

Magnetic Cell Separation for Elimination of Corneal Stromal Fibroblast Contamination in Corneal Endothelial Cell Cultures

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Human corneal endothelial cells (HCECs) are responsible for the major water-pumping function of the cornea, from corneal stroma to the aqueous humor, and any damage to these cells may lead to corneal decompensation. When the endothelium functions adequately, it regulates stromal hydration and thus both corneal thickness and transparency which are critical for optimal function. This function is accomplished by an ATP-dependent endothelial pump function, in addition to the presence of focal tight junctions which act as a barrier.

HCEC density decreases with aging and with various diseases such as bullous keratopathy and Fuchs' endothelial dystrophy. The conventional treatment for this condition is corneal transplantation using a full-thickness donor cornea. More recently an alternative surgical approach has become popular; Descemet's stripping endothelial keratoplasty (DSEK) which selectively replaces the corneal endothelium without the need for large circumferential corneal incisions or use of sutures. Regardless of the procedure, an entire fresh and high-quality donor cornea with viable endothelium is required. However, many patients cannot receive such transplants in numerous countries due to shortage of donor corneas. Therefore, alternative sources of tissue procurement by exploiting engineering approaches to expand HCECs *in vitro* and fabricate transplantable sheets of HCEC would be of immense benefit.

HCECs are very difficult to culture *in vitro* and even in the case of a successful culture, their slow replication can easily be hindered by rapidly growing stromal corneal fibroblasts (SCFs) that may have been co-isolated in some cases. Enzymatic dissociation for removing HCECs may also release stromal keratocytes into the culture system which can also turn into rapidly growing SCFs. This tissue contamination

is detrimental to HCECs cultures which generally require 14 to 21 days to establish and will also adversely impact the development of tissue-engineered constructs where pure populations of cultivated and functional corneal endothelial cells are required. Furthermore, fibroblastic contamination in these cultures will interfere with the critical barrier and pump function of cultivated corneal endothelial cells.

Peh et al have recently described the use of a magnetic cell separation technique to deplete contaminating SCFs from corneal endothelial cell cultures using antifibroblast magnetic microbeads.¹ In their study, the experimentally mixed cultures of SCFs and HCECs were tagged with antifibroblast magnetic microbeads, subjected to separation within a magnetic field, and then separated into "labeled" and "flow-through" fractions. The magnetic cell separation (MAC)-separated cells were left to adhere for at least one day to enable them to establish their morphology. Postseparation assessment of cultured fluorescent cells from both the "labeled" and "flow-through" fractions of the separated mixtures was performed to determine the efficacy of the separation. They observed a separation efficacy of 96.88% and concluded that this technique would be useful for eliminating contaminating SCFs within a culture of corneal endothelial cells.

Conflicts of Interest

None.

REFERENCES

1. Peh GS, Lee MX, Wu FY, Toh KP, Balehosur D, Mehta JS. Optimization of human corneal endothelial cells for culture: the removal of corneal stromal fibroblast contamination using magnetic cell separation. *Int J Biomater* 2012;2012:601302. Epub 2012 Jan 12.