



Cecsi Cells Can Be Cryopreserved and Transplanted as a Cell Suspension

sample [9], and the RNeasy kit (Qiagen, Valencia, CA) was used to extract total RNA from the cells because the amount of total RNA from a single donor cornea is limited. For the purpose of analysis, three distinct samples of corneal endothelial RNA were prepared. There were a total of nine donor eyes, with 5 male and 4 female donors, a mean donor age of 62.8 ± 4.8, and a mean donor corneal endothelial cell density of 2734.0 ± 382.0 cells/mm². Cells on culture dishes, 6-well culture plates, or monkey corneal buttons in 24-well plates were fixed at room temperature for 10 min in 4% paraformaldehyde (PFA) in phosphate-cushioned saline (PBS) [10]. To prevent nonspecific binding, samples were incubated for 30 minutes at room temperature in 10% normal donkey serum following two washes with PBS [11].

Then, examples were hatched for an hour at room temperature with the showed essential antibodies and washed twice with PBS. After that, the cells were washed twice in the dark and incubated for one hour with the designated secondary antibodies. Using B4G12 cells as a positive control, the immunostaining conditions for tight junction protein-1, Na⁺ K⁺ATPase alpha-1 subunit (ATPIA1), N-cadherin, transcription factor H⁺TX2, and DAH⁺ were determined [12].

Discussion

The true value of regenerative cell therapy lies in its capacity to supply numerous patients worldwide with safe and effective cells. The latest thing is to treat illnesses that are generally incurable. The application of the technology to patients with limited access to surgical care would be the next logical step. To this end, we created a cell product that can be transplanted at a low cost to treat patients with bullous keratopathy who do not have access to donor tissue.