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Optimizing Pluripotent Stem Cell Spheroid Growth in Liter Scale Bioreactors Through the Use of Constant Medium Perfusion

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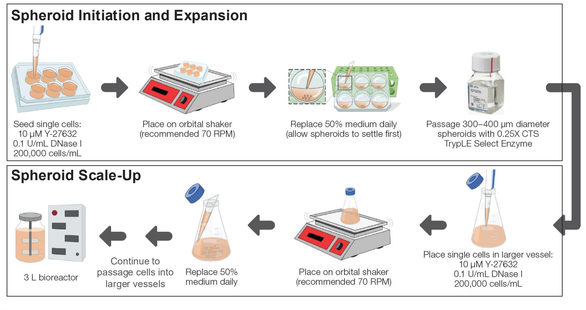
Abstract / Introduction

In order to efficiently meet clinical manufacturing needs requiring high quantities of pluripotent stem cells (PSCs) for downstream differentiation, the development and optimization of PSC culture methods in large volume bioreactors is critical. We have previously shown how PSCs can be expanded as spheroids in 3L stirred-tank reactors (STRs). These types of bioreactors use impellers that continuously mix the medium but also generate shear stress that may negatively affect PSC growth. Additionally, current protocols that rely on gravity sedimentation of PSC spheroids and manual aspiration of spent medium are impractical beyond a 3L culture scale. Notably, STRs can be combined with perfusion systems to constantly supply fresh medium and mitigate the need to manually exchange spent medium. Here, we describe our efforts to optimize PSC spheroid growth in liter scale bioreactor cultures by balancing stir speeds, shear stress effects, and medium exchange rates using constant perfusion of StemScale PSC Suspension Medium. Cells were initially seeded into STRs 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. Shear was detrimental to spheroid growth at high speeds, though this could be minimized with shear protectants (e.g., Poloxamer 188). Our results indicated that the addition of 0.1 – 0.2% Poloxamer 188 enabled spheroids to grow larger and more uniform in size, resulting in greater cell yields. We further evaluated spheroid growth by using tangential flow depth filtration (TFDF) and alternating tangential flow (ATF) perfusion systems to perform medium exchanges. We determined that both systems were able to support spheroid expansion with similar efficiencies. Taken together, with these optimized parameters we observed yields of up to 20 billion PSCs ($10x10^6$ cells/mL, equaling ~80-fold expansion) capable of maintaining pluripotency (>95% OCT4+/NANOG+ cells) after 10 days in culture. Overall, these results demonstrate that PSC culture in perfused STRs is an effective means to improve PSC expansion workflows.

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

Long sedimentation times contribute to undesirable PSC spheroid aggregation and unhealthy cultures

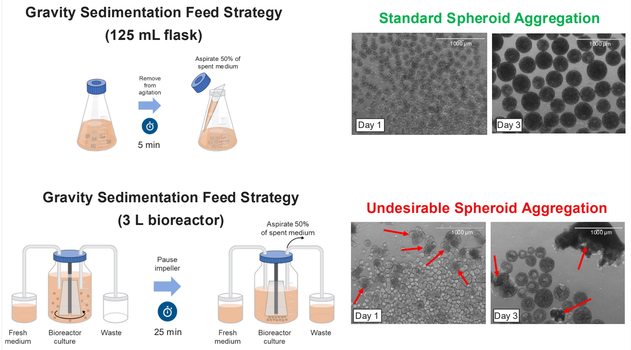


Figure 1. Gravity sedimentation allows spheroids to settle to the bottom of the vessel so that spent medium can be replaced from the top of the vessel. This approach is effective in small (< 1 L) vessels, as spheroids only require 5 – 10 minutes to settle. However, in large (> 1 L) vessels, spheroids 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.

Multiple bioreactor parameters must be optimized to enhance spheroid growth

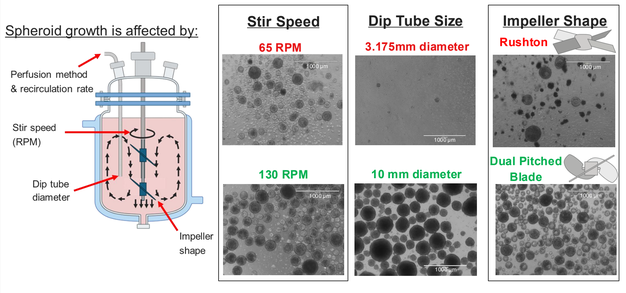
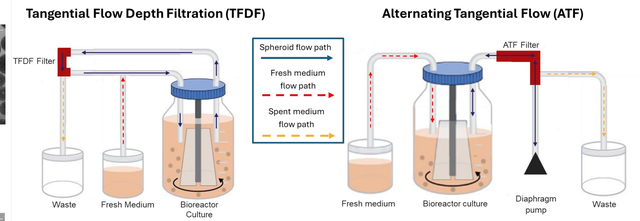
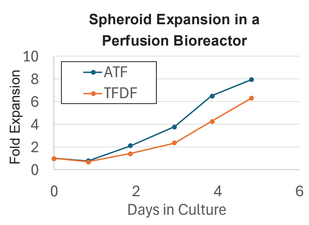


Figure 2. The high shear generated in a perfusion bioreactor can make it difficult to maintain healthy, uniform spheroid cultures. Thus, optimization of several bioreactor parameters is necessary to minimize shear stress. We observed that a speed of 130 RPM was optimal to initiate spheroid formation. 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)

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





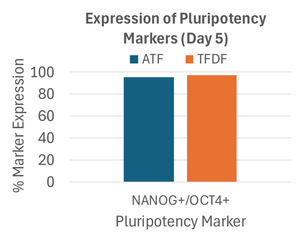


Figure 3. TFDF perfusion utilizes a closed loop to perfuse media, while ATF perfusion utilizes an alternating flow path to perfuse media. When we evaluated both perfusion methods, we observed similar rates of cell expansion over a 5-day period. The harvested cells were also found to maintain pluripotency, 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.

Shear protectants can help promote the growth of uniform PSC spheroids

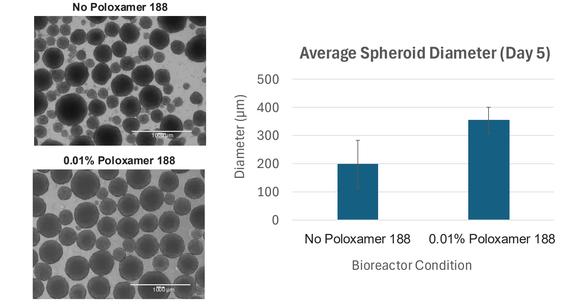
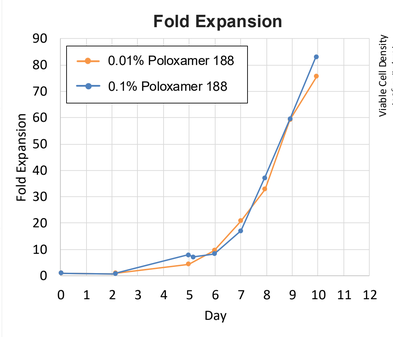


Figure 4. The high shear environment within a bioreactor leads to the formation of small spheroids that are non-uniform in size. To counteract these detrimental effects, we supplemented our cell culture medium with Poloxamer 188, a shear protectant that can minimize the damage induced by hydrodynamic 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)

Extended culture time significantly improves cell expansion



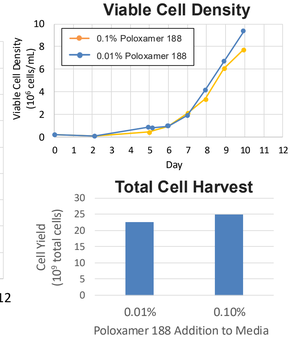


Figure 5. After confirming the perfusion systems could support spheroid growth in Figure 4, we extended the culture duration to allow spheroids to grow closer to our 300 – 400 um diameter recommendation. The bioreactors were seeded with cells and continually perfused with medium containing either 0.01% 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.

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