Characterisation and development of a novel human retinal model for the study of diabetic retinopathy

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Aims

Currently, there is no model of the human retina that encompasses more than two different cell types

We aim to characterise a novel triple-cell culture human retinal model to allow for vital advancements in the study of diabetic retinopathy

Cell types used in triple culture

Retinal microvascular endothelial cells (HRMVEC)

Primary human ACBRI 181 (Cell Systems, USA). Cells were grown in Cell Systems culture (CS-C) medium

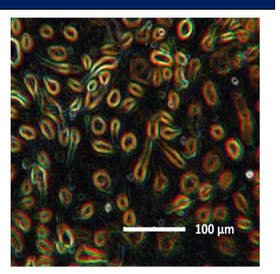

Figure 1- HRMVEC's

Müller glial cells (MIO-M1) Human MIO-M1 (Institute of Ophthalmology and Moorfields Eye Hospital, UK), grown in DMEM (+ glutamine, 10% FBS and 1% penicillin-streptomycin)

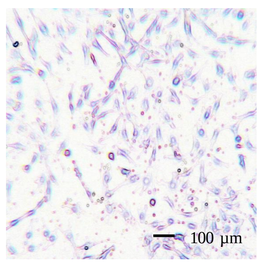

Figure 2- MIO-M1

Retinal pigment epithelial cells (RPEs) Human ARPE-19 (ATCC® CRL-2302, USA), grown in DMEM (+ glutamine, 10% FBS and 1% penicillin-streptomycin)

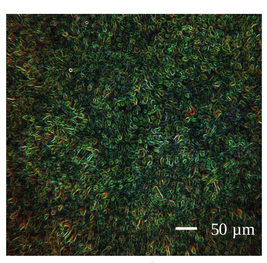

Figure 3- RPE's

Triple culture assessment

- Cell morphology for each cell type was analysed using cell staining (Hematoxylin) & ImageJ analysis
- Gene expression for vascular endothelial growth factor (*VEGF*) and pigment epithelial derived factor (*PEDF*) were assessed for each cell type
- RNA was extracted & reverse transcribed cDNA run on a BioRad CFX connect using SYBR Green I for each primer set
- Raw threshold cycles were normalised against *β-actin* housekeeper gene and analysed using the $2^{-\Delta\Delta CT}$ method and presented as fold change (FC)

Triple culture formation

- Multi-culture medium (MC Medium) consisting of 2:1, DMEM with a stable glutamine source: CS-C medium
- Co-culture schematic (Fig 4), RPEs were incubated for 28 days for maturation, MIO-M1 were seeded ($1x10^5$)(day 28) & HRMVECs were seeded ($1x10^5$ cells per cm^2)(day 30), model optimum on day 32-37

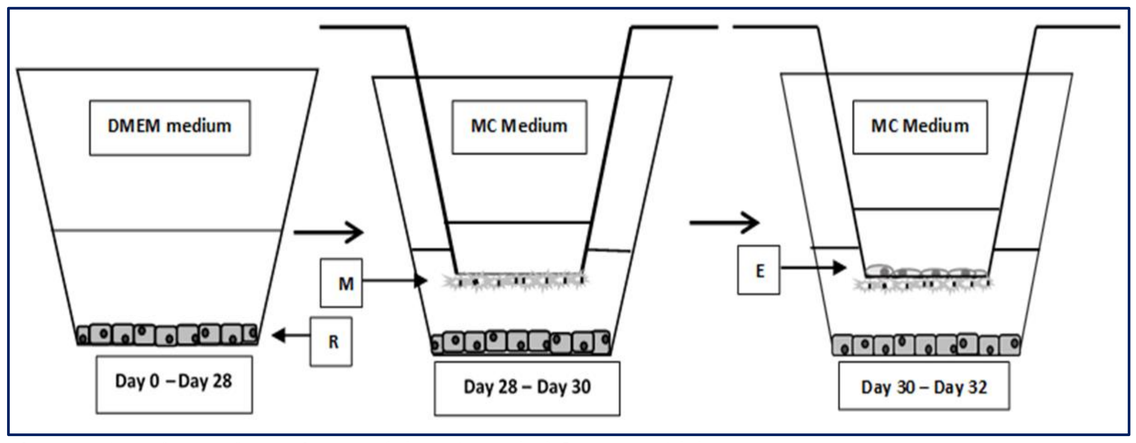

Figure 4- Co-culture schematic. RPEs (R), MIO-M1 (M) & HMVREC (E)

Results

- Cell morphologies were uniform across all culture formations (Table 1)

Table 1- Mean and standard deviation of cell diameter (µM). P-value determined using one way ANOVA.

Culture Formation	Cell Type		
	ARPE-19 (n=300)	MIO-M1 (n=300)	HMVREC (n=300)
Solo	17.5 (6.1)	62.7 (13.3)	98.9 (24.4)
Dual		61.9 (10.6)	97.8 (28.0)
Dual	18.1 (4.3)		96.0 (22.9)
Dual	17.9 (4.5)	63.8 (13.9)	
Triple	18.2 (5.3)	62.5 (14.3)	-
P-value	0.48	0.54	0.66

- *PEDF* expression was upregulated for both RPE (+5.2FC, $p<0.01$) and MIO-M1 (+7.5FC, $p<0.05$) in triple compared to solo-culture (Fig 5A)
- Triple vs solo-culture: *VEGF* expression was significantly decreased within HMVREC ($p<0.05$)(Fig 5B). Possible role of MIO-M1 in HMVREC *VEGF* expression (MIO-M1+HMVREC, $p<0.05$; RPE+HMVREC, $p=0.11$)

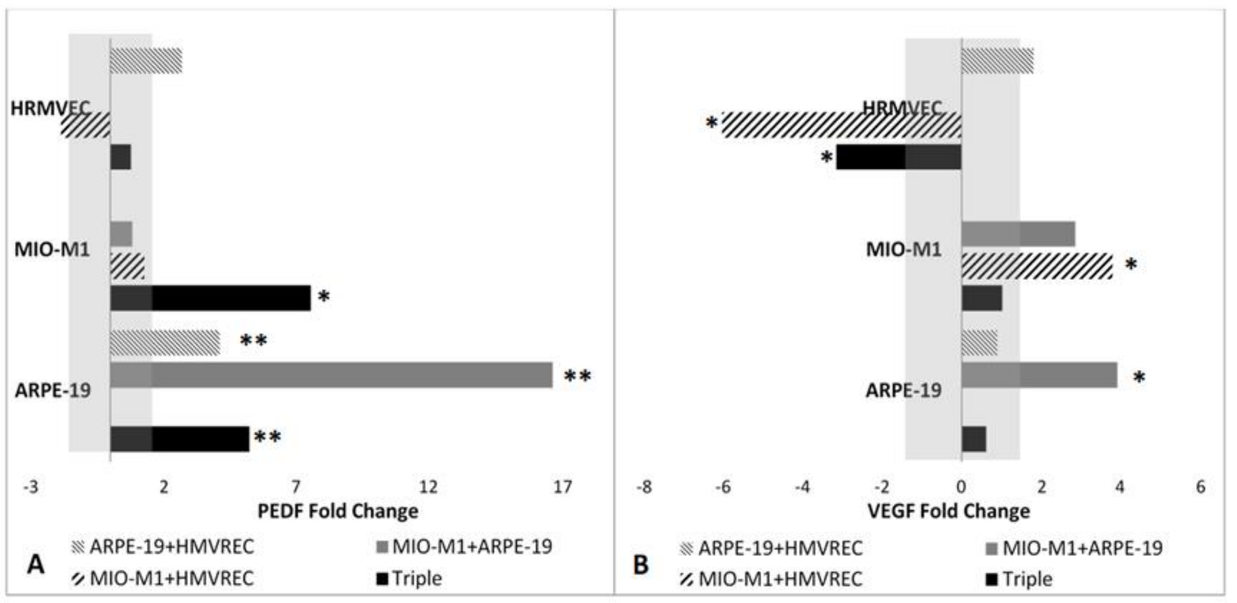

Figure 5A/B- Gene expression profiles, showing fold change (FC), *$p<0.05$, **$p<0.001$. A- *PEDF* FC. B- *VEGF* FC.

Conclusions

Results indicate Müller glial cells may have a regulatory role in the HMVREC expression of *VEGF*, demonstrating the importance of using multiple cell culture systems for assessment of retinal disease i.e. diabetic retinopathy and the evaluation of possible treatments.

Acknowledgements

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