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AUTHORS

AUTHORS (LAST NAME, FIRST NAME): Ratnayaka, J Arjuna[1]; Lynn, Savannah A.[1]; Griffiths, Helen[1]; Scott, Jenny[1]; Cree, Angela[1]; Lotery, Andrew J.[1]
INSTITUTIONS (ALL):
1. Clinical and Experimental Sciences., University of Southampton, Southampton, England, United Kingdom.
Commercial Relationships Disclosure (Abstract): J Arjuna Ratnayaka: Commercial Relationship: Code N (No Commercial Relationship) | Savannah Lynn: Commercial Relationship: Code N (No Commercial Relationship) | Helen Griffiths: Commercial Relationship: Code N (No Commercial Relationship) | Jenny Scott: Commercial Relationship: Code N (No Commercial Relationship) | Angela Cree: Commercial Relationship: Code N (No Commercial Relationship) | Andrew Lotery: Commercial Relationship: Code N (No Commercial Relationship)
Study Group:

ABSTRACT

TITLE: An ex-vivo platform for manipulation and study of Retinal Pigment Epithelial (RPE) cells in long-term culture.
ABSTRACT BODY:
Purpose: Impairment of the Retinal Pigment Epithelium (RPE) is strongly correlated with degenerative retinas including Age-related Macular Degeneration (AMD). Studies to elucidate dynamic intracellular processes underlying chronic degeneration of the RPE are limited by poor access of microscopes in the retinal space. Here we combine the use of an ex-vivo platform with live-confocal and ultrastructural imaging to study these events in individual RPE cells of mouse and human origin over long time periods. Our experimental model system provides a powerful tool to recapitulate chronic degenerative mechanisms in early AMD.
Methods: Confluent monolayers of RPE cells were grown on a synthetic porous support which mimics the Bruch's membrane. Cultures were maintained overs several months. We analysed morphology and barrier properties of the RPE monolayer, including expression of junctional complexes, trans-epithelial resistance (TER) as well as directional secretion of key RPE proteins. We used a combination of live-confocal microscopy, immunofluorescence, transmission electron microscopy (TEM), ELISA and biochemical approaches.
Results: Ultrastructural studies show formation of a monolayer with features typical of RPE cells, including melanin pigmentation, apical microvilli and basal infoldings. Ultrastructural mapping of lysosomes and mitochondria provided convenient readouts of key organelles linked with RPE dysfunction at nanoscale resolution. A mobile custom-designed chamber allowed longitudinal analysis of live-cellular physiology using organelle-specific probes LysoSensor blue/yellow and MitoTracker in long-term cultures. For the first time we show that primary mouse RPE cells can be cultured for several weeks with an average TER measurement of 55 ±0.69 Ω/cm2. Directionally secreted proteins VEGF (Vascular Endothelial Growth Factor) and Aβ (Amyloid beta) were quantified using ELISA.
Conclusions: Our ex-vivo model system which mimics the RPE/Bruch's complex can be subject to a high degree of experimental manipulation, and is a powerful tool to investigate dynamic intracellular events as well as ultrastructural changes associated with chronic RPE degeneration in the ageing retina. This tool may be utilized to study RPE physiology at single-molecule resolution, providing mechanistic insights into early AMD.
(No Image Selected)

DETAILS

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TRAVEL GRANTS and AWARDS APPLICATIONS

AWARDS: