Generation of Microglia From Human Pluripotent Stem Cells for Neurodegenerative Disease Modeling

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INTRODUCTION

Microglia are critical modulators of neurodegenerative disease. Since microglia are of mesodermal origin, current human pluripotent stem cell (hPSC)-derived neuroectodermal differentiation models do not give rise to this critical cell type. Based on the publications of Abud et al.[1] and McQuade et al.[2], we have developed media that robustly produce functional microglia-like cells. These cells are distinct from other mononuclear cell types (e.g. monocytes or macrophages) and express characteristic protein and gene markers across multiple different embryonic stem (ES) and induced pluripotent stem (iPS) cell lines maintained on either mTeSR™1, TeSR™-E8™, or mTeSR™ Plus. Additionally, we show that these cells can be co-cultured with brain organoids and display a reactive-type morphology in response to injury.

METHODS

Microglia Differentiation: hPSCs maintained in either mTeSR™1, TeSR™-E8™, or mTeSR™ Plus were differentiated into hematopoietic progenitor cells (HPCs) using STEMdiff™ Hematopoietic Kit (Catalog #05310) for 12 days. On day 12, the HPCs were collected and differentiated using the STEMdiff™ microglia culture system for 28 - 34 days (Figure 1). At the end of the maturation stage (day 34), the cells were characterized by flow cytometry. Expression of microglia-specific genes were assessed by qPCR. IBA1 and PU.1 expression were assessed by immunocytochemistry. Functional characterization was performed using 1 µg/mL pHrodo™ Red Zymosan Bioparticles™ over a 12-hour incubation period on an Incucyte® S-3 Live-Cell Analysis System.

Monocyte Selection and Differentiation: CD14+ monocytes were isolated with EasySep™ Human CD14 Positive Selection Kit II (Catalog #17858) from human peripheral blood leukapheresis packs. hPSCs maintained in mTeSR™1 were differentiated using STEMdiff™ Hematopoietic Kit for 7 days. The cultures were switched to STEMdiff™ Monocyte Kit (Catalog #05320). On days 17 - 23, the cells expressed high levels of CD14 fluorescence intensity and were either analyzed by flow cytometry or further differentiated to M1-activated macrophages.

M1-Activated Macrophage Differentiation: iPS cell-derived and blood leukapheresis-isolated monocytes were differentiated in ImmunoCult™-SF Macrophage Medium (Catalog #10961) for a total of 6 or 8 days. The macrophages were activated to the M1-phenotype with 10 ng/mL LPS and 50 ng/mL IFN-γ at day 4 or 6 for 2 days before being collected for flow cytometry.

hPSC-Derived Microglia and Cerebral Organoid Co-Culture: 2.5 - 5 x 10^5 microglia were added to one well containing a single 200-day-old dorsal forebrain patterned brain organoid (BORGs) produced using STEMdiff™ Dorsal Forebrain Organoid Kit for 10 days in STEMdiff™ Neural Organoid Maintenance Medium. An injury was inflicted on the co-culture by piercing the organoid with a 25G needle on day 7.

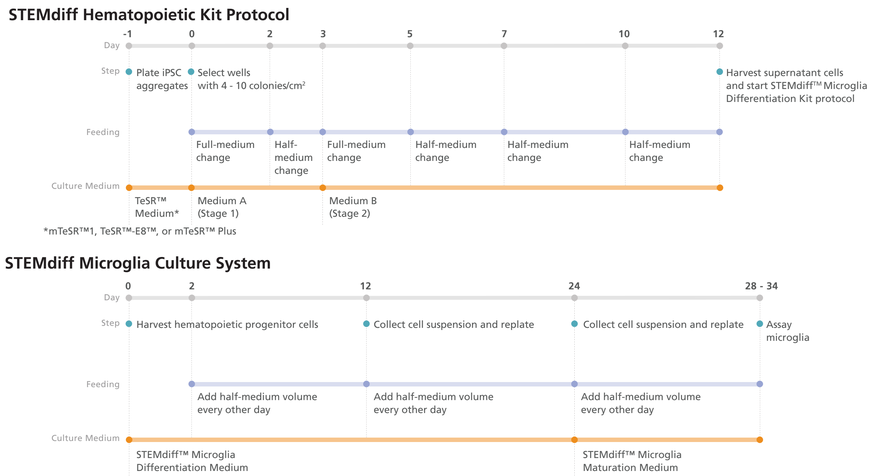
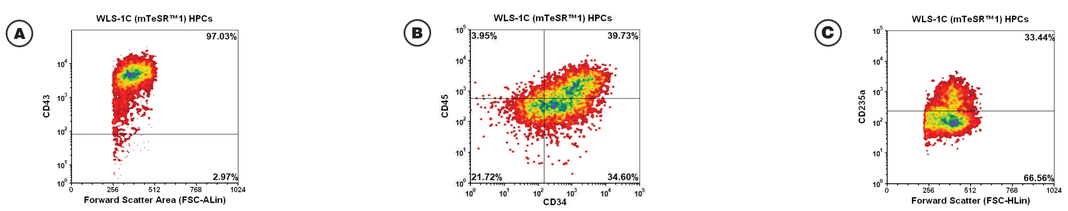


FIGURE 1. Workflow for hPSC-Derived Hematopoietic Progenitor Differentiation and Microglia Differentiation and Maturation

RESULTS



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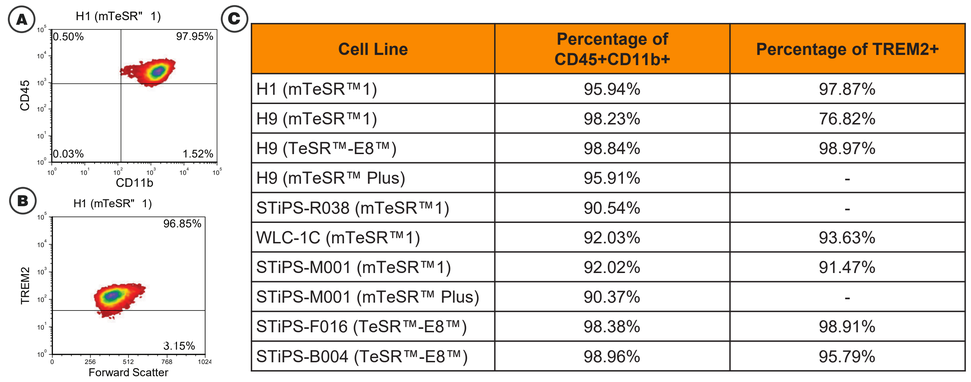
Cell Line	Percentage of CD43+	Percentage of CD34+/CD45+	Percentage of CD235a+
H1 (mTeSR™1)	95.77	36.16	33.15
H7 (mTeSR™1)	97.50	24.53	56.45
H9 (mTeSR™1)	97.97	32.30	43.35
WLS-1C (mTeSR™1)	98.18	49.90	12.01
STiPS-M001 (mTeSR™1)	-	60.76	58.38
H1 (TeSR™-E8™)	-	48.48	-
H9 (TeSR™-E8™)	99.05	39.34	31.03
STiPS-F016 (TeSR™-E8™)	98.53	39.05	33.19
STiPS-BO04 (TeSR™-E8™)	97.81	40.32	18.84

FIGURE 2. STEMdiff™ Hematopoietic Kit Robustly Generates a Starting Population of > 95% CD43+ HPCs by Day 12
(A-C) Representative flow cytometry plots for CD43 versus forward scatter **(A)**, CD34 and CD45 co-staining **(B)**, and CD235a versus forward scatter **(C)** in HPCs generated using the WLS-1C (iPS) cell line maintained in mTeSR™1. **(D)** Percentage of CD43- and CD45/CD34-expressing HPCs generated from STEMdiff™ Hematopoietic Kit across all cell lines tested.



Cell Line	Percentage of CD45+CD11b+	Percentage of TREM2+
H1 (mTeSR™1)	95.94%	97.87%
H9 (mTeSR™1)	98.23%	76.82%
H9 (TeSR™-E8™)	98.84%	98.97%
H9 (mTeSR™ Plus)	95.91%	-
STiPS-R038 (mTeSR™1)	90.54%	-
WLC-1C (mTeSR™1)	92.03%	93.63%
STiPS-M001 (mTeSR™1)	92.02%	91.47%
STiPS-M001 (mTeSR™ Plus)	90.37%	-
STiPS-F016 (TeSR™-E8™)	98.38%	98.91%
STiPS-B004 (TeSR™-E8™)	98.96%	95.79%

FIGURE 3. Optimized Microglia Media Consistently Generate Microglia-Like Cells Expressing Expected Markers by Day 34.
Representative flow cytometry plots for CD45 and CD11b co-staining **(A)** and TREM2 **(B)** versus forward scatter in microglia generated using the H1 (ES) cell line maintained in mTeSR™1. **(C)** The percentage of CD45/CD11b co-expression and TREM2 expression from the STEMdiff™ Microglia Culture System across all cell lines tested. **(D, E)** Representative images of microglia stained with IBA1 and PU.1 taken at 20X; scale bars = 50 µm.

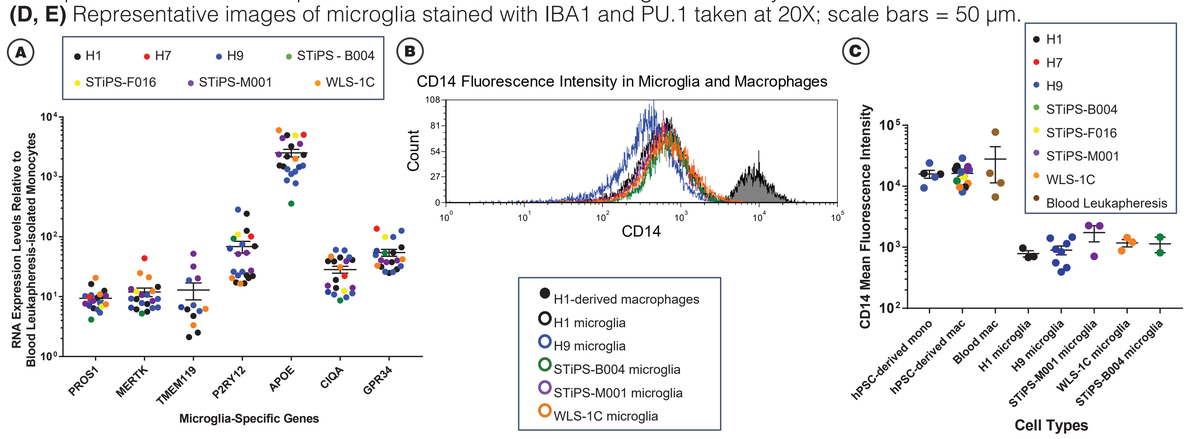


FIGURE 4. Cells Generated Using STEMdiff™ Microglia Culture System at Day 34 are Distinct From Other Mononuclear Cell Types.
(A) RNA from microglia derived using multiple ES and iPS cell lines shows increased expression of microglia-specific genes relative to blood CD14+ monocytes (n = 13 - 22). **(B)** Representative flow cytometry histogram of CD14 fluorescence intensity for microglia derived from ES (H1 and H9) and iPS (STiPS-B004, STiPS-M001, and WLS-1C) cell lines (unshaded peaks) and H1-derived macrophages (shaded peak). **(C)** Microglia generated from multiple ES and iPS cell lines have significantly lower mean intensity of CD14 fluorescence compared to hPSC-derived monocytes ('hPSC-derived mono'), hPSC-derived M1-activated macrophages ('hPSC-derived mac'), and blood M1-activated macrophages ('Blood mac') (n = 2 - 14, p = 0.0002 by two-way ANOVA). The bars show the mean and standard error, and the dots show the results of individual experiments.

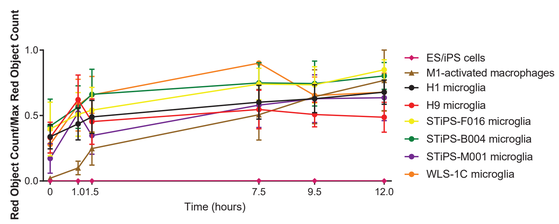


FIGURE 5. STEMdiff™ Microglia Culture System Generates Functional Microglia Capable of Phagocytosis at Day 34
The amount of pHrodo™ Red Zymosan Bioparticles™ taken up by microglia, blood leukapheresis-isolated M1-activated macrophages, and ES or iPS cells was measured over a 12-hour time period on an Incucyte® Live-Cell Analysis System. The red object count was divided by the maximum red object count of each experiment.

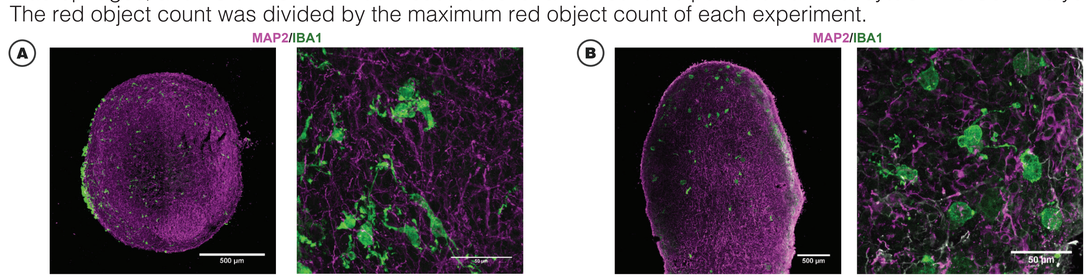


FIGURE 6. hPSC-Derived Microglia Incorporate into BORGs and Display an Activated Morphology Upon Injury
(A) Co-cultures with microglia and BORGs stained with IBA1 for microglia (green) and MAP2 for neurons (magenta). The microglia integrate among the neurons and display an unactivated morphology with extended processes (arrow). **(B)** After a needle-stick injury to the organoid, the microglia display an activated amoeboid morphology as shown by IBA1 staining.

Summary

STEMdiff™ Hematopoietic Progenitor Kit robustly generates HPCs suitable for microglia differentiation.

STEMdiff™ Microglia Culture System robustly generates microglia-like from HPCs across multiple cell lines. These cells:

- Express expected microglia markers after 34 days
- Have significantly different gene expression patterns and CD14 fluorescent intensity from other mononuclear cells
- Are capable of phagocytosis
- Can be co-cultured with and can integrate into brain organoids for disease modeling

References
1. Abud EM et al. (2017) Neuron 94(2): 278–93.e9.
2. McQuade A et al. (2018) Mol Neurodegener 13: 67.