Application note

Differentiation of induced pluripotent stem cells (iPSCs) into dopaminergic neurons

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The ability to generate dopaminergic neurons from iPSCs holds immense potential for both research and therapy. iPSC-derived dopaminergic neurons provide a valuable model for studying the mechanisms underlying neurodegenerative diseases such as Parkinson's disease and the dysregulation of dopaminergic signaling in psychiatric disorders like schizophrenia. As research progresses, the translation of these findings into clinical applications holds the promise of transformative impacts on patient care and disease management.

Differentiation of induced pluripotent stem cells (iPSCs) into dopaminergic neurons requires specific conditions to obtain the desired cell type. This application note will describe the method we use to differentiate human iPSCs into functional dopaminergic neurons, using a variety of media types that utilize a series of Qkine growth factors and cytokine supplements at different stages of key differentiation steps.

Introduction

Dopaminergic neurons are specialized nerve cells responsible for the synthesis, storage, and release of the neurotransmitter dopamine. These neurons play a pivotal role in several critical brain functions, including motor control, reward, motivation, and regulation of mood [1]. Dopaminergic neurons are primarily located in the substantia nigra and the ventral tegmental area of the brain.

They project to various brain regions, including the striatum, prefrontal cortex, and limbic system, forming complex networks that influence a variety of behaviors and physiological processes [2].

Dopaminergic neurons are crucial in motor control, as evidenced by their degeneration in Parkinson's disease, a condition characterized by motor symptoms such as tremors, rigidity, and bradykinesia. In psychiatric disorders, dysregulation of dopaminergic signaling is implicated. For instance, hyperactivity of dopaminergic pathways is associated with the positive symptoms of schizophrenia, while diminished dopamine function is linked to anhedonia and lack of motivation seen in depression [2].

Differentiating iPSCs into dopaminergic neurons is an area of research with significant implications for treating neurological disorders, especially Parkinson's disease.

Methods

Cell Culture and maintenance

Human iPSCs were cultured in a E8-like media in 6-well plates coated with vitronectin (Qk120) (5 µg/ml) using a weekend free media changing process. Passages were performed using 0.5 µM EDTA every 3-4 days and split using a 1:6 ratio to lift cells to ensure high quality iPSCs before starting the differentiation process. For further information on this process, please see our guide to Weekend-free human induced pluripotent stem cell culture using Qkine's thermostable FGF-2 (bFGF), together with our animal origin-free TGF-β1 and vitronectin, for improved colony homogeneity

iPSC differentiation to midbrain-specified floor plate progenitor cells

When iPSCs reached the desired confluence and quality, they were passaged using Accutase® and seeded into a

Geltrex-coated 24-well plate at a density of $3.8x10^5$ cells / ml. They were then allowed to adhere until achieving 90-100% confluency, forming a monolayer.

Neural induction and dopaminergic specification

iPSCs are first directed to become neural progenitor cells (NPCs) by dual SMAD inhibition, using SB431542, which inhibits pathways that maintain pluripotency and promotes neural differentiation towards the development of dopaminergic neurons using key molecules like fibroblast growth factor 8 (FGF8), which are critical for the midbrain dopaminergic neuron specification.

Maturation and Maintenance

The final stages involve the maturation of these cells into functional dopaminergic neurons (figure 1), which can be supported by factors such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor b3 (TGFb3).

Day 1: Media refreshed using Induction Media 1

Induction media 1

85%	Knockout DMEM
15%	Knockout serum replacement
1x	Glutamax
1x	MEM Non-Essential Amino Acids (NEAA)
10μM	2-Mercaptoethanol
100nM	LDN193189
10μM	SB431542

Days 2 & 3: Media refreshed using Induction Media 2

Induction media 2
Induction media 1 plus

100nM	SAG hydrochloride
2μM	Puromorphamine
100ng/ml	Human FGF8a (Qk059)

Day 4 & 5: Media refresh using Induction Media 3

Induction media 3
Induction media 1 plus

100nM	SAG hydrochloride
2μM	Puromorphamine
100ng/ml	Human FGF8a (Qk059)
3μM	CHIR99021

Day 6 & 7: Media refersh using Induction Media 4

Induction media 4

63.2%	Knockout DMEM
11.6%	Knockout serum replacement
24.8%	Neurobasal medium
0.13%	N-2 supplement
0.26%	B-27 Supplement
1x	Glutamax
1x	MEM Non-Essential Amino Acids (NEAA)
10μM	2-Mercaptoethanol
100nM	LDN193189
100nM	SAG hydrochloride
2μM	Puromorphamine
100ng/ml	Human FGF8a (Qk059)
3μM	CHIR99021

Day 8 & 9: Media refreshed using Induction Media 5

Induction media 5

42.3%	Knockout DMEM
7.64%	Knockout serum replacement
49.3%	Neurobasal medium
0.26%	N-2 supplement
0.51%	B-27 Supplement
1x	Glutamax
1x	MEM Non-Essential Amino Acids (NEAA)
10μM	2-Mercaptoethanol
100nM	LDN193189
3μM	CHIR99021

Day 10 & 11: Media refreshed using Induction Media 6

Induction media 6

21%	Knockout DMEM
3.8%	Knockout serum replacement
74%	Neurobasal medium
0.38%	N-2 supplement
0.74%	B-27 Supplement
1x	Glutamax
1x	MEM Non-Essential Amino Acids (NEAA)
10μM	2-Mercaptoethanol
100nM	LDN193189
3μM	CHIR99021

Day 12 & 13: Media refreshed using Induction Media 7

Induction media 7

96%	Neurobasal medium
1x	B-27 Supplement
1x	Glutamax
10μM	DAPT
500μM	Dibutyryl cyclic-AMP sodium salt
200μM	Ascorbic acid
3μM	CHIR99021
20ng/ml	Human BDNF (Qk050)
20ng/ml	Human GDNF (Qk051)
1ng/ml	Human TGFb3 (Qk054)

Day 14 - 19: Media refreshed using maturation media

Maturation Media

96%	Neurobasal medium
1x	B-27 Supplement
1x	Glutamax
10μM	DAPT
500μM	Dibutyryl cyclic-AMP sodium salt
200μM	Ascorbic acid
20ng/ml	Human BDNF (Qk050)
20ng/ml	Human GDNF (Qk051)
1ng/ml	Human TGFb3 (Qk054)

Day 20: Floor plate sphere isolation passaging

Cells were passaged using a combination of Accutase® and Papain to detach them, then washed and filtered with DMEM/F-12 containing DNase I. Afterward, they were washed and filtered again with DMEM/F-12 alone. The cells were then seeded at a density of 8.645 x 10^5 cells/ ml into a Geltrex-coated 24-well plate containing Seeding Media.

Seeding Media

96%	Neurobasal medium
1x	B-27 Supplement
1x	Glutamax
10μM	DAPT
500μM	Dibutyryl cyclic-AMP sodium salt
200μM	Ascorbic acid
20ng/ml	Human BDNF (Qk050)
20ng/ml	Human GDNF (Qk051)
1ng/ml	Human TGFb3 (Qk054)
10μM	Y-27632 2HCl

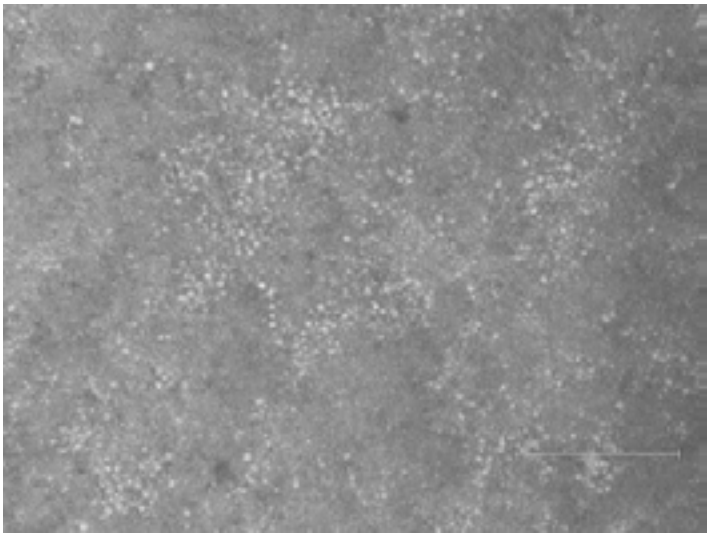


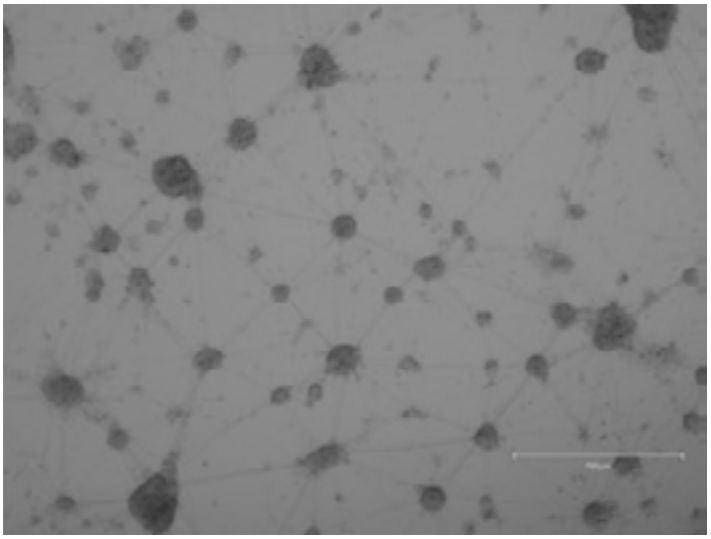
Figure 1. Day 20 maturing midbrain-specified floor plate progenitor cells differentiating into floor plate spheres prior to sphere isolation passage (scale bar = 300 μM)

Day 21-30, 32 and 34: Media refreshed using Maturation Media. As prepared on Day 14-19

Results

At Day 35, mature dopaminergic neurons could be visualized. (figure 2).

a.



b.

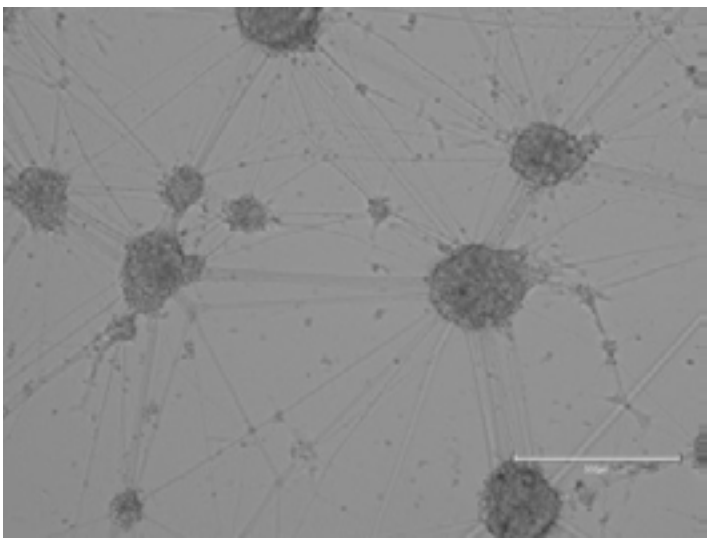


Figure 2. Day 35 differentiated mature dopaminergic neurons (a, scale bar = 750μM, b, scale bar = 300μM).

Conclusion

The differentiation of iPSCs into dopaminergic neurons represents a significant tool for advancement in regenerative medicine and neuroscience. The ability to reliably achieve consistent high-quality results can enhance our understanding of the fundamental processes governing neural development and degeneration, and lead toward innovative treatments for neurodegenerative diseases like Parkinson's.
The data presented in this application note demonstrate that using Qkine animal origin-free growth factors FGF8a (Qk059), BDNF (Qk050), GDNF (Qk051) and TGFb3 (Qk054) can support the differentiation of iPSCs into mature dopaminergic neurons within 35 days.

Further information

Qkine growth factors are manufactured to the highest of quality standards and are free from animal-derived contaminants, delivering low endotoxicity and high purity. At Qkine, we are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex neural stem cell culture. We are a science-led team, please reach out with any questions or requests to support@qkine.com

References

[1] O Klein, M. et al. Dopamine: Functions, Signaling, and Association with Neurological Diseases. Cellular and Molecular Neurobiology. 2019 Jan;39(1):31-59. doi: 10.1007/s10571-018-0632-3

[2] Zhuang, Y. et al. Mechanism of dopamine binding and allosteric modulation of the human D1 dopamine receptor. Cell Research 2021 May;31(5):593-596. doi: 10.1038/s41422-021-00482-0.

For more information

Please contact our team: **customerservice@qkine.com** if you would like to discuss commercial or academic collaborations, supply agreements or any aspects of growth factor optimization and other products.

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To explore our high-purity, animal-free bioactive proteins visit qkine.com

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