Differentiation of Human Pluripotent Stem Cells to Retinal Pigmented Epithelium using Purified Extracellular Matrix Proteins

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Summary: Human pluripotent stem cells were found to generate retinal pigmented epithelial cells on multiple substrates and extracellular matrix proteins in defined culture conditions that may be translatable to scaffold designs for transplantation efforts.

An appealing use of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) is to create retinal pigmented epithelium (RPE) to treat age-related macular degeneration (AMD), an incurable retinal disease that is a leading cause of blindness1. RPE derived from hESCs (hESC-RPE) and iPSCs (iPSC-RPE) express essential RPE markers and can rescue visual function in animal models2. However, current culture methods are inefficient and use animal-derived products, making them not suitable for translation to the clinic. Furthermore, we found that differentiating iPSCs yielded significantly fewer RPE than hESCs did. Because the extracellular matrix (ECM) can affect differentiation, we hypothesized that certain ECM proteins and ECM-based substrates may improve iPSC-RPE differentiation.

We selected several purified ECM proteins and substrates, based on the ECM environment surrounding RPE in vivo, and tested their ability to support iPSC-RPE differentiation and maintenance. The selected ECM proteins and substrates included laminins (LM), collagens (Coll), fibronectin (FN), vitronectin (VN), elastin, and Matrigel. iPSCs differentiated on nearly all substrates developed pigmented regions (Fig. 1a). iPSC differentiation on Matrigel and mouse LM-111 resulted in relatively high levels of pigmentation (Fig. 1b). iPSC-RPE cultured on several substrates developed confluent monolayers with normal RPE morphology and expression of key RPE genes.

iPSCs and hESCs differentiated entirely on mouse laminin-111 produced RPE-like cells expressing RPE proteins and displaying functional RPE activity. Laminin-111 and other ECM proteins investigated in this study may assist with future transplantation scaffold designs by providing peptide sequences for use in clinically-relevant, synthetic generation and maintenance of RPE from human pluripotent stem cells.