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# PSC Suspension Culture Eases the Transition to 3D Differentiation Workflows

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## Abstract / Introduction

Pluripotent stem cells (PSCs) have unlimited expansion capacity and can be differentiated into any cell type, making them powerful tools to study development and disease. The emergence of PSC-derived organoid cultures has further improved the physiological relevancy of these cell models. However, conventional PSC culture is based on adherent culture techniques that have limited scalability potential and require the dissociation and reaggregation of PSCs for many organoid differentiation methods. To address these workflow limitations, StemScale™ PSC suspension medium was developed to support PSC growth in suspension which can reduce hands-on time and enable rapid scale-up from small culture vessels to bioreactors while maintaining key stem cell attributes. Indeed, PSCs cultured for >30 passages maintained stemness marker expression (eg. *NANOG*, *OCT4*), trilineage differentiation potential and a stable karyotype. Moreover, RNAseq indicated the global transcriptome of PSCs cultured in StemScale was highly concordant with conventional adherent medium PSC cultures (correlation coefficient >0.95). A cohort of 230 differentially enriched genes was detected in adherent medium cultures that were primarily associated with ECM and focal adhesin KEGG pathways. Suspension culture enriched genes were not associated with any KEGG pathway and principal component analysis suggested transcriptome differences were driven less by the culture medium and more PSC-line specific. Furthermore, we discuss how PSC suspension culture may ease the transition into 3D differentiation protocols leading to lineage dependent and lineage agnostic benefits. For example, we observed that differentiating PSCs in suspension culture may shorten the time to produce dopaminergic neurons from 35 to 25 days and electrophysiology suggested these cells also achieve a higher maturation level as compared with matched 2D controls. Likewise, PSCs could be differentiated into cytotoxic CD56+CD3-CD16+ natural killer cells, capable of killing patient derived tumoroids, in a more scalable manner. Overall, this data suggests PSC suspension culture is well-suited for scalable PSC workflows including 3D differentiation and for generating more reliable, reproducible, and human-relevant cell models.

## Methods

**PSC Culture**. PSCs were maintained in Gibco™ StemFlex™ or StemScale™ PSC suspension medium. Adherent cells were passaged with Versene™. PSC spheroids were dissociated with StemPro™ Accutase™ and replated with Y-27632 for serial passaging. PSC seeding density and spent medium was exchanged per product manual instructions.

**RNA sequencing**. Total RNA was isolated using Purelink™ RNA mini kits. RNA integrity and concentration was determine using 4200 Tapestation system. First strand cDNA synthesis was performed using SuperScript™ IV VILO with ezDnase. Bulk RNA sequencing libraries were generation using the Ion Chef™ System using Chef-ready reagents and sequencing on the Ion GeneStudio S5 system.

**Differentiation**. Neuronal induction, expansion differentiation were performed using the Gibco PSC Neural Induction kit, B-27 and B-27 Plus supplements and CultureOne™ supplement. Natural Killer cell differentiation was performed as outline in Figure 5A using CTS™ StemScale medium, CTS StemPro-34 medium, CTS-NK-Xpander medium and a selection of PeproGMP growth factors and cytokines.

## Results

### Growing PSCs in suspension culture

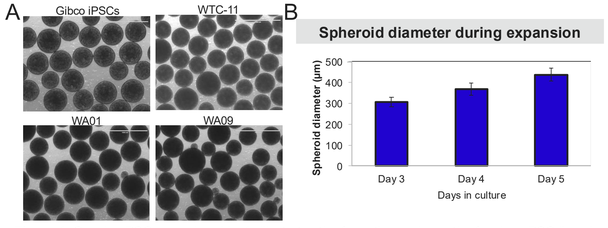



**Figure 1**. Growing PSCs as spheroids. A, illustrates the formation and growth of various PSC lines in suspension. PSCs aggregate into clusters with 24hrs. B, these PSC spheroids are uniform in size and get larger with time in culture.

## Results

### Maintenance of PSC suspension cultures

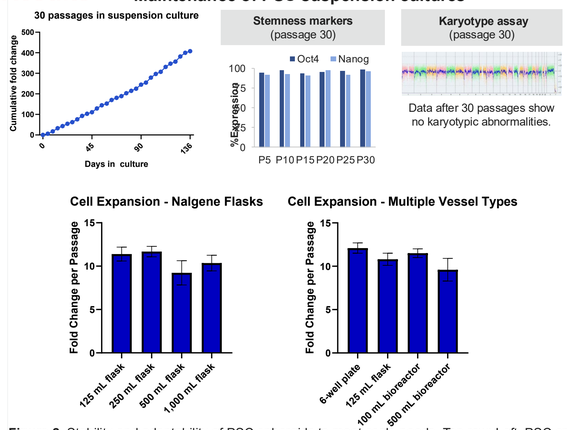



**Figure 2**. Stability and adaptability of PSC spheroids to meet scale needs. Top row: Left, PSC spheroids were serially cultured in StemScale suspension medium and show consistent growth at each passage. Middle, Stemness markers OCT4 and NANOG maintained a high level of expression in culture for nearly 140 days. Right, these cells maintained a normal karyotype after 30 passages. Bottom row: Left, comparable growth per passage was observed in a range of culture flasks demonstrating how one can scale the culture volume to meet their desired cell yields. Right, PSC spheroids showed comparable growth in 6-well plates, flasks and mini bioreactors indicating adaptability to a wide range of culture formats.

### High concordance of adherent and suspension transcriptomes

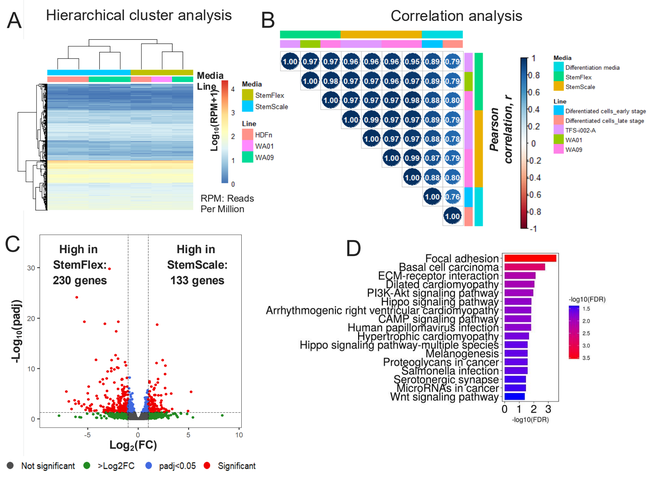
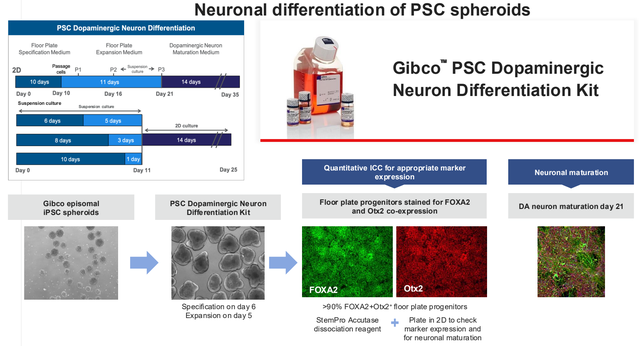



**Figure 3**. Transcriptomic analysis of adherent and suspension PSCs. A-B, Hierarchical clustering and correlation analysis of global transcriptomes demonstrates a high degree (r> 0.95) of similarity between multiple PSC lines and both adherent and suspension PSC media systems. C, Differential gene expression (DEG) analysis detected upregulated gene signatures in each media. StemFlex upregulated DEGs were mostly associated with cell adhesion pathways. D, Upregulated Stemscale DEGs were not statistically associated with any pathways (not shown)

## Results (continued)

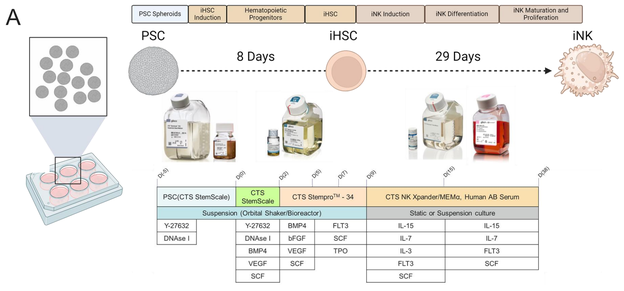
### Neuronal differentiation of PSC spheroids




Gibco™ PSC Dopaminergic Neuron Differentiation Kit

**Figure 4**. Neuronal differentiation of PSC spheroids. Top left, Overview of neuronal differentiation using adherent or suspension PSC cultures. The original 2D protocol required ~35days whereas 3D protocols wherein PSC spheroids are directly differentiation with the same reagents requires only ~25 days to achieve the same or greater level of maturation.

### Differentiation PSC spheroids into immune cells



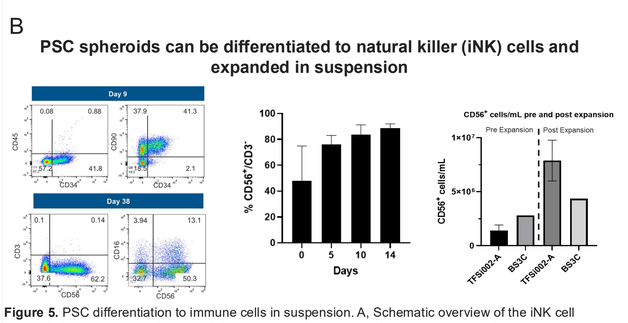




**Figure 5.** PSC differentiation to immune cells in suspension. A, Schematic overview of the iNK cell differentiation process starting with 3D suspension PSCs, B, iPSCs cultured in StemScale Medium were differentiated to CD56+ natural killer cells (iNKs) in suspension without feeder cells. iNK cells could be subsequently expanded with maximum cell yields observed around 14 days after post-differentiation.

## Results (continued)

### iNK cells kill patient-derived cancer tumoroids

HuCo1044-GFP tumoroids grown in OncoPro Tumoroid Culture Medium

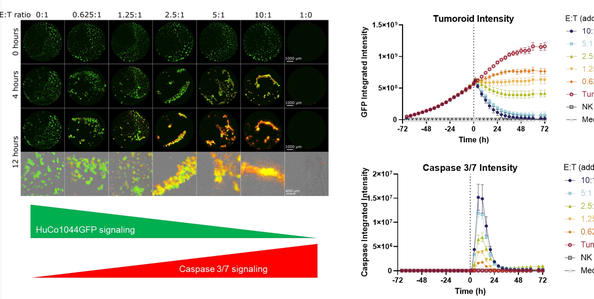



**Figure 6.** 3D culture derived iNK cells efficiently target patient derived tumoroids cells. iNK cells were co-cultured with HuCo1044-GFP tumoroids for 72 hours. The GFP signal (visualized in green, viable cells) from the colorectal tumoroids progressively decreased once the iNKs were introduced into the culture system, accompanied by an increase in caspase-based signaling (cell death marked by Caspase 3/7, visualized in red) demonstrating functional cytotoxicity of these iPSC derived cells.

## Summary

- PSCs maintained in suspension culture are transcriptomically comparable to adherent culture systems
- Suspension cultures maintain stemness and differentiation potential
- 3D differentiation shows lineage dependent benefits, such as faster timing and improved functional maturity
- PSCs can be propagated in a variety of small or large vessel/volume formats
- Easier path to more scalable methods

## Acknowledgements

We would like to thank Chris Yankaskas, Colin Paul and Logan Wilson for assistance with human cancer derived tumoroid culture experiments.

## Trademarks/licensing



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