Simplifying Progress

Applying a DoE Approach to Optimize hiPSC Culture Conditions in the Ambr®15 Bioreactor

Amin Vossoughi[1]*, David Splan[1], Donald Traul[2], Art Hamfeldt[3], Denise van Rossum[4], Mark Szczypka[1]

[1]Corporate Research, Sartorius Stedim North America, Ann Arbor, MI, USA
[2]Cell Culture Technologies, Sartorius Stedim North America, Bohemia, NY, USA
[3]Senior Data Scientist, Sartorius Stedim North America, Bohemia, NY, USA
[4]Advanced Therapies, Sartorius Stedim North America, Bohemia, NY, USA
*Corresponding author: amin.vossoughi@sartorius.com

Introduction

The fundamental discovery and generation of human induced pluripotent stem cells (hiPSCs) in the Yamanaka and Thomson laboratories in 2007 has tremendously advanced the field of cell therapy and regenerative medicine. Despite the multiple advantages of hiPSCs, such as pluripotency, self-renewal, and low immunogenicity, these cells are notoriously difficult to propagate in 3D cultures due to high sensitivity to shear stress, special culture media requirements, and their susceptibility to differentiation in distressing culture conditions.

The Ambr®15 is a fully-automated cell culture bioreactor that consists of 24 or 48 mini bioreactors (15 mL) that can be utilized to optimize cell culture parameters by implementing a design of experiments (DoE) approach with MODDE®13 software. The scalability of this platform is a significant advantage that reduces the process cost prior to culture scale-up. In this study, a complete portfolio of Sartorius products, including cell culture media, bioreactors, and DoE software, was utilized to optimize the hiPSCs 3D culture conditions.

Experimental Approach

- A complete portfolio of Sartorius products was used to optimize the hiPSCs culture conditions
- Two hiPSCs media – NutriStem® hPSC XF and NutriStem® hPSC 3D ACF – were evaluated
- The effect of stirring speed on aggregate formation was analyzed for each media type
- The effect of seeding density on cell propagation was investigated for each media type

Figure 1: *Schematic of the Sartorius Products Used in the Project: (a) MODDE®13 software, (b) NutriStem® hPSC XF and NutriStem® 3D ACF Media, (c) Ambr®15 Bioreactor System*

- Four Ambr®15 bioreactors were used in the study
- Two seeding densities of 1.25e5 cells/mL and 5e5 cells/mL were tested
- The effect of stirring at 600 rpm and 800 rpm was evaluated for each media type
- Low seeding densities were studied at 600 rpm, and high seeding densities were studied at 800 rpm

Bioreactor	Seeding Density [cell/mL]	Media Type	Stirring Speed
ambr1	5e5	NutriStem® XF	600
ambr2	5e5	NutriStem® 3D ACF	600
ambr3	1.25e5	NutriStem® XF	800
ambr4	1.25e5	NutriStem® 3D ACF	800

Table 1: *DoE Using MODDE®13*

Description	Low	High
Density	1.25e5 cells/mL	5e5 cells/mL
Volume	15 mL	15 mL
Stir speed	600 rpm	800 rpm
pH set point	7.4	
DO set point	50%	
Temperature	37° C	
Duration	4 days	
Enzyme	Accutase®	

Table 2: *Culture Parameters*

Result

Aggregate Formation and Sizing

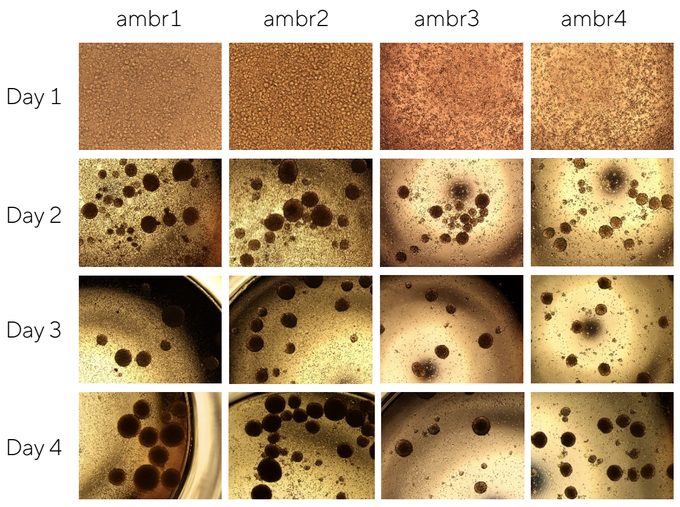

Figure 2: *Images of iPSC Aggregates Formed in 4x Ambr®15 Bioreactors Under Different Culture Conditions. As the Cultivation Progresses, Aggregate Size Increases in All Conditions*

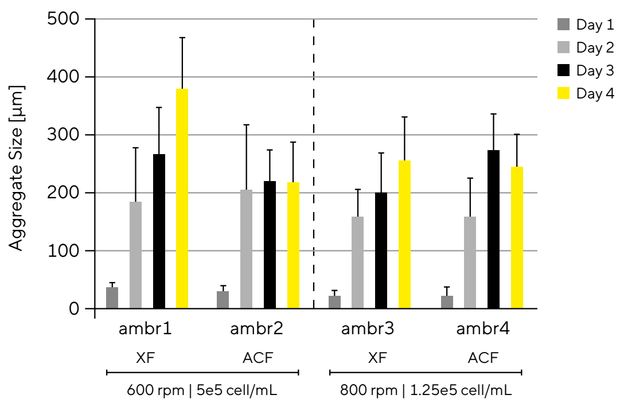

Figure 3: *iPSC Aggregate Size Increase Over 4 Days of Culture in Different Conditions. Conditions With High Cell Density and Low Stirring Speed (ambr1) Represented the Highest Aggregate Size*

Aggregate Concentration and Viability

Aggregates were dissociated into single cells using Accutase® and cell counts were performed using a NucleoCounter® NC-200™ cell counter. All culture conditions increased in cell number throughout the culture with cells cultured in NutriStem® hPSC 3D ACF at high density and high stirring speed (ambr4) showing the highest fold increase.

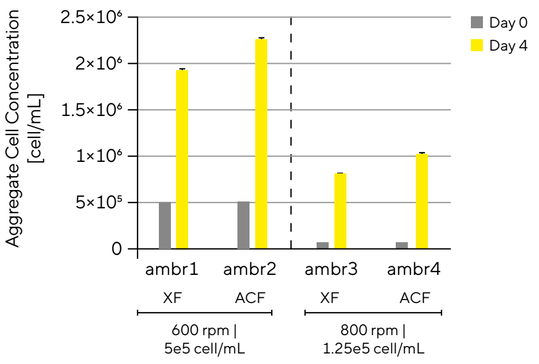

Figure 4: *Aggregate Cell Concentration on Day 0 and Day 4. NutriStem® hPS 3D ACF (ACF) Performed Better Compared to NutriStem® hPSC XF (XF)*

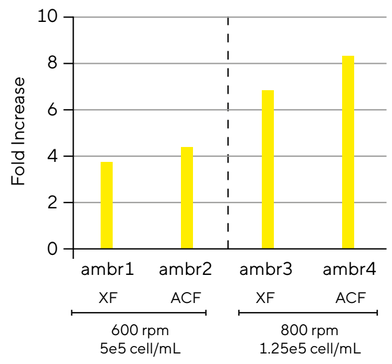

Figure 5: *Increase in Cell Counts in Each Bioreactor at the End of the Culture*

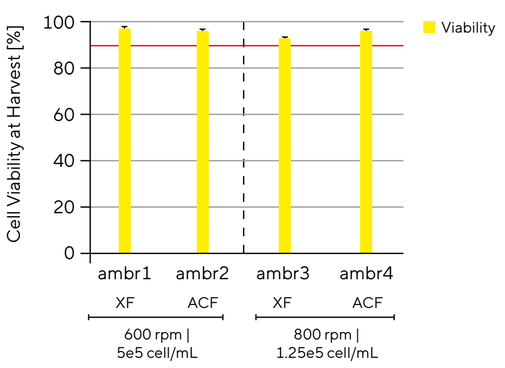

Figure 6:
Cell Viability on Harvest Day in Each Bioreactor. All Bioreactors Maintained > 99% Viability on Harvest Day

Single Cell Concentration and Viability

Single cells detached from aggregates contribute to the decline in cell harvest in the culture. Therefore, their count is of great importance.

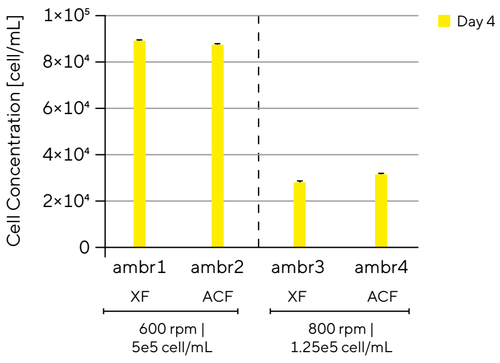

Figure 7: *Single Cell Concentration in Each Bioreactor on the Harvest Day*

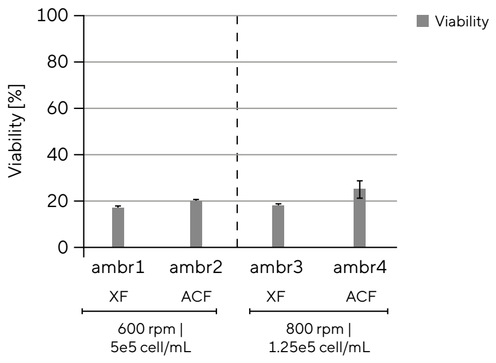

Figure 8: *Single Cell Viability on Harvest Day. Single Cells Contribute to Most of the Dead Cells in Culture*

MODDE 13® Results

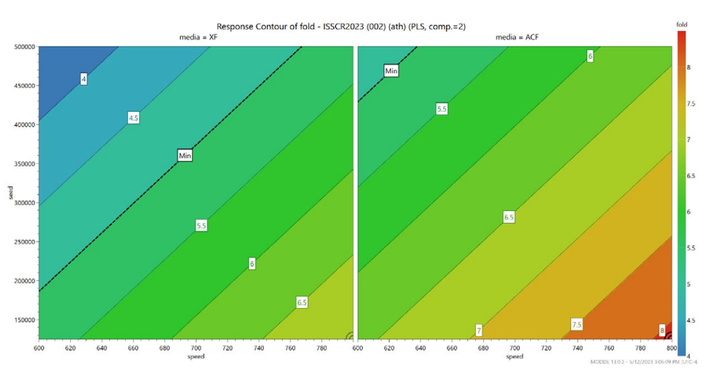

Figure 9:
Response Contour Plot. ACF Media Performed Better Compared to XF Media. Desirable Results Lie in the Red Region Which Relates to Lower Seeding Density (1.25e5 cells/mL), ACF Media and Higher Stirring Speed (800 rpm)

Conclusion

This study was able to optimize the iPSC culture conditions on the Ambr®15 platform using Sartorius product portfolio, including media, bioreactor, and MODDE®13 software. The major conclusions from this study includes:

- The NutriStem® hPSC 3D ACF media led to higher fold increase in cells compared to NutriStem® hPSC XF media in both datasets
- Both seeding densities tested resulted in a successful aggregate formation
- Aggregate size increased over the culture period ranging from 70 µm to 400 µm with cells cultured in NutriStem® hPSC XF at low density and stirring speed (ambr1) reaching the highest diameter
- Aggregate viability in all conditions was > 90% on harvest day
- MODDE®13 results imply that higher speed, lower seeding density, and NutriStem® hPSC 3D ACF media are in favor of cell growth