Culture maturation enhances the expression of key genes in stem cell derived RPE - a step on the road of realizing a viable cellular therapy for AMD

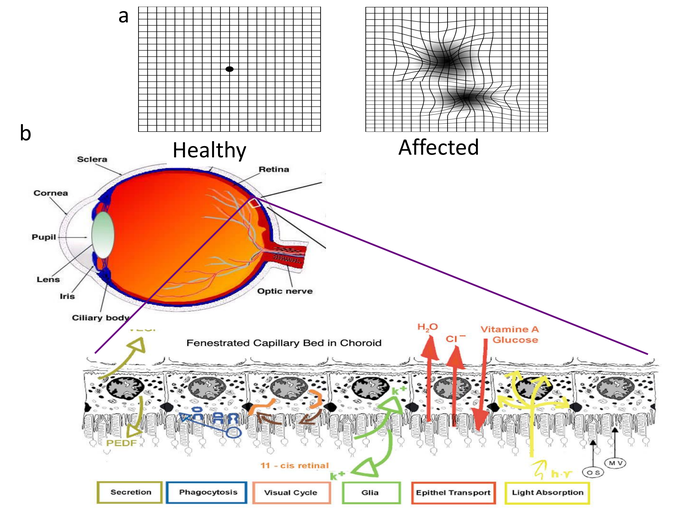
Abdullah Al-Ani[1,2], **Saud Sunba**[1,2], Muhammad Hafeez[1], Derek Toms[1] and Mark Ungrin[1]

Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary[1]

Cumming School of Medicine, University of Calgary[2]

RPE degeneration leads to vision problems

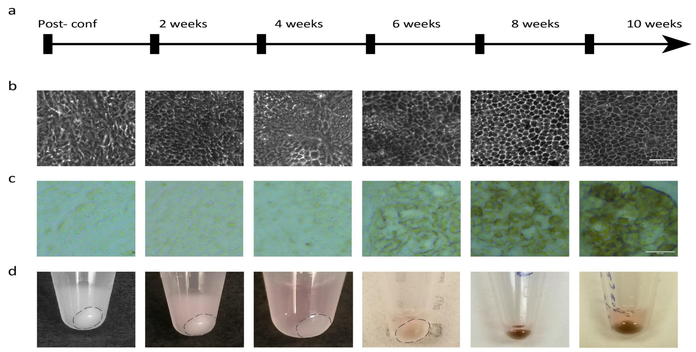
Age-related macular degeneration (AMD) is the leading cause of blindness in the industrial world and is often initiated by the dysfunction of the retinal pigment epithelium (RPE), the monolayer that trophically supports photoreceptor survival and function. RPE transplantation is a promising avenue to a potentially curative treatment for early stage AMD patients; however, RPE maturation is often overlooked as a quality control parameter. This project focuses on improving existing RPE transplantation to improve their success for AMD patients through broader understanding of RPE maturation.



(A) Visual representation of the effects of AMD on vision, through emphasis on deterioration of central vision. (B) RPE is outlined as part of the retina, where it supports retinal photoreceptors. RPE receive nutrients from the choroid and perform supportive functions such as secreting factors, phagocytosis, and visual cycle.

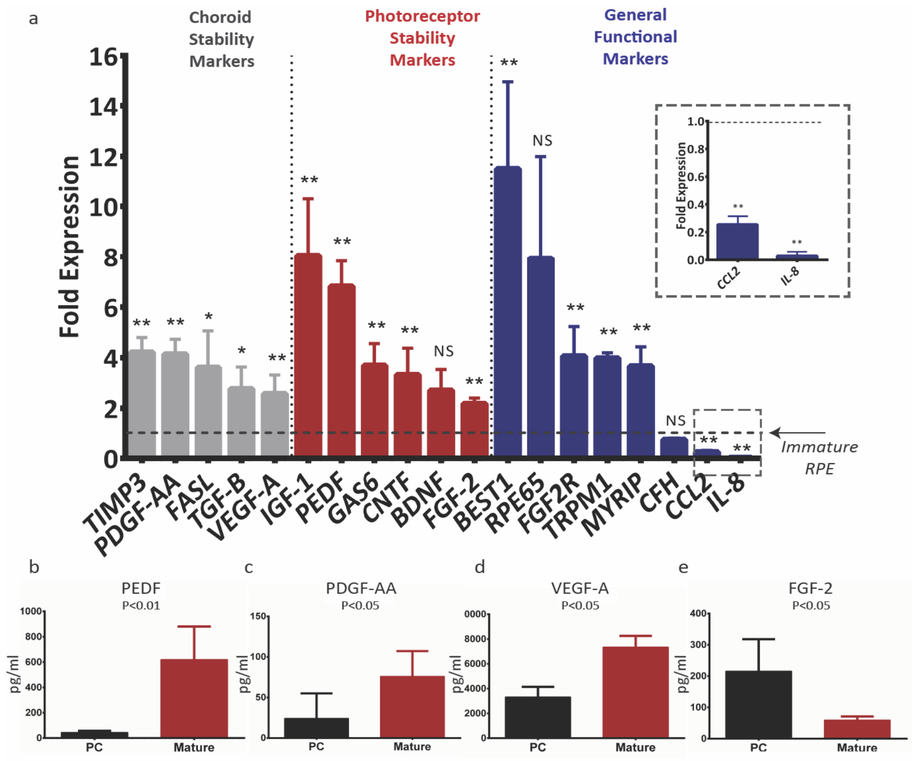
We hypothesize that longer RPE culture times result in superior RPE performance compared to immature RPE due to enhanced cellular niche and signaling.

RPE maturation enhances morphology and pigmentation



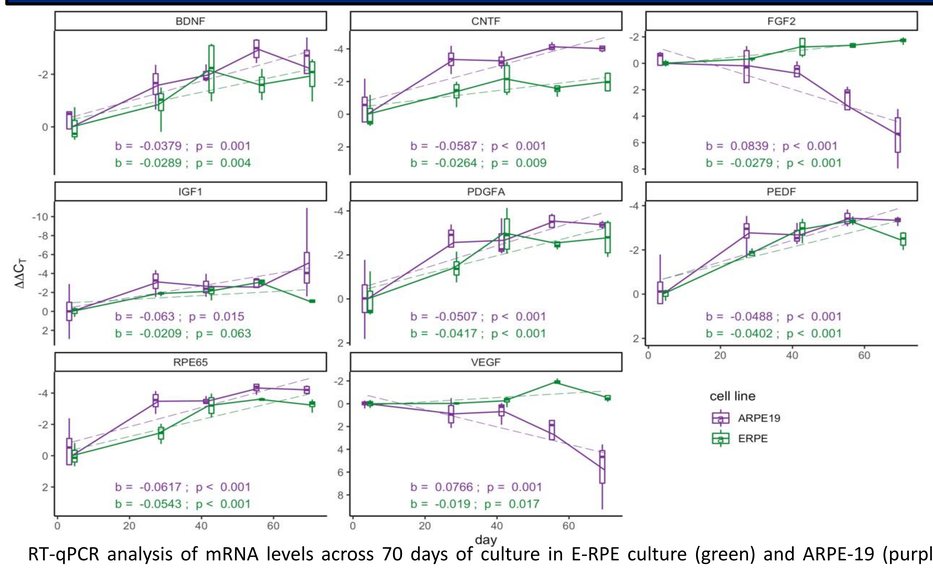
(a) the experimental timeline where ARPE-19 and ES-derived RPE were sampled for mRNA. (b) ES-derived RPE over time. (c) representative micrographs of the progressive pigmentation of the ES-derived RPE culture as it matures. (d) cellular pellets of ES-derived RPE demonstrate progressive pigmentation as a function of time.

hES-RPE Maturation upregulates the expression of desirable trophic factors



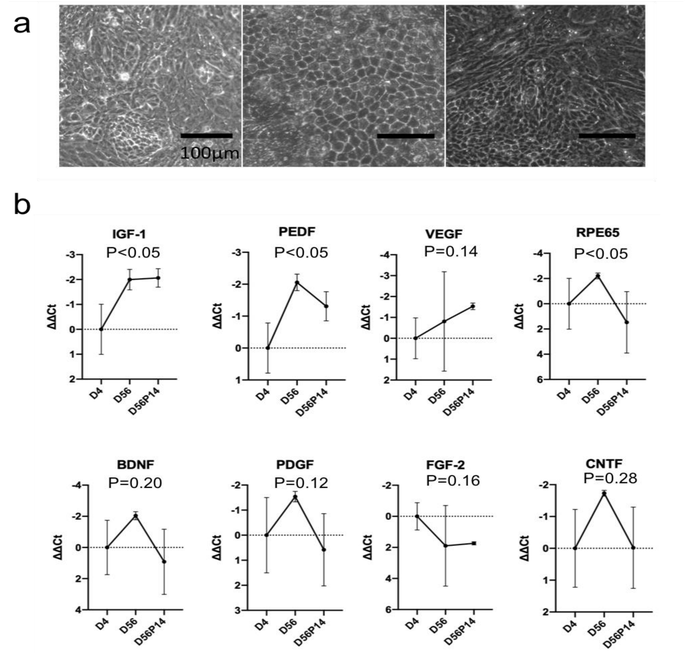
ES-derived RPE upregulate the mRNA levels of key RPE genes that enable RPE cells to act as effective niche cells for the retina. The upregulation of *PEDF*, *CNTF*, *IGF-1* and *BDNF* is of therapeutic value.
(a) mRNA levels of key RPE genes between the immature (post-confluent culture) and mature (6-8 weeks in culture) ES-derived RPE cells (n=5). The amount of PEDF (b) and PDGF (c), VEGF-A (d), and FGF-2 (e) proteins secreted (pg/mL) by our immature and mature ES-derived RPE cultures into the conditioned media (N=5).

mRNA levels of genes important to RPE function maximize after 42 days in culture



RT-qPCR analysis of mRNA levels across 70 days of culture in E-RPE culture (green) and ARPE-19 (purple). Results were normalized to an endogenous reference gene (*PPIA*) and are presented as ΔΔCt means (n = 4) ± standard deviation at each time point. A linear regression model (dashed line) was used to describe the relationship between ΔΔCt values and days of maturation of each gene for both RPE cell sources; regression coefficients, denoted as b, and associated p-values, to reject the null hypothesis that b = 0, are shown on each graph.

Markers of maturation are lost after passaging hES-RPE



(**a**) Morphology of E-RPE cells at confluence, four days after seeding (left), at 56 days of maturation (centre) and at a further 14 days after passaging (right). Scale bar represents 100 µm. (**b**) RT-qPCR analysis at three time points (4 days, 56 days and 56 + 14 days after passaging). Results were normalized to *PPIA* and represent mean ΔΔCt values (n = 3 or 4) ± standard deviation plotted on a negative Y-axis (higher expression at the top). Kruskal-Wallis one-way ANOVA test was used to compare ΔΔCt values of the various maturation points within each gene.

Conclusion: 6-8 week old RPE shows promise for therapeutic efficacy

- *In Vitro* maturation ES-derived RPE leads to differential expression of genes that carry out crucial RPE functions
- ES-derived RPE displays superior morphology and pigmentation as they mature.
- Mature RPE cultures upregulate therapeutically relevant neurotrophic factors such as *PEDF, IGF-1, CNTF, BDNF.*
- Passaging ES-RPE cells following maturity leads to decreased mRNA expression of RPE factors.
- We recommend that RPE cultures are matured for 6-8 weeks before being utilized for *in vitro* and *in vivo* studies related to Age-related macular degeneration.

References

Strauss O (2005) The retinal pigment epithelium in visual function. Physiol Rev 85:845-81
Wong, W.L., Su, X., Li, X., Cheung, C.M.G., Klein, R., Cheng, C.-Y., and Wong, T.Y. (2014). Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob. Heal. 2, e106–e116.

Acknowledgments

This project was supported by the following agencies and the Lydia Sikora Memorial Award, Queen Elizabeth II PhD award, MARKIN USRP award. We would also like to acknowledge the Biomedical Engineering graduate program and members of the Ungrin lab.

UNIVERSITY OF CALGARY
CUMMING SCHOOL OF MEDICINE

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

Alberta Children's Hospital Research Institute

LEADERS IN MEDICINE