Characterization of Engineered Macrophages and other Myeloid Cells Differentiated from CD34+ Hematopoietic Progenitor Cells Derived from Pluripotent Stem Cells

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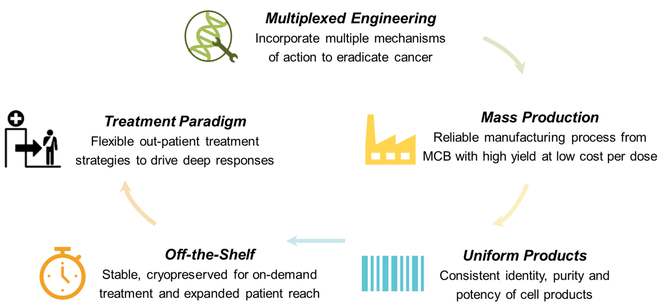
Executive Summary

Human induced pluripotent stem cells (hiPSCs) exhibit unlimited self-renewal potential and have the capacity to differentiate into somatic cell derivatives of all three germ layers, making them an ideal source for generation of cellular therapies. hiPSCs also address the inherent variability that arises from the use of patient- or donor-sourced cells which can lead to drug product inconsistencies that impact safety, efficacy, and therapeutic utility. To leverage these attributes, we have established a platform that enables multiplexed engineering of hiPSCs at the single-cell level and have developed a proprietary stage-specific differentiation protocol to support definitive hematopoiesis for the derivation of CD34+ hematopoietic progenitor (iCD34) cells. We have previously shown that these iCD34 cells exhibit efficient differentiation and expansion to lymphoid lineages, including Natural Killer (NK) and αβ T-cells

Additionally, iCD34 cells can efficiently give rise to myeloid populations including myeloid progenitor cells and macrophages. To this end, we have established a process for differentiating our engineered iCD34 cells into myeloid populations. Leveraging key aspects of definitive hematopoiesis, the iCD34 cells expand and differentiate into myeloid progenitor and uniformly express known macrophage markers including CD45, CD11b and CD14.

Graphical Representation



Conclusions

- iMacs can be made in uniform and consistent manner from hiPSCs, express canonical macrophage markers and display a gene expression profile like their peripheral blood derived counterparts.
- iMacs can acquire further specification based on environmental stimuli and can be broadly classified into M1-like/pro-inflammatory and M2-like/anti-inflammatory populations.
- Importantly, iMacs demonstrate macrophage function such as phagocytosis and the secretion of polarization specific cytokines.
- iMacs display biodistribution to various tissues, including the lung, following intravenous tail vein dosing.
- Collectively, the data confirm that the iCD34 cells generated from our hiPSC platform are multipotent hematopoietic progenitor cells and can efficiently expand and differentiate into lymphoid and myeloid lineages.

Results

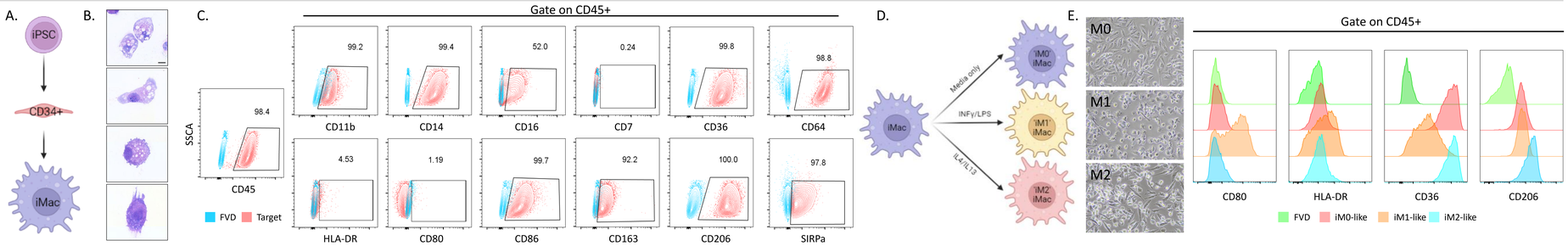


Figure 1. Derivation, cell surface phenotype and polarization of Fate iPSC derived Macrophage. (A) iMacs are produced by directed differentiation of iPS cells to CD34+ hematopoietic progenitors and then to CD45+, CD11b+, CD14+ macrophages. (B) Giemsa stain of iPSC derived macrophages. (C) Flow cytometry of select macrophage markers in iMacs. (D) Schematic illustrating method to polarize iMacs with pro-inflammatory (M1) or anti-inflammatory (M2) stimuli. (E) Macrophages acquire further specification based on environmental stimuli and display M1-like/pro-inflammatory or M2-like/anti-inflammatory morphological changes and polarization specific cell surface marker expression. Cartoons created using BioRender.com.

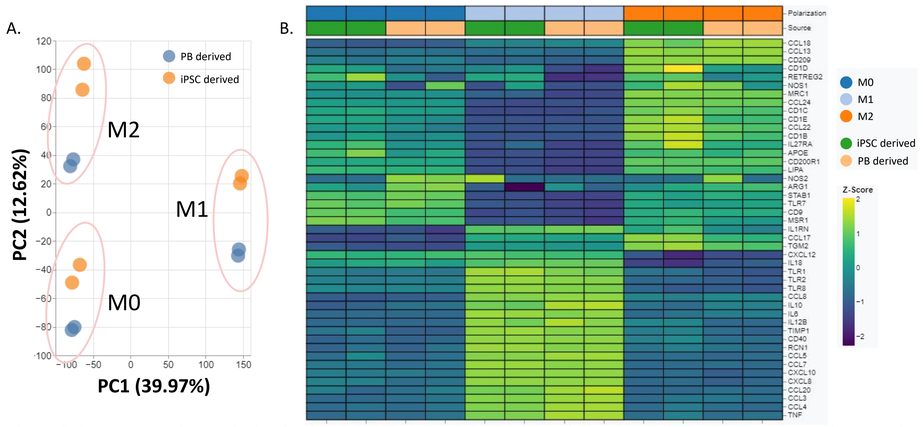


Figure 2. Gene expression analysis of polarized iPSC derived and Peripheral Blood (PB) derived macrophages. iMacs and PB derived macrophages were polarized as in Figure 1D then analyzed through Bulk RNA sequencing. (A) Principal component analysis reveals primary clustering by polarization and not source. (B) Heatmap analysis of differentially expressed genes between macrophage source (iPSC or PB) and polarization state (M0, M1, M2).

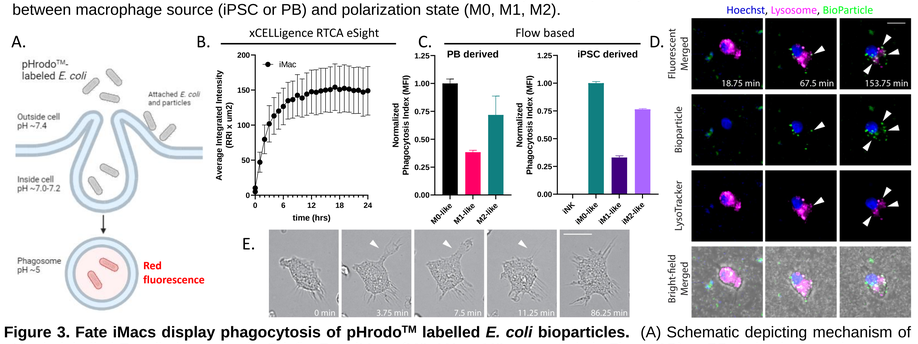


Figure 3. Fate iMacs display phagocytosis of pHrodo™ labelled *E. coli* bioparticles. (A) Schematic depicting mechanism of pHrodo-labelled E. coli phagocytosis. Briefly, Fate iMacs (M0, M1 or M2 generated via Figure 1D) or PB derived macrophages are incubated with pHrodo™ labelled E. coli bioparticles and analyzed on respective platforms. (B) Red Average Integrated Intensity (e.g., red fluorescence per um^2) measured by an xCELLigence RTCA eSight, over a 24 hr period (C) pHrodo fluorescence measured via flow cytometry after 1.5-hour incubation and (D) live cell imaging of iM2 phagocytosis using a Keyence Live Cell Imager depicting overlap of pHrodo labelled Bioparticle and lysosome. (E) Keyence live cell imaging of lamellipodia extension and retraction in iMacs incubated with pHrodo labelled E. coli bioparticle.

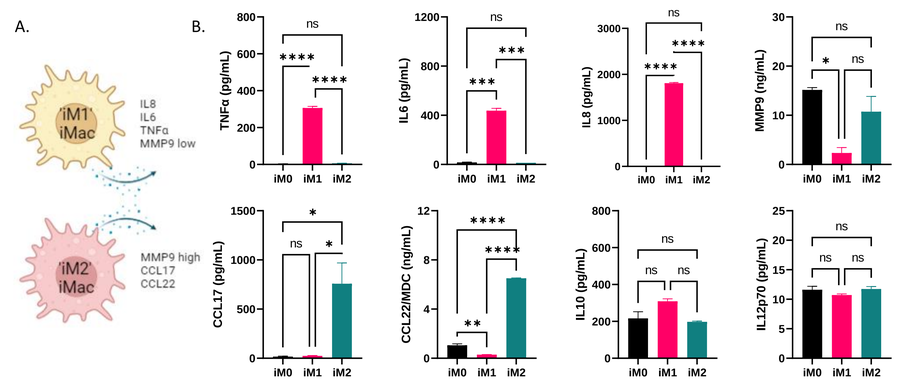


Figure 4. Fate iMacs display environmental specific cytokine release based on polarization state. Briefly, iMacs were polarized following Figure 1D. (A) Schematic depicting cytokine release profile of pro-inflammatory and anti-inflammatory polarized iMacs. Following polarization supernatant was collected and analyzed by R&D Quantikine ELISA kits for selected cytokines. (B) Quantified TNFα, IL6, IL8, MMP9, CCL17, CCL22, IL10, and IL12p70. Statistical analysis performed by One-way ANOVA with Tukey post-hoc test correction. *p<0.0332, **p<0.0021, ***p<0.0002, ****p<0.0001.

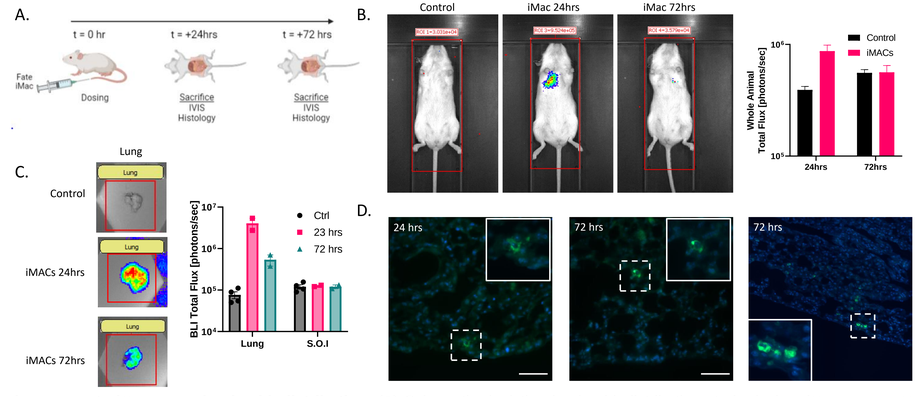


Figure 5. Fate iMac Lung *in vivo* biodistribution. (A) Schematic depicting in vivo biodistribution study design. iMacs were I.V. dosed at time 0 and analyzed by in situ and ex vivo imaging and histology at 24 and 72 hours. (B) Whole body in situ IVIS imaging at 24 and 72 hours. (C) Ex vivo IVIS imaging of Lung at 24 and 72 hours. (D). Lung histology for GFP-iMacs at 24 and 72 hours.