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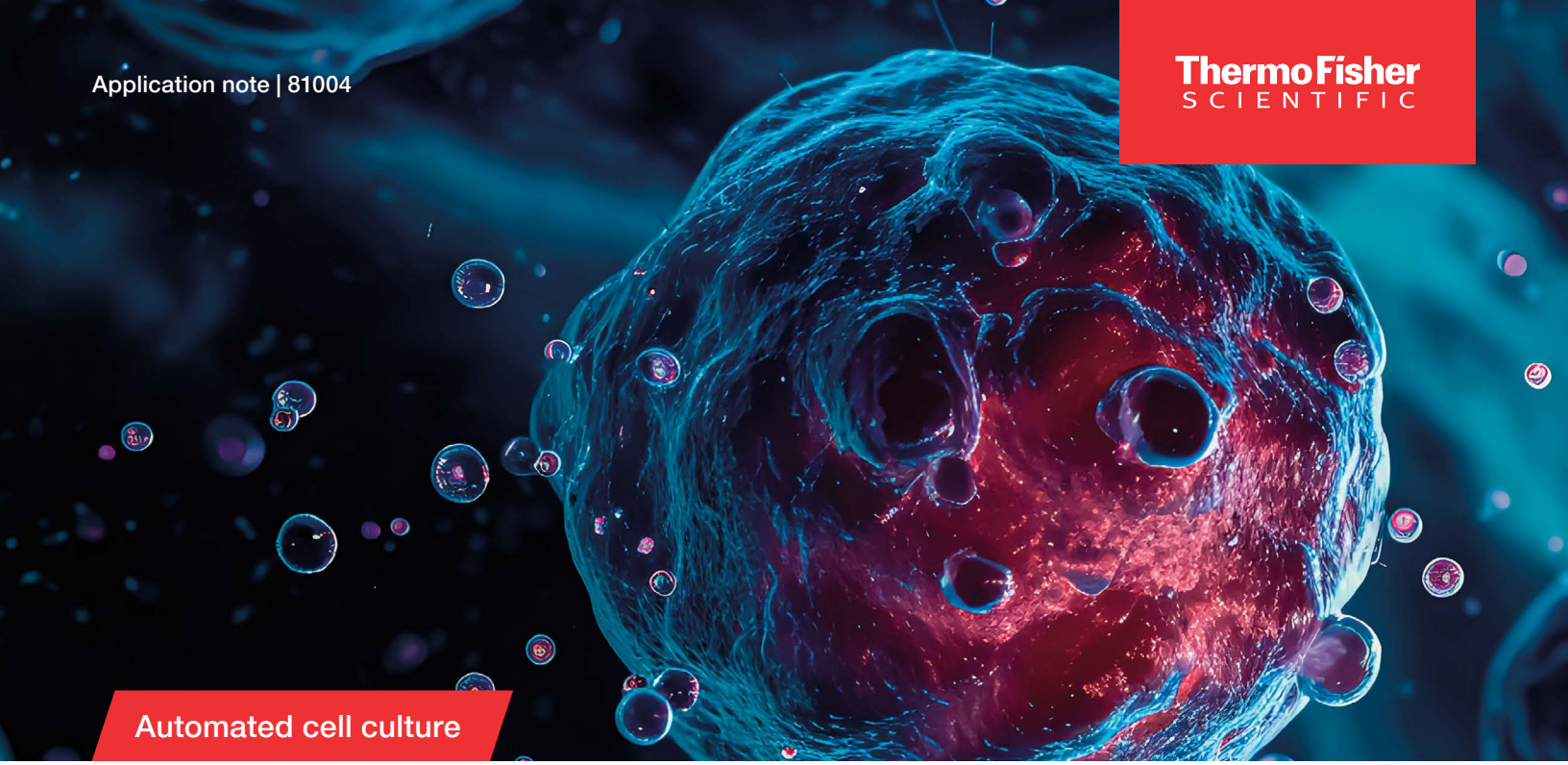

Automated cell culture

Automated small-scale suspension cell culture for bioprocess development

Automating CHO suspension cell culture for media formulation screening using 96 deep-well plates and a Tecan Fluent Automation Workstation

Keywords

Automated cell culture, suspension cell culture, CHO cell culture, bioprocess development, high-throughput screening, media formulation screening, laboratory automation workstation

Introduction

Optimizing cell culture media and supplements is crucial for biopharmaceutical production, particularly during process development. Fine-tuning the composition of the culture media and adjusting the incubation conditions are vital steps to enhance yields.

Currently, suspension cell screening is predominantly conducted in shake flask systems with limited throughput and scalability. The use of small-scale culture systems suitable for high throughput screening can significantly enhance the number of samples and formulations that can be tested within a single experiment for more efficient, cost-effective evaluation of optimal growth conditions.

An evaporation study was conducted before performing cell expansion experiments to identify the labware combinations that minimized media loss and ensured optimal experimental conditions. The growth performance of Gibco™ ExpiCHO-S™ Cells was then evaluated to assess the suitability of 96 deep-well plates for automated small-scale culture in process development. CHO cells are widely used for expressing biotherapeutic proteins, and account for about 70% of licensed biologics production. The Gibco™ ExpiCHO™ Expression System Kit is specifically designed for high-density

suspension cultures in a chemically defined, serum-free medium. Several investigations were carried out to optimize process parameters, such as the sample volume and agitation rate, to accelerate cell growth and ensure robust data.

This study describes automated cell seeding and handling of 96 deep-well plates on the Tecan® Fluent® Automation Workstation. Furthermore, integration of a Thermo Scientific™ Cytomat™ 2 C450-LiN Automated Incubator, featuring two Thermo Scientific™ tower shakers with the Fluent workstation, enables true orbital shaking for the dynamic incubation of suspension cell cultures.

Materials and methods

Automation workstation

Experiments were conducted on a Tecan Fluent 780 workstation with an integrated Tecan Multiple Channel Arm (MCA) 384 for pipetting and a Tecan Robotic Gripper Arm (RGA) robotic manipulator for plate handling (Figure 1A). The use of an Extended Volume Adapter (EVA) on the MCA allowed parallel pipetting of up to 96 channels, accommodating volumes of up to 500 µL per tip. A Cytomat 2 C450-LiN automated incubator (Figure 1B) equipped with two 7-position tower shakers (52 mm pitch, 3 mm oscillation amplitude) was integrated with the system, enabling storage and shaking incubation of deep-well plates throughout the workflow. The incubator was set to 37 °C and 8% CO_2 to provide an optimal, stable environment for cell growth. The system's ContraCon™ automated decontamination feature simplifies cleaning and eliminates variability in disinfection without using harmful gases.

The Fluent system was equipped with a vertical laminar flow HEPA hood with Bigneat UV light to ensure a clean environment for sterile liquid handling. Scripts for cell seeding, plate shuttling, and plate incubation were developed using Tecan® FluentControl® software.

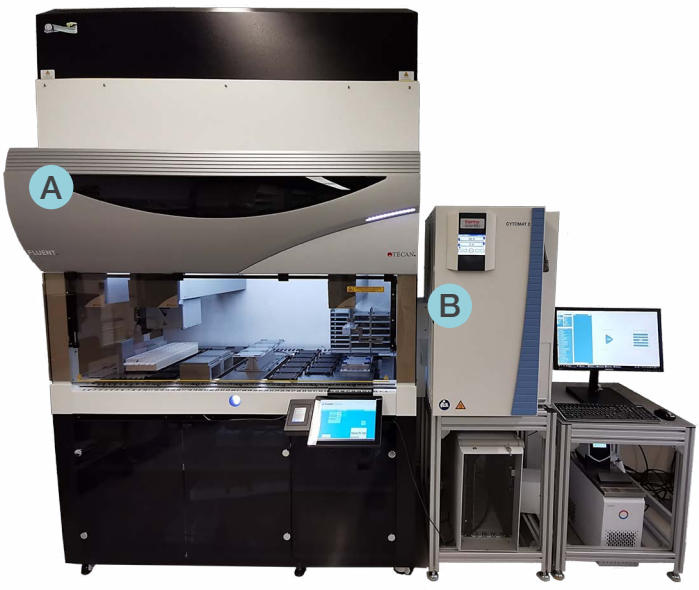

Figure 1. A Fluent automation workstation (A) was integrated with a Cytomat 2 C450-LiN automated incubator (B) to automate the preparation and incubation of 96 deep-well plates.

Evaporation testing

A preliminary experiment was performed to evaluate evaporation during shaking incubation across various types of 96 deep-well plates (Table 1), each covered with a Cytiva™ universal polystyrene lid.

Table 1. Specifications of the labware used for evaporation testing.

Brand	VWR	J.T. Baker	BRAND	Axygen
Vendor	VWR	VWR	VWR	Corning
Part No.	732-3324	43001-0020	391-5705	P-DW-20-C-S
Well capacity	1 mL	2 mL	2 mL	2 mL
Well shape	Round	Square	Square	Round
Bottom shape	U-bottom	U-bottom	U-bottom	U-bottom

Changes during incubation were monitored by adding 0.6 mL of water to each well and storing the plates in the central positions of the tower shakers. The plates were weighed before incubation, and again after 1, 4, and 7 days of shaking incubation at 1,000 rpm with a 3 mm oscillation amplitude. Plate weights were recorded over time, and the average remaining weight per well was calculated for each time point.

Cell expansion in an Erlenmeyer flask (manual protocol)

One vial containing 1 x 10^7 ExpiCHO-S cells (Part No. A29127) was thawed according to the manufacturer's protocol. The cells were expanded for 4 days in 30 mL Gibco™ ExpiCHO™ Expression Medium (Part No. 29100-01) in a 125 mL Corning™ Erlenmeyer flask (Corning Part No. 430421) on a 25 mm amplitude Infors™ HT Celltron™ shaking platform set to 120 rpm. The shaker setup was placed inside a standard cell culture incubator maintained at 37 °C with 8% CO_2.

Cell densities and viabilities were determined on days 0, 2, and 4. On day 4, the final day of the pre-expansion process, the cells were centrifuged at 300 x *g* and resuspended in fresh expansion media. They were then adjusted to a concentration of 3 x 10^5 cells/mL before being seeded into 96 deep-well plates.

Cell expansion in 96 deep-well plates (automated protocol)

Unless stated otherwise, aliquots of the cell suspension were transferred from INTEGRA Biosciences™ sterile troughs (INTEGRA Biosciences Part No. 6327) to sterile, non-adhesive Axygen™ 96 deep-well plates (Sigma Part No. P-DW-20-C-S) using 500 µL filtered disposable tips on the EVA adapter. Two mixing cycles were performed at the source trough before each aspiration step to ensure homogenous cell distribution. The plates were then covered with the Cytiva polystyrene lids (Cytiva Part No. 7704-1001) and transferred to the humidified automated incubator set at 37 °C, 8% CO_2 and stable relative humidity above 90%. Shaking incubation was carried out with a 3 mm oscillation amplitude at either 600 or 900 rpm. Cell densities and cell viabilities were determined at different time points post seeding.

1. Parameter optimization

Initially, process parameters were investigated by evaluating different incubation volumes and shaking speeds. A separate plate was prepared for each tower shaker, consisting of 4 rows filled with different starting volumes, ranging from 0.3 mL/well in row B to 1.2 mL/well in row H (Figure 2A).

A single row of tips was mounted on the EVA to transfer aliquots of 0.3 mL per tip and per pipetting iteration, to achieve the final volumes per well outlined in Figure 2. The plates were covered with lids and positioned in the middle positions of either the left or right tower shaker before initiating shaking at 600 rpm or 900 rpm, respectively (Figure 2B). Cell densities across the different conditions were assessed 3 and 5 days post-seeding, with 1 well sampled per condition at each time point.

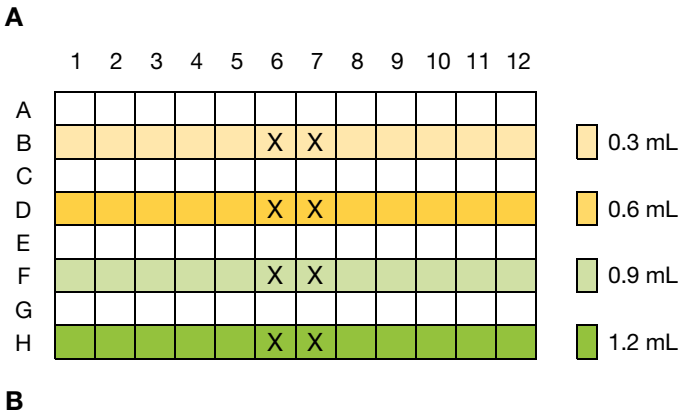

Figure 2. Plate preparation and positioning for growth condition screening. (A) Four distinct starting volumes were tested, each represented by a separate row. The analyzed wells are indicated by an X. (B) The plates were incubated in the central sections of the towers, which were set to different shaking frequencies.

2. Scale-up and growth consistency testing

A follow-up experiment evaluated the consistency of growth kinetics between plates incubated in different stacker positions, and between individual wells of the same plate. Three deep-well plates, each containing 1.2 mL/well, were incubated in 1 shaker, while 7 plates containing 0.6 mL/well were incubated in the second shaker (Figure 3B). Both shakers were set to 900 rpm during incubation. Cell densities were determined on days 3 and 5 post-seeding.

For the 0.6 mL/well setup, 1 center well was sampled from each plate at each time point (Figure 3A) to assess growth kinetic consistency across plates. For the 1.2 mL/well setup, 5 wells were sampled per plate at each time point, including 4 corner wells and 1 central well (Figure 3A) to evaluate consistency between wells of the same plate.

Cell counting

To assess cell concentration and viability, the samples were diluted at a 1:4 ratio in culture media and analyzed using a Beckman Coulter™ Vi-CELL™ XR Cell Viability Analyzer with the trypan blue dye exclusion method.

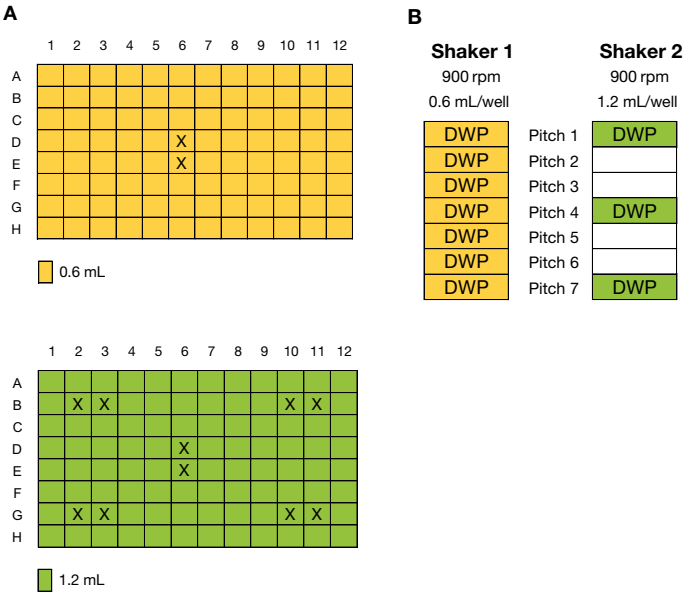

Figure 3. Plate preparation and positioning for growth consistency testing. (A) All wells of the plates were either filled with 0.6 mL or 1.2 mL of cell suspension. The analyzed wells are indicated by an X. (B) Shaker 1 was fully loaded with plates, while the top, middle and bottom positions were used for Shaker 2.

Table 2: Evaporation testing results across various labware types.

Brand		VWR	J.T. Baker	BRAND	Axygen
Vendor		VWR	VWR	VWR	Corning
Part No.		732-3324	43001-0020	391-5705	P-DW-20-C-S
Well capacity		1 mL	2 mL	2 mL	2 mL
Well shape		Round	Square	Square	Round
Bottom shape		U-bottom	U-bottom	U-bottom	U-bottom
D0	Weight plate dry (g)	148.3	116.3	115.5	94.4
	Weight plate filled (g)	205.6	173.4	172.4	151.8
	Weight/well (g)	0.60	0.60	0.59	0.60
	Recovery (%)	**100.0**	**100.0**	**100.0**	**100.0**
D1	Weight plate (g)	204.3	173.0	171.6	151.2
	Weight/well (g)	0.58	0.59	0.58	0.59
	Recovery (%)	**97.7**	**99.2**	**98.6**	**99.0**
D4	Weight plate (g)	200.6	171.7	169.8	150.1
	Weight/well [(g)	0.54	0.58	0.57	0.58
	Recovery (%)	**91.3**	**97.0**	**95.3**	**97.1**
D7	Weight plate (g)	196.1	169.8	166.9	149.7
	Weight/well (g)	0.50	0.56	0.54	0.58
	Recovery (%)	**83.3**	**93.6**	**90.3**	**96.3**

Results

Evaporation testing

Evaporation testing was conducted to evaluate the performance of various 96 deep-well plates under shaking incubation conditions, including VWR™, J.T.Baker™, BRAND™, and Axygen plates, each covered with universal polystyrene lids. The Axygen plates exhibited the lowest evaporation rates with the given lid, making them the most suitable choice for subsequent experiments. The weight recovery percentages for the Axygen plates were consistently high, with 99.0% on day 1, 97.1% on day 4, and 96.3% on day 7, demonstrating minimal average evaporation over the total incubation period.

Pre-expansion in an Erlenmeyer flask (manual protocol)

The pre-expansion of ExpiCHO-S cells in an Erlenmeyer flask showed robust exponential growth (Figure 4).

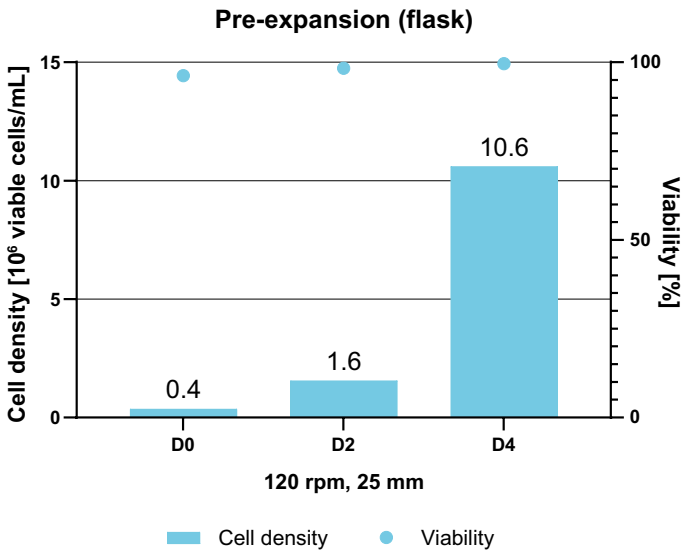

Figure 4. ExpiCHO-S cell expansion in an Erlenmeyer flasks over 4 days.

The initial cell concentration was 0.36 x 10^6 viable cells/mL with a viability of 96.2%. After 4 days of incubation in the shake flask, the cell density increased to 10.6 x 10^6 viable cells/mL, with a viability exceeding 99%. This high proliferative activity confirmed the suitability of the ExpiCHO-S cell line for subsequent automated processes.

Cell expansion in 96 deep-well plates (automated protocol)

1. Process parameter screening

The process parameter investigation experiments were crucial in identifying the ideal conditions for cell growth in 96 deep-well plates. Various starting volumes and shaking speeds were tested to determine their impact on cell density and viability. Incubation at the lower shaking speed of 600 rpm resulted in lower cell densities on day 5 across all tested volumes compared to incubation at 900 rpm (Figure 5).

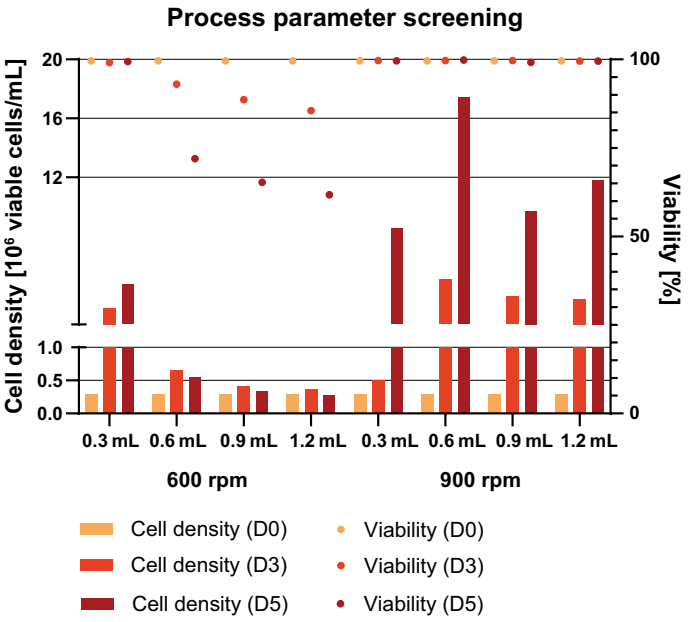

Figure 5. Impact of shaking speed and starting volume on ExpiCHO-S cell growth and viability in 96 deep-well plates.

While a shaking speed of 600 rpm supported substantial cell proliferation for the smallest test volume (0.3 mL/well), poor cell growth was observed for higher volumes. Furthermore, increasing the starting volume led to a marked decrease in cell viability by day 3, with an even more pronounced decline by day 5. This inverse correlation between cell viability and incubation volume suggests insufficient sample agitation for larger volumes, resulting in cell death likely due to a lack of proper media oxygenation.

Alternatively, incubation at 900 rpm resulted in robust cell growth and consistently high cell viability across all volumes tested. Notably, a starting volume of 0.6 mL/well yielded the most rapid cell amplification, reaching high cell densities in a shorter time frame. Nevertheless, a higher absolute cell yield per well was observed with a starting volume of 1.2 mL/well, despite the slower growth rate and therefore lower yield per volume. These findings suggest that while 0.6 mL is optimal for rapid cell expansion, 1.2 mL is preferable when a larger cell yield per well or plate is required.

The results indicate that the shaking speed and starting volume are critical parameters that need to be carefully optimized to balance the rate of cell growth and the total cell yield. The ability to fine-tune these parameters allows greater flexibility in experimental design and can significantly enhance the efficiency of process development.

2. Growth consistency assessment

The growth consistency testing was designed to evaluate the uniformity of cell growth kinetics across plates and wells when incubated in different stacker positions within the same shaker. Seven plates, each containing 0.6 mL/well, were incubated in 1 shaker (Figure 6), while 3 of the 96 deep-well plates, each containing 1.2 m L/well, were incubated in the other shaker (Figure 7). Both shakers were set to 900 rpm during incubation.

For the 0.6 mL/well setup, 1 center well was sampled per plate at each time point, to evaluate growth consistency between plates in the same shaker. Cell densities were highly uniform across all 7 plates, showing only minor variations (Figure 6). All sampled wells maintained cell viability above 95% at all time points. The results demonstrate high consistency in cell growth kinetics across plates in different stacker positions of the same shaker. Additionally, cell densities on days 3 and 5 roughly matched those observed during parameter screening with the same settings, underscoring the robustness of both the cell line and the automation equipment across different runs.

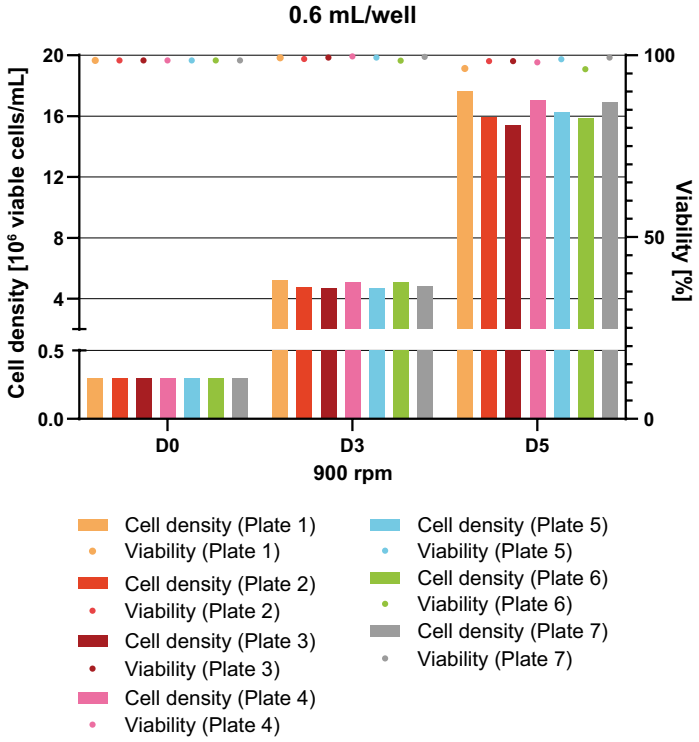

Figure 6. Consistency of ExpiCHO-S cell growth across multiple 96 deep-well plates with 0.6 mL starting volume.

For the 1.2 mL/well setup, 5 wells were sampled per plate at each time point, including 4 corner wells and 1 central well, to evaluate growth consistency between wells of the same plate. Consistent growth was demonstrated, with minimal variation between plates, and between corner and central wells of the same plate (Figure 7). All sampled wells maintained cell viability above 95% at all time points, highlighting the suitability of the selected process parameters for ExpiCHO-S cell expansion.

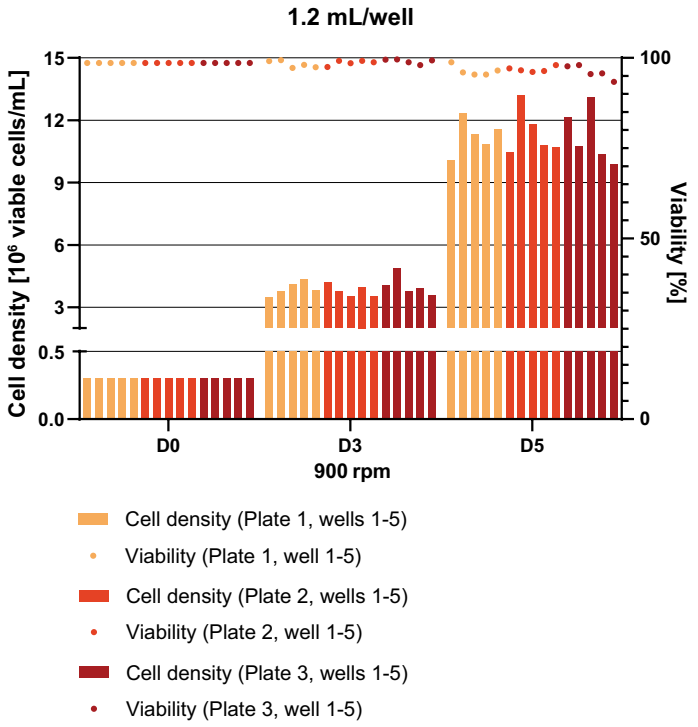

Figure 7. consistency of ExpiCHO-S cell growth within 96 deep-well plates with 1.2 mL starting volume.

On the final day of incubation (day 5), cell densities for the 1.2 mL/well setup were between 9.87 x 10^6 and 13.20 x 10^6 viable cells/mL, and between 15.39 x 10^6 and 17.65 x 10^6 for the 0.6 mL/well setup. This corresponds to coefficients of variation (CVs) of 8.9% for the higher volume (n=15, 5 wells, 3 plates), and 4.5% for the lower volume (n=7, 1 well, 7 plates), which are commendable values considering biological variation and minor differences in starting volumes. Like the parameter screening assessment, the observed growth rates were slower for 1.2 mL/well (Ø density/well on day 5: 11.29 x 10^6) compared to 0.6 mL/well (Ø density/well on day 5: 16.44 x 10^6), again suggesting a suboptimal sample agitation, and therefore media oxygenation, leading to a decrease in proliferative activity. Although cell counts per volume were higher for the 0.6 mL/well setup, the total cell yield per well was greater for the 1.2 mL/well starting volume. The average total cell yields per well can be obtained by multiplication of sample volume x average cell concentration, which results in a total cell yield of 9.87 x 10^6 viable cells/well for the 0.6 mL/well setup, and 13.55 million viable cells/well for 1.2 mL/well on day 5.

Conclusion

This study successfully demonstrated the effectiveness of the automated small-scale suspension cell culture system using a Fluent automation workstation integrated with a Cytomat 2 C450-LiN automated incubator. This system enables true orbital shaking, ensuring uniform growth conditions across different plate positions and minimizing variability. The use of 96 deep-well plates allowed consistent and reproducible CHO cell cultivation, showing high plate-to-plate and well-to-well consistency, and a performance comparable to traditional shake flask systems. Critical process parameters were identified and optimized to enhance cell growth performance and maximize yield.

The results presented here highlight the system's potential for high throughput media formulation screening and automated incubation, providing a scalable and cost-effective solution for bioprocess development. The automated platform proved to be a reliable tool for generating reproducible data, which is crucial for accelerating the development and optimization of biopharmaceutical production processes.

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