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/EDTA.

Established hCECs propagated in the four media exhibited striking morphological and proliferative differences. Cultivated hCECs were found to express both ZO-1 and Na+/K+ATPase at P1, P2 and P3, as shown.

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

<table>
<thead>
<tr>
<th>Medium 1</th>
<th>Medium 2</th>
<th>Medium 3</th>
<th>Medium 4</th>
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<tbody>
<tr>
<td>Fetal Bovine Serum 10%</td>
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<tr>
<td>2.75 µg/ml Transferrin</td>
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<tr>
<td>0.5 µg/ml Hydrocortisone</td>
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<td>0.1 µg /ml Cholera Toxin</td>
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<tr>
<td>0.5 µg/ml Insulin</td>
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<tr>
<td>2.5 ng/ml Selenium</td>
<td>2.5 ng/ml Selenium</td>
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<tr>
<td>5 µg/ml BPE</td>
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<tr>
<td>0.5% DMSO</td>
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<td>0.5% DMSO</td>
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<tr>
<td>0.08% Chondroitin Sulphate</td>
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RESULTS
Enhancement of hCECs attachment
Morphology of attached hCECs on (A) uncoated and (B) FNC coated (culture surface at 48hrs and 42hrs. C) Analysis of cell attachment with and without FNC coating using hCELIquent real-time impedance-based cell analyzer system (Roche).

Morphology of cultured hCECs at P0, P1 & P4
Morphology at attached hCECs on uncoated and FNC coated culture surface at 6hrs and 42hrs. C) Analysis of cell attachment with and without FNC coating using hCELIquent real-time impedance-based cell analyzer system (Roche).

Medium 1 - Ophthalmic TM (B) Day 2
Medium 2 - Ophthalmic TM (A) Day 2
Medium 3 - Ophthalmic TM (A) Day 2
Medium 4 - Ophthalmic TM (A) Day 2

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 became highly heterogeneous with subsequent passages (P4, as shown), with the appearance of long spindle-shaped fibroblastic-like cells.

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.
- Development of corneal substitute is currently underway using primary hCECs propagated in either M2 or M4 for up to the second passage.

References