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Optimization of Pluripotent Stem Cell Spheroid Growth in Liter Scale Bioreactors via Continuous Medium Perfusion

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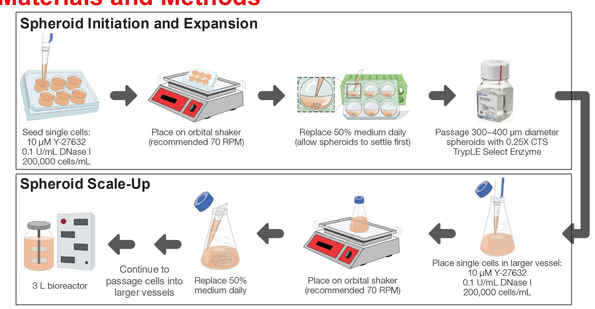
Abstract / Introduction

The development of pluripotent stem cell (PSC) culture protocols in multi-liter bioreactors is important for efficiently scaling PSC yields to manufacturing levels. We have previously demonstrated that PSCs can be expanded as spheroids in 3 L stirred-tank bioreactors (SBRs). These systems use impellers that continuously mix the culture medium but also generate shear stress, which may negatively affect PSC growth. Additionally, protocols that rely on gravity sedimentation of PSC spheroids and manual aspiration of spent medium become impractical beyond ~3 L culture scale. SBRs can be combined with perfusion systems to continuously supply fresh medium and eliminate the need for manual medium exchange. Here, we describe our ongoing efforts to optimize PSC spheroid growth in perfusion-fed, multi-liter bioreactor cultures by balancing stir speed, shear stress, and medium exchange rates. PSCs were seeded into dual pitch blade horizontal impeller-based SBRs at low stir speeds (i.e., low RPM) to promote spheroid formation. The RPM was then gradually increased to prevent spheroid aggregation and maintain culture homogeneity. Unsurprisingly, shear stress was detrimental to spheroid growth at higher speeds; however, this effect was mitigated by the addition of Pluronic. Specifically, 0.1–0.2% Pluronic enabled the formation of larger, more uniform spheroids, resulting in increased cell yields. We further evaluated spheroid growth using tangential flow depth filtration (TFDF) and alternating tangential flow (ATF) perfusion systems for medium exchange. Both systems supported spheroid expansion with similar efficiencies. Under optimized conditions, we observed yields of up to 25 billion PSCs in 2 L cultures (~12 × 10^6 cells/mL; ~80-fold expansion) after 10 days, while maintaining high levels of pluripotency marker expression (>95% $OCT4^+$/$NANOG^+$ cells). Overall, these results demonstrate that PSC culture in perfused SBRs is a promising approach for meeting manufacturing-scale demands.

Materials and Methods



Spheroid Scale-Up

- Seed single cells at 200,000 cells/mL.
- Include 10 µM Y-27632 and 0.1U/mL DNase I.
- 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 1: Long sedimentation times contribute to undesirable PSC spheroid aggregation and unhealthy cultures

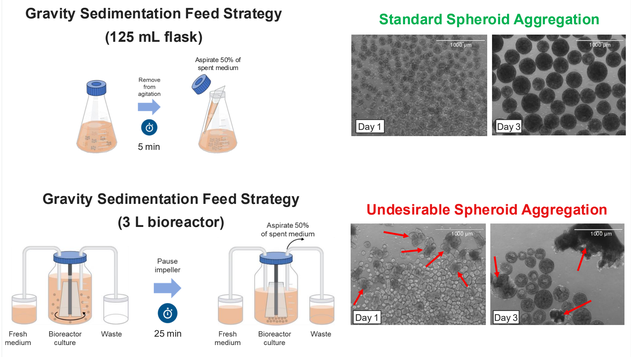


Figure 1. Gravity sedimentation allows spheroids to settle to the bottom of the vessel so that spent medium can be replaced from the top of the vessel. This approach is effective in small (< 1 L) vessels, as spheroids only require 5 – 10 minutes to settle. However, in large (> 1 L) vessels, spheroids require significantly more time (> 25 min) to settle. This extended time causes spheroids to aggregate into large clumps. At these larger culture volumes, perfusion feeding is required to maintain healthy cultures.

Figure 2: Optimizing multiple bioreactor parameters enhances spheroid growth

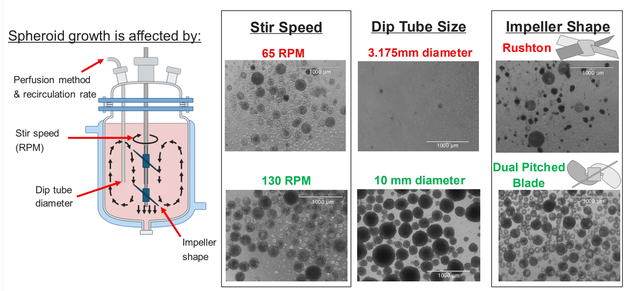


Figure 2. The high shear generated in a perfusion bioreactor can make it difficult to maintain healthy, uniform spheroid cultures. Thus, optimization of several bioreactor parameters is necessary to minimize shear stress. We observed that a speed of 130 RPM was optimal to initiate spheroid formation. After spheroids have formed, stir speeds can be increased to exert greater control of spheroid size over the culture duration. Secondly, choosing a dip tube of appropriate diameter to remove spent medium from the bioreactor is also important. If the tube diameter is too small, spheroids will be physically damaged and fail to grow. Furthermore, we found that using a dual pitched blade impeller contributed to maintaining uniform spheroid morphology. Pitched blade impellers tend to have gentler mixing and a low shear environment more favorable for spheroid growth compared to Rushton impellers.

Results (continued)

Figure 3: Tangential Flow Depth Filtration (TFDF) and Alternating Tangential Flow (ATF) perfusion systems support PSC spheroid growth

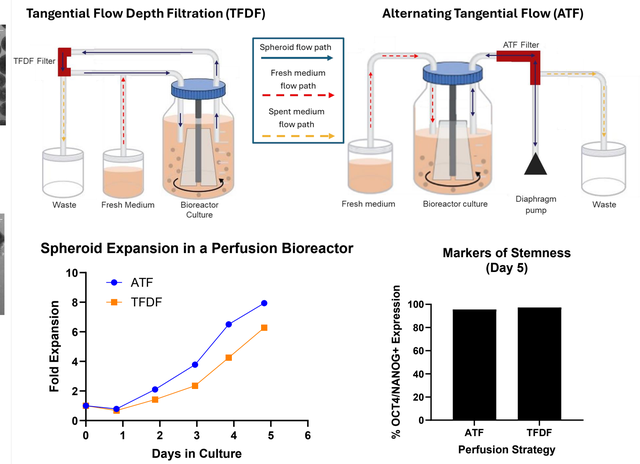


Figure 3. TFDF perfusion utilizes a closed loop to perfuse media, while ATF perfusion utilizes an alternating flow path to perfuse media. When we evaluated both perfusion methods, we observed similar rates of cell expansion over a 5-day period. The harvested cells were also found to maintain pluripotency, as assessed via flow cytometric analysis of NANOG+ and OCT4+ antibody expression. Taken together, these results suggest that both TFDF and ATF perfusion can support similar spheroid growth rates in stirred tank bioreactors.

Figure 4: Shear protectants promote the growth of uniform PSC spheroids

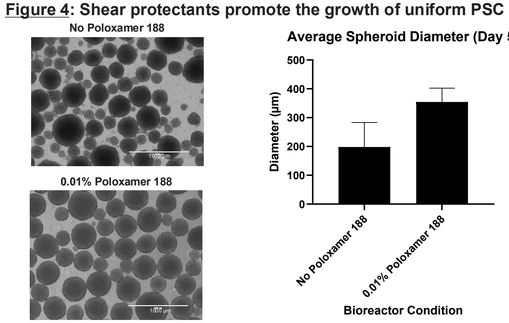


Figure 4. The high shear environment within a bioreactor leads to the formation of small spheroids that are non-uniform in size. To counteract these detrimental effects, we supplemented our cell culture medium with Poloxamer 188, a shear protectant that can minimize the damage induced by hydrodynamic forces within the bioreactor. We found that addition of 0.01% Poloxamer 188 can improve spheroid morphology by enabling spheroids to grow larger and more uniform in size, resulting in increased cell expansion.

Results (continued)

Figure 5: Extended culture time significantly improves cell expansion

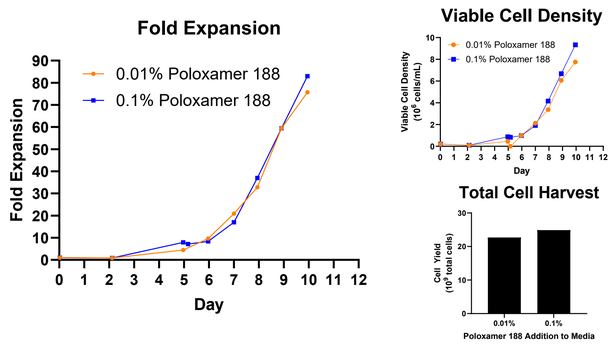


Figure 5. After confirming the perfusion systems could support spheroid growth in Figure 4, we extended the culture duration to allow spheroids to grow closer to our 300 – 400 um diameter recommendation. The bioreactors were seeded with cells and continually perfused with medium containing either 0.01% Poloxamer 188 or 0.1% Poloxamer 188 for 10 consecutive days. These bioreactors saw significant cell growth of approximately 80-fold expansion, or $10x10^6$ cells/mL. Ultimately, this resulted in the harvest of ~20 billion cells

Conclusions

1) Medium perfusion of PSC cultures is necessary to avoid the cell aggregation issues caused by gravity sedimentation in liter-scale suspension cultures.
2) Stir speed, impeller shape, and dip tube size are features of perfusion bioreactors that should be optimized for maximum cell expansion.
3) Addition of shear protectants can reduce the detrimental effects of the high shear environment in a liter-scale suspension culture.

Acknowledgements

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