Supplementary Information

Microcarrier-Based Culture of Human Pluripotent Stem-Cell-Derived Retinal Pigmented Epithelium

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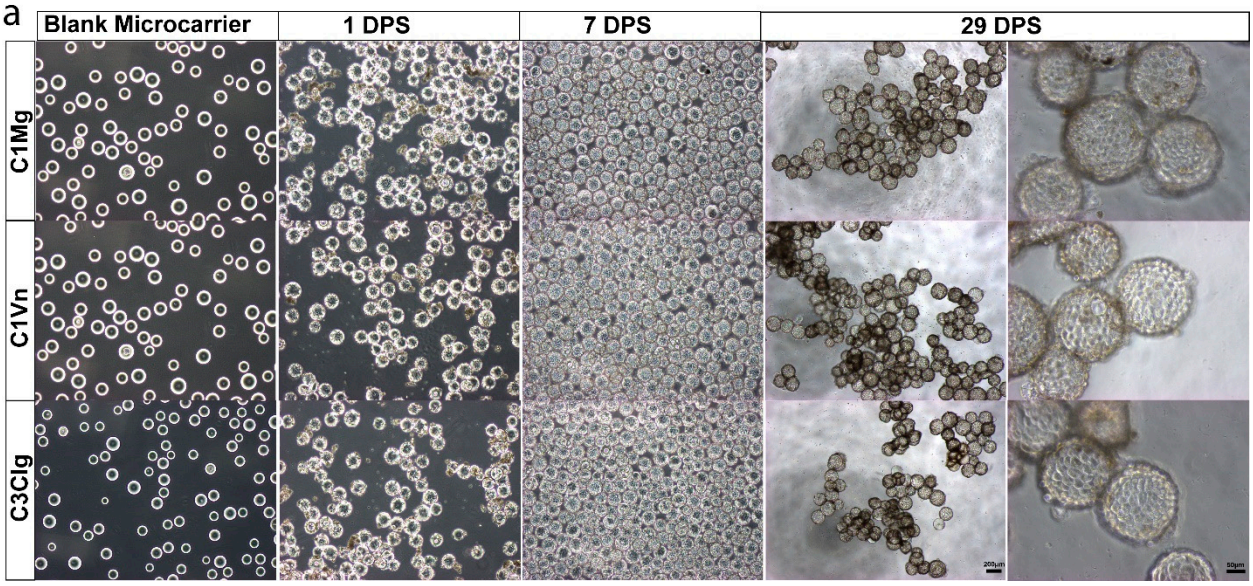

Figure S1. mcRPE mature and pigment over 30-day culture period**.** Phase contrast images demonstrate attachment of hESC-RPE cells to Matrigel and recombinant human vitronectin coated Cytodex 1 as well as pre-coated collagen Cytodex 3 microcarriers. mcRPE display cobblestone morphology, phase bright borders and pigmentation 29DPS.

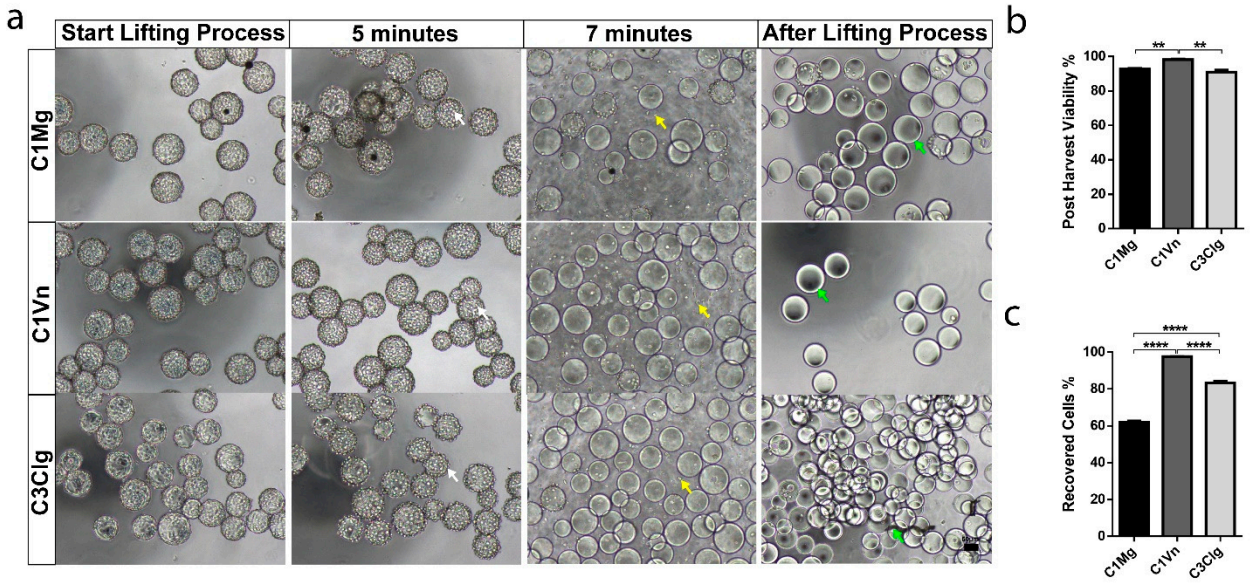

Figure S2. Harvesting hESC-RPE cells from microcarriers using a xeno-free enzymatic disassociation reagent. **a)** mcRPE were collected at a density of $2x10^5$ cells per well and exposed to TrypLE enzyme for a period of 7 min. Cells were still attached at 5 min (white arrows) and were completely dissociated after mechanical perturbation at 7 minutes. Dissociated cells were clearly visualized post perturbation (yellow arrow) and harvested by filtration to separate microcarriers from cells. Empty microcarriers (green arrow) after filtration process demonstrates appropriate harvesting of hESC-RPE cells. **b)** Viability of harvested hESC-RPE cells were assessed using Acridine Orange/DAPI exclusion on an NC200. All three conditions demonstrate high viability with C1Vn exhibiting significantly greater viability than C1Mg and C3Clg (**$p<0.05$). **c)** Cell recovery was quantified using Acridine Orange/DAPI and compared to initial starting density equating to an approximate $2x10^5$ cells. All three conditions were amenable to cell recovery but C1Vn exhibited a significantly greater percentage of recovered cells compared to C1Mg and C3Clg. C1Mg was the least favorable coating for cell recovery (****$p<0.001$). Statistical analysis, one way ANOVA, Tukey's correction.

Table S1. Calculations to determine the required surface area to meet the estimated patient demand and approaches to achieve.

*Surface Area Required to Supply $8x10^9$ RPE Cells**	$\frac{8\times10^9\ \boldsymbol{RPE\ cells}}{\boldsymbol{year}}\times\frac{\boldsymbol{cm^2}}{\boldsymbol{2.1\times10^5\ RPE\ cells}}\times\frac{\boldsymbol{1\ year}}{\boldsymbol{12\ months}}=\frac{\boldsymbol{3,174cm^2}}{\boldsymbol{month}}$
Approaches to Achieve $\frac{3,174cm^2}{month}$	
T-75 Flasks	$\frac{3,174cm^2}{month}\times\frac{1\ flask}{75cm^2}\cong\frac{\boldsymbol{42\ flasks}}{\boldsymbol{month}}$
T-225 Flasks	$\frac{3,174cm^2}{month}\times\frac{1\ flask}{225cm^2}\cong\frac{\boldsymbol{14\ flasks}}{\boldsymbol{month}}$
Cytodex 1	$\frac{3,174cm^2}{month}\times\frac{g\ of\ Cytodex1}{4400cm^2}\times\frac{L}{5\ g\ of\ Cytodex^{**}}=\frac{\boldsymbol{144mL\ Bioreactor\ Volume}}{\boldsymbol{month}}$
Cytodex 3	$\frac{3,174cm^2}{month}\times\frac{g\ of\ Cytodex3}{2700cm^2}\times\frac{L}{5\ g\ of\ Cytodex^{**}}=\frac{\boldsymbol{235mL\ Bioreactor\ Volume}}{\boldsymbol{month}}$

* Estimate of the required cells assumes that only half of the projected number of AMD patients in the United States receives an RPE cell-based therapy [36].

** Microcarrier cultures may contain 1-5g of Cytodex/L [21].