Development of a new 3D pluripotent stem cell suspension culture medium with a simplified protocol that yields highly efficient spheroid nucleation and robust expansion

Michael Akenhead, Rhonda Newman, Bob Scott, David Kuninger
Thermo Fisher Scientific, *Frederick, MD, United States*

ABSTRACT

As the use of pluripotent stem cells (PSCs) in therapeutic and screening applications continues to expand, a key bottleneck is the efficient generation of high-quality PSCs. Three-dimensional (3D) suspension culture offers key advantages for scale up over two-dimensional (2D) adherent culture: overall cost; reduced footprint and hands on time; and compatibility with closed systems. In addition, expansion of PSCs in 3D culture consumes less media (and plasticware) than the same number of cells grown in 2D culture. Together, these features make 3D culture an attractive path for cost-effectively and rapidly generating large quantities of cells required for downstream applications. However, there are a number of barriers for moving from 2D to 3D PSC culture which limit their wider adoption. In particular, there is a lack of effective culture systems and protocols which support this 3D PSC culture and scale up. To help address this, we have recenty developed a new 3D suspension culture medium – Gibco™ StemScale™ PSC Suspension Medium, which promotes the efficient self-aggregation of singularized PSCs into spheroids, without the need for microcarriers. Spheroids grown in this media maintain robust cell expansion (≥ 8 fold). In addition, the viability and pluripotency remain high (≥ 90% of cells) for spheroids cultured over consecutive passages. We also demonstrate our new system is compatible with a variety of 3D culture vessels (from well plates to bioreactors) through the optimization of straightforward parameters. We have found PSCs can be maintained in small volume suspension cultures, or readily scaled up into large volume culture vessels, with data shown for 500 mL cultures. The basic workflow is as follows: single cells are seeded at a concentration of ~ 150,000 cells/mL and cultured 4 – 5 days under constant agitation with periodic medium exchanges. Spheroids are typically visible by day 2 and are passaged once the average spheroid diameter is between 300 – 400 um. We demonstrate PSC spheroids can be taken directly into differentiation conditions, facilitating the formation of distinct 3D tissue like structures. Alternatively, PSC spheroids can be dissociated into single cells for downstream applications. Singularized cells can be replated into 2D, cryopreserved, and/or reseeded back into 3D conditions for continued expansion.

INTRODUCTION

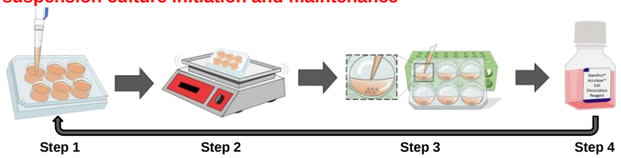
Adapting adherent cultures to suspension cultures can sometimes be a difficult process. We formulated StemScale PSC Suspension Medium to simplify the passaging and feeding of PSC suspension cultures.

Key benefits of the StemScale PSC Suspension Medium system include:

1) High expansion potential: achieve 5 – 10X pluripotent cell expansion per passage.
2) Simplified workflow: obtain consistent nucleation of spheroids without the need to use microcarriers or cell strainers.
3) Vessel compatibility: scalable across multiple culture vessel formats.
4) Cell line compatibility: promotes formation of spheroids with multiple ESC and iPSC lines.
5) Flexible culture schedule: enables users the flexibility to skip daily feeding of cultures.

METHODS

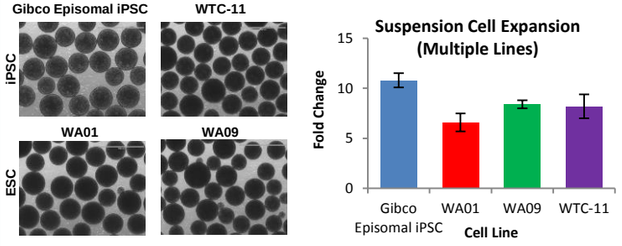
Figure 1. The StemScale PSC Suspension Medium workflow simplifies suspension culture initiation and maintenance



Step 1: single cells are seeded into culture vessels containing StemScale PSC Suspension Medium. The recommended seeding density of 150,000 cells/mL can be utilized for any size culture vessel. It is important to include 10 uM Y-27632 during seeding, as spheroids are unable to form in absence of a ROCK inhibitor on Day 0.
Step 2: place culture vessels onto a platform capable of providing constant agitation. An orbital shaker platform may be utilized for well plates and shaker flasks, while a magnetic stirrer may be utilized for spinner flasks and bioreactors. Spheroids must remain in suspension for the duration of the culture via constant agitation.
Step 3: periodically feed cultures with 50% medium replacement. To feed cultures, the spheroids must settle via gravity sedimentation for 5 minutes. Once spheroids have settled, the spent medium can be carefully removed and replaced with an equal volume of fresh medium. Cultures can be fed every-day or every-other-day.
Step 4: passage spheroids using StemPro Accutase. Spheroids are ready to be passaged when the average spheroids diameter is ~400 um. This generally occurs after 4 – 5 days of expansion in StemScale PSC Suspension Medium. Spheroids can be dissociated into single cells after 10 – 15 minutes of exposure to StemPro Accutase. Once spheroids have been dissociated into single cells, these single cells can be reseeded into suspension cultures for continued expansion. Alternatively, the dissociated single cells can also be utilized for various downstream applications.

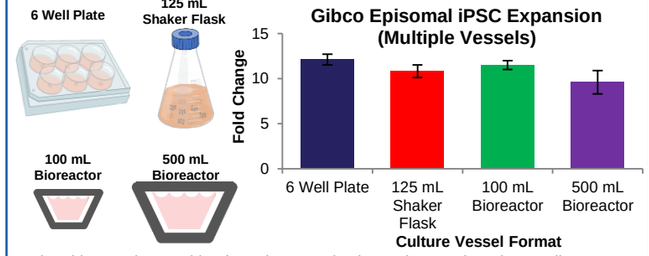
RESULTS

Figure 2. StemScale PSC Suspension Medium promotes the formation of spheroids in cell lines which experience difficulty adapting into suspension cultures



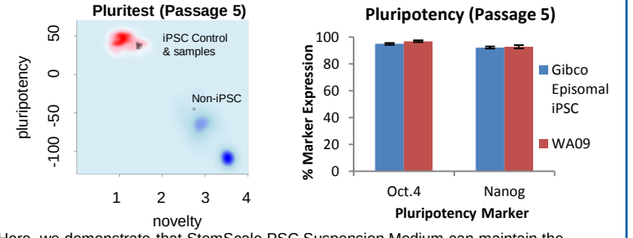
When transitioning from adherent to suspension culture, some cell lines experience difficulty forming spheroids. In order to address this, StemScale PSC Suspension Medium was designed to improve the nucleation of single cells into spheroids. Here, we demonstrate that StemScale PSC Suspension Medium is capable of forming spheroids in iPSC and ESC cell lines, some of which do not easily transition into suspension culture. Expansion of these spheroids is cell line dependent, with the average fold change between 5 – 10 fold for the four cell lines tested here.

Figure 3. StemScale PSC Suspension Medium is compatible in a variety of different culture vessels, from small-scale to large-scale



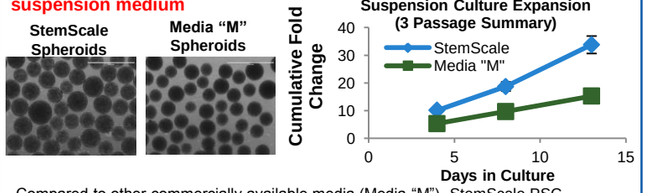
Spheroids must be capable of consistent nucleation and expansion when scaling up from small-scale to large-scale culture formats. Here, we demonstrate that StemScale PSC Suspension Medium is capable of expanding spheroids in a variety of different culture vessel formats. Both small-scale (6 well plate and 125 mL shaker flask) and large-scale (100 mL and 500 mL bioreactors) were evaluated. Using Gibco Episomal iPSCs, we obtained at least 8 fold expansion in all culture vessel formats.

Figure 4. StemScale PSC Suspension Medium maintains the pluripotency of cells expanded as spheroids across multiple passages



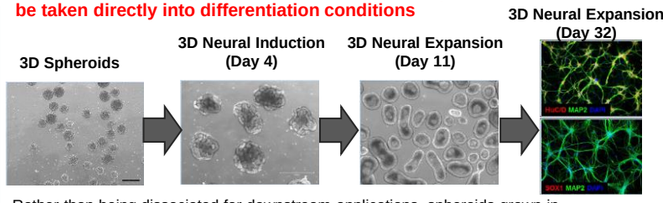
Here, we demonstrate that StemScale PSC Suspension Medium can maintain the pluripotency of cells grown as spheroids. Gibso Episomal iPSC and WA09 cell lines expanded as spheroids over 5 consecutive passages were found to remain pluripotent, as assessed by PluriTest (A). Furthermore, the expression of Oct4 and Nanog markers is >90% for these same cell lines, as assessed by flow cytometric analysis (B).

Figure 5. StemScale PSC Suspension Medium enhances spheroid expansion to a greater degree than other commercially available suspension medium



Compared to other commercially available media (Media "M"), StemScale PSC Suspension Medium shows greater expansion potential over multiple consecutive passages. Media "M" generated fold changes of 3 – 5X, while StemScale PSC Suspension Medium generated fold changes of 8 – 12X. This greater expansion enables researchers to scale-up quickly and more effectively, saving both time and money.

Figure 6. Spheroids grown in StemScale PSC Suspension Medium can be taken directly into differentiation conditions



Rather than being dissociated for downstream applications, spheroids grown in StemScale PSC Suspension Medium can be taken directly into differentiation conditions. Here we show spheroids differentiating into neurons with a high expression of phenotypic markers (HuCD and Map2).

CONCLUSIONS

1) StemScale PSC Suspension Medium offers a simplified workflow for suspension culture, which does not require the use of microcarriers or cell strainers during culture or passaging.
2) StemScale PSC Suspension Medium is scalable across multiple culture vessel formats, from small-scale to large-scale vessels.
3) StemScale PSC Suspension Medium supports high-scale generation of PSCs in suspension, with an average 5 – 10X cell expansion per passage.
4) StemScale PSC Suspension Medium maintains the pluripotency of cells expanded as spheroids across multiple passages
5) StemScale PSC Suspension Medium supports downstream differentiation as 3D spheroids.

REFERENCES

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TRADEMARKS/LICENSING