A Reliable, Efficient, and Matrix-Free Method to Generate Midbrain Organoids from Human Pluripotent Stem Cells

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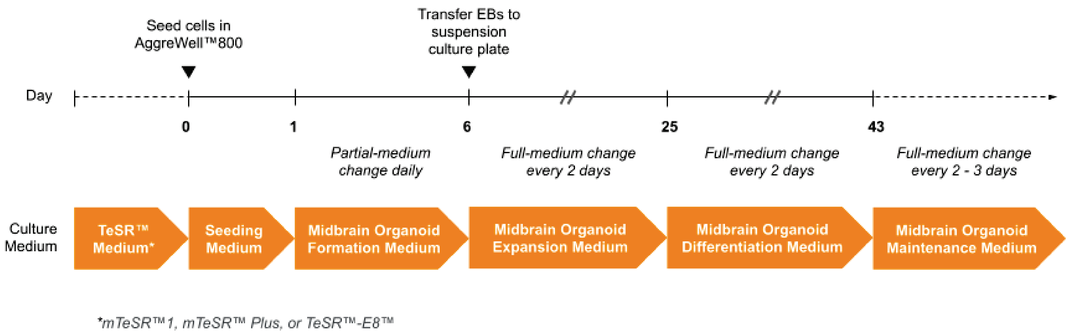
INTRODUCTION

Dopamine circuits originating from the midbrain have diverse functions that include regulating motor function, affect, and high-level executive function. Disruption of these circuits and/or dopaminergic neurons is implicated in diseases such as Parkinson's, schizophrenia, depression, and addiction. Animal models have been useful for studying these diseases, but the effectiveness of resulting clinical treatments has been variable. Human pluripotent stem cell (hPSC)-derived organoids permit the study of brain development, as well as disease etiology and progression in physiologically relevant tissue. Here we describe a matrix-free system to generate human midbrain organoids which can be applied to disease modeling of pathologies involving dopaminergic neurons.

METHODS

Midbrain Organoid Differentiation

hPSCs maintained in mTeSR™ Plus, mTeSR™1, or TeSR™-E8™ were dissociated and seeded at a density of 3 x 10^6 cells/well in Seeding Medium (Formation Medium + 10 µM rho-kinase inhibitor (ROCKi)) in AggreWell™800 plates. Cultures were fed daily with Formation Medium. After 6 days, organoids were transferred to a 6-well plate in Expansion Medium. Organoids were fed every 2 - 3 days until day 25, at which point organoids were fed with Differentiation Medium until day 43. From day 43 onward, organoids were fed every 2 - 3 days with Maintenance Medium.



*mTeSR™1, mTeSR™ Plus, or TeSR™-E8™

FIGURE 1. Workflow for STEMdiff™ Midbrain Organoid Kit Assembloid Formation With Microglia

hPSCs maintained in mTeSR™ Plus, mTeSR™1, or TeSR™-E8™ were differentiated into CD43-expressing hematopoietic progenitor cells (HPCs) using STEMdiff™ Hematopoietic Kit for 12 days and then further differentiated using the STEMdiff™ Microglia culture system for 28 - 34 days. The cells were characterized by flow cytometry for CD45 and CD11b expression and by immunocytochemistry by IBA1 and PU.1 expression. Day 80 midbrain organoids were co-cultured with 250,000 hPSC-derived microglia in 12-well culture plates for 8 days using Midbrain Organoid Maintenance Medium.

RESULTS

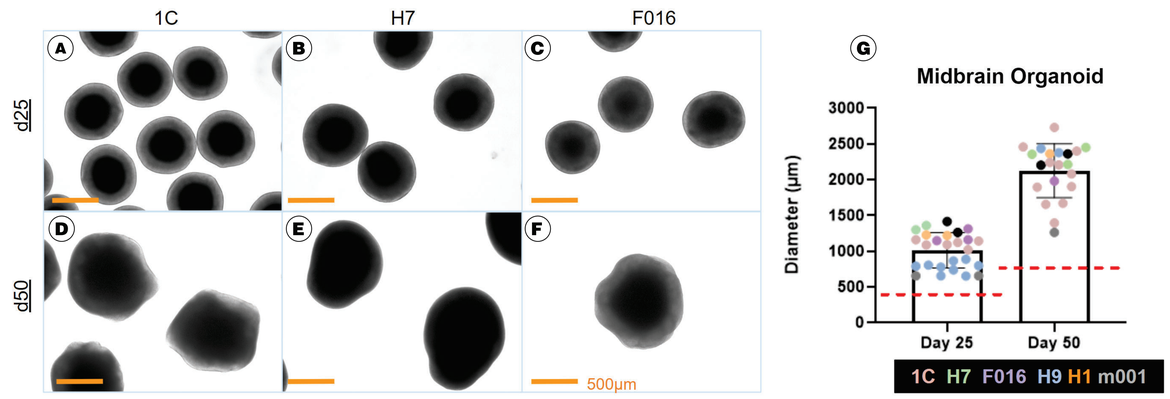


FIGURE 2. The STEMdiff™ Midbrain Organoid Kit Supports the Generation of Midbrain Organoids From Multiple Cell Lines

(A-F) Representative phase contrast images showing 25 and 50 days in vitro (DIV) midbrain organoids differentiated from three pluripotent stem cell lines. **(G)** Diameter measurements of midbrain organoids at 25 (1013 ± 247 µm) and 50 (2126 ± 377 µm) DIV. (mean ± SD; 25 DIV n = 21, 50 DIV n = 26).

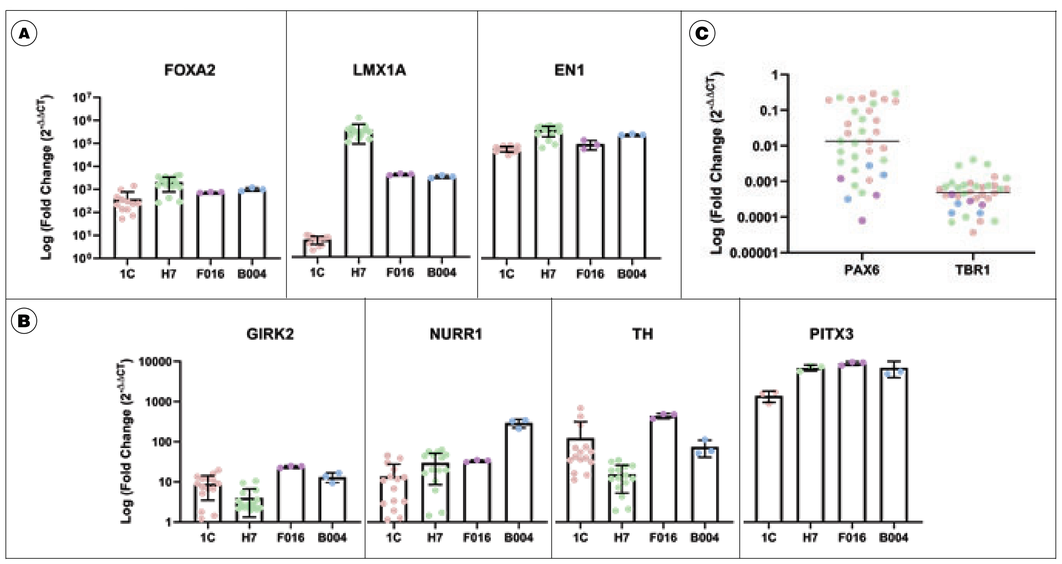


FIGURE 3. Midbrain Organoids Express Midbrain Floorplate and Dopaminergic Marginal Zone Genes

Single organoids differentiated from multiple cell lines were harvested for RNA at 25 and 50 DIV. **(A)** Midbrain floorplate precursor markers FOXA2, LMX1A, and EN1 were elevated in midbrain organoids at 25 DIV. **(B)** More mature marginal zone dopaminergic markers NURR1, TH, GIRK2, and PITX3 were elevated at 50 DIV. ΔΔCT (Fold Change) of midbrain organoids normalized to TBP housekeeping gene and respective dorsal forebrain organoid control (mean ± SD; n = 3 - 15). **(C)** Expression of dorsal forebrain markers PAX6 and TBR1 were downregulated in 25 DIV midbrain organoids. ΔΔCT of midbrain organoids normalized to TBP and respective PSC control (mean ± SD; n = 3 - 15 organoids).

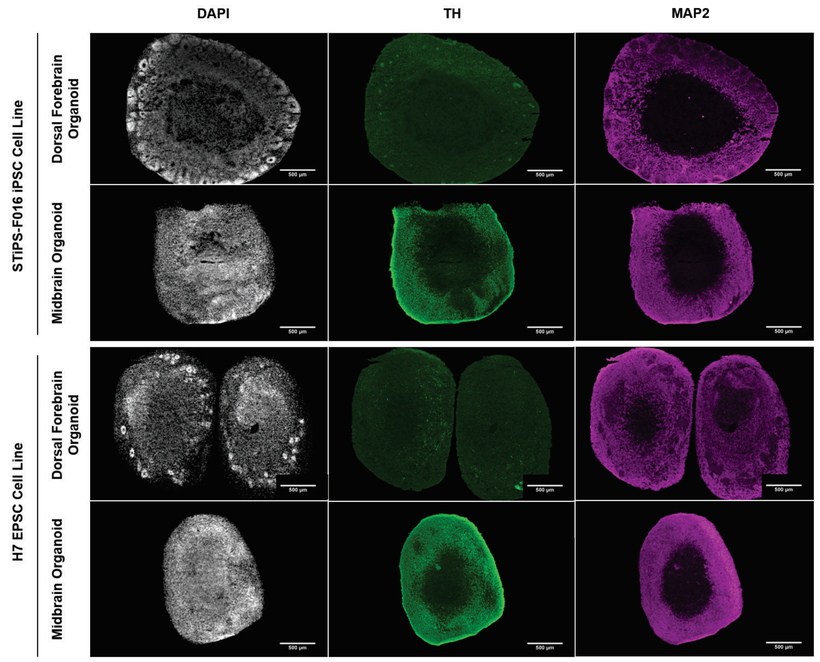


FIGURE 4. Midbrain Organoids Express Catecholaminergic Protein Tyrosine Hydroxylase

Representative images of 50 DIV dorsal forebrain and midbrain organoids differentiated from two hPSC lines. Both dorsal forebrain and midbrain organoids generate neurons that expressed MAP2, but only midbrain organoids expressed the catecholaminergic neuron-specific marker TH.

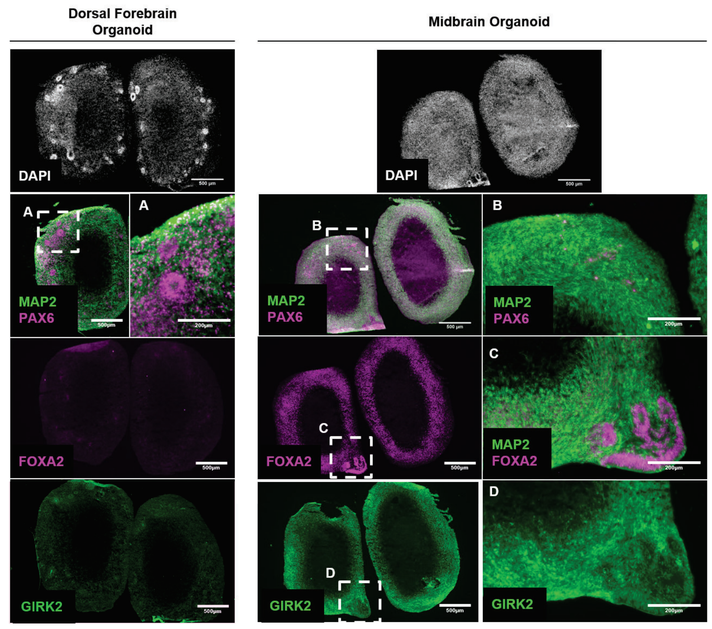


FIGURE 5. Midbrain Organoids Express Markers for Midbrain Floorplate Progenitors and Mature Marginal Zone Dopaminergic Neurons

Representative images of 50 DIV dorsal forebrain and midbrain organoids. Dorsal forebrain organoids demonstrated elevated expression of dorsal cortical progenitor marker PAX6 organized radially (inset A). Midbrain organoids expressed comparatively less PAX6 (inset B). Midbrain organoid markers showed elevated expression of floorplate progenitor marker FOXA2, that was arranged around pseudo-ventricular regions (inset C), and marginal zone dopaminergic neuron marker GIRK2 that expanded from FOXA2-expressing progenitor zones (inset D).

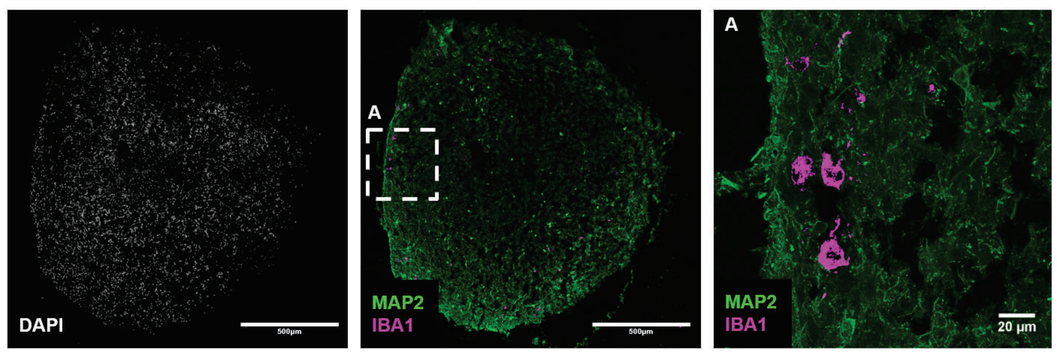


FIGURE 6. Midbrain Organoids Can Form Assembloids With Microglia

Assembloids were formed between midbrain organoids and microglia cells. Day 80 midbrain organoids were co-cultured with 250,000 hPSC-derived microglia in 12-well culture plates for 8 days using Midbrain Organoid Maintenance Medium. After 8 days of co-culture, organoids were sectioned and stained for microglia marker IBA1. IBA1+ cells were observed within organoids.

Summary

- The STEMdiff™ Midbrain Organoid Kit can reliably generate midbrain organoids from multiple diverse hPSC lines that express markers relevant to midbrain tissue development. Midbrain organoids can also be used to generate assembloid cultures with microglia generated using the STEMdiff™ Microglia Culture System.