Neural induction of human pluripotent stem cells in XenoFree culture systems

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ABSTRACT

In this study, we describe ways to accomplish neural fate induction of pluripotent stem cells (hPSC) in xeno-free conditions. These methods facilitate generation of neural stem/progenitor cells in scales appropriate to meet the increasing cellular needs of the neuroregenerative field.
One of the key components in neural differentiation is the B-27® supplement, which has traditionally contained ingredients that are of animal origin. In order to generate xeno free conditions, we developed B27® supplement XenoFree CTS™, a XenoFree matrix, a XenoFree dissociating reagent and a culture system that would suit the need of cell therapy requirements.
Using such a system, in conjunction with small molecules to efficiently direct hPSC fate down neural lineages, neural stem cells were derived and fully characterized from integration free human iPSC lines (BS3-III and iPSC6.2).
The resulting cells can be leveraged in generating neuronal, astrocyte and oligodendrocyte lineages and in downstream applications focusing on neural disease.

INTRODUCTION

There has been great progress in the generation of induced pluripotent stem cells (iPSC) in recent years. The concern for potential genomic modifications by insertion of viral transgenes in the cellular genome has been mitigated by non-integrating expression systems such as Episomal vectors and RNA Sendai virus vectors. Concurrently, the supporting culture systems for reprogramming and expansion are evolving from feeder-based to feeder-free conditions and from freeder-free conditions to humanized (XenoFree) culture systems. A remaining critical step in the iPSC workflow involves differentiation of pluripotent cells to the desired cell type without exposure to xenogenic components.

MATERIALS AND METHODS

Generation of iPSC:
- Episomal iPSC Reprogramming Vectors (Cat No. A14703)
- CytoTune®-iPSC Sendai Reprogramming Kit (Cat No. A1378001)

Culture of iPSC in XenoFree condition :
- Vitronectin (VTN-N) substrate (Cat No. A14701SA)
- Essential 8™ Medium (Cat No. A14666SA)

XenoFree differentiation of iPSC to NSC, Proliferation of NSC, differentiation of NSC to Neurons, Culture of human neurons:
- B-27® Supplement XenoFree CTS™ (Cat No. A1486701)
- Neurobasal® Medium CTS™ medium (Cat No. A13712)
- KnockOut™ DMEM/F-12 CTS™ Medium (Cat No. A13708)
- N-2 CTS™ Supplement(Cat No. A1370701)
- Glutamax I (Cat No. 35050)
- bFGF (Cat No. CTP0261)
- EGF (Cat No. PHG0311)
- CELLstart™ CTS™ substrate(Cat No. A10142)
- TrypLE™ Select CTS™(Cat No. A12859)

Detail Method is available on Life Technologies webpage. Visit lifetechnologies.com and find protocol located under B-27® Supplement XenoFree CTS™ or appropriate product name.

RESULTS

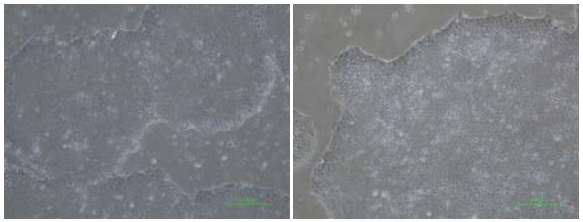

Figure 1. iPSC cultured in XenoFree condition (in Essential 8™ medium on recombinant Vitronectin substrate) . Phase contrast images of hiPSC generated by episomal vector (Left) and by Sendai virus (Right)

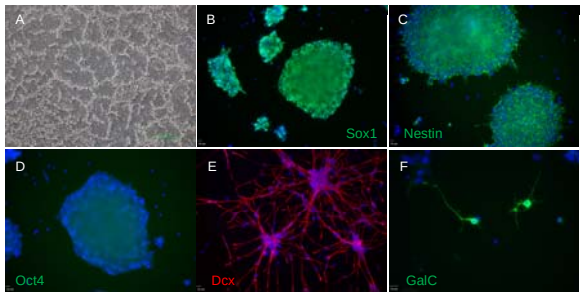

Figure 2. iPSC were differentiated in Xenofree neural induction medium made with B-27® Supplement XenoFree CTS™ and small molecules. Derived NSC (A) were characterized with phenotype marker with Sox1(B) and Nestin (C). The population was devoid of cells with pluripotent marker of Oct4(D). Upon further differentiation, neurons (E, DcX – Red) and Oligodendrocytes (F, GalC – Green) were obtained. All nuclei were stained with Dapi (blue).

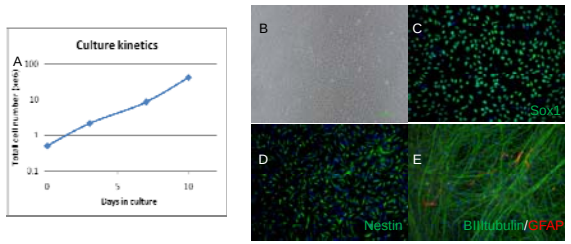

Figure 3. Xenofree proliferation of NSC. (A) Culture kinetics of neural stem cells in proliferation medium made with B-27® Supplement XenoFree CTS™. After 10 days in culture, 40e6 cells could be produced out of 0.5e6 cells. B-D) NSC proliferated (p3) in B-27® Supplement XenoFree CTS™ (B) were characterized with NSC phenotype marker of Sox1 (C) and Nestin (D) and further differentiated to neurons and astrocytes (E, BIIItubulin – Green, GFAP - Red)

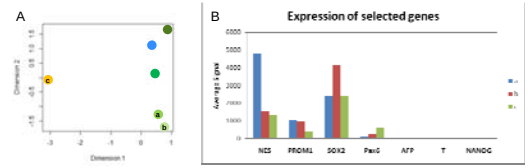

Figure 4. Microarray data profiling of NSCs. (A) Multidimensional scaling (MDS) indicates NSC derived in B-27® Supplement XenoFree CTS™ (b) shared most of gene expression profile to the one derived in B-27® supplement (a). NSC isolated from fetal cortex (c). (B) All samples picked up neural stem cells genes (NES, Prom1, Sox2) with few expression on non neural genes (AFP, T, Nanog)

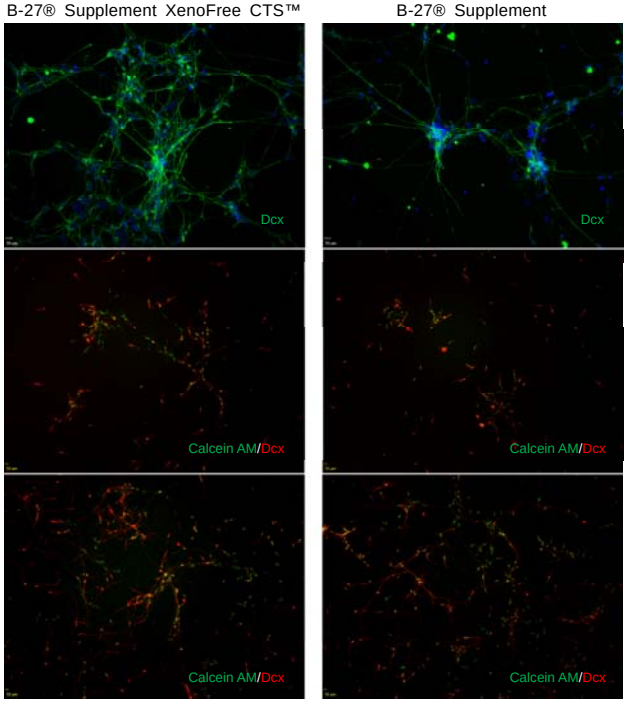

Figure 5 Culture of human neurons in XenoFree condition. iPSCs were differentiated to neurons and neurons were cultured in respective medium for a week (upper panel). Neurons were stained with Dcx (green) and nuclei was stained with DAPI (blue) . Human hippocampal neurons (middle panel) and cortical neurons (lower panel) were cultured in respective medium for a week, live cells were stained with Calcein AM (Green) and neurons were stained with Dcx (red).

CONCLUSIONS

1.B-27® Supplement XenoFree CTS™ is critical component for neural induction of PSC and the culture of NSC or neurons.
2.To obtain XenoFree conditions, a XenoFree variation of B-27® Supplement was developed and the performance for the intended use were examined.
3.With B-27® supplement XenoFree CTS™, the following cultures can be obtained in XenoFree conditions.

- Neural differentiation of hPSC
- Proliferation of neural stem cells
- Culture of primary neurons and neurons from PSC

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