Corneal endothelial cells (CECs) exist at the innermost layer of the cornea and play an essential role in the maintenance of corneal transparency via their barrier and pump functions. Because human CECs (HCECs) have severely limited proliferative ability in vivo, endothelial cell loss due to dystrophy, trauma, or surgical intervention results in a compensatory enlargement of the remaining HCECs. However, when the loss of HCECs is too great, it often leads to irreversible corneal enlargement of the remaining HCECs. However, when the loss of HCECs is too great, it often leads to irreversible corneal endothelial dysfunction. In such cases, keratoplasty is currently considered an ideal method of reconstructing the corneal endothelial layer of patients with endothelial dysfunction. Some groups have reported methods for delivering HCECs to the posterior cornea via “cell injection into the anterior chamber” has been considered an ideal method of reconstructing the corneal endothelial layer of patients with endothelial dysfunction. Some groups have reported methods for delivering HCECs to the posterior cornea: via the magnetic attachment of iron powder or superparamagnetic microspheres incorporated into HCECs. Although these methods have been shown to work in a rabbit transplantation model or in an organ culture model of the human eye, they have yet to be applied in the clinical setting. At present, we are focused on developing a cell-injection therapy combined with the use of a ROCK inhibitor. Compared with the above-described cell-delivery methods of other groups, we theorize that the use of ROCK-inhibitor Y-27632 is both simpler and easier for application in the clinical setting.

The anterior chamber of the eye, as Y-27632 reportedly promotes cell adhesion and proliferation and inhibits the apoptosis of primate CECs in culture.

The different binding properties of cultured human corneal endothelial cell (cHCEC) subpopulations (SPs).

**METHODS.** Each SP was prepared by controlling the culture conditions or by using magnetic cell separation, and then confirmed by staining with several cell-surface markers. Binding abilities of HCEC SPs were examined by adding the cells to culture plates immobilized with collagens, laminins, or proteoglycans, and then centrifuging the plates. Adhered cells were then evaluated by phase-contrast microscopy.

**RESULTS.** The cHCECs were bound to laminin-511, laminin-411, and Type-IV collagen in a concentration-dependent manner, yet weakly bound to Perlecan, Agrin, and TSP-1. Comparison of the influence of cell-suspension vehicles on cHCEC attachment showed that cells suspended in Opti-MEM-I or Opeguard-MA were bound to laminin, yet no binding was observed in cells suspended in BSS-Plus. Next, we compared the adherent properties of HCEC SPs. Both the fully differentiated, mature cHCEC SP and the epithelial-to-mesenchymal-transitioned (EMT)-phenotype SP were found to attach to laminin- or collagen-coated plates. Interestingly, the binding properties to laminins differed among those SPs. Although the level of cells adhered to the laminin-411–coated plate was the same among the cHCEC SPs, the fully differentiated, mature cHCEC SP was significantly more tightly bound to laminin-511 than was the EMT-phenotype SP.

**CONCLUSIONS.** The findings of this study suggest that the binding ability of cHCECs to major Descemet’s membrane components is distinct among cHCEC SPs, and that Opti-MEM-I and Opeguard-MA are useful cell-suspension vehicles for cell-injection therapy.

Keywords: corneal endothelial cell, cultured cell subpopulation, binding property

---

**Cornea**

The Different Binding Properties of Cultured Human Corneal Endothelial Cell Subpopulations to Descemet’s Membrane Components

Munetoyo Toda,1 Morio Ueno,2 Jun Yamada,2,3 Asako Hiraga,1 Hiroshi Tanaka,2 Ursula Schlötzer-Schrehardt,4 Chie Sotozono,2 Shigeru Kinoshita,1 and Junji Hamuro2

1Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
2Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
3Department of Ophthalmology, Meiji University of Integrative Medicine, Kyoto, Japan
4Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany

Correspondence: Munetoyo Toda, Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-ku, Kyoto, 602-8566, Japan; munetoda@koto.kpu-m.ac.jp.

Submitted: June 8, 2016
Accepted: July 20, 2016

Citation: Toda M, Ueno M, Yamada J, et al. The different binding properties of cultured human corneal endothelial cell subpopulations to Descemet’s membrane components. Invest Ophthalmol Vis Sci. 2016;57:4599–4605. DOI:10.1167/iovs.16-20087

PURPOSE. To clarify the adherent properties of cultured human corneal endothelial cell (cHCEC) subpopulations (SPs).

**METHODS.** Each SP was prepared by controlling the culture conditions or by using magnetic cell separation, and then confirmed by staining with several cell-surface markers. Binding abilities of HCEC SPs were examined by adding the cells to culture plates immobilized with collagens, laminins, or proteoglycans, and then centrifuging the plates. Adhered cells were then evaluated by phase-contrast microscopy.

**RESULTS.** The cHCECs were bound to laminin-511, laminin-411, and Type-IV collagen in a concentration-dependent manner, yet weakly bound to Perlecan, Agrin, and TSP-1. Comparison of the influence of cell-suspension vehicles on cHCEC attachment showed that cells suspended in Opti-MEM-I or Opeguard-MA were bound to laminin, yet no binding was observed in cells suspended in BSS-Plus. Next, we compared the adherent properties of HCEC SPs. Both the fully differentiated, mature cHCEC SP and the epithelial-to-mesenchymal–transitioned (EMT)-phenotype SP were found to attach to laminin- or collagen-coated plates. Interestingly, the binding properties to laminins differed among those SPs. Although the level of cells adhered to the laminin-411–coated plate was the same among the cHCEC SPs, the fully differentiated, mature cHCEC SP was significantly more tightly bound to laminin-511 than was the EMT-phenotype SP.

**CONCLUSIONS.** The findings of this study suggest that the binding ability of cHCECs to major Descemet’s membrane components is distinct among cHCEC SPs, and that Opti-MEM-I and Opeguard-MA are useful cell-suspension vehicles for cell-injection therapy.

Keywords: corneal endothelial cell, cultured cell subpopulation, binding property
In culture, HCECs tend to enter into cell-state transition (CST), such as epithelial-to-mesenchymal transition (EMT) or fibrosis, thus resulting in the production of different subpopulations (SPs). Our group has successfully developed a method for definitively discriminating those SPs via their cell-surface markers and we have established culture methods that allow us to obtain a high content of the CD44-SP with nearly identical phenotypes to fresh HCECs in vivo (i.e., fully differentiated mature SP and an SP with an EMT-phenotype). CD44 plays diverse critical roles in CST and the maintenance of stem-cell features. CD44 is known to be the key to distinguish differentiated cHCECs from either in-differentiated cells or hCECs with CST.7 CD44 ablation increased metabolic flux to mitochondrial respiration and concomitantly inhibited entry into glycolysis.8

In cell-injection therapy, one of important steps for therapeutic efficacy is the attachment of HCECs to the Descemet's membrane. In this present study, we examined the binding ability of cHCECs to major Descemet's membrane components that distribute to the endothelial face; that is, laminin-511, -411, Type-IV collagen, and proteoglycans via the centrifugation cell-adhesion assay previously reported by Friedlander et al.9 and other groups.10–12 We also compared the influence of different cell-suspension vehicles and the adherent properties of HCECs between a fully differentiated mature SP and an SP with an EMT-phenotype.

MATERIALS AND METHODS

Human Endothelial Cell Donors

The human tissue used in this study was handled in accordance with the tenets set forth in the Declaration of Helsinki. Human donor corneas were obtained from the SightLife (Seattle, WA, USA) eye bank, and informed written consent for eye donation for research was obtained from the next of kin of all deceased donors. All tissues were recovered under the tenets of the Uniform Anatomical Gift Act (UAGA) of the particular state in which the donor consent was obtained and the tissue was recovered. All donor corneas were preserved in Optisol GS (Chiron Vision, Irvine, CA, USA) and imported via international air transport for research purposes. Donor information accompanying the donor corneas showed that they were all considered healthy and absent of any corneal disease, and that all donors had no past history of chromosomal abnormality.

Cell Culture of HCECs

The HCECs obtained from 30 human donor corneas at distinct ages were cultured according to published protocols, with some modifications.13 Briefly, the Descemet's membranes with the CECs were stripped from donor corneas and digested at 37°C with 1 mg/mL collagenase A (Roche Applied Science, Penzberg, Germany) for 2 hours. The HCECs obtained from a single donor cornea were seeded in one well of a Type-I collagen-coated six-well plate (Corning, Corning, NY, USA). The culture medium was prepared according to published protocols.13 Briefly, basal medium was prepared with Opti-MEM-I (Life Technologies Corporation, Carlsbad, CA, USA), 8% fetal bovine serum (FBS), 5 mg/mL epidermal growth factor (Life Technologies), 20 µg/mL ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA), 200 mg/L calcium chloride (Sigma-Aldrich), 0.08% chondroitin sulfate (Wako Pure Chemical Industries, Osaka, Japan), and 50 µg/mL gentamicin. The HCECs were cultured at 37°C in a humidified atmosphere containing 5% CO₂, and the culture medium was changed twice per week. When they reached confluence, the HCECs were passaged at ratios of 1:3, after the treatment with 10× TrypLE Select (Life Technologies) for 12 minutes at 37°C. The HCECs at passages two through five were used for all experiments.

Cell Attachment Assay

Cell attachment to laminins, Type-IV collagen, proteoglycans, and glycoprotein was examined via the centrifugation cell-attachment assay described previously, with some modifications (Supplementary Fig. S1). Laminin-521, -511, -411, and -332 were purchased from VERTIAS Corporation (Tokyo, Japan). Perlecan, Agrin, Nidogen-1, TSP-1, and Fibulin-5 (FBNL5) were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Type-IV collagen was purchased from BD Biosciences (San Jose, CA, USA).

Each well of a 96-well U-shaped plate (MaxiSorp; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was coated with 40 µL laminin, proteoglycan, or glycoprotein solution at 4°C overnight. The wells were washed three times with PBS(−), and then blocked with 1% BSA in 0.1 M NaHCO₃ (pH 8.0) at room temperature (RT) for 1 hour. The cultured HCECs were suspended in Opti-MEM-I, Opeguard-MA (Senjyu Pharmaceutical, Osaka, Japan), or BSS-Plus (Alcon Laboratories, Fort Worth, TX, USA) at the concentration of 1×10⁵ cells/mL and 0.1 mL of the cell suspension was added into each well and incubated for 10 minutes. The plate was then centrifuged at 200g for 2 minutes. Bright-field Z stack images were captured by use of a BZ-9000 microscope (Keyence Corporation, Osaka, Japan) at 2x objective magnification, and omnifocal images were created from those images using BZ-II Analyzer software (Keyence). According to the description by Friedlander et al.,9 the cells were centrifuged into a pellet at the bottom of the well on nonadhesive substrates, and as the adhesiveness of the substrate increased, more cells bound to the substrates along the wall of the well, which could be detected in a circular area with a measurable diameter. On strongly adhesive substrates, cells were distributed more or less uniformly on the well (Fig. 1).

Flow Cytometry Analysis of the cHCECs

Cultured HCECs were collected from the culture dish by TrypLE Select treatment as described above and suspended at a concentration of 4×10⁶ cells/mL in FACS buffer (PBS containing 1% BSA and 0.05% NaN₃). An equal volume of antibody solution was added and incubated at 4°C for 2 hours. The antibody solutions were as follows: FITC-conjugated anti-human CD26 mAb, PE-conjugated anti-human CD166 mAb, PerCP-Cy 5.5 conjugated anti-human CD24 mAb, PE-Cy 7-conjugated anti-human CD44 mAb (all from BD Biosciences), and APC-conjugated anti-human CD105 mAb (eBioscience, Inc., San Diego, CA, USA). For study of integrin expression, AlexaFluor 488-conjugated anti-human CD49f mAb, PerCP-Cy 5.5 conjugated anti-human CD49f mAb, PE-Cy 5.7-conjugated anti-human CD44 mAb, CD105 mAb (eBioscience, Inc., San Diego, CA, USA), and APC-conjugated anti-human CD105 mAb (eBioscience, Inc., San Diego, CA, USA) were used. After washing with FACs buffer, the cHCECs were analyzed with the FACScanto II Flow Cytometry Analyzer System (BD Biosciences).

Isolation of HCEC SPs by Magnetic Bead Cell Sorting

The HCECs were detached with TrypLE Select as described above, and the CD44+ HCEC SP (the effector SP) was isolated using anti-human CD44 MicroBeads and the deplet05 program of an autoMACS Pro Separator (Miltenyi Biotec, Bergisch...
Results

Binding Strength of cHCECs to Laminin-332, -411, -511, -521, and Type-IV Collagen

As mentioned above, one of the important steps for therapeutic efficacy in cell-injection therapy is the successful attachment of cHCECs to the Descemet’s membrane. The Descemet’s membrane is composed of mainly Type-IV collagen, laminin, fibronectin, and proteoglycan/glycoprotein.14,15 Hence, we examined the attachment ability of cHCECs to the Descemet’s membrane components listed in the Table. As a method for assessing that attachment ability, we performed a centrifugation cell-adhesion assay,16–19 with some modification. The cHCECs were detached from the culture dishes, as described above, and cell suspension was prepared at a concentration of $1 \times 10^5$ cells/mL. Next, 100 μL cell suspension was added to each well of the 96-well U-bottomed plates coated with laminin-511, laminin-411, Type-IV collagen, or the other Descemet’s membrane listed in the Table. The culture plate was stored at RT for 10 minutes and then centrifuged at 200g for 2 minutes. The attached cells were evaluated under a BZ-9000 microscope system, as described above.

As is shown in Figure 1A, the cHCECs were uniformly distributed on the bottom of the wells coated with laminin-521 or -511, whereas cHCECs formed pellets at the bottom of the negative-control BSA-coated wells. In the laminin-411 or -532 coated wells, the cHCECs formed a circular pellet. These results suggest that the cHCECs bound more strongly to laminin-521 and -511 than to laminin-411 and -532.

We next attempted to compare the binding ability of cHCECs to laminin-521 and to laminin-511 by decreasing the coating concentration of laminins. As is shown in Figure 1B, the cHCECs were bound to laminin-521 and -511 in a concentration-dependent manner, yet in this experiment also, no discernible difference was found between these two substrates.

Next, we examined the binding ability of the cHCECs to Type-IV collagen. The centrifugation attachment assay was performed in a similar manner to that with the Type-IV collagen-coated plates. As is shown in Figure 1C, the cHCECs were bound to Type-IV collagen in a concentration-dependent manner. The minimum concentration of both laminin-521 and -511 to detect cell attachment was 4 pM (5 ng/mL), whereas that of Type-IV collagen was 200 ng/mL, although it is important to keep in mind that a difference of immobilization efficiency possibly exists between laminins and collagen.

Binding Strength of cHCECs to Agrin, TSP-1, and Perlecan

We also examined the binding strength of cHCECs to proteoglycan/glycoproteins that reportedly exist in Descemet’s membrane (i.e., Agrin, Nidogen-1, FBLN5, TSP-1, and Perlecan).14,15 No cell binding was observed when the coating concentrations of these proteoglycan/glycoproteins were equally 5 nM, as in Figure 1A (data not shown), but cell bindings to Agrin, TSP-1, and Perlecan were observed at coating concentrations as high as 400 nM (Fig. 2). These results suggest that the cHCECs bind to these components, yet the binding affinity is considerably lower than that to laminins.

Influence of Cell-Suspension Vehicle on cHCEC Binding

For cell-injection therapy, the selection of the cell-suspension vehicle may be a critical aspect. Therefore, we compared the effects of cell-suspension vehicles Opti-MEM-I, Opeguard-MA, and BSS-Plus on the attachment of cHCECs to Descemet’s membrane. The purity of the isolated effector SP was higher than 95% in all cases.
membrane components. Opti-MEM-I (used in Figs. 1, 2) is the base medium for the HCECs culture. Opeguard-MA and BSS-Plus are intraocular irrigating solutions routinely used in the clinical setting. Laminin-411 was used for the Descemet’s membrane component, as it is considered that when laminin-411 with mid affinity for cHCECs is used, it is more detectable than laminin-511 with high affinity.

The cHCECs were bound to laminin-411 when using Opti-MEM-I and Opeguard-MA, yet not when using BSS-Plus (Fig. 3).

### Binding Properties of cHCEC SPs

The tendency of HCECs to enter into CST, such as EMT or fibrosis, during cultivation produces different SPs with distinct surface cluster-of-differentiation (CD) markers from cHCECs, and we have now developed the appropriate method to definitively discriminate those SPs with regard to their cell-surface markers. To clarify the adherent properties of these SPs, we compared the binding ability of cHCEC SPs via a centrifugation cell-adhesion assay. A purified cHCEC SP exhibiting no sign of CST with hexagonal morphology (the fully differentiated mature HCEC subpopulation) was prepared by magnetic bead cell sorting (MACS) with human-CD44 magnetic beads by negative selection as described above. As is shown in Figure 4A, flow cytometry analysis revealed that the effector SP separated by MACS with CD44 magnetic beads was purified to over 90% purity and that Na+/K+ ATPase and ZO-1 were expressed in those cells (Fig. 4B).

To gain SP with EMT-phenotype (CD166⁺, CD44+++ and CD24⁺), the MACS-positive selection with human-CD44 magnetic beads was not performed, to avoid the modulation of the expression intensity of CD44 on the cell surface by the direct interaction with CD44 magnetic beads. Instead, the SPs generated in subsequent cultures were used (Figs. 4C, 4D).

Both the fully differentiated mature HCEC SP and the EMT-phenotype SP were found to attach to laminin- or collagen-coated plates (Fig. 5A). Interestingly, the binding properties to laminins were different among these two SPs. Although the

---

### Table. Distribution of Basement Membrane Components in Adult Descemet’s Membrane

<table>
<thead>
<tr>
<th></th>
<th>Stromal Face</th>
<th>Endothelial Face</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type-IV collagen chains</td>
<td>α1, α2, α3-α6</td>
<td>21-26</td>
</tr>
<tr>
<td>Laminin chains</td>
<td>None</td>
<td>α1, α2, β1, γ1</td>
</tr>
<tr>
<td>Possible laminin isoforms</td>
<td>None</td>
<td>411, 511</td>
</tr>
<tr>
<td>Nidogen-1/entactin-1</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Nidogen-2/entactin-2</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Perlecan</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Netrin-4/b.netrin</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Matrilin-4</td>
<td>−</td>
<td>−/+</td>
</tr>
<tr>
<td>Type VIII Collagen</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Type XII Collagen</td>
<td>−</td>
<td>+ (Short form)</td>
</tr>
<tr>
<td>SPARC/BM-40/osteonectin</td>
<td>−/+</td>
<td>−</td>
</tr>
<tr>
<td>Type XVIII Collagen</td>
<td>−</td>
<td>−/+</td>
</tr>
<tr>
<td>Thrombospondin-1</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Fibronecin</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Vimentin</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Grading is as follows: −, lack of staining; −/+ weak staining in some cases; + distinct staining in most or all cases. Modified from Kabosova et al.³⁴

* Some cases were negative.

---

**Figure 3.** The influence of the cell-suspension vehicle on cHCEC binding. Cultured HCECs suspended in Opti-MEM-I, Opeguard-MA, or BSS-Plus were added to 96-well U-bottom culture plates coated with 1.25 or 5 nM laminin-411, and then centrifugation cell-adhesion assays were performed as in Figure 1.

**Figure 2.** Binding of the cHCECs to proteoglycans and glycoproteins in the centrifugation cell-adhesion assay. The cHCECs suspended in Opti-MEM-I were added to 96-well U-bottom culture plates coated with 400 nM Agrin, Nidogen-1, FBLN5, TSP-1, or Perlecan, and the centrifugation assays were performed as in Figure 1.
FIGURE 4. Phase-contrast images and cell-surface marker expression of the cHCECs used in Figure 5. (A) Phase-contrast images and FACS analysis of the mature cHCEC SP purified by MACS CD44-negative selection. FACS analysis was performed as follows: cHCECs were detached from the culture dish and analyzed with regard to the expression of CD166, CD24, CD44, CD105, and CD26 with a FACS Canto II flow cytometer as described in Materials and Methods. After gating for CD166^+CD24^- (R1), the following 3 SPs were defined: CD166^+CD24^-CD44^-/CD105^- SP (gate 1 = G1), CD166^+CD24^-CD44^+/CD105^- SP (G2), and CD166^+CD24^-CD44^+/+CD105^- SP (G3). (B) The cHCECs shown in (A) were stained with ZO-1 and Na^+/K^-ATPase. Nuclei were stained with DAPI. (C) Phase-contrast images and FACS analysis of the EMT-phenotype SP. (D) The cHCECs shown in (C) were stained with ZO-1 and Na^+/K^-ATPase. Nuclei were stained with DAPI.

FIGURE 5. Comparison of the binding abilities of cHCEC SPs to Descemet’s membrane components. The binding abilities of cHCEC SPs to laminins and Type-IV collagen were compared via centrifugation cell-adhesion assay. The adhesion index was calculated as follows: Adhesion Index = (Area subtratum - Area BSA)/Area BSA.
In this study, we investigated the binding properties of chHCECs via centrifugation cell-adhesion assay. The chHCECs were found to be more strongly bound to laminin-521 and -511 than to laminin-411 and -332, thus suggesting that these cells have high affinity to the laminin α5 subunit. These cells were also bound to Type-IV collagen in a concentration-dependent manner. The minimum concentrations necessary for the observed cell binding in this study are as follows: laminin-521 and -511, 4 μM (3 ng/mL); laminin-411, 1.25 nM (2.85 μg/mL); and Type-IV collagen, 250 ng/mL. Perlecan, TSP-1, and Agrin binding were observed only at the concentrations at 400 nM. It is important to keep in mind that a difference of immobilization efficiency possibly exists between laminins and collagen, and proteoglycans/glycoproteins, as it appears that chHCECs most strongly bind to laminin-521/511 during short-duration incubation. Yamaguchi et al.17 reported that the bindings of HCECs to laminin-5 (laminin-332) and to Type-IV collagen were not significant in their adhesion assay. Taken together, it is conclusive that laminin-521 and -511 have higher affinity for the chHCECs than does Type-IV collagen, because laminin-521 and -511, more than laminin-332, were strongly bound to these cells in our assay.

In the above-cited study, the authors also reported that laminin-5 (laminin-332) promoted the proliferation of chHCECs, whereas Type-IV collagen did not. Thus, the binding affinity and bioactivity are not necessarily correlated. Okumura et al.16 reported that laminin-511 and -521 enhance cell proliferation, yet did not compare them with laminin-332. Thus, elucidation of the biological effect of these components after binding is needed.

Human CECs express several integrins (i.e., α2β1, α3β1, α5β1, and α6β1). Recently, Okumura et al.16 reported that HCECs binding to laminin-511 are mediated by integrin α5β1 and α6β1. Integrin α5β1 and α6β1 reportedly have higher affinity to laminin-511 than laminin-332 or 411.18 In our experiment, despite having higher affinity to laminin-511, the expression of integrins α3 and α6 on the fully differentiated mature chHCEC SPs was lower than that of the EMT-phenotype SP whereas integrin α2 subunit expression was higher in the former SP than that in latter SP. In addition, integrin β1 expression was not significantly different between those two SPs (data not shown). It may be possible that the high expression of integrin α2 subunit raises the α2β1 complex known as the collagen-binding integrin,18 resulting in a lower expression of the α5β1 and α6β1 complex. This clearly indicates the presence of distinction in the ratio of integrin α2β1 versus α5β1 and α6β1 among SPs, the proportion of which significantly differed among chHCECs (Toda et al., unpublished data). The discrepancy of our findings from those of Okumura et al.16 might be explained by their usage of chHCECs heterogeneous in the composites of HCEC SPs. In addition, findings using chHCECs not defined in their composites of SPs produces controversial results in scientific terms. Thus, most of the results thus-far reported should be strictly reexamined on the grounds of the biochemical features and composites of chHCEC SPs.

For “cell-injection therapy,” selection of the optimal cell-suspension vehicle is important for therapeutic efficacy. In this study, we compared the effect of three cell-suspension vehicles (Opti-MEM-I, Opeguard-MA, and BSS-Plus) on the attachment of HCECs to laminin. Opeguard-MA was comparable to Opti-MEM-I. Because the composition formula of Opti-MEM-I is not disclosed by the manufacturer, Opeguard-MA, an intraocular irrigating solution routinely used in the clinical setting, might be the better choice of cell-suspension vehicle for cell infusion therapy. We are currently in the process of attempting to confirm the results using in vivo murine models with cryoinjured corneal endothelium.
Acknowledgments

The authors express their sincere appreciation to John Bush for his excellent work in reviewing the manuscript. Supported by the Highway Program for Realization of Regenerative Medicine from Japan Agency for Medical Research and Development, AMED and JSPS KAKENHI Grant Numbers JP26293376, JP25670756, and JP26670759.

Disclosure: M. Toda, None; M. Ueno, None; J. Yamada, None; A. Hiraga, None; H. Tanaka, None; U. Schlötzer-Schrehardt, None; C. Sotozono, None; S. Kinoshita, None; J. Hamuro, None

References


