Comparison of Growth and Monolayer Formation of Primary Bovine RPE and IPE on Polyurethane (PU) Membranes

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Introduction

Age-related macular degeneration (AMD) results in the deterioration of the retinal pigment epithelial (RPE) layer under the macula. A potential surgical treatment involves the replacement of the diseased RPE cells with healthy cells 1. Clinically, this requires identification of the appropriate cells to use and the optimal substrate for use as a transplant vehicle. Iris pigment epithelium (IPE) is derived from the same embryonic origin as RPE and has been shown to have several of the same functions. IPE can be harvested more easily than RPE. The aim of this study is to compare the behavior of primary bovine RPE and IPE cultured on polyurethane (PU) membranes with the objective of optimizing the cell/substrate combination for transplantation.

Materials and Methods

The PU used in this study was a b9™ (Biomer Technology Ltd.) membrane with thickness <100 µm. Freshly harvested bovine RPE (bRPE) and IPE (bIPE) cells were seeded on the b9 and tissue-culture polystyrene (TCPS) as control at 3.33 x 10⁴ cells/ml cultured with MEM media. The serum concentration was reduced from 20% to 5% and retinoic acid added when the cells reached 90% confluence in order to encourage differentiation. Cell morphology was assessed by phase contrast microscopy at day 25. Substrates were fixed at 25 days and stained for F-actin and the marker of tight junction formation; zonula occludens-1 (ZO-1). Nuclei were stained with DAPI. Cytokeratin staining was used to confirm the epithelial phenotype.

Results

The PU membrane supported the growth of bRPE (Fig 1) and bIPE (Fig 2) cells. Both cell types demonstrated good epithelial morphology, confirmed by cytokeratin expression. Nuclei were dispersed evenly throughout the layer and there was evidence of formation of junctional proteins ZO-1 at the cell borders.

Discussion and Conclusions

The primary IPE cells were shown to attach, grow and form a monolayer of epithelial cells on the PU membranes in much the same way as the RPE cells. It was found that the morphology of both cell type was better when the cells were seeded directly on the PU substrates rather than expanding the cells on TCPS prior to subculturing onto the PU membranes. These results are encouraging and justify further study of the functional behaviour of the IPE cells on the PU.

References


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Disclosures

The authors have nothing to disclose.