WHITE PAPER | PBS-MINI, PBS-3, & PBS-15 S.U.B.

Aggregate Perfusion Medium Exchange in Vertical-Wheel® Bioreactors for Scalable PSC Production

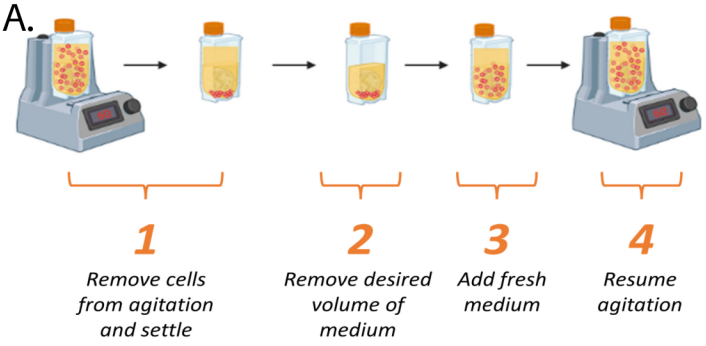
INTRODUCTION

Scale-up of quality-assured pluripotent stem cells (PSCs) to meet increasing demand for lifesaving therapies is heavily studied in cell therapy bioprocessing. While emphasis has been placed on the development of large-scale bioreactor technologies, success hinges on the ability to identify key process parameters that impact the yield and quality of cells at large scale. Aggregate size homogeneity is a critical quality attribute in PSC expansion that significantly affects downstream differentiation efficiency and lineage bias. This attribute is impacted by the medium exchange (MX) regime used during expansion. MXs in small scale bioreactors are commonly performed by stopping agitation to allow aggregates to settle, manually removing spent medium, replacing with fresh medium, and finally resuming agitation to resuspend settled aggregates. When culture samples were assessed directly after MX, we found that aggregate merging during settlement greatly increased aggregate size heterogeneity, even when settled in small-scale reactors (0.1-0.5L) for a short period of time. This MX method is more detrimental in large-scale bioreactor cultures where the required time for aggregate settlement is significantly greater. Using a filter perfusion MX system within the PBS Vertical-Wheel® (VW) bioreactor, we demonstrate how to reproducibly scale up PSC aggregate production from 0.5L to 15L scales while maintaining control of aggregate size homogeneity. Characterization of this perfusion method was performed in PBS-0.5 Mini vessels. It was found that this perfusion feeding protocol reliably produced cells with consistent growth rates/ yields and metabolic rates, resulting in more homogeneous distributions of smaller, more spherical aggregates compared to a control MX method of settled aggregates. Scale-up of this perfusion method into 3L and 15L VW bioreactors resulted in consistent growth kinetics, metabolite profiles, and aggregate morphologies across scales and demonstrated that perfusion feeding in VW bioreactors is a scalable method for high yield and quality maintenance of large-scale PSC production.

SETTLING METHOD FOR SMALL SCALE MX

MX is an important aspect of cell culture and can impact cell quality. MXs for PSC culture in small bioreactors typically involves settlement of cells either as aggregates or on microcarriers for a period to remove spent medium. While this method is commonly used, the settling of PSCs impacts aggregate homogeneity in culture and is not optimal for high quality PSC scale-up.

A.



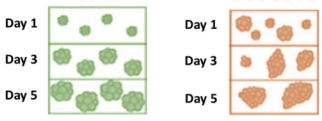
B.

PBS Vessel Volume (L)	Predicted Settling Time (mins)
0.1	5 (experimental)
0.5	9
3	14
15	26
80	43

Figure 1. (A) Overview of common MX process for PSC aggregate culture in small-scale. (B) Table depicting predicted settlement times for MXs in larger scale reactors performed with the settlement procedure. There is a significant increase in settlement time as vessel volume increases. Stokes' Law was used for theoretical calculations within minimum aggregate diameters of 60-100µm on Day 3.

IMPACT OF SETTLING ON AGGREGATE CULTURE HOMOGENEITY

A.



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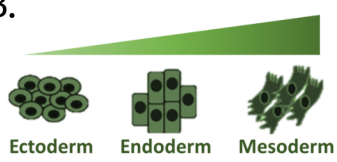

Figure 2. (A) The ideal aggregate culture involves growth of uniform, spherical, independent aggregates. (B) Aggregate size is a critical quality attribute in PSC expansion that impacts cell quality, phenotype, growth and differentiation potential. Different aggregate sizes have different lineage biases during differentiation.

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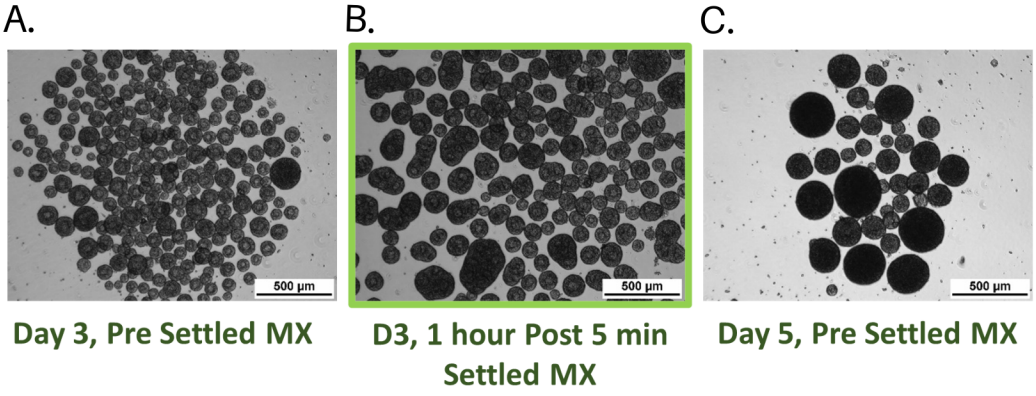

Figure 3. (A) Aggregates before first MX. (B) Aggregate merging is noticeable one hour post medium exchange by settlement. Images show non-spherical aggregates of varying size. (C) Aggregates regain sphericity, but heterogeneity caused by merging remains. Aggregate settlement, even for a short time (5 mins), results in aggregate merging and decreased homogeneity in aggregate size for the remainder of the culture.

SCALABLE MX USING PERFUSION

Perfusion is an MX method that has been widely used for process intensification in non-stem cell culture (Mendes et al., 2022). It not only enables maintenance of higher cell densities, but also creates a more homogenous culture environment and requires no interruption to agitation for medium exchange (Kropp et al., 2016).

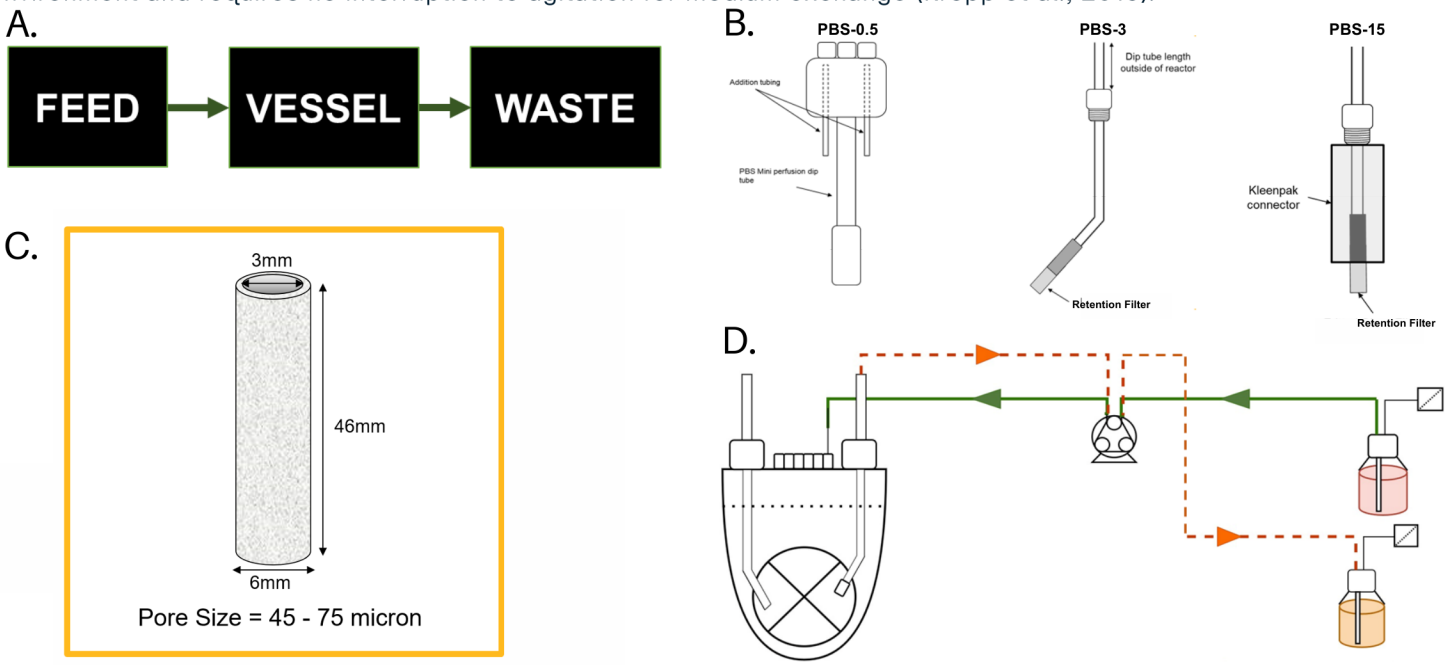

Figure 4. (A) Basic principle of perfusion: continuous addition of feed and removal of waste medium. (B) Adaptation of the dip tube/cell retention device into the PBS-0.5, -3 and -15 bioreactors, respectively (modified dip tubes for PBS-0.5 are not available from PBS Biotech at this time). (C) In situ porous plastic filter used for cell retention. (D) Perfusion schematic of the PBS-3. Key components: media & waste reservoirs, peristaltic pump, and tubing. Additional details on the perfusion system set-up for the PBS-3 are available by contacting PBS Biotech.

IMPACT OF PERFUSION MX ON CULTURE HETEROGENEITY

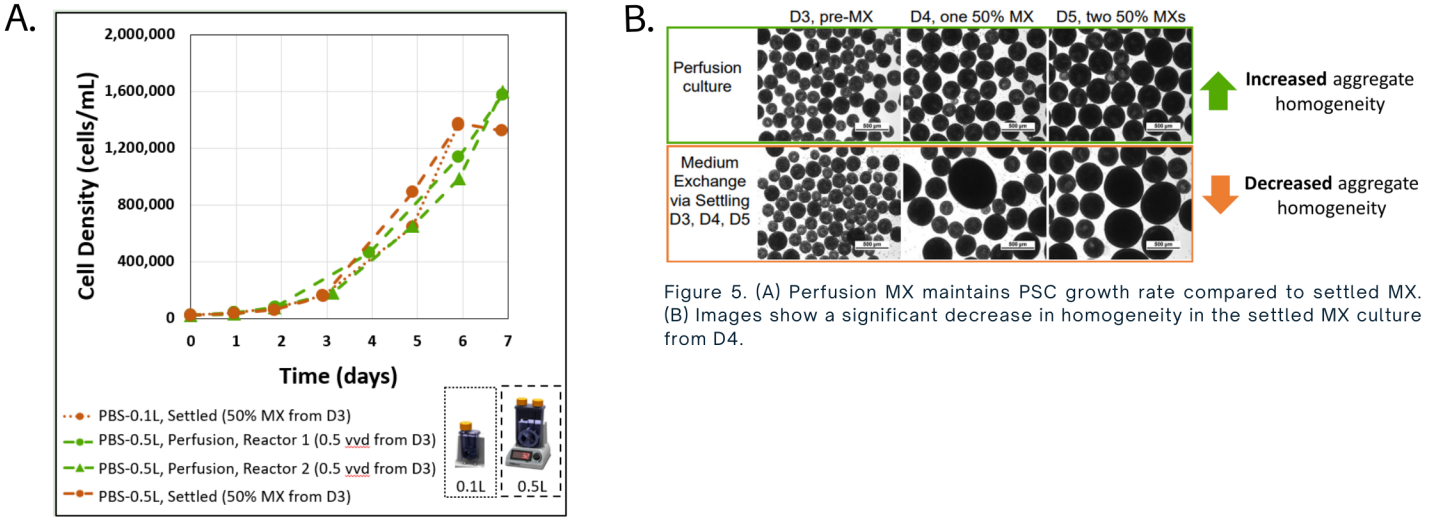

Figure 5. (A) Perfusion MX maintains PSC growth rate compared to settled MX. (B) Images show a significant decrease in homogeneity in the settled MX culture from D4.

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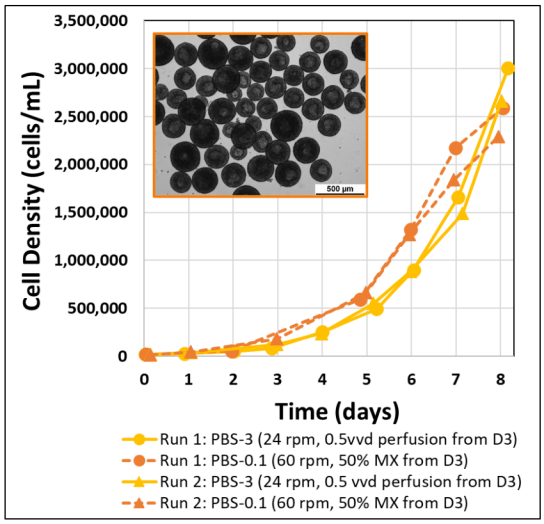
LINEAR SCALE-UP OF PSC AGGREGATE CULTURE TO 10L WORKING VOLUME

A.



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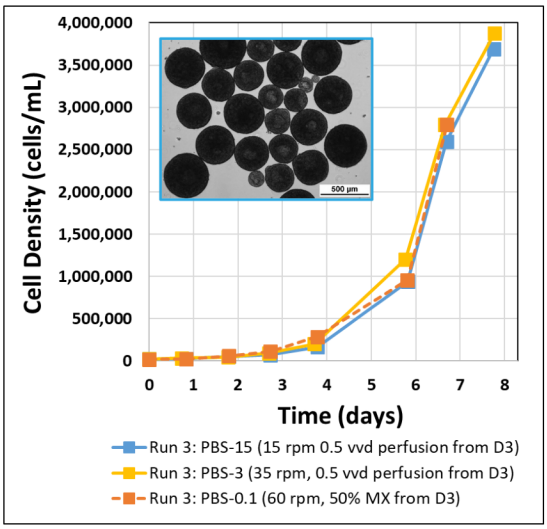

Figure 6. (A) Perfusion MX resulted in reproducible, robust and scalable growth kinetics between runs 1 and 2 performed by different operators. Image is a representative sample of homogenous aggregate morphology in PBS-3 culture on Day 5. (B) Perfusion MX enables scalability to 10L working volume in run 3 performed by a third operator. Image is a representative sample of aggregate morphology in PBS-15 culture on Day 5. All runs produced over 2 x 10^6 cells/mL by Day 8. PBS-15 yielded >3 x 10^6 cells/mL by Day 8 for a lot size of >30 billion cells.

CONCLUSION

- Commonly practiced settled MX methods are not scalable for high quality PSC aggregate culture due to the impact on aggregate size homogeneity
- Implementation of perfusion MX in PBS Vertical Wheel bioreactors enables linear scale-up of PSC aggregate culture to the PBS-15 and a yield of 3 x 10^6 cells/ mL
- Comparisons between settled MX and perfusion MX in the PBS-0.5 demonstrate that this Perfusion MX protocol reliably produces cells with consistent growth kinetics and greater aggregate size homogeneity compared to settled MX controls
- For information on the 60 Micron Plastic (PE) Retention Filter and additional guidance on PSC perfusion set-up using PBS-3 Vertical-Wheel Bioreactors, go to www.pbsbiotech.com/manuals or contact sales@pbsbiotech.com

ORDERING INFORMATION

Product	Part Number
PBS Mini Bioreactor Base Unit	FA-UNI-B-501
PBS-0.1 Mini Single-Use Vessels (4-pack)	FA-0.1-D-001
PBS-0.5 Mini Single-Use Vessels (4-pack)	FA-0.5-D-001
PBS-3 Vertical-Wheel Bioreactor, SUS	IA-3-B-711
PBS-3 Single-Use Vessel, Floored, SUS	FA-3-D-704-L
PBS-15 Vertical-Wheel Bioreactor	IA-15-B-501
PBS-15 Single-Use Vessel	IA-15-D-504-L
PBS-3 Dip Tube Assembly	IA-3-BA-007
60 Micron Plastic (PE) Retention Filter	IC01036

For more information, please contact your account manager at **sales@pbsbiotech.com**.

To place an order, please contact customer service at **customer.service@pbsbiotech.com**.

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