14]. Especially,

GAG can bind with growth factors and morphogens related with developmental and repair processes, such as VEGF, Wnt, TGF-βs, and IGFBP [15, 16]. The retention of the GAG after decellularization is important to regenerate organs [17]. As a possible scaffold for corneal remodeling and as an alternative tissue source for corneal replacement, decellularization of corneal tissue has attracted considerable attention [18–21]. Recently, several research groups have successfully prepared acellular corneal stroma using several detergents and enzymes [14, 21–23]. However, there are two main obstacles that must be overcome before application: one is the shortage of corneal supply and the other is the lack of a standardized decellularized protocol.

Despite increased corneal donation in western countries, the corneal supply does not meet the demand in many other countries. To create allograft rejection-free decellularized cornea, a donor cornea is required. Since 2011, small incision lenticule extraction using a femtosecond laser has become clinically available as an alternative to laser in situ keratomileusis [24]. The corneal lenticules extracted during small incision lenticule extraction can be used for preparation of acellular cornea, rather than a donor cornea.

An ideal decellularization protocol should completely remove cellular material and antigen molecules while retaining the structural and functional proteins of the extracellular matrix without disrupting the overall tissue matrix. However, most decellularization protocols are toxic and destructive to the tissue matrix. A more effective protocol for removing cell components is more destructive to the extracellular matrix composition. Complete cell component removal methods would alter the extracellular matrix composition and cause some degree of ultrastructure disruption. However, there is currently no reliable or standardized protocol for the decellularization of human corneal lenticules, which have different physiological properties with full thickness cornea. Herein, a specific optimized decellularization method for corneal lenticule must be determined to minimize these undesirable effects and achieve complete cell removal.

In this study, we decellularized corneal lenticules created by a femtosecond laser using several reagents with different conditions and compared the efficacy to optimize the protocol. After selecting the optimized method, processed corneas were evaluated with respect to their biological, physical, and ultrastructural properties.

2. Methods

2.1. Human Corneal Lenticule Preparation. Human corneal tissues were obtained from Santa Lucia International Eye Bank (Manila, Philippines). Approval from the Institutional Review Board of the Hospital Ethics Committee was obtained for the study, and the Declaration of Helsinki was followed. Corneal tissue was positioned under the VisuMax femtosecond laser system (Carl Zeiss Meditec AG, Jena, Germany) using an artificial anterior chamber, and samples were regularly cut to 8 mm wide and 100 μm thick.

2.2. Decellularization Processes. Fresh lenticules were decellularized using various concentrations of Triton X-100, sodium dodecyl sulfate (SDS), and trypsin-EDTA (TE) dissolved in hypertonic (100 mM), isotonic (50 mM), and hypotonic Tris buffer (10 mM, pH 7.2). Samples were incubated in each solution for 2 days at 37°C with continuous shaking (100 rpm), and 50 U/mL DNase I (Sigma, MO, USA) and 1 U/mL RNase A (Sigma) were added to each Tris buffer for 1 day at 37°C. Decellularized corneal lenticules (DCLs) were washed with phosphate-buffered saline (PBS) and stored in Optisol™ (Chiron Ophthalmics, Irvine, CA, USA) at 4°C until use (Table 1).

Table 1: Decellularizing process. Corneal lenticules were decellularized by various conditioned solutions. A lenticule incubated with each decellularizing solution, followed by washing with PBS. The lenticules were incubated with DNase I and RNase A to remove nucleic acid. After washing with each tonic buffer, the lenticules were stored in Optisol at 4°C until use.

Number	Process	Time	Temp.
1	Washing with PBS	1 h	37°C
2	Incubation with each decellularizing solution	48 h	37°C
3	Washing with each tonic buffer	1 h	37°C
4	Incubation with DNase I and RNase A	24 h	37°C
5	Washing with each tonic buffer	1 h	37°C
6	Store in Optisol		4°C

2.3. Comparison of Decellularization Efficacy. Decellularization efficacy was compared with histological methods. DCLs were acquired using different three reagents and three tonic buffers (Table 2). Tissues were fixed with 4% paraformaldehyde in PBS (pH 7.4) overnight. Samples were dehydrated through a graded ethanol series, cleared with xylene, and mounted in paraffin. Slides were prepared in 4 μm sections, and the sections were dewaxed, rehydrated, and labeled with Alexa Fluor 488 conjugated anti-vimentin (Abcam, Cambridge, UK) and then counterstained with 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI). Fluorescence images were acquired using an Eclipse 80i (Nikon, Tokyo, Japan). After processing, we compared the degree of staining.

2.4. Measurement of DNA and GAG Contents. Of all protocols, the decellularization methods, which efficiently removed nuclear materials examined as detected by immunofluorescent staining, were quantitatively tested for sample DNA and GAG contents. The lenticules were lyophilized in a freeze dryer (FD5512, IlShin, Gyeonggi-do, Korea) and weighed. The DCLs were digested in 0.2 M sodium phosphate buffer (pH 6.4) containing 125 μg/mL papain (Sigma), 10 mM cysteine hydrochloride (Sigma), 0.1 M sodium acetate (Junsei, Tokyo, Japan), and 2 mM EDTA (Sigma) for 3 h at 65°C as described previously [25]. The DNA content was measured by using a DNA quantitation kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol. Briefly, 5 μL solution of dissolved lenticules (10 mg/mL) was added to 1 mL TEN buffer (100 mM Tris, 2 M NaCl, 10 mM EDTA, pH 7.4) containing Hoechst 33528 (1 μg/mL). Fluorescence

Table 2: Comparison of decellularizing agents. To extract effective decellularizing conditions, the DCLs were stained with DAPI for detecting remained nucleic acid. "+" showed over 10 positively stained per view, "±" showed few positively stained with DAPI, "–" did not show any nuclear stain in all fields ($n = 10$).

Decellularizing agent	Concentration	Tonicity	DAPI
Triton X-100	0.1%	Hypo	+
		Iso	+
		Hyper	+
	0.25%	Hypo	+
		Iso	+
		Hyper	+
	0.5%	Hypo	+
		Iso	+
		Hyper	+
SDS	0.1%	Hypo	+
		Iso	+
		Hyper	+
	0.25%	Hypo	+
		Iso	+
		Hyper	+
	0.5%	Hypo	±
		Iso	–
		Hyper	–
Trypsin-EDTA	0.1%	Hypo	+
		Iso	+
		Hyper	+
	0.25%	Hypo	–
		Iso	+
		Hyper	+
	0.5%	Hypo	–
		Iso	–
		Hyper	–

intensity was read using a 460 nm emission filter with a microplate reader (FLUOstar OPTIMA, BMG LABTECH, Ortenberg, Germany). The DNA contents were calculated from a standard curve determined by using calf thymus DNA.

Sulfated GAG in the DCL was measured with a Blyscan™ glycosaminoglycan assay kit (Biocolor, County Antrim, UK). GAG quantification was performed according to the manufacturer's protocol. Absorbance was measured using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 656 nm.

2.5. Electron Microscopy (EM). The extracellular matrix composition was evaluated by electron microscopic examination. For scanning electron microscopy (SEM), fresh and decellularized tissues were fixed with 2% glutaraldehyde in 0.1 M PBS at 37°C for 1 h, washed with PBS twice, and dehydrated through a graded series of ethanol. All specimens were critical point dried in an HCP-2 (Hitachi, Tokyo, Japan) and coated with platinum using an HCP-2 (Hitachi). Images were observed and captured using an S-4300 (Hitachi). For transmission electron microscopy (TEM), specimens were fixed, dehydrated, and embedded in Epon 812 (Sigma-Aldrich). Ultrathin sections (70 nm) were generated by using an EM UC7 (Leica, Wetzlar, Germany) and stained with 2% uranyl acetate and 1% phosphotungstic acid (Sigma-Aldrich), pH 3.2. TEM images were captured at 100 kV on a HT7700 Bio-TEM (Hitachi).

2.6. Evaluation of Transmission of DCLs. To evaluate the optical properties of various DCLs, transmissions of the DCLs were measured with a UV-visible spectrophotometer (Molecular Devices). The DCL was laid on the center of a well surface (48-well plate, Corning Inc., Corning, NY, USA), and wrinkles were removed. The values were measured from 300 to 700 nm wavelength at 10 nm intervals. The graphs were made by subtracting the value of empty wells from those of samples.

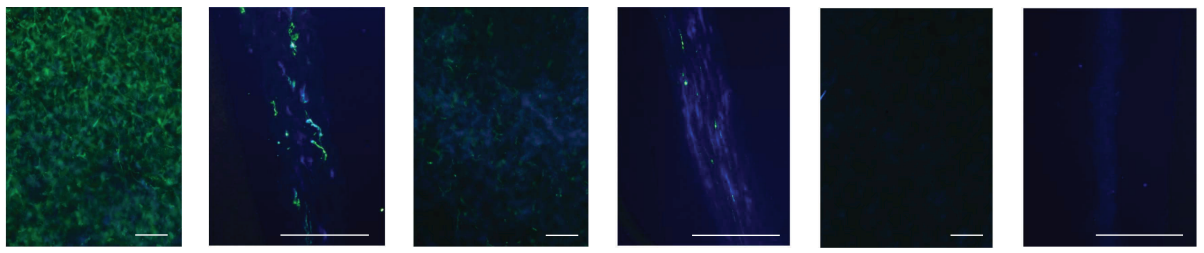
2.7. Culture of Human Limbal Epithelial Cells (HLECs). HLECs were cultured from remnant human limbal tissues. After removing the iris, endothelium, and extra conjunctiva, the tissues were incubated with dispase II (4 mg/mL) and trypsin-EDTA for 10 min at 37°C sequentially. Next, the tissues were incubated with 5 mg/mL collagenase I (Worthington Biochemical Co., Lakewood, NJ, USA) for 1 h at 37°C followed by mechanically removing superficial epithelium. After incubation with collagenase I, cells were collected and plated on collagen-coated dishes (IWAKI, Tokyo, Japan) at density of 1×10^4 cells/cm^2. The cells were remained with CnT-PR and used before passage 3. To isolate keratocytes, stromal tissues were further incubated with 5 mg/mL collagenase I (Worthington Biochemical Co.) for 3 h at 37°C. The cells were collected and plated on a tissue culture dish (Corning) with DMEM (low glucose)/MCDB-201 containing 2% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA), 10 ng/mL epidermal growth factor, 10 ng/mL platelet-derived growth factor, 1 kU/mL leukocyte inhibitory factor, 1% insulin-transferrin-selenium, and 1% antibiotics (Sigma-Aldrich).

In the attachment assay for the DCLs, the cells were reseeded on DCLs at a density of 1×10^5 cells/cm^2. After 60 min, the cells were fixed with 4% paraformaldehyde and stained with DAPI and fluorescein isothiocyanate- (FITC-) conjugated phalloidin. The images were captured using a Nikon eclipse Ti. The area of cells was calculated using ImageJ software (NIH, Bethesda, MD, USA), and the number of attached cells was counted by unit area ($250 \times 250\ \mu$m). To evaluate the viability of cells on DCL, the cells were seeded in quadruplicate onto 48-well plates (Corning) with 1×10^5 cells/cm^2. After 24 h, cell growth was measured using the cell counting kit-8 (CCK-8) following the manufacturer's instructions (Dojindo Molecular Technologies Inc., Kumamoto, Japan). The supernatants were transferred into 96-well plates (Corning). Cell viability was measured by reading the absorbance at 405 nm on a 96-well plate reader (VersaMax, Molecular Devices).

To reconstruct a stratified epithelium on DCL, a DCL was laid on a 24-well culture insert (0.4 μm pore size, Corning),

Table 3: Reverse transcript PCR primers.

Gene	Sequence	Annealing ˚C	Cycles
GAPDH	5′-GAGTCAACGGATTTGGTCGT-3′ 5′-TTGATTTTGGAGGGATCTCG-3′	54˚C	20
ABCG2	5′-GTTTATCCGTGGTGTGTCTGG-3′ 5′-CTGAGCTATAGAGGCCTGGG-3′	58˚C	20
Vimentin	5′-GAGAACTTTGCCGTTGAAGC-3′ 5′-TCCAGCAGCTTCCTGTAGGT-3′	56˚C	20
CK1	5′-AGGAGGTGGACGTGGTAGTG-3′ 5′-AGGAGGCAAATTGGTTGTTG-3′	54˚C	30
CK3	5′-GGCAGAGATCGAGGGTGTC-3′ 5′-GTCATCCTTCGCCTGCTGTAG-3′	59˚C	30
CK4	5′-CTACAACCTCAGGGGGAACA-3′ 5′-GCTCAAGGTTTTTGCTGGAG-3′	55˚C	30
CK5	5′-CTTGTGGAGTGGGTGGCTAT-3′ 5′-CCACTTGGTGTCCAGAACCT-3′	56˚C	30
CK12	5′-ACATGAAGAAGAACCACGAGGATG-3′ 5′-TCTGCTCAGCGATGGTTTCA-3′	56˚C	30
CK13	5′-GATCCAGGGACTCATCAGCA-3′ 5′-AAGGCCTACGGACATCAGAA-3′	56˚C	30
CK14	5′-TTCTGAACGAGATGCGTGAC-3′ 5′-GCAGCTCAATCTCCAGGTTC-3′	55˚C	30
CK15	5′-GGAGGTGGAAGCCGAAGTAT-3′ 5′-GAGAGGAGACCACCATCGCC-3′	58˚C	30



(a) (b) (c) (d) (e) (f)

Figure 1: Remained cellular components after decellularization. Remained nuclear and cell component were visualized with DAPI and vimentin after decellularized with hypotonic solution containing 0.5% Triton X-100 (a and b), 0.5% SDS (c and d), and 0.5% TE (e and f). The lenticules were cross sectioned for precise observation, respectively (b, d, and f). DAPI and vimentin stain of TE decellularized lenticules showed no positive signal. Original magnification is ×200 (a, c, and e) and ×400 (b, d, and f). Scale bar indicates 50 μm.

and HLECs were seeded at a density of 1×10^5 cells/cm^2. The cells were cultured for 2 days until confluence. The DCLs were then positioned at the air-liquid interface and further cultured for 2 weeks to induce stratification. The bottom well was filled with 450 μL keratocyte-conditioned medium containing aprotinin (163 μg/mL, Sigma-Aldrich), and the medium was refreshed every 2 days.

2.8. Biocompatibility of DCLs. To evaluate the biocompatibility of the DCLs, male ICR mice weighing 25–30 g and male New Zealand white rabbits weighing 1–1.5 kg were used. All animals were treated in accordance with the ARVO statement on the use of animals in ophthalmic and vision research. All animals were maintained and treated according to the Kyungpook National University Animal Care guidelines.

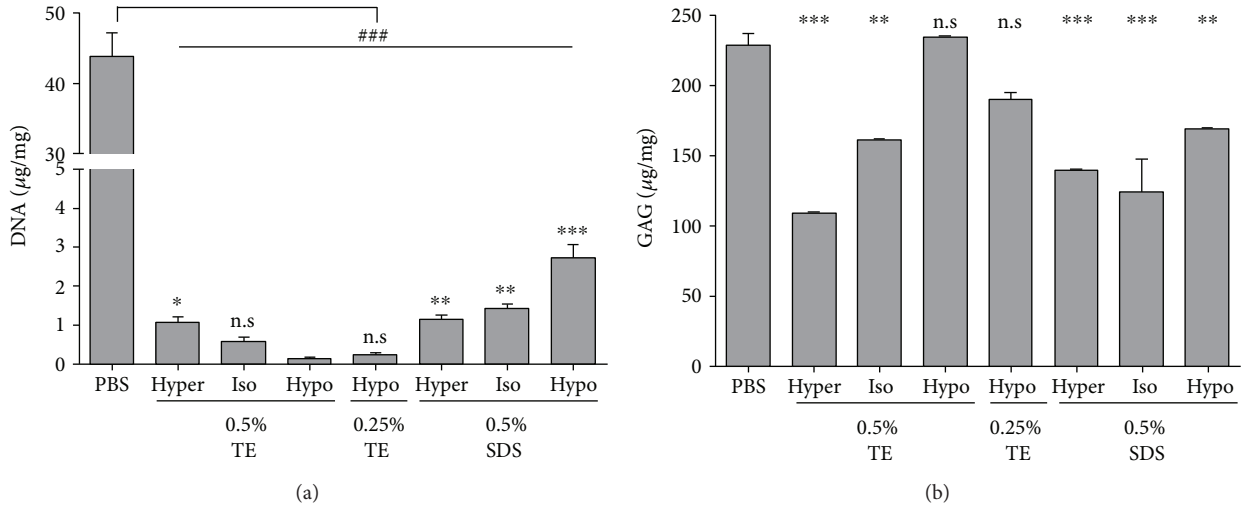


Figure 2: DNA contents and GAG retentions of the DCL. The DNA and GAG contents of DCL under various conditions were measured. The results indicated that the DCL treated with hypotonic 0.5% TE showed the lowest DNA contents (a) and DCL treated with hypertonic 0.5% TE showed the highest GAG contents (b). NC indicates nitrocellulose membrane. Scale bar indicates 50 μm. The values shown are the means ± SEMs ($n = 4$). Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.005$ versus hypotonic 0.5% TE. n.s is not significant.

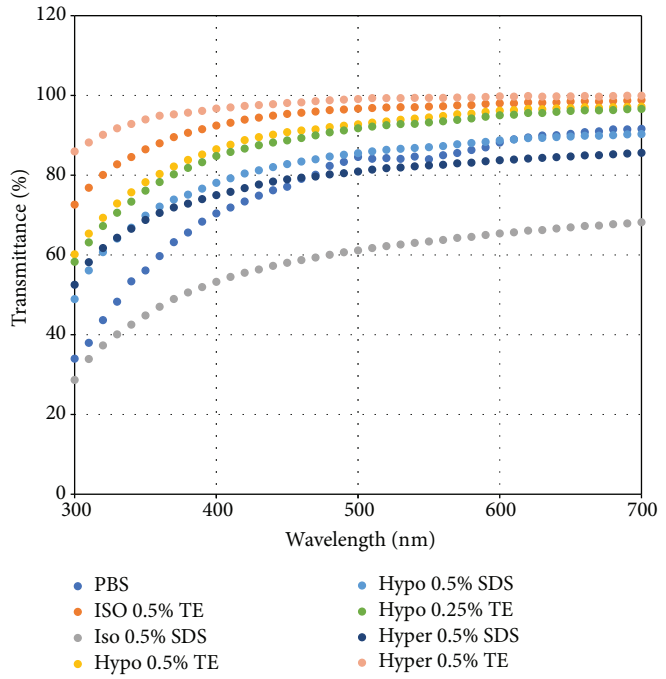


Figure 3: Transmittance of DCLs. Transmittance of the DCL was measured by visible spectrophotometry (wavelength, 300–700 nm). The results indicated that the DCL treated with hypertonic 0.5% TE showed the highest transmittance.

This study was conducted to evaluate the immunogenic potential and tissue response by subcutaneous implantation for 12 weeks in 6-week-old male ICR mice. Healthy animals were randomly assigned into TE-treated groups or the untreated human corneal lenticule group consisting of five animals. The animals were subcutaneously implanted under the dorsal skin. The animals were observed daily for 12 weeks; after the experiments, mice were sacrificed and processed for histopathological evaluation.

Adult New Zealand white rabbits (male, 1–1.5 kg, 6 weeks old) (Daehan Biolink, Seoul, Korea) were used. Recipient animals were anesthetized by intramuscular injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) and 0.5% proparacaine ophthalmic solution (Alcon Laboratories Inc., Fort Worth, TX, USA). Only one eye was operated in each animal. The cornea was incised in the corneal stroma to approximately half depth, and a corneal pocket was created with a spatula. Decellularized corneal discs (diameter of 3 mm) were

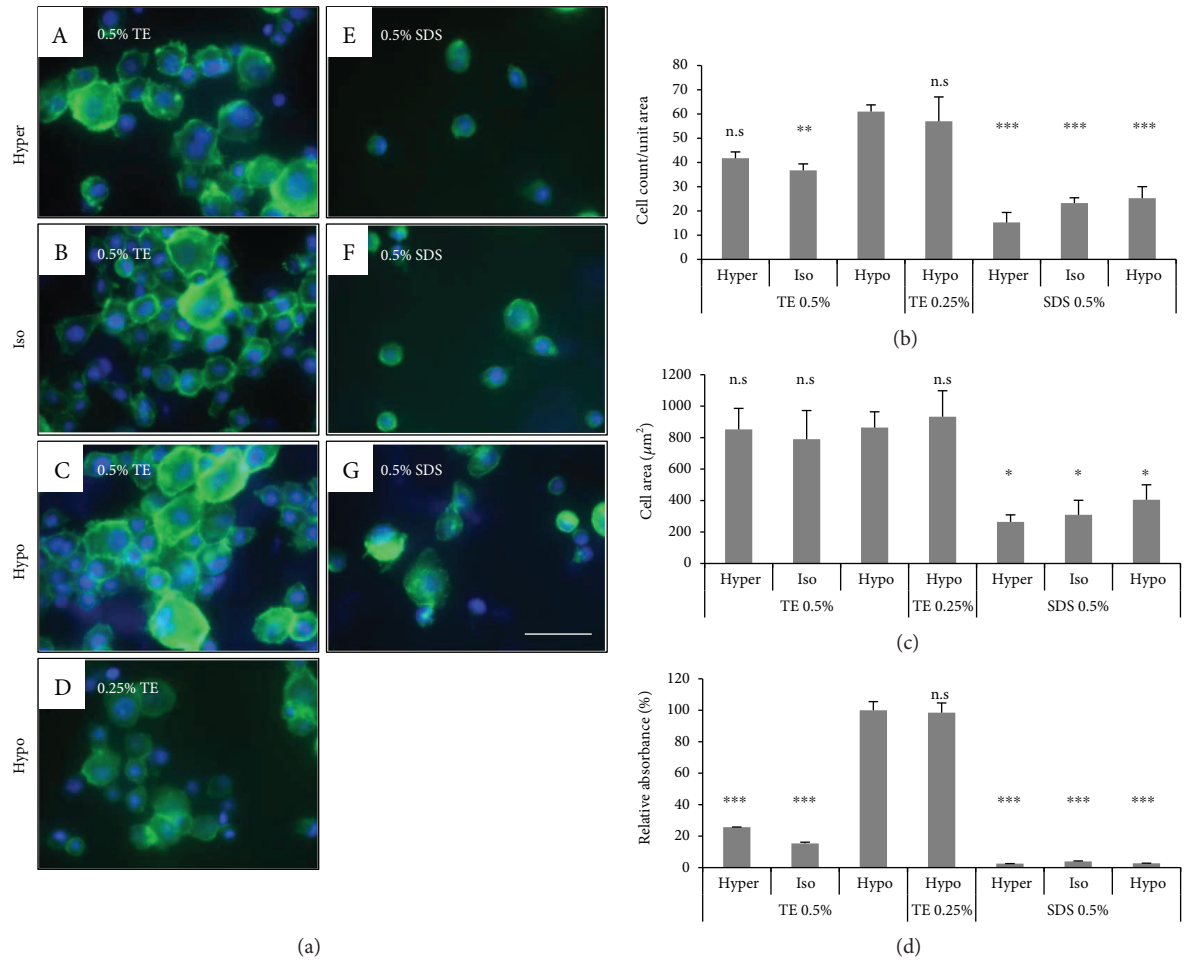


Figure 4: *In vitro* biocompatibility of DCL treated under various conditions. HLEC attachment, spreading, and proliferating activity on the DCLs were evaluated. The cells were seeded on DCL treated with 0.5% TE under various tonic conditions and visualized with DAPI (blue) and phalloidin (green). The count and spreading area of attached cells in TE-treated DCL was higher than on SDS-treated DCL (b and c). Additionally, the proliferating activity of HLECs on DCLs showed the highest rate in hypotonic TE (0.25 and 0.5%). The values shown are the means ± SEMs ($n = 4$). Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.005$ versus hypotonic 0.5% TE. n.s is not significant.

inserted into the corneal pockets and sutured with 10-0 ethilon for the fixation of corneal lenticule. Two months after implantation, the rabbits were sacrificed with an overdose of pentobarbital sodium.

2.9. Reverse Transcript PCR. To evaluate marker gene expression of corneal epithelial cells, total RNA from stratified cells cultured on the DCL was extracted using RNAspin Mini according to the manufacturer's protocol (GE Healthcare Bio-Sciences AB, Little Chalfont, UK). Equal amounts (1 μg) of total RNA were reverse transcribed into cDNA by using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The transcribed complementary DNAs were used for real-time polymerase chain reaction (RT-PCR). The PCR primers and annealing temperatures for target genes were designed based on published human gene sequences (Table 3). The amplified products were visualized by 1% agarose gel electrophoresis and ethidium bromide staining, and GAPDH was used as an internal loading control. Images were captured on an ImageQuant LAS 4000 (GE Healthcare).

2.10. Statistical Analysis. Data are presented as the means ± standard error of the mean. As all data were shown to be not normally distributed, analysis of variance was used to determine significant differences between samples. $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Evaluation of Lenticules Decellularized with Various Methods. Remaining nucleic acid and cytoskeleton were visualized by immunofluorescence using DAPI and anti-vimentin. 0.5% SDS in hypertonic and isotonic buffer, 0.25% TE in hypotonic buffer, and 0.5% TE in all tonicities showed negative staining of DAPI, while some DCL by 0.5% SDS in hypotonic buffer weakly stained with DAPI (Table 2) (Figure 1).

3.2. Comparison with SDS and the TE Decellularizing Method

3.2.1. DNA and GAG Contents. DNA contents were evaluated by Hoechst 33528 staining. DCL in 0.5% hypotonic

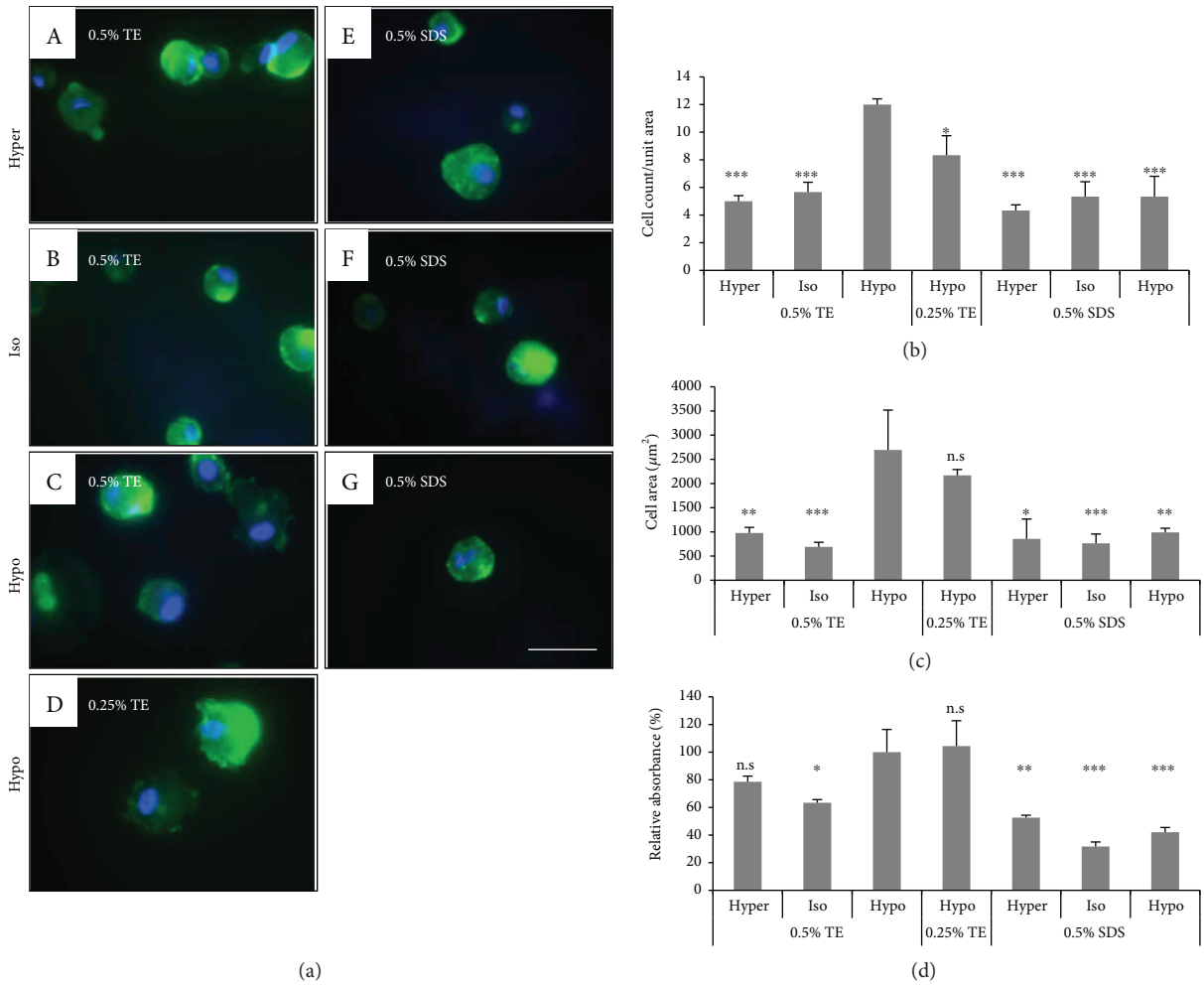


FIGURE 5: *In vitro* biocompatibility of DCL treated under various conditions. Human keratocyte attachment, spreading, and proliferating activity on the DCLs were evaluated. The cells were seeded on DCL treated with 0.5% TE under various tonic conditions and visualized with DAPI (blue) and phalloidin (green). The count and spreading area of attached cells in TE-treated DCL was higher than on SDS-treated DCL (b and c). Additionally, the proliferating activity of keratocytes on DCLs showed the highest rate in hypotonic TE (0.25 and 0.5%). The values shown are the means ± SEMs ($n = 4$). Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.005$ versus hypotonic 0.5% TE. n.s is not significant.

buffer showed DNA contents of $0.14 \pm 0.05\,\mu$g per mg of dried DCL, while 0.5% SDS in hypotonic buffer showed as $2.72 \pm 0.43\,\mu$g. In this study, DCLs of 0.25 and 0.5% TE in hypotonic buffer showed lower DNA contents than the others (Figure 2(a)).

As shown Figure 2(b), remaining GAG contents in 0.5% TE in hypotonic buffer showed the highest level of $234.40 \pm 1.10\,\mu$g/mg. However, DCLs excluding 0.25 and 0.5% TE in hypotonic buffer showed GAG contents less than $200\,\mu$g/mg.

3.2.2. Optical Properties. As shown in Figure 3, we examined the transmittance of the DCL. The DCL prepared in hypertonic 0.5% TE showed the best transmittance, while isotonic 0.5% SDS showed the lowest transmittance. Interestingly, DCLs prepared in TE showed better transparency than those in SDS (Figure 3).

3.2.3. In Vitro Biocompatibility. To evaluate the biocompatibility of a HLEC and human keratocyte on the DCLs, we examined cell attachment, spreading area, and proliferating activity. The cells were fixed at 1 h after seeding in DCL at various conditions and then visualized with DAPI and FITC-conjugated phalloidin. TE-treated DCL showed a higher number of attached HLEC than SDS-treated DCL. The numbers of the cells on 0.25% and 0.5% TE in hypotonic buffer were 61 ± 2.75 and 57 ± 10.03 cells/unit area, respectively. Similarly, the spreading area of the cells on TE-treated DCLs was larger than that on SDS-treated DCLs. The cell surface of TE-treated DCL was expanded to $670.56–1097.78\,\mu m^2$/cell, but on SDS-treated DCL was $216.89–500.10\,\mu m^2$/cell (Figure 4). In the case of the keratocyte, TE-treated DCL showed a higher number of attached keratocytes than SDS-treated DCL. The numbers of the cells on 0.5% TE in hypotonic buffer were 12 ± 1.41 cells/unit

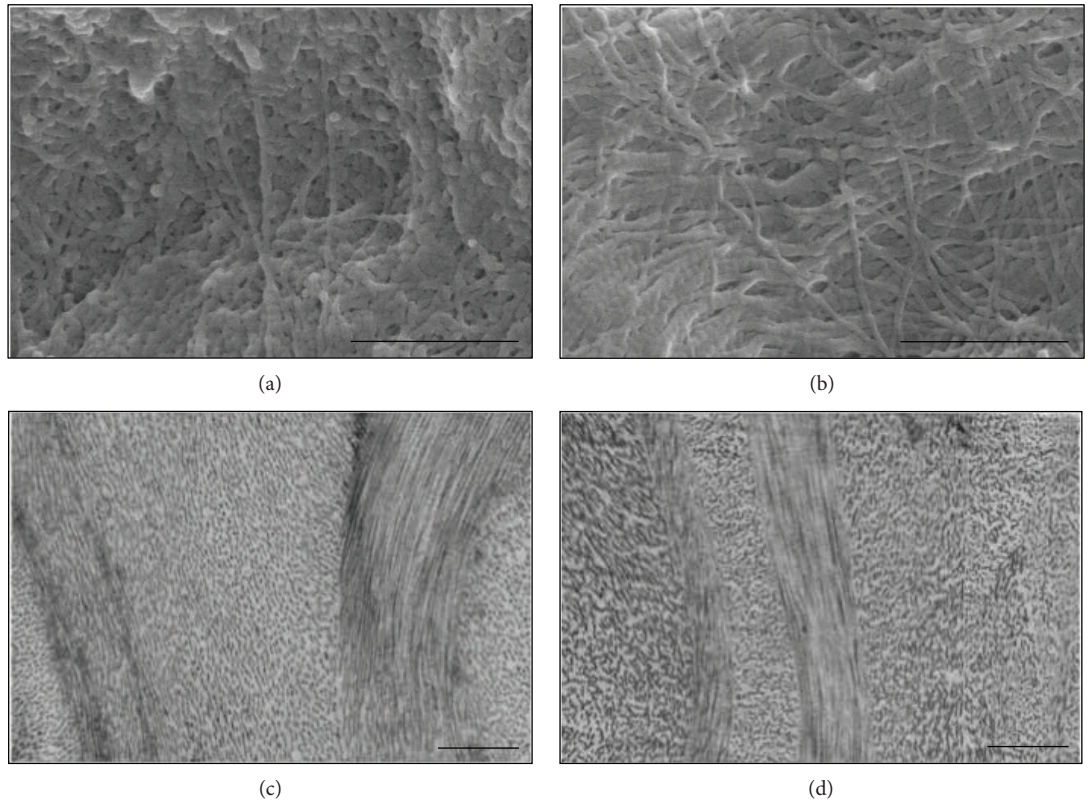


Figure 6: Electron microscopic images of lenticules. Collagen fibril structure of fresh lenticule (a and c) and DCL (b and d). SEM images (a and b) and TEM images (c and d) showing entire collagen microfibrils.

area. The spreading area of the cells on 0.5% TE-treated DCLs was larger than that on SDS-treated DCLs. The cell surface of 0.5% TE-treated DCL was expanded to 1866.24–4027.43 μm^2/cell, but on SDS-treated DCL was 462.73–1522.34 μm^2/cell (Figure 5).

To evaluate proliferation activity, HLEC and keratocyte were analyzed with a CCK-8 assay kit at 24 h after seeding on the DCLs. Notably, proliferating activity of cells on the DCL was only detected in hypotonic TE groups, but showed very low rates in other groups (Figures 4(d) and 5(d)).

3.3. Electron Microscopy. Surface characterization by SEM imaging showed that extracellular matrices in the DCL were not disturbed, and natural collagen bundles were well preserved after decellularization with 0.5% trypsin-EDTA. In the TEM images, collagen fibers are well aligned without damage and showed native matrix orientation (Figure 6).

3.4. Examination of Biocompatibility. Biocompatibility testing of a DCL was performed by insertion into the rabbit corneal stromal layer. The DCL was transparent during the implant periods. Optical coherence tomography examination (Spectalis, Heidelberg Engineering, Franklin, MA, USA) was performed at 2 and 4 weeks after surgery on four rabbit eyes and showed that the inserted DCL was settled stably without degradation and haze. Hematoxylin and eosin staining of the cornea collected at 4 weeks revealed no infiltration of inflammatory cells or vascularization, and the DCL remained acellular up to 4 weeks after surgery (Figure 7).

Unlike interlamellar transplantation, numerous infiltrated inflammatory cells, such as neutrophils and lymphocytes, were detected at 2 weeks after surgery in the subcutaneous implantation model. However, at 12 weeks, the decellularized lenticule exhibited less inflammation with fewer cells than in the untreated model. The untreated lenticule showed numerous infiltrated cells and fibrotic hyperplasia until 12 weeks (Figure 8).

3.5. Generation of a Cell Sheet. The ability of the DCL to support corneal epithelial cell growth was examined *in vitro*. After air-liquid interface culture, HLECs formed stratified epithelium containing 3-4 cell layers. We analyzed the gene expression of the constructed cornea anterior lamellar at 2 weeks after air-liquid culture. RT-PCR showed that stratified epithelial cells strongly expressed makers of epithelial cell progenitor cells, such as CK 5, 14, ABCG2, and vimentin (Figure 9).

4. Discussion

In this study, we demonstrated the feasibility of femtosecond laser-cut DCL as an alternative scaffold for corneal tissue bioengineering. Femtosecond laser in corneal surgery has several advantages such as its precision and versatility. We obtained very uniform and customized corneal lenticules. Furthermore, the use of femtosecond laser-cut DCL for corneal replacement can overcome the lack of donor cornea because donor cells can be procured during refractive

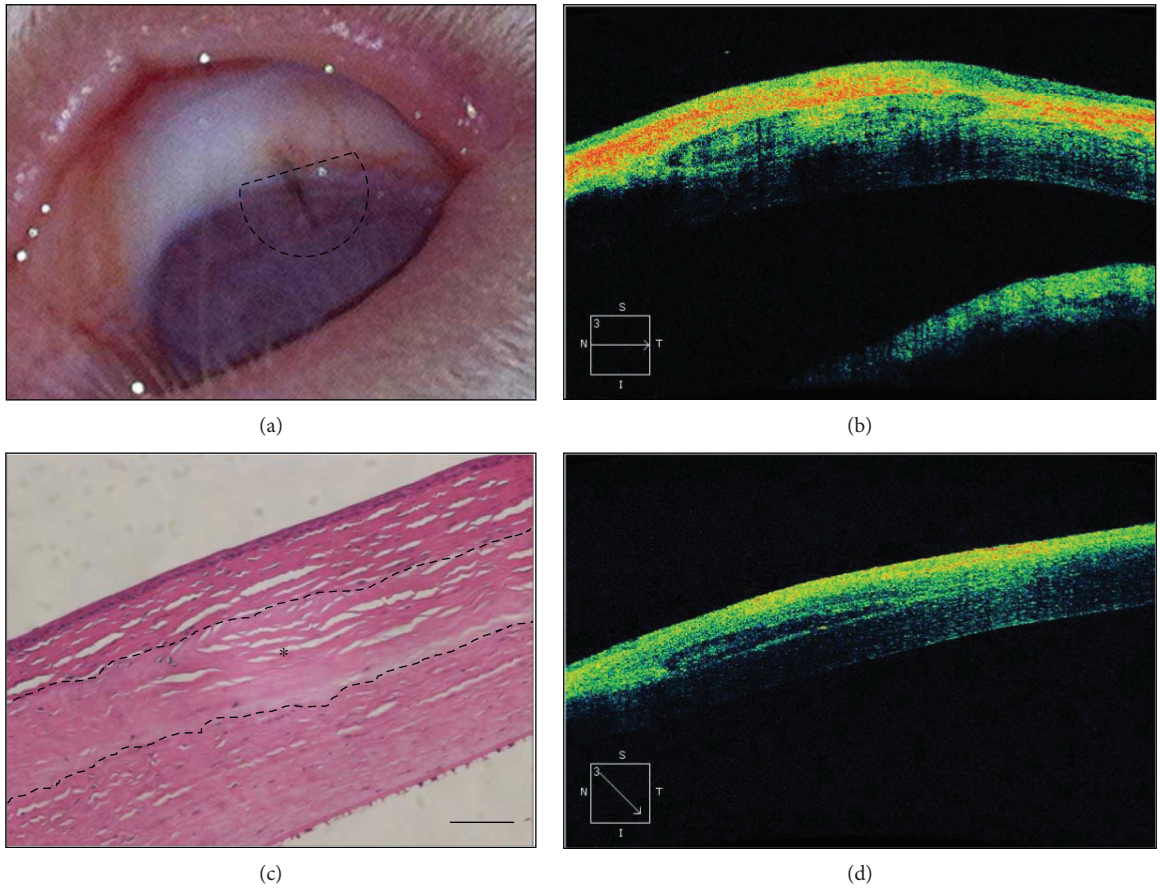


Figure 7: Xenograft of decellularized lenticules into the rabbit corneal stromal layer. DCL was inserted into the stromal layer and fixed with a suture. The slit lamp image shows that the transplanted decellularized lenticule is cleared at 2 weeks after operation. The dotted area indicates the inserted site of the lenticule (a). OCT image showing a transplanted DCL in the stromal layers at 2 (b) and 4 (d) weeks after operation. The DCL was located at the stromal layer. Hematoxylin and eosin staining was performed to detect infiltration of immune cells. There was no infiltration of immune cells in or around the DCL at up to 4 weeks (c). Original magnification is ×200, and scale bar indicates 50 μm (c). *Implanted DCL.

surgery. We should perform various studies to evaluate the safety and efficacy of this method before applying femtosecond laser-cut DCL to clinical cases. We found very promising results. Decellularized corneal tissue as a scaffold for corneal regeneration should maintain the essential properties of the native cornea, such as intact extracellular matrix composition, transparency, and biocompatibility under a harsh decellularization process. However, most decellularization methods use ionic and/or nonionic detergent, which cannot maintain such properties. Many studies have attempted to limit toxicity to maximize the protection of the extracellular composition. However, no comparison studies have optimized the characteristics of the reagents. In this study, we focused on developing an optimized decellularization method for minimizing the toxicity of the reagent without compromising decellularization efficacy. We compared the three reagents most frequently used in each decellularization protocol. A strong ionic detergent such as SDS is commonly used for decellularization of an organ, as it effectively solubilizes cellular membranes and completely removes cells [6, 19, 20, 26, 27]. In the previous report, lenticules which were decellularized with 0.1% SDS combined with 2 U nuclease showed the best decellularizing efficacy [28]. However, SDS causes denaturation of proteins related to the structure of the tissues and causes corneal haze [29, 30]. We also examined that lenticules, which treated with 0.5% SDS, did not maintain morphology of the lenticule in this study. Nonionic detergents, such as Triton X, are also frequently used for corneal decellularization. These detergents are considered milder than ionic treatments, as they target lipid-lipid and lipid-protein interactions, but low decellularization efficacy has been reported [19]. Chelating agents such as EDTA aid in cell dissociation by separating metal ions [29]. These agents are often used in combination with enzymes or detergents because of their lower efficacy for superficial cell removal. Here, we combined trypsin with EDTA, a chelating agent. When we compared the three reagents, TE showed the best efficacy in decellularization. Decellularization efficacy mostly depends upon the tissue, organ, and species of tissue. Unlike a very thick organ or tissues, corneal tissue has a thin, lamellae structure. The decellularization protocol using TE is the most appropriate for preserving the unique collagenous structure.

To increase decellularization efficacy, we evaluated several osmolar conditions with TE. Tonicity can affect the decellularization protocol. Decellularization by immersion in hypotonic or hypertonic solutions can increase decellularization

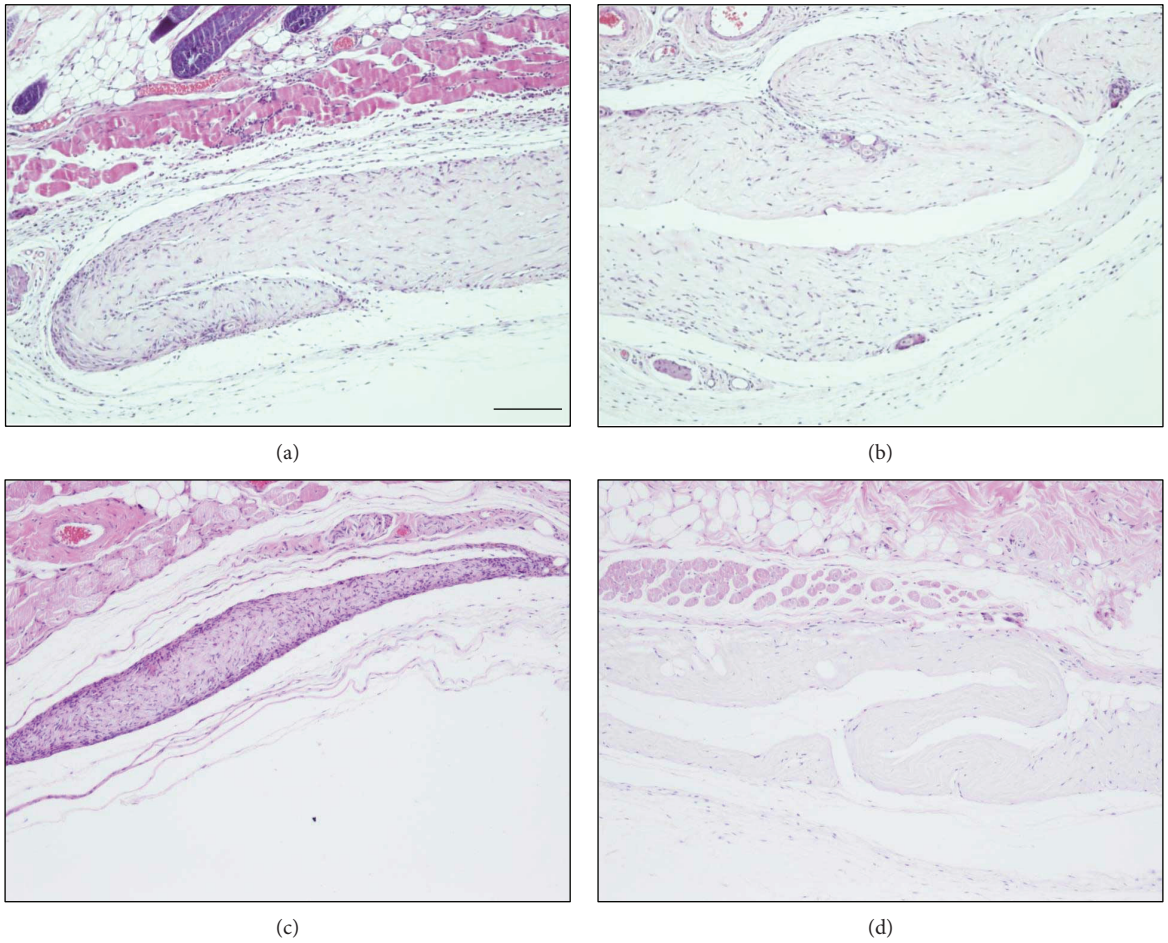


Figure 8: Subcutaneous implantation of the DCL and untreated lenticules in the experimental mouse model. Two weeks after implantation with naive lenticule or DCL, marked inflammatory cells were observed. However, at 12 weeks, DCLs exhibited relatively fewer inflammatory reactions (d) compared to naive lenticules (c). Original magnification is ×100. Scale bar indicates 100 μm.

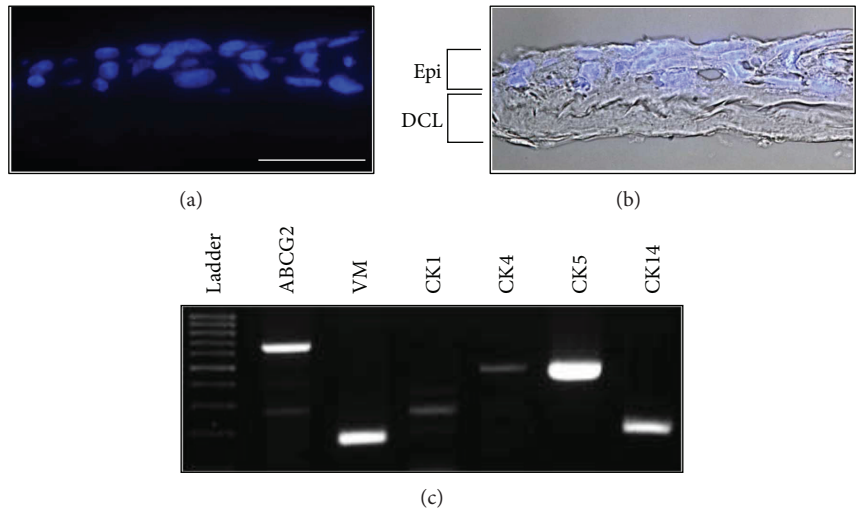


Figure 9: Human limbal epithelial cells on the DCL. Epithelial cells formed a stratified multilayer on the DCL. The cells visualized with DAPI (a) and merged with the bright field image (b). RT-PCR data showed that stratified epithelium cultured on DCL expressed progenitor and corneal epithelial cell markers (c). Scale bar indicates 50 μm.

efficacy [31–34]. Hypotonic solutions can lyse cells via osmotic shock and increase reagent penetration, while hypertonic saline can detach DNA from proteins [32]. As shown in our study, TE in hypotonic Tris buffer exhibited the highest decellularization efficacy.

By optimizing the reagents and conditions, we found that hypotonic TE solution showed the best results in the decellularization protocol. To confirm its efficacy and safety, we evaluated hypotonic TE-treated DCL for three properties: extracellular composition, transparency, and biocompatibility. Hypotonic TE-treated DCL was transparent and biocompatible, like native corneal tissue, and preserved the extracellular matrix. Additionally, we successfully constructed an epithelial cell sheet with DCL. Hypotonic TE-treated corneal tissue showed promising results for applications as a scaffold for regenerative cornea.

Taken together, we established a novel method for the decellularization of corneal tissue using a femtosecond laser and suggested that DCL can be used as a scaffold for the partial substitution of diseased cornea including epithelial, stromal, and endothelial conditions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2015R1D1A3A01020112 and NRF-2015R1D1A1A01060876) and the Industrial Technology Innovation Program (no. 10048358) funded by the Ministry of Trade, Industry and Energy (MI, Korea).

References

[1] B. D. Ayres and C. J. Rapuano, "Refractive power of the cornea," *Comprehensive Ophthalmology Update*, vol. 7, no. 5, pp. 243–251, 2006.

[2] F. E. Kruse and C. Cursiefen, "Surgery of the cornea: corneal, limbal stem cell and amniotic membrane transplantation," *Developments in Ophthalmology*, vol. 41, pp. 159–170, 2008.

[3] K. McColgan, "Corneal transplant surgery," *Journal of Perioperative Practice*, vol. 19, no. 2, pp. 51–54, 2009.

[4] G. R. J. Melles, L. Remeijer, A. J. M. Geerards, and W. H. Beekhuis, "The future of lamellar keratoplasty," *Current Opinion in Ophthalmology*, vol. 10, no. 4, pp. 253–259, 1999.

[5] K. Nishida, "Tissue engineering of the cornea," *Cornea*, vol. 22, Supplement 1, pp. S28–S34, 2003.

[6] W. Liu, K. Merrett, M. Griffith et al., "Recombinant human collagen for tissue engineered corneal substitutes," *Biomaterials*, vol. 29, no. 9, pp. 1147–1158, 2008.

[7] Y. Minami, H. Sugihara, and S. Oono, "Reconstruction of cornea in three-dimensional collagen gel matrix culture," *Investigative Ophthalmology & Visual Science*, vol. 34, no. 7, pp. 2316–2324, 1993.

[8] N. Vázquez, M. Chacón, C. A. Rodríguez-Barrientos et al., "Human bone derived collagen for the development of an artificial corneal endothelial graft. *In vivo* results in a rabbit model," *PLoS One*, vol. 11, no. 12, article e0167578, 2016.

[9] X. Cheng and P. M. Pinsky, "Mechanisms of self-organization for the collagen fibril lattice in the human cornea," *Journal of the Royal Society Interface*, vol. 10, no. 87, article 20130512, 2013.

[10] W. K. Grier, E. M. Iyoha, and B. A. C. Harley, "The influence of pore size and stiffness on tenocyte bioactivity and transcriptomic stability in collagen-GAG scaffolds," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 65, pp. 295–305, 2017.

[11] M. Alaminos, M. D. C. Sa´nchez-Quevedo, J. I. Mun~oz-A´vila et al., "Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold," *Investigative Ophthalmology & Visual Science*, vol. 47, no. 8, pp. 3311–3317, 2006.

[12] M. González-Andrades, I. Garzón, M. I. Gascón et al., "Sequential development of intercellular junctions in bioengineered human corneas," *Journal of Tissue Engineering and Regenerative Medicine*, vol. 3, no. 6, pp. 442–449, 2009.

[13] E. Vardar, H. M. Larsson, E. M. Engelhardt et al., "IGF-1-containing multi-layered collagen-fibrin hybrid scaffolds for bladder tissue engineering," *Acta Biomaterialia*, vol. 41, pp. 75–85, 2016.

[14] P. M. Crapo, T. W. Gilbert, and S. F. Badylak, "An overview of tissue and whole organ decellularization processes," *Biomaterials*, vol. 32, no. 12, pp. 3233–3243, 2011.

[15] B. Zhao, T. Katagiri, H. Toyoda et al., "Heparin potentiates the *in vivo* ectopic bone formation induced by bone morphogenetic protein-2," *Journal of Biological Chemistry*, vol. 281, no. 32, pp. 23246–23253, 2006.

[16] S. P. Seto, T. Miller, and J. S. Temenoff, "Effect of selective heparin desulfation on preservation of bone morphogenetic protein-2 bioactivity after thermal stress," *Bioconjugate Chemistry*, vol. 26, no. 2, pp. 286–293, 2015.

[17] Y. Yu, A. Alkhawaji, Y. Ding, and J. Mei, "Decellularized scaffolds in regenerative medicine," *Oncotarget*, vol. 7, no. 36, pp. 58671–58683, 2016.

[18] Z. Wu, Y. Zhou, N. Li et al., "The use of phospholipase A_2 to prepare acellular porcine corneal stroma as a tissue engineering scaffold," *Biomaterials*, vol. 30, no. 21, pp. 3513–3522, 2009.

[19] Y. Fu, X. Fan, P. Chen, C. Shao, and W. Lu, "Reconstruction of a tissue-engineered cornea with porcine corneal acellular matrix as the scaffold," *Cells Tissues Organs*, vol. 191, no. 3, pp. 193–202, 2010.

[20] Y. Hashimoto, S. Funamoto, S. Sasaki et al., "Preparation and characterization of decellularized cornea using high-hydrostatic pressurization for corneal tissue engineering," *Biomaterials*, vol. 31, no. 14, pp. 3941–3948, 2010.

[21] Y. G. Xu, Y. S. Xu, C. Huang, Y. Feng, Y. Li, and W. Wang, "Development of a rabbit corneal equivalent using an acellular corneal matrix of a porcine substrate," *Molecular Vision*, vol. 14, pp. 2180–2189, 2008.

[22] A. L. de Araujo and J. A. Gomes, "Corneal stem cells and tissue engineering: current advances and future perspectives," *World Journal of Stem Cells*, vol. 7, no. 5, pp. 806–814, 2015.

[23] P. Vavken, S. Joshi, and M. M. Murray, "TRITON-X is most effective among three decellularization agents for ACL tissue engineering," *Journal of Orthopaedic Research*, vol. 27, no. 12, pp. 1612–1618, 2009.

[24] A. Ivarsen, S. Asp, and J. Hjortdal, "Safety and complications of more than 1500 small-incision lenticule extraction procedures," *Ophthalmology*, vol. 121, no. 4, pp. 822–828, 2014.

[25] V. Barbaro, S. Ferrari, A. Fasolo, D. Ponzin, and E. Di Iorio, "Reconstruction of a human hemicornea through natural scaffolds compatible with the growth of corneal epithelial stem cells and stromal keratocytes," *Molecular Vision*, vol. 15, pp. 2084–2093, 2009.

[26] M. Hirayama, M. Ogawa, M. Oshima et al., "Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ," *Nature Communications*, vol. 4, p. 2497, 2013.

[27] J. R. Sharpe, S. M. Daya, M. Dimitriadi, R. Martin, and S. E. James, "Survival of cultured allogeneic limbal epithelial cells following corneal repair," *Tissue Engineering*, vol. 13, no. 1, pp. 123–132, 2007.

[28] G. H.-F. Yam, N. Z. B. M. Yusoff, T.-W. Goh et al., "Decellularization of human stromal refractive lenticules for corneal tissue engineering," *Scientific Reports*, vol. 6, no. 1, article 26339, 2016.

[29] K. Pang, L. Du, and X. Wu, "A rabbit anterior cornea replacement derived from acellular porcine cornea matrix, epithelial cells and keratocytes," *Biomaterials*, vol. 31, no. 28, pp. 7257–7265, 2010.

[30] S. Sasaki, S. Funamoto, Y. Hashimoto et al., "In vivo evaluation of a novel scaffold for artificial corneas prepared by using ultrahigh hydrostatic pressure to decellularize porcine corneas," *Molecular Vision*, vol. 15, pp. 2022–2028, 2009.

[31] S. R. Meyer, B. Chiu, T. A. Churchill, L. Zhu, J. R. Lakey, and D. B. Ross, "Comparison of aortic valve allograft decellularization techniques in the rat," *Journal of Biomedical Materials Research. Part A*, vol. 79, no. 2, pp. 254–262, 2006.

[32] B. Yang, Y. Zhang, L. Zhou et al., "Development of a porcine bladder acellular matrix with well-preserved extracellular bioactive factors for tissue engineering," *Tissue Engineering Part C: Methods*, vol. 16, no. 5, pp. 1201–1211, 2010.

[33] E. Yoeruek, T. Bayyoud, C. Maurus et al., "Reconstruction of corneal stroma with decellularized porcine xenografts in a rabbit model," *Acta Ophthalmologica*, vol. 90, no. 3, pp. e206–e210, 2012.

[34] E. Yoeruek, T. Bayyoud, C. Maurus et al., "Decellularization of porcine corneas and repopulation with human corneal cells for tissue-engineered xenografts," *Acta Ophthalmologica*, vol. 90, no. 2, pp. e125–e131, 2012.