COMPARISON OF FOUR CULTURE MEDIA FOR THE ISOLATION AND PROPAGATION OF HUMAN CORNEAL ENDOTHELIAL CELLS

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INTRODUCTION

The corneal endothelium is the innermost layer of the cornea. It is crucial in the maintenance of corneal transparency through the regulation of corneal hydration. This unique layer of cells is not known to actively proliferate *in vivo*. Hence, excessive corneal endothelial cell-loss due to endothelial dystrophy, accidental or surgical trauma leads to stromal edema, corneal clouding, and eventually the loss of visual acuity. To restore vision in such cases, the endothelium can be replaced with healthy donor cornea through corneal transplantation. However, there is a global shortage of donor corneas.

A potential strategy to circumvent the shortage of donor corneas lies in the development of tissue-engineered corneal substitute, and the ability to be able to consistently cultivate human corneal endothelial cells (hCECs) *in vitro* is critical. The cultivation and expansion of hCECs has been widely explored, and a range of complex serum-supplemented culture media has been reported to support the isolation and expansion of hCECs, some with more success than others.

AIM

To compare and characterize the isolation and expansion of hCECs *in vitro* using four previously reported serum-supplemented culture media (Table 1).

METHODS

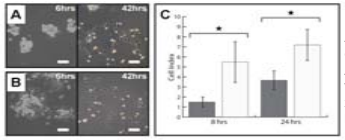
Paired research grade corneas were procured from the Lions Eye Institute for Transplant and Research (Tampa, USA). Isolation of hCECs involved a two-step peel-and-digest method. The Descemet's membrane (DM), together with the corneal endothelium, was carefully peeled off from the corneal stroma under a dissecting stereomicroscope. The peeled DM-endothelial layers were subsequently subjected to an enzymatic digestion using collagenase (2mg/mL) for at least 2 hours, and further dissociated using TrypLE™ Express for approximately 5 minutes. Isolated hCECs were plated equally onto culture dishes coated with FNC coating mix® in four conditions (Table 1). Established hCECs in these conditions were expanded for two passages and analyzed for their propensity to proliferate (Click-iT™ EdU), their general morphology, as well as their expression of markers characteristics of corneal endothelial cells: ZO-1 and Na^+K^+ATPase.

	Medium 1 *(Ishino et al., IOVS 2004)*	**Medium 2** *(Zhu & Joyce, IOVS 2004)*	**Medium 3** *(Li et al., IOVS 2007)*	**Medium 4** *(Bednarz et al., Br J Ophthalmol 2001)*
Basal medium	DMEM	OPTI-MEM-I	Ham's F12/DMEM	Ham's F12 : M199
Fetal Bovine Serum	10%	8%	5%	5%
Mitogens	2 ng/ml bFGF	20 ng/ml NGF | 5 ng/ml EGF	2 ng/ml EGF	10 ng/ml bFGF
Other supplements	-	100 µg/ml BPE 20 µg/ml Ascorbic Acid 200 µg/ml $CaCl_2$ 0.08% Chondroitin Sulphate	0.5% DMSO 5 µg/ml Insulin 2.75 µg/ml Transferrin 2.5 ng/ml Selenium 0.5 µg/ml Hydrocortisone 0.1 µg /ml Cholera Toxin	20 µg /ml Ascorbic Acid 5 µg/ml Insulin 2.75 µg/ml Transferrin 2.5 ng/ml Selenium

Table 1: Formulation of the four culture media used in the culture of human corneal endothelial cells.

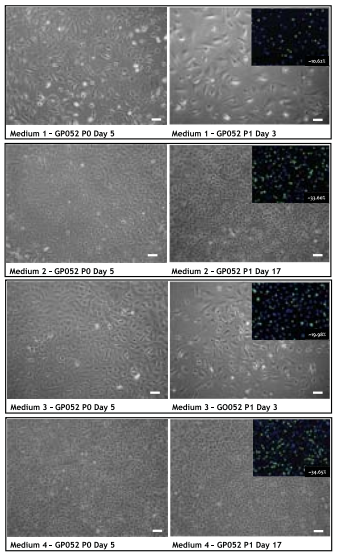
RESULTS

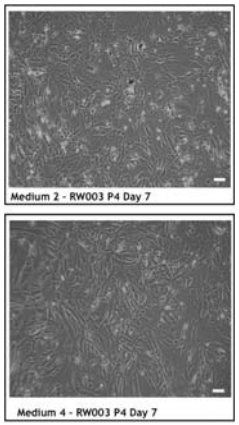
Enhancement of hCECs attachment (Scale bar = 100µm)



Morphology of attached hCECs on **A)** uncoated and **B)** FNC coated culture surface at 6hrs and 42hrs. **C)** Analysis of cell attachment with and without FNC coating using xCELLigence real-time impedance-based cell analyzer system (Roche).

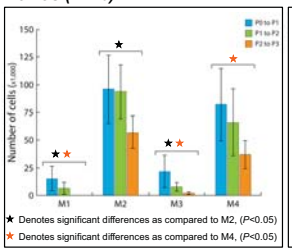
Morphology of cultured hCECs at P0, P1 & P4 (Scale bar = 100µm)





Corneal endothelial cells cultured in either M2 or M4 has a greater propensity to proliferate when compared to M1 or M3, and exhibited hexagonal or polygonal morphology at low passages (P0, P1). However, hCECs cultured in the proliferative media become highly heterogeneous with subsequent passages (P4, as shown), with the appearance of long spindle-shaped fibroblastic-like cells.

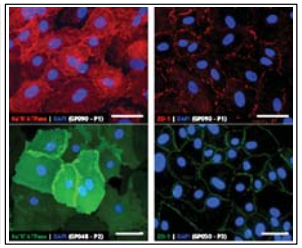
Growth dynamics of cultured hCECs (n = 5)



★ Denotes significant differences as compared to M2, (P<0.05)

★ Denotes significant differences as compared to M4, (P<0.05)

Characterization of cultured hCECs (Scale bar = 50µm)



Proliferation pattern of hCECs in four media. M1, and in most culture of M3 were unable to support the proliferation of hCECs. M2 and M4 stimulated robust cell proliferation as illustrated.

Expression of markers characteristic of the human corneal endothelium. Cultivated hCECs were found to express both ZO-1 and Na^+K^+ATPase at P1, P2 and P3, as shown.

CONCLUSION

- The use of FNC coating on culture surfaces significantly enhanced the attachment and subsequent culture of hCECs.
- Established hCECs propagated in the four media exhibited striking morphological and proliferative differences.
- **M1** and **M3** were unable to actively support the propagation of hCECs beyond the first two passages.
- Although hCECs cultured in **M2** and **M4** were significantly more proliferative, cellular heterogeneity and the loss of hCECs unique polygonal/hexagonal morphology arose in subsequent passages.
- Cultured hCECs (up to P3) express markers indicative of the human corneal endothelium: tight junction ZO-1 and Na+K+ATPase pump.
- Development of corneal substitute is currently underway using primary hCECs propagated in either **M2** or **M4** for up to the second passage.

References

1) Ishino et al., (2004). *IOVS*. 45: 800-806.

2) Zhu and Joyce, (2004). *IOVS*. 45: 1743-1751.

3) Li *et al.*, (2007). *IOVS*. 48: 614-620.

4) Bednarz *et al.*, (2001). *Br J Ophthalmol*. 85: 1416-1420.

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