A next-generation PSC suspension culture system for optimal expansion and scale-up

Introduction

As the use of pluripotent stem cells (PSCs) in research, drug discovery, and therapeutic applications continues to expand, the need to create large numbers of high-quality cells in a robust and cost-effective manner becomes ever more pressing. Media that enable scalable culture and support efficient downstream differentiation while minimizing material, labor, and production requirements will greatly facilitate the use of PSCs for these applications. To address these challenges, three-dimensional (3D) suspension culture systems have been adopted that offer advantages for scale-up over 2D adherent culture. Notably, suspension cultures have a lower overall cost, reduced footprint and hands-on time, and greater compatibility with closed systems compared to adherent culture systems. Furthermore, suspension culture systems consume less media and plasticware than the same number of cells grown in adherent culture systems. For these reasons, suspension culture systems are more desirable for generating large quantities of PSCs. However, current suspension culture systems are suboptimal due to relatively low cell expansion capability, cumbersome protocols, and inconsistency across cell lines. To address these challenges, we have developed Gibco™ StemScale™ PSC Suspension Medium. Here we describe how researchers can use this medium to readily transition their existing adherent cultures to suspension cultures to realize a range of benefits as compared to standard monolayer cultures.

Product overview

StemScale PSC Suspension Medium has been designed to simplify passaging and feeding of PSC suspension cultures (Figure 1) while maximizing cell expansion. Key benefits of the StemScale PSC Suspension Medium system include:

- **High expansion potential**—achieve 5–10x cell expansion per passage, making high-scale generation of PSCs cost-efficient
- **Simplified workflow**—initiate spheroid formation across multiple cell lines, without the use of microcarriers or cell strainers during culture or passaging
- **Compatible with multiple culture formats**—scale PSC cultures across multiple vessel formats: multiwell plates, shake flasks, and bioreactors
- **Flexible feeding schedule**—choose to feed cultures every day or every other day, providing more freedom when expanding PSCs

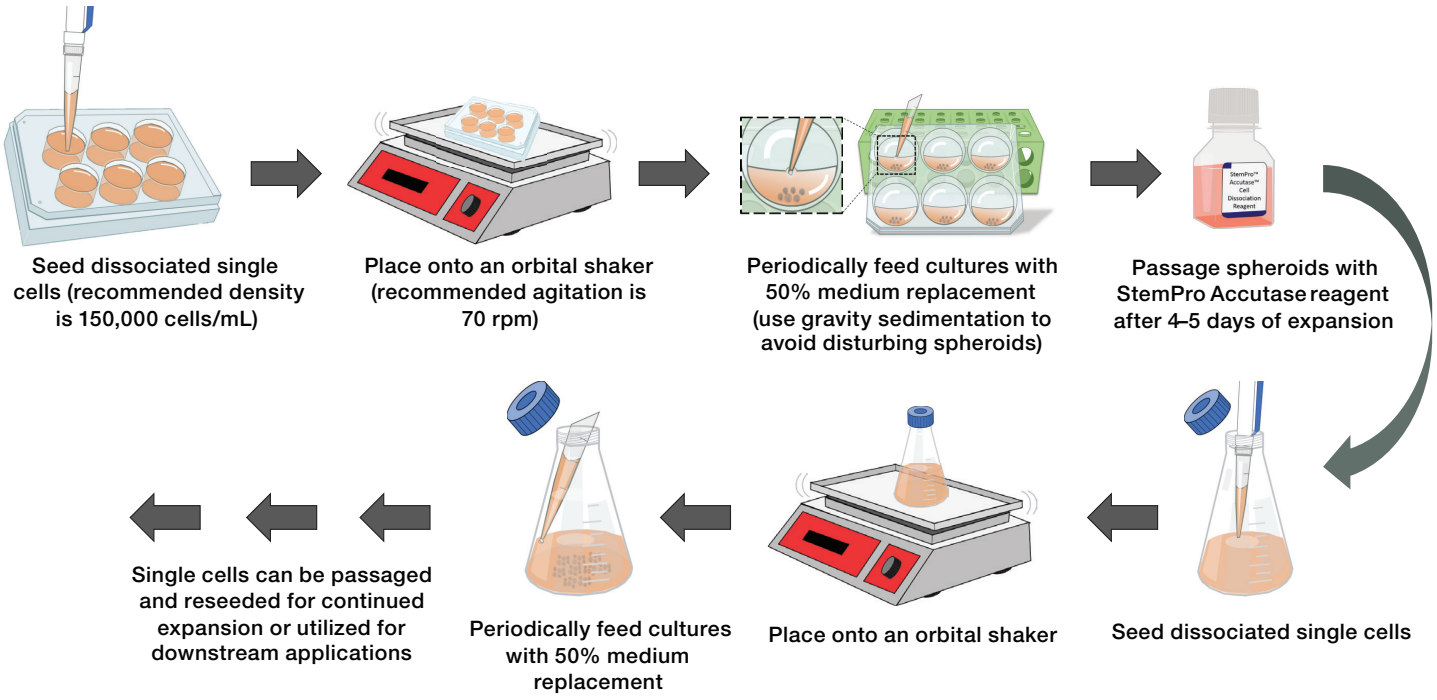

Figure 1. Simplified workflow for adapting adherent cultures to suspension cultures using StemScale PSC Suspension Medium. After initiation of cultures in StemScale medium, cells are fed periodically using a 50% medium exchange (every day or every other day). After 4–5 days of growth, spheroids are passaged using Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent, with no need for cell strainers. Dissociated cells can continue to be expanded, replated in monolayers for characterization, or cryopreserved. For detailed instructions on transitioning to suspension cultures using StemScale PSC Suspension Medium, refer to the instructions in the user guide.

Expansion capability of PSCs cultured in StemScale medium

Common challenges of PSC suspension culture include the inconsistent growth of cell lines adapted to suspension culture, and the inability of some cell lines to reliably form spheroids when transitioning to suspension culture from adherent culture. StemScale PSC Suspension Medium was designed to improve the nucleation of single cells into spheroids and maximize cell growth across lines (Figure 2). Depending on how easily the cell line transitions to suspension culture, typically 5–10x cell expansion can be consistently generated per passage, supporting reliable and rapid expansion of cells during scale-up. Additionally, since StemScale PSC Suspension Medium does not require the use of cell strainers during passaging, it allows for transition across multiple vessel types of increasing scale without sacrificing expansion capability (Figure 2B, C).

Expandability (adherent vs. suspension)

In comparison to adherent culture systems, growing PSCs as self-aggregating spheroids in StemScale PSC Suspension Medium allows users to culture greater numbers of cells in a shorter amount of time, while reducing the amount of media and plasticware consumed. Vast differences in cost, time, and cell number are evident between cells grown in adherent culture and suspension culture starting from a single well of a 6-well plate (Table 1). Extrapolating growth rates, suspension cultures in StemScale PSC Suspension Medium can be scaled up to a 3 L bioreactor within 3 weeks, achieving 4.5 x 10^9 cells. In contrast, a typical adherent culture system would take 4 weeks, requiring substantially more culture vessels and a larger incubator footprint, as well as up to 3x more medium. Ultimately, this leads up to a 5x cost increase as compared to cells grown in suspension using StemScale PSC Suspension Medium.

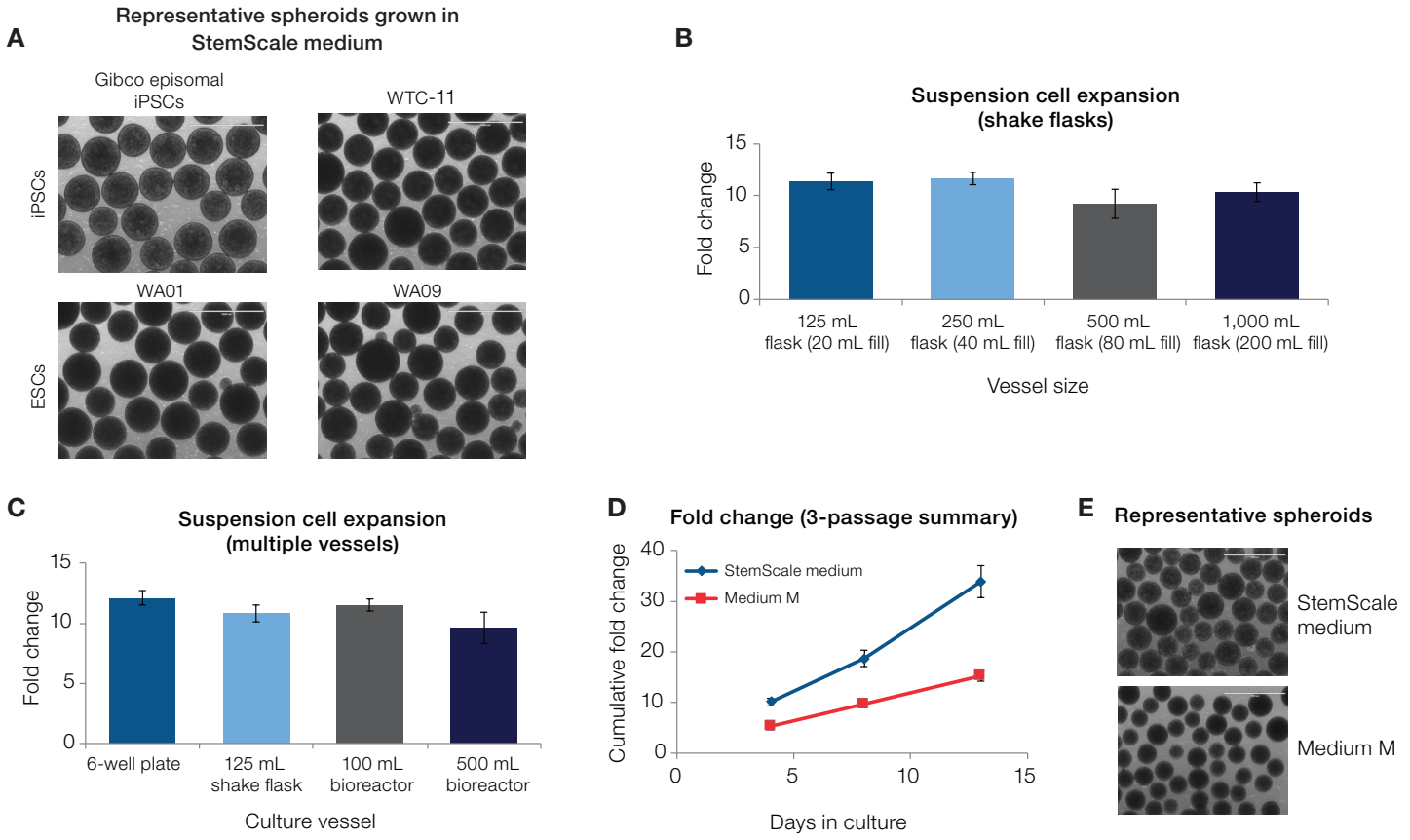

Figure 2. Compatibility of multiple cell lines in StemScale PSC Suspension Medium. (A) Multiple cell lines grown in StemScale PSC Suspension Medium are capable of forming spheroids. **(B)** Cell lines are capable of achieving 5–10x expansion per passage, depending on the ease with which cells nucleate into spheroids. **(C)** Additionally, this growth is consistent across multiple types of culture vessels, from small (well plates) to large (bioreactors). **(D, E)** When compared against a medium from another supplier (Medium M), StemScale PSC Suspension Medium can generate up to 3x expansion across the same period of time. Data for all panels were generated with 2 replicates.

Table 1. Scale-up in suspension culture systems (using StemScale PSC Suspension Medium) vs. scale-up in adherent culture systems (using Gibco™ Essential 8™ Medium).

	Suspension scale-up			Adherent scale-up		
Total days in culture	**Culture vessel format**	**Average cell yield***	**Medium consumed**	**Culture vessel format**	**Average cell yield***	**Medium consumed**
3 days	6-well plate (single well)	1.5 x 10^6 cells	6 mL	6-well plate (single well)	1.5 x 10^6 cells	6 mL
7 days	6-well plate (full plate)	18 x 10^6 cells	30 mL	100 mm dish	9 x 10^6 cells	48 mL
11 days	100 mL bioreactor	150 x 10^6 cells	200 mL	150 mm dish	22 x 10^6 cells	72 mL
15 days	500 mL bioreactor	750 x 10^6 cells	1,000 mL	Nunc TripleFlask Treated Cell Culture Flask	78 x 10^6 cells	800 mL
19 days	3 L bioreactor	4.5 x 10^9 cells	6,000 mL	2-layer Nunc Cell Factory System	300 x 10^6 cells	1,200 mL
23 days	–	–	–	10-layer Nunc Cell Factory System	1.5 x 10^9 cells	6,000 mL
27 days	–	–	–	30-layer Nunc Cell Factory System	4.5 x 10^9 cells	18,000 mL

* Values are estimated from data based on 4 days of expansion per culture vessel format. Values for the 3 L bioreactor and 30-layer Thermo Scientific™ Nunc™ Cell Factory™ System have been extrapolated from the smaller bioreactor and Nunc Cell Factory System formats listed in the table.

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Cell characterization

Cells expanded in StemScale PSC Suspension Medium grow as self-aggregating spheroids. Cells obtained from these spheroids after dissociation have been demonstrated to maintain pluripotency across multiple consecutive passages. Pluripotency was confirmed using the PluriTest™ characterization assay as well as flow cytometric analyses of Oct4 and Nanog expression. Additionally, these cells were found to maintain a normal karyotype, assessed using the Applied Biosystems™ KaryoStat™ Assay (Figure 3).

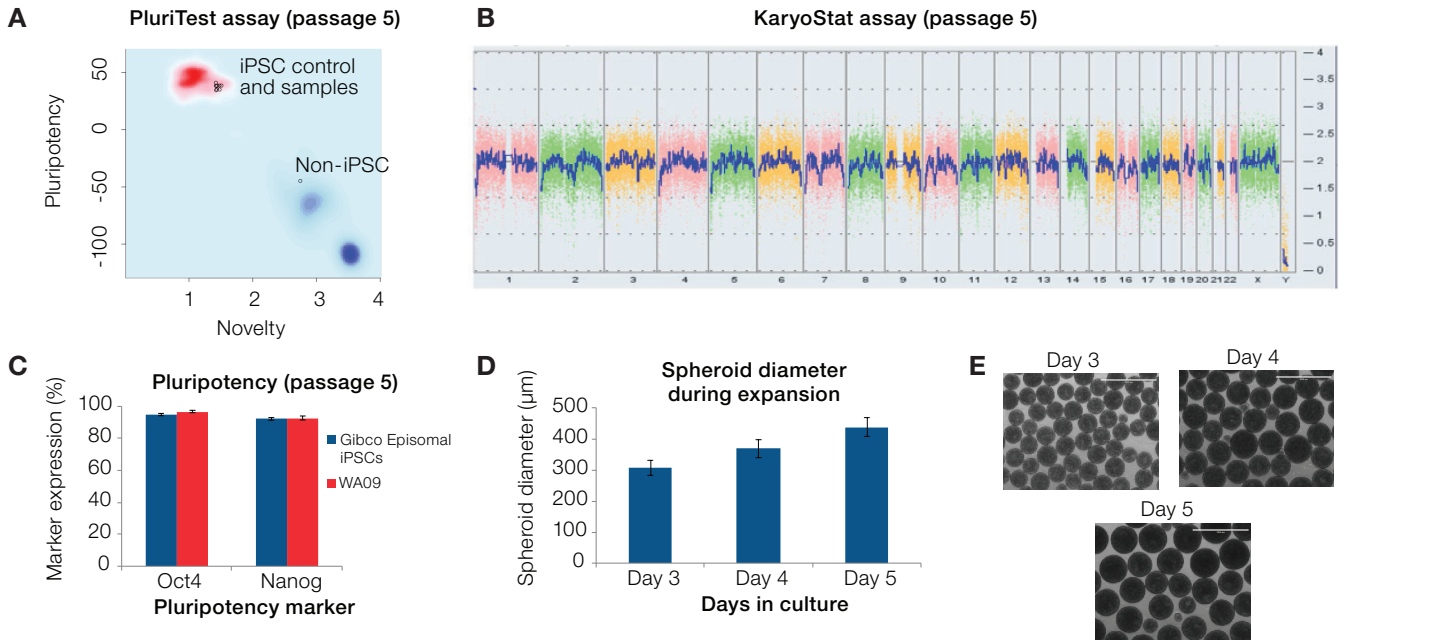

Figure 3. Characterization of spheroids grown in StemScale PSC Suspension Medium. Spheroids grown in StemScale PSC Suspension Medium maintain pluripotency and a normal karyotype, as assessed by **(A)** PluriTest and **(B)** KaryoStat assays. **(C)** High (>90%) expression of Oct4 and Nanog markers is observed in cells obtained from spheroids grown for five consecutive passages. **(D, E)** The characterizations described in **A**–**C** were performed on spheroids grown to our recommended passaging metric of an average diameter of ~400 µm. Cells grown as spheroids to an average diameter of 400 µm remain highly viable and pluripotent. This diameter can be achieved by growing spheroids in StemScale PSC Suspension Medium for 4–5 days. Data for all panels were generated with 2 replicates.

Conclusions

- Rapid suspension culture scale-up is possible when using StemScale PSC Suspension Medium due to the typical 5–10x cell expansion per passage.
- StemScale PSC Suspension Medium simplifies the workflow for suspension culture, removing the cumbersome use of microcarriers or cell strainers during culture or passaging.
- A wide variety of culture vessel formats, from small vessels (well plates and shake flasks) to large vessels (bioreactors), are compatible with StemScale PSC Suspension Medium.
- Cells expanded as spheroids in StemScale PSC Suspension Medium maintain pluripotency and a normal karyotype across multiple passages.
- StemScale PSC Suspension Medium provides a more flexible culture schedule while expanding PSCs, due to the option to feed cultures every day or every other day.

Learn more at **thermofisher.com/stemscale-elearn**

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