Table 1 - Ideal hydrogel characteristics for differentiation and maturation of human iPSC-derived retina.
Polymerizable under biocompatible conditions
Low mechanical strength with high water swelling
Large average pore size with interconnected geometry
Low crosslinking density
Sufficient attachment domains
Scaffold degradation timed to new tissue formation
Benign degradation products

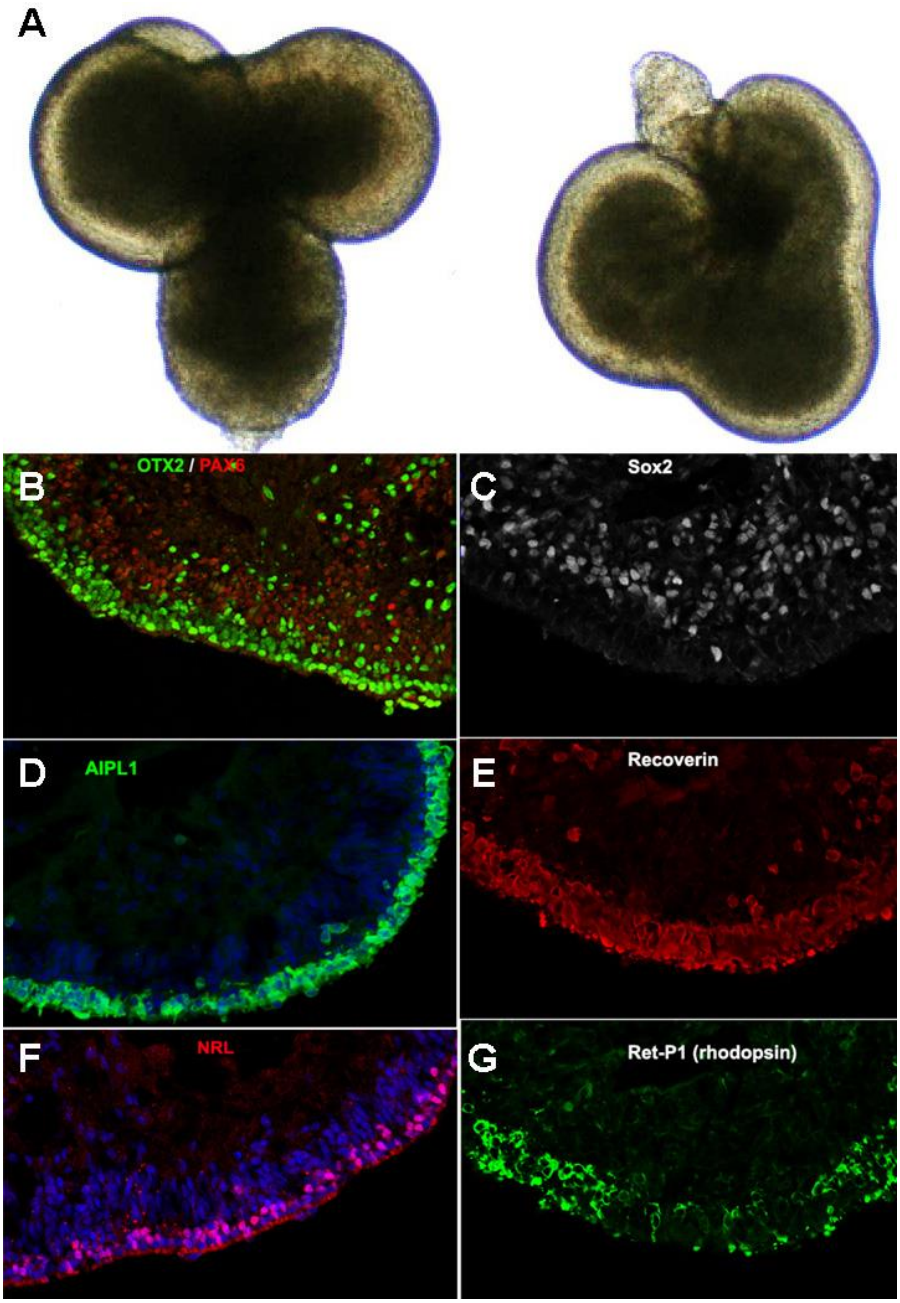

Figure 1: Neuro-Retinal Organoid Culture.
A. Representative images of human retinal organoids following 6 weeks of low-attachment culture. B-G Representative ICC images of 3-month organoids showing expression of retinal stem cell marker, SOX2 and PAX6, and photoreceptor markers, OTX2, AIPL1, RCVRN, NRL and Rhodopsin (Ret-P1 antibody) in a typical laminated pattern.

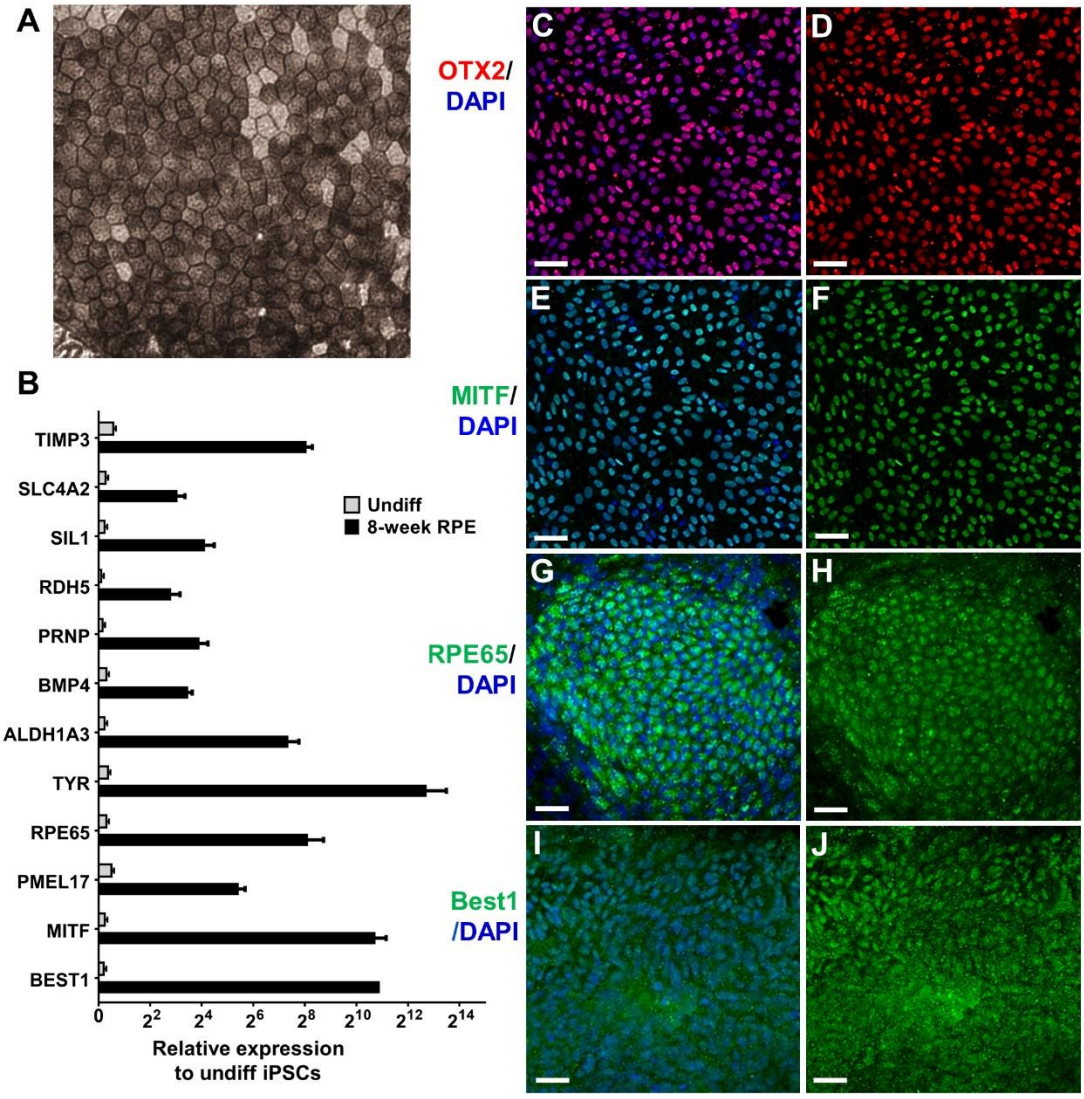

Figure 2: RPE differentiation of human iPSCs

(A) Representative brightfield microscopy image showing RPE cultures exhibiting typical cobblestone morphology and pigmentation at 8 weeks of differentiation. (B) QRT-PCR data showing the expression of various immature and mature RPE genes including BEST1, PMEL17, RPE65, MITF, TYR, ALDH1A3, BMP4, PRNP, RDH5, SIL1, SLC4A2 and TIMP3 in cells. (C-F) ICC analysis showing most of the cells in culture expressed RPE-specific transcription factors OTX2 (C, D) and MITF (E, F). (G-J) RPE-specific markers RPE65 (G, H) and Bestrophin (I, J) were also expressed in cells at 8 weeks of culture. DAPI (blue) stains the nuclei of the cells. Scale bars= 20µm.

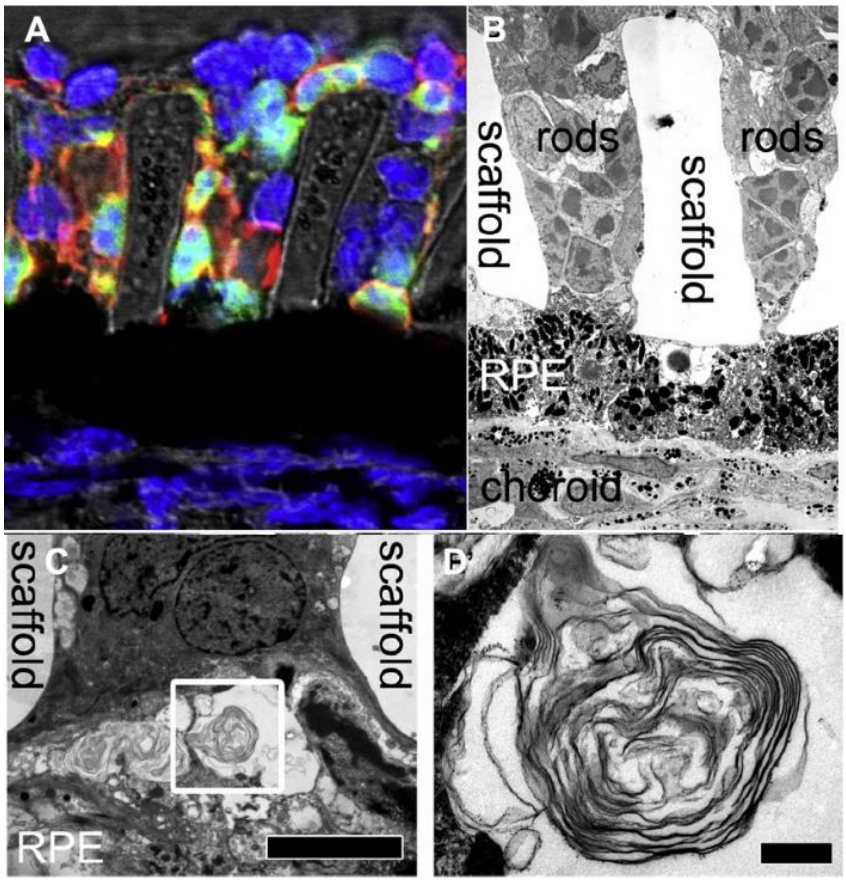

Figure 3: Disc formation *in vitro* in the Polymer-Retina RPE sandwich. (A) shows mouse retinal cells growing in the pores of the scaffold cultured against the pigmented RPE on a filter. (B) TEM of the bioengineered tissue. (C-D) show higher magnification view of the disc structures.

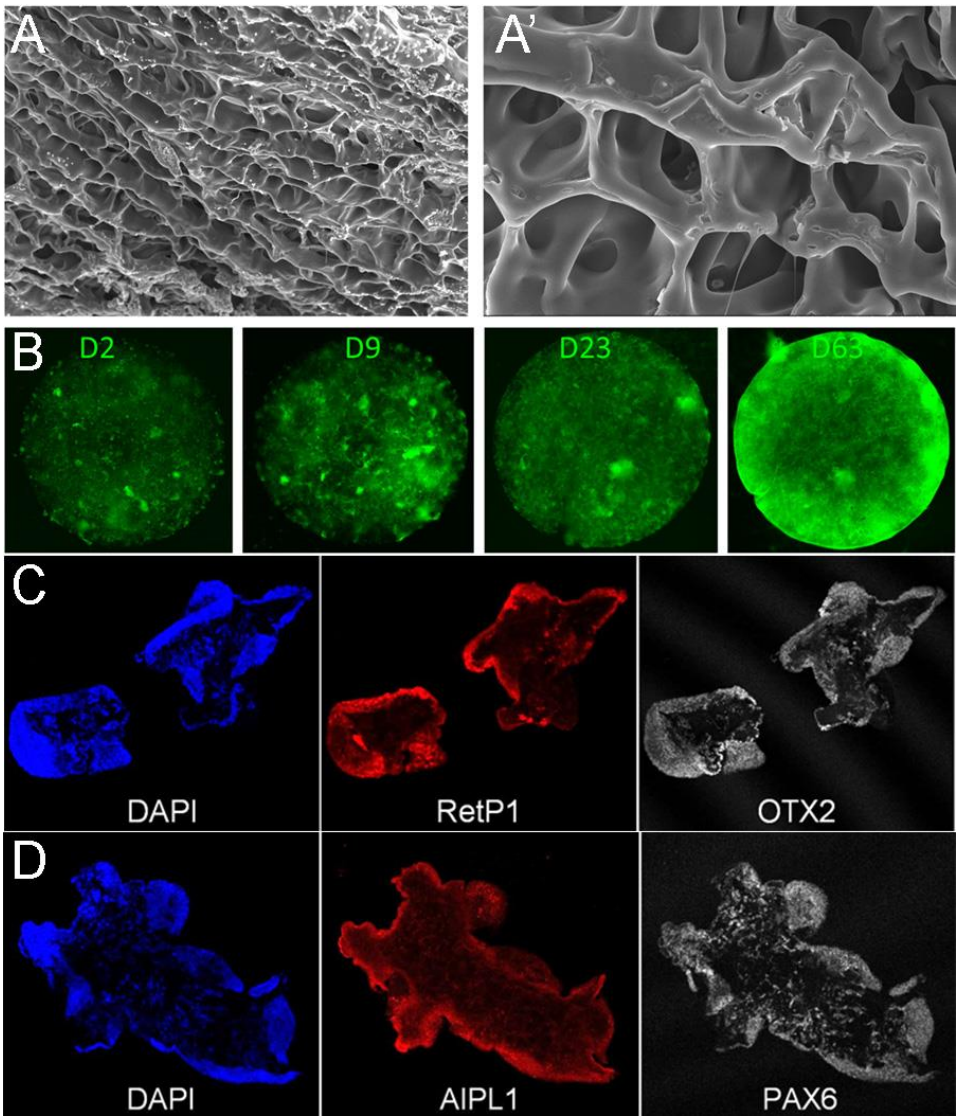

Figure 4: GelMA-based bioink characterization. A-A' are representative SEM scans of the hydrogel polymerized using 0.4% LAP and 30sec UV exposure. The hydrogels have uniform interconnected pores ranging in size from 15-35µm. (B) Live culture images of GFP-expressing hiPSC-derived retinal cells in GelMA hydrogel over 2 months. The images belong to a single disc imaged at days 2, 9, 23 and 63 following polymerization confirming biocompatibility and the ability of the retinal stem cells to proliferate and thrive in the hydrogel. (C-D) ICC images of a cellularized neural-retina-bioink hydrogel scaffold at 3 months. We observe lamination of photoreceptors marked by OTX2, AIPL1 and RetP1 on the outside of the organoid and Pax6-expresisng inner retinal neurons on the inner layers.

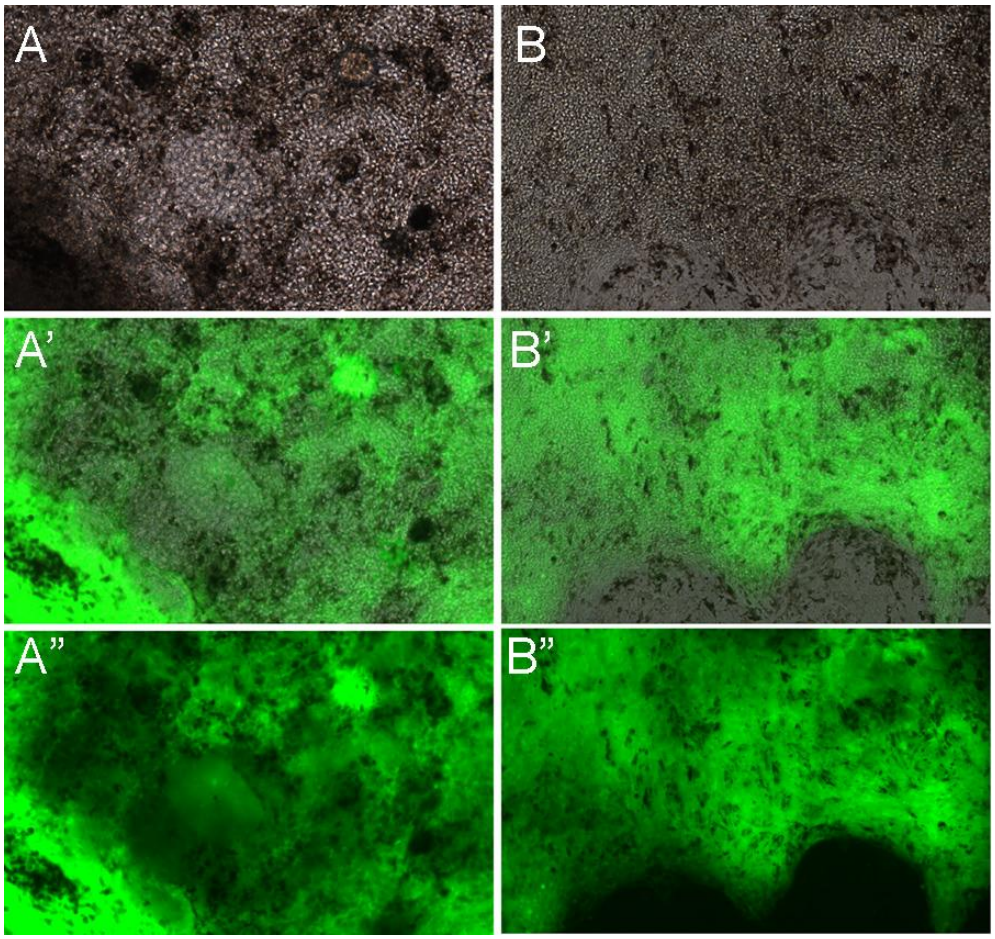

Figure 5: Neural Retina-Bioink matrix cultured over RPE monolayer. (A-A" and B-B") Representative images showing two regions of co-culture of GFP-expressing human iPSC-derived retinal cells polymerized in GelMA-based bioink over a layer of non-GFP expressing human ESC-derived RPE monolayer 7 days following co-culture. A, B show the brightfield images with pigmented RPE cells and A" and B" show the GFP-fluorescence emanating from the neural retinal cells within the GelMA scaffolding with merged views in A' and B'.

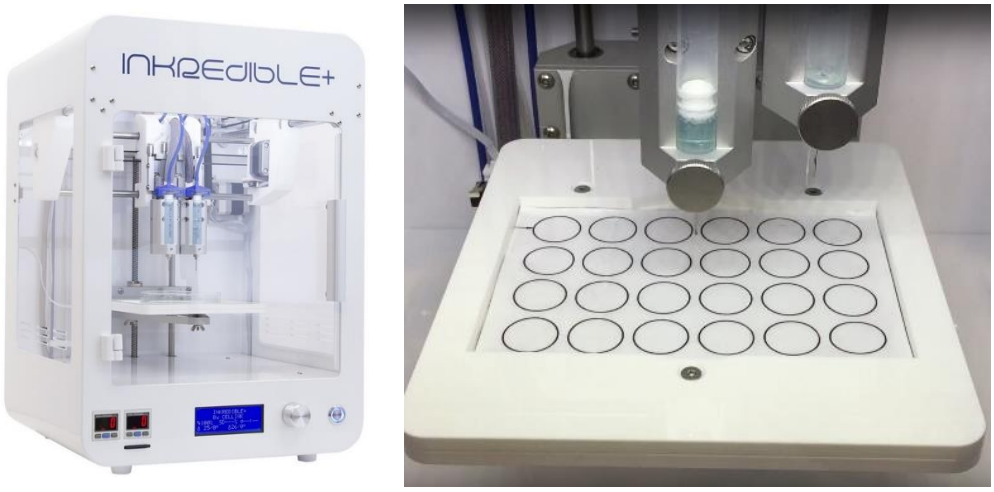

Figure 6 – Dual-head Inkredible+ bioprinter performing XY calibration for multi-well printing. The printer has HEPA filters and UV light to maintain a clean environment. The software is customizable to adapt to multi-well printing to user specifications by editing the gcode files.