Comparison of 2D and 3D neuronal differentiation of patient specific induced pluripotent stem cells

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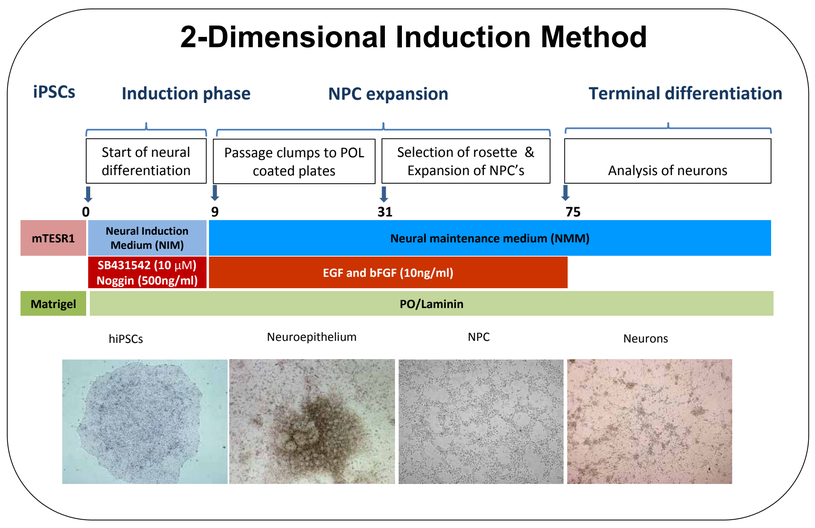
Introduction

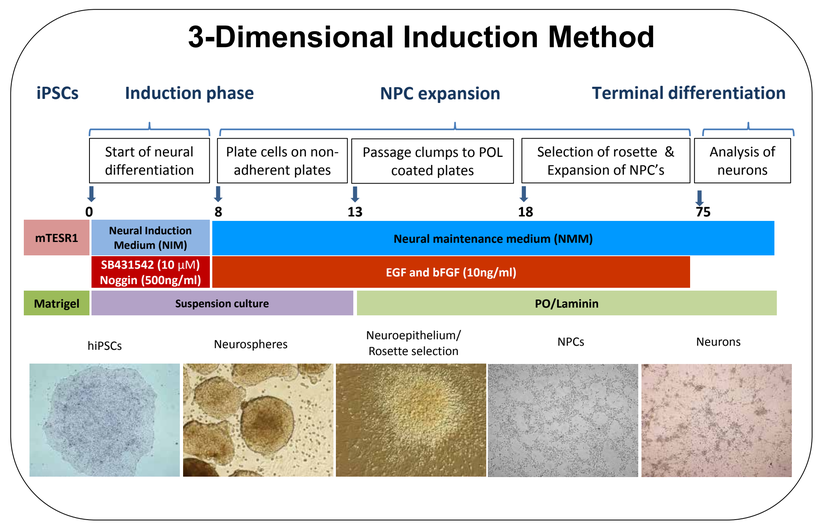
Patient-derived induced pluripotent stem cells (iPSCs) are providing a useful tool to model the pathology of certain diseases, especially when the affected tissue is hard to study, like in the central nervous system (CNS). Pluripotent cells, carrying the genotype of a given patient, can be differentiated *in vitro* to neural precursor cells (NPCs) which are then used to obtain neuronal and glial cells in culture. These cells offer a valuable platform which allows the investigation of the patient-specific pathomechanism of a disease. Furthermore, NPCs and their derivates can be used for in vitro drug testing assays. Finally, these cells serve as an unlimited source of immune compatible cells for cell replacement therapies to treat neurodegenerative disease such as Autism Spectrum Disorder or Alzheimer's disease.

Our aim was to develop a highly efficient and reproducible system to generate neurons from human iPSCs with different genetic background. In this study we compare two different induction methods: Monolayer(2D) and Embryoid body formation (3D) with two different genetic background cell lines, along with various small molecules and recombinant proteins in a step-wise manner, which selectively induced neural differentiation of iPSCs into NPCs and, later, into neural linages. The treatment induced neural rosette formation followed by the occurrence of neuroepithelial cells (NEPs) resulting in a homogenous population of NPCs. At the NPC stage all small molecules were withdrawn allowing for the terminal differentiation of mature post-mitotic neurons.

Methods

iPSCs of two known genetic backgrounds were directed towards neural fate using Noggin and a small molecule SB431542 on monolayer level (i.e 2-dimensional induction) and Embryoid formation level (i.e 3-dimensional induction). At the end of induction phase, neural rosettes containing neuroepithelial progenitors cells (NEP) were de-segregated and seeded onto poly-ornithine/laminin coated plates in defined media supplemented with N2, B27 and growth factors. For efficient neural differentiation growth factors were simply withdrawn from the medium.





Results

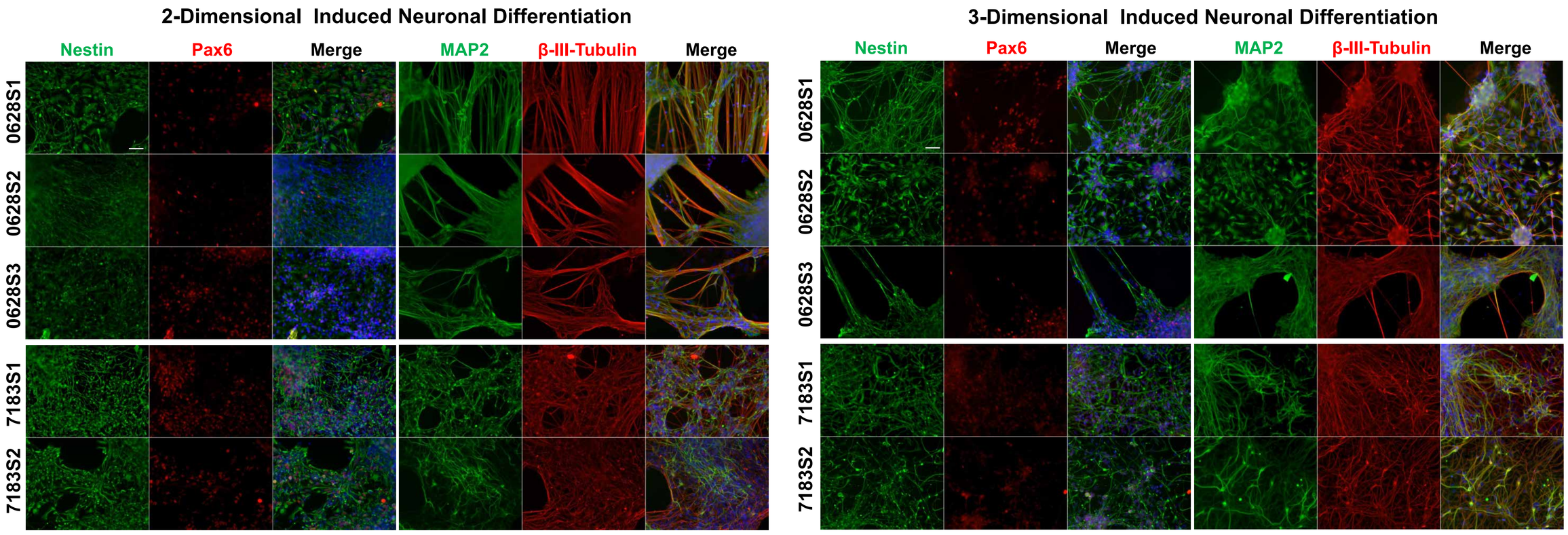


Figure 1: Differentiated neurons widely expressed neuronal markers such as [β-III-Tubulin&Map2]
Cells with two different genetic background 0628 (upper panel) and 7183 (lower panel) were maintained for 21 days in media supplemented with N2 and B27 without any growth factors, thereby directing to terminal differentiation. The medium contains DMEM/F12, 1%NEAA, 1%N2, 2%B27. Two induction methods: 2-Dimensional Induced Neuronal Differentiation (left panel) and 3-Dimensional Induced Neuronal Differentiation (right panel) were compared.
Scale bar: 100μm

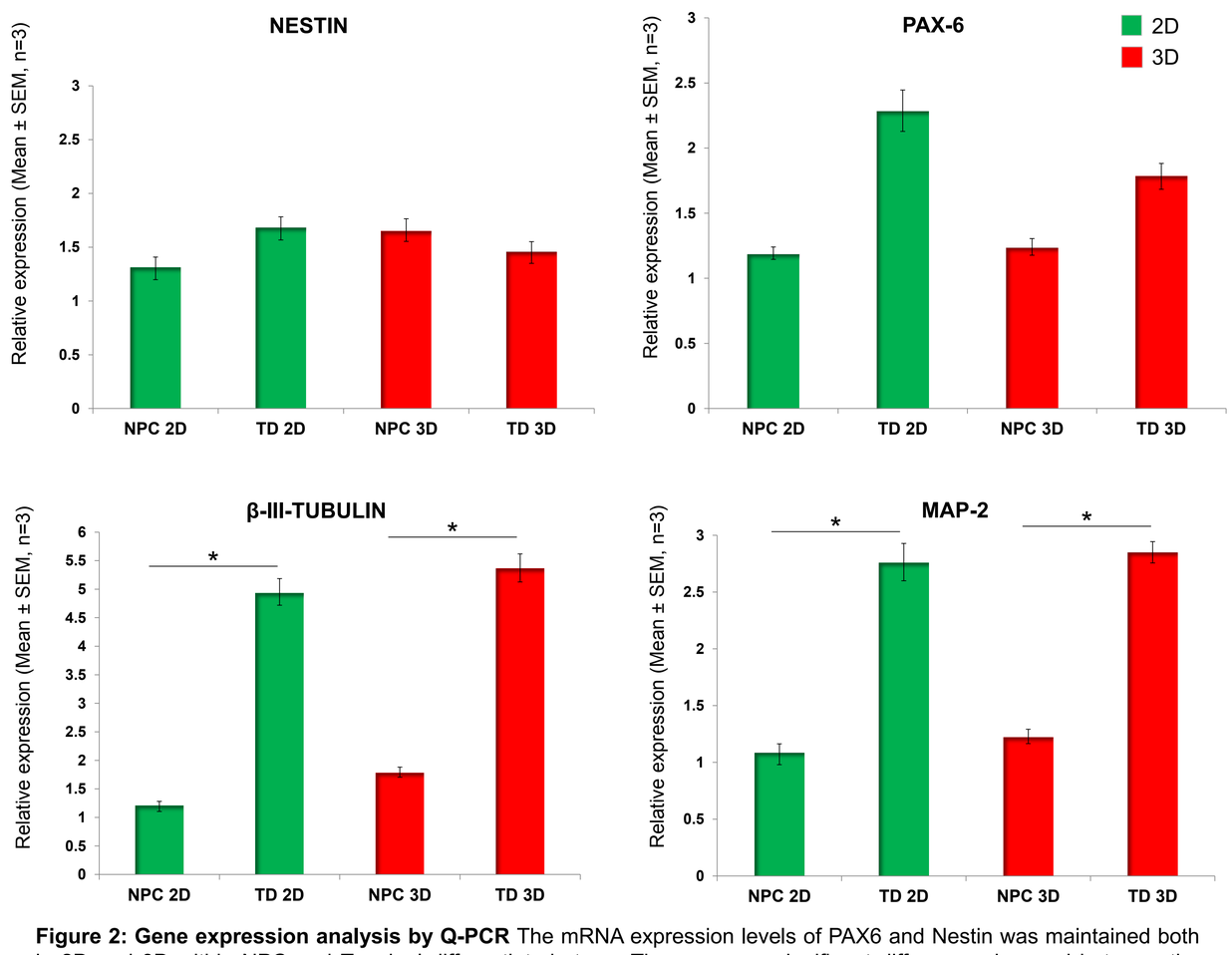


Figure 2: Gene expression analysis by Q-PCR The mRNA expression levels of PAX6 and Nestin was maintained both in 2D and 3D within NPC and Terminal differentiated stage. There was no significant difference observed between the induction methods. However, Beta-III-Tubulin is upregulated by 3-fold increase in week 3 (TD) compared to NPC stage both in 2D and 3D inductions methods. Likewise, MAP-2 is upregulated in week 3 compared to the NPC stage. Abbreviations: TD= terminal differentiation for 21 days; NPC stage = neuronal precursor cells plated for 4 days in N2B27 medium supplemented with 10ng/ml of FGF-2 and EGF. * significant difference ($P < 0.05$)

Conclusion

The human induced-pluripotent stem cells (iPSC) with two known genetic backgrounds were successfully generated into the neural lineage using both 2-dimensional and 3-dimensional induction methods via dual inhibition of SMAD signaling. Although no significant difference was observed between two different culture systems. However, we could firmly say that the number of mature neurons both in 2D and 3D method was increasing over time on terminal differentiation samples. The used time point for terminal differentiation was week 3.

Neural progenitor cells generated from this prototype may be used for in vitro drug testing and cell-based assays with the potential prospect of clinical transplantation applications for Alzheimer's disease.

Future Work

In future we plan to investigate the electrophysiological properties by using multi-electrode arrays (MEA). A specific neuronal subtype was not studied within this experimental approach, due to the lack of time points in terminal differentiation. However this will be considered for further experiments.

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