

Large-scale Expansion of Pluripotent Stem Cells as Spheroids in a New Xeno-Free Suspension Culture Medium

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Abstract

Scalable and efficient expansion of cell cultures are an important need for pluripotent stem cell (PSC)-derived allogeneic cell therapies. The large-scale production of high-quality PSCs can be achieved by three-dimensional (3D) suspension culture, wherein PSCs are cultured as aggregates or spheroids. However, adoption of 3D PSC suspension culture in clinical workflows is limited by the lack of commercial options for PSC suspension culture media. To address this, we developed the new GMP manufactured Gibco™ Cell Therapy Systems (CTS) StemScale™ PSC Suspension Medium to support PSC-based clinical manufacturing workflows. CTS StemScale is xeno-free and enables single cells to self-aggregate into 3D spheroids for efficient cell expansion. CTS StemScale supports both induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), with cell line-dependent growth in the range of 5X – 10X expansion per passage. When cultured over multiple consecutive passages, these spheroids have been demonstrated to maintain pluripotency, genomic stability, and trilineage differentiation potential. This suspension culture approach enables easy scale-up in various cell culture vessel sizes, from small-scale (<100 mL) culture vessels to large-scale (>1L) culture systems inclusive of bioreactors. Notably, 450 million cells were expanded to 5 billion cells in 5 days by using this PSC culture medium to grow spheroids within a bioreactor. To better enable future scale-up or other downstream applications, we also cryopreserved these cells at high densities which would minimize the number of cryovials required to thaw. Cells thawed from these vials showed high viability and were able to form spheroids which were capable of expanding at normal rates. Ultimately, cells grown in CTS StemScale have the flexibility to differentiate as 3D spheroids, dissociate into single cells and be utilized in downstream applications, or be cryopreserved as single cells for future use.

Introduction

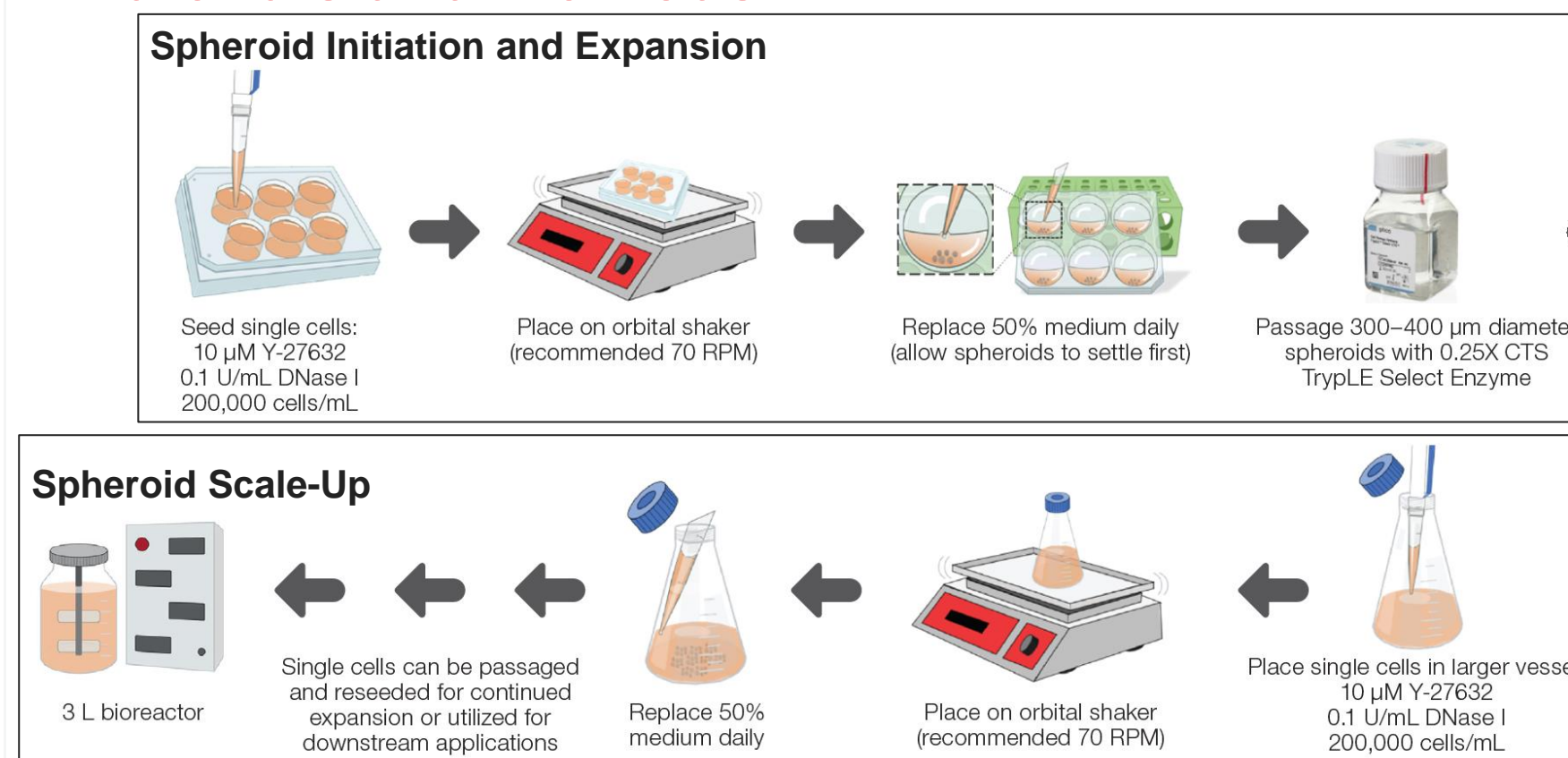
The generation of high-quality pluripotent stem cells (PSCs) is an important aspect in the that is compatible with clinical workflows. To address this, we developed Gibco™ development of PSC-based therapies and for clinical manufacturing applications. However, critical roadblocks exist which can make it difficult to obtain the desired quality of cells. In particular, the ability to obtain the large number of cells necessary to perform these applications can be a significant limiting factor.

Three-dimensional (3D) suspension culture is one solution to enable the rapid scale-up of large cell quantities in a short amount of time. However, suspension culture has its own drawbacks as well. Notably, there are a lack of commercial options available for PSC suspension culture media Cell Therapy Systems (CTS) StemScale™ PSC Suspension Medium to support PSC-based clinical manufacturing workflows.

The benefits of CTS StemScale PSC Suspension Medium include:

- Enhanced expansion capability versus other PSC suspension media, reducing manufacturing time and cost.
- Scalable expansion of PSCs with a simplified workflow that does not require a cell strainer.
- Consistent growth of multiple PSC lines.
- Supported regulatory filings with GMP manufacturing, traceability of raw materials, and regulatory documentation.

Materials and methods



Spheroid Initiation and Expansion

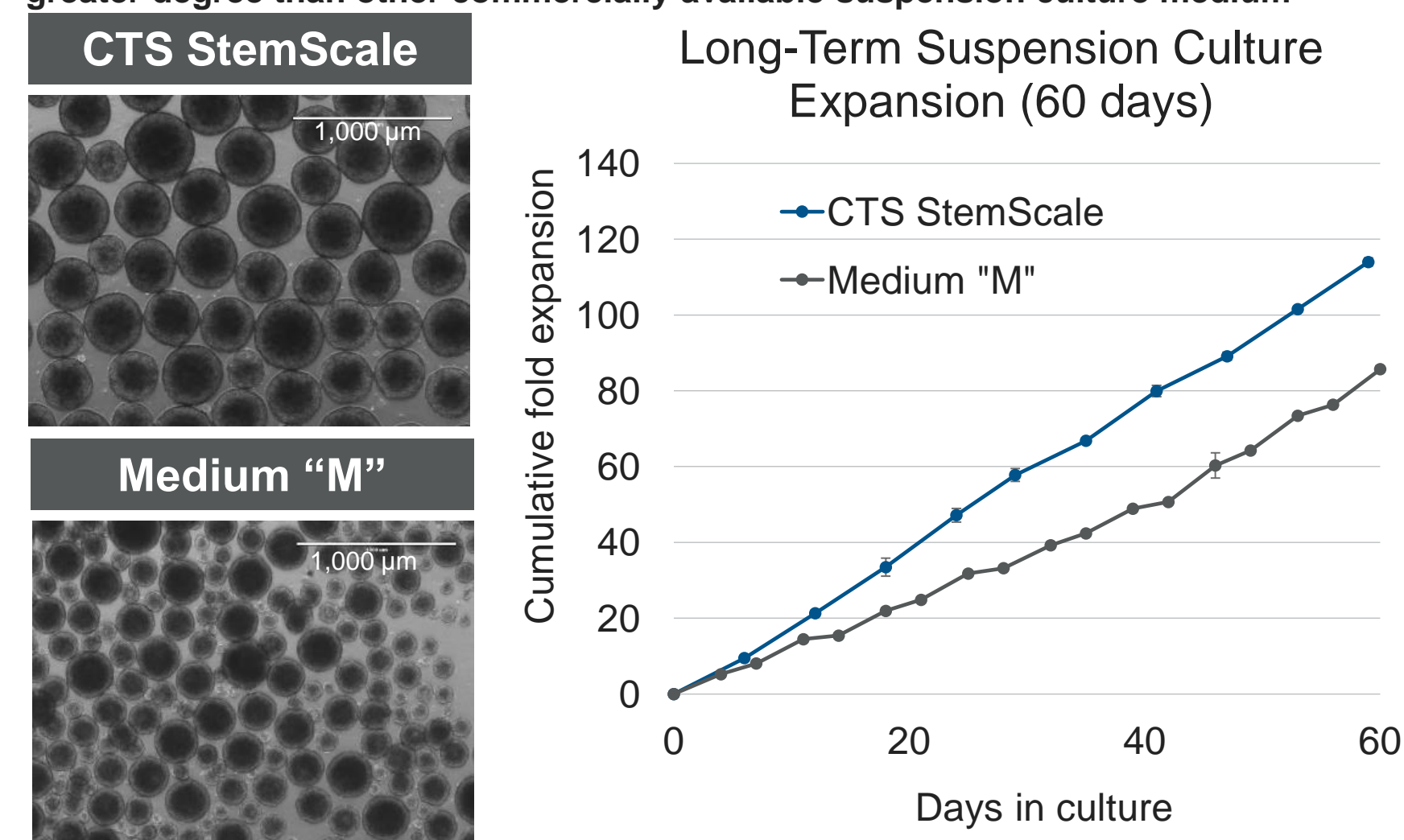
- Seed single cells at 200,000 cells/mL.
- Include 10 μM Y-27632 and 0.1U/mL Dnase I.
- Grow on an orbital shaker (70 RPM using a 19 mm orbit diameter).
- Feed daily with 50% medium replacement.
- Passage when spheroids are 300 – 400 μm in diameter.
- Use diluted CTS TrypLE Select to passage (0.25X CTS TrypLE at small-scale).

Spheroid Scale-Up

- Use the cell yields from smaller vessels to seed into larger vessels.
- 125 mL shake flask → 100 mL spinner flask → 500 mL spinner flask → 3L bioreactor.
- Scale-up is flexible and can be initiated in any sized vessel, depending on cell quantity.
- Ask us for more recommendations on scaling up to liter-scale vessels!

Results

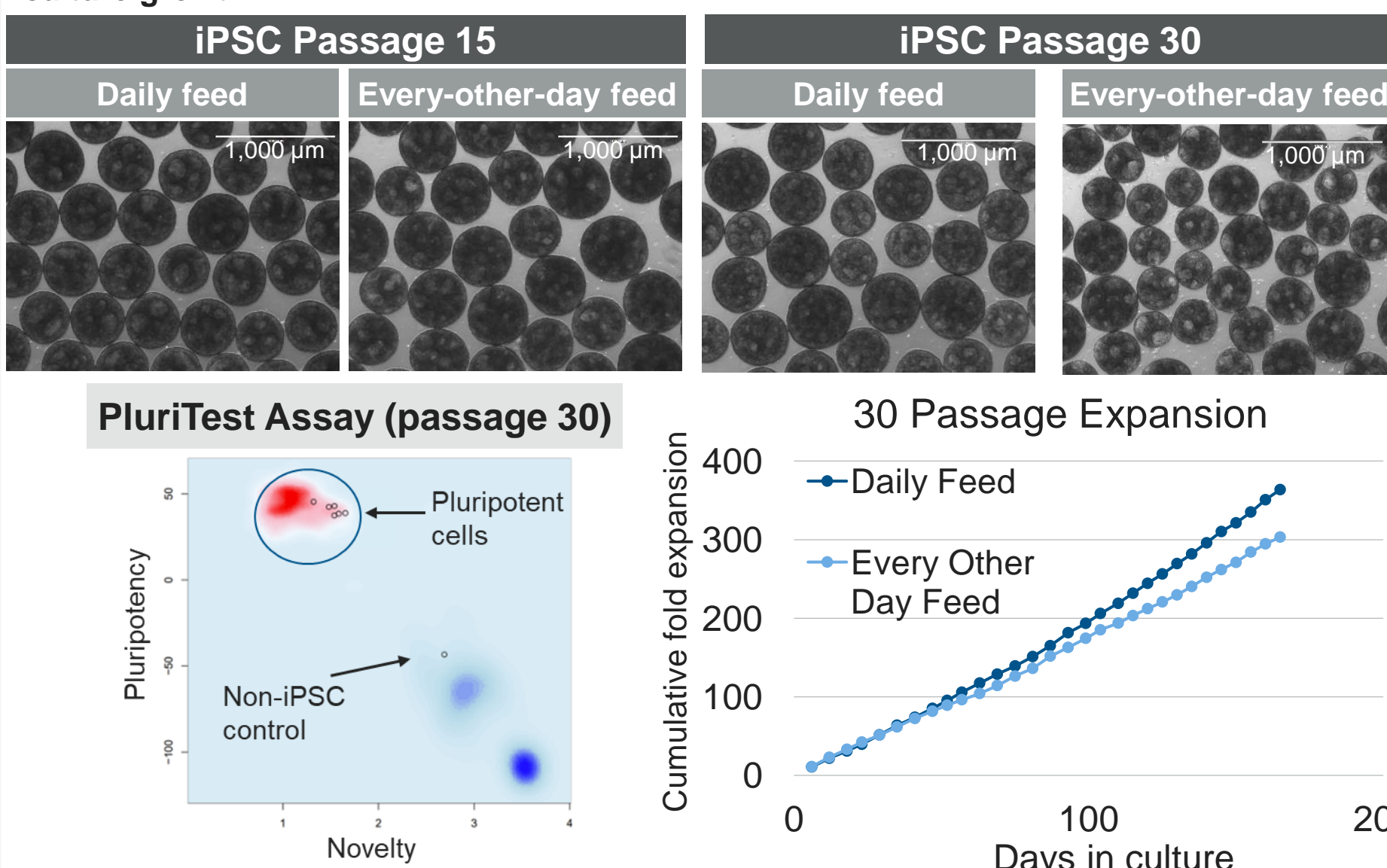
Figure 2. CTS StemScale PSC Suspension Medium enhances spheroid expansion to a greater degree than other commercially available suspension culture medium



Compared to other commercially available suspension culture media, CTS StemScale PSC Suspension Medium shows greater expansion potential over multiple consecutive passages. CTS StemScale exhibits 25% greater cell expansion over a 60-day culture period. Additionally, CTS StemScale requires fewer passages over the 60-day culture period: 10 passages using CTS StemScale, compared to 18 passages using Medium "M".

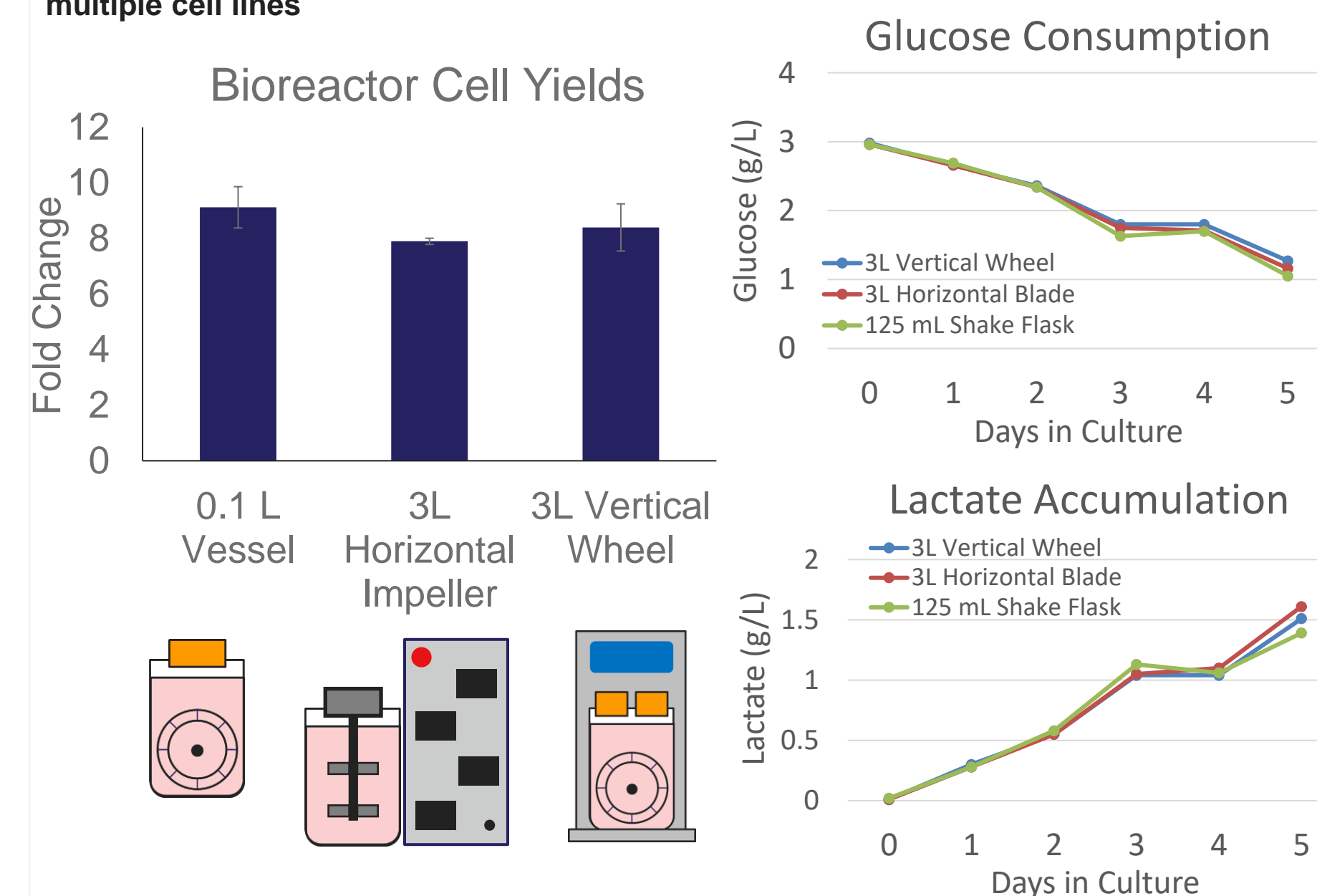
Results (continued)

Figure 3. Daily feeding with CTS StemScale Medium promotes consistent long-term culture growth



Spheroid can consistently grow in CTS StemScale over 30 consecutive passages when fed daily. The expansion potential of the spheroids begins to decrease over time when fed every other day. Regardless of feed strategy, all spheroids remain pluripotent after 30 consecutive passages. Additionally, while the data is not shown on this poster, the cells were also found to remain genomically stable via the KaryoStat assay.

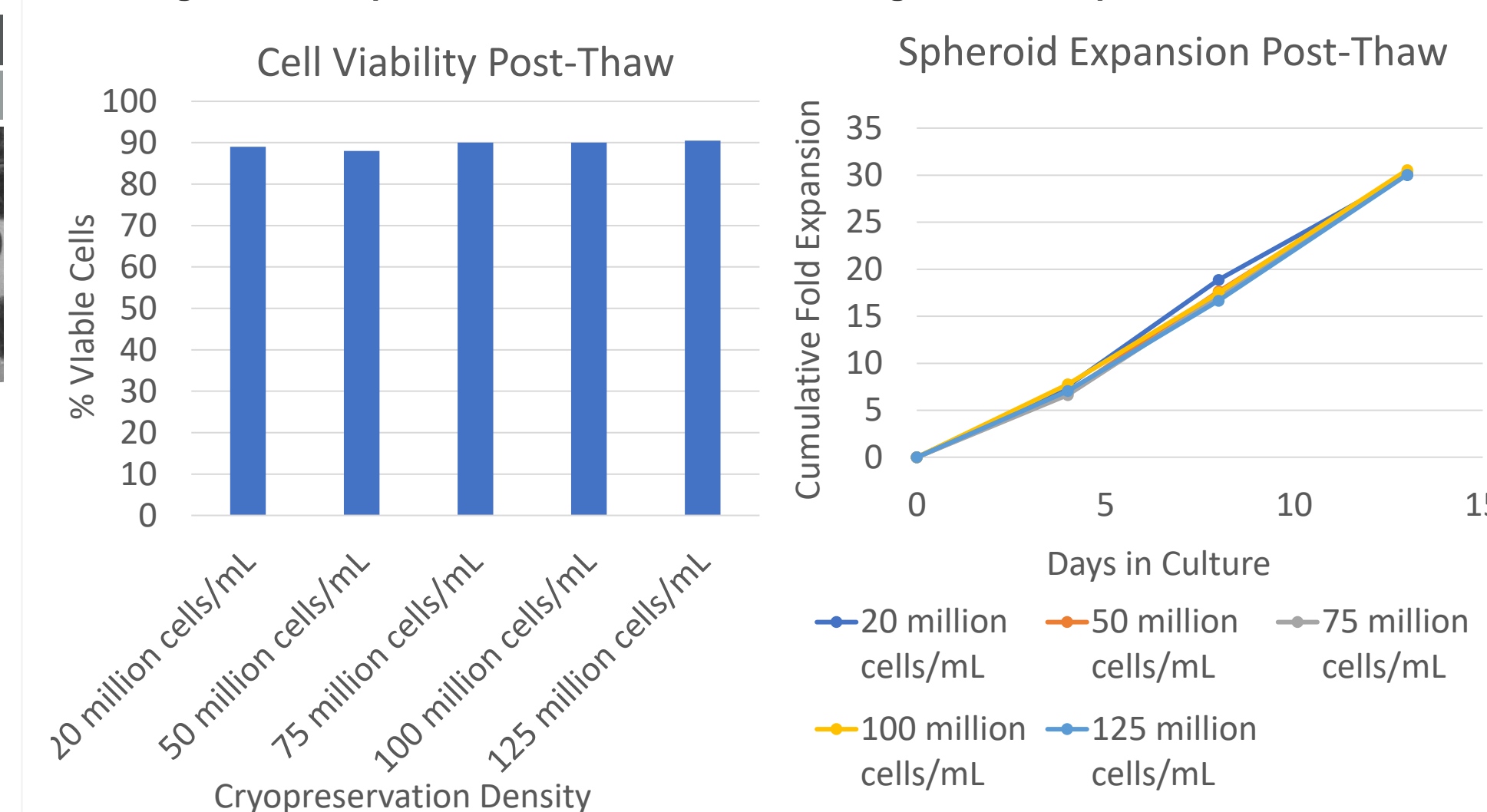
Figure 4. CTS StemScale PSC Suspension Medium shows consistent growth across multiple cell lines



CTS StemScale PSC Suspension Medium supports the growth of spheroids in liter-scale bioreactors. Growth is possible in different types of bioreactors, including horizontal blade impeller bioreactors and vertical wheel impeller bioreactors. Bioreactor cell yields show fold expansion comparable to small-scale vessels.

Results (continued)

Figure 5. Cells harvested from spheroids can be cryopreserved at high densities to directly seed large-scale suspension culture vessels according to future experiments



Cells obtained from dissociated spheroids are capable of being cryopreserved at high densities using CTS PSC Cryomedium. Cells maintained a high viability immediately after thaw. Notably, cells from all cryopreservation densities also showed similar rates post-thaw. These proof-of-concept results indicate it is possible to cryopreserve cells at high densities that enable large-scale culture vessels (e.g., 500 mL) to be seeded directly on demand.

Conclusions

- CTS StemScale PSC Suspension Medium spheroid nucleation is optimized to promote efficient growth in suspension cultures.
- CTS StemScale PSC Suspension Medium supports periodic media exchanges and the ability to passage cells without the need for a cell strainer.
- CTS StemScale PSC Suspension Medium is amenable to vessel sizes from 6-well plates to larger bioreactors.
- Cells from spheroids grown in CTS StemScale PSC Suspension Medium can be cryopreserved or utilized for other downstream applications.

Acknowledgements

We would like to thank Bob Scott, Lauren Sangenario, Michael Derr, and Richard Josephson for assistance with proliferation and differentiation studies.

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