Development and use of a scale-down model with the DynaSpin Single-Use Centrifuge

Centrifuge

Introduction

As cell culture processes for monoclonal antibody (mAb) production intensify and increase in scale, single-use centrifuges like the Thermo Scientific™ DynaSpin™ Single-Use Centrifuge are becoming more attractive as harvest solutions than traditional two-stage depth filtration. This application note describes the development of a scale-down model for the DynaSpin Single-Use Centrifuge and how it can be used to narrow down the operational conditions for a large-scale harvest. Several factors must be considered when designing a harvest operation for a typical cell culture process, including operation time, centrate clarity, product yield, and cost. Since it is not always possible to perform all of the desired process parameter testing at scale using a manufacturing-scale centrifuge, a small-scale centrifuge with higher throughput capacity is desirable.

Our methodology followed that of Westoby et al. [1], who developed scale-down models for large stainless steel disk stack centrifuges using sigma theory. With sigma theory, or the concept of equivalent settling area, the separation efficiency of a continuous flow centrifuge can be compared to that of a batch centrifuge. Aside from the physical characteristics of the centrifuge rotor (fin count, radius, angle), it is important to determine a so-called correction factor (*c*) to account for the non-ideal flow of fluid between the disk fins. The correction factor is determined empirically by measuring separation efficiency under various conditions and comparing those to conditions run on a batch centrifuge, which is assumed to provide ideal separation. We conclude with further discussion on how to use this scale-down model to inform the next stage of purification (i.e., depth filtration).

Materials and methods

The cell lines listed in Table 1 were used to determine the correction factor for the DynaSpin Single-Use Centrifuge. The cell lines and culture conditions were selected to recreate conditions found in standard fed-batch cell culture for mAb production.

Table 1. Cell lines and culture conditions used to develop a scale-down model for the DynaSpin centrifuge.

Cell line	Final viability (%)	Packed cell volume (%)	Turbidity (NTU*)
Gibco™ ExpiCHO-S™ Cells	68–98	3–30	1,400–7,313**
CHO-K1 GS cells	64	8	2,100
Cellca	82	10	3,500
CHO Edge cells	90	4.5	1,500
Sellexis CHO-K1 cells	78	7.5	3,000

* NTU: nephelometric turbidity units.

** Cultures from standard fed-batch and intensified processes were used.

Cultures were spun down using the DynaSpin centrifuge at flow rates from 2 L per minute (LPM) to 11 LPM and rotor speeds from 1,500 rpm to 3,600 rpm to produce a sufficient range of separation efficiencies to generate the model. Bioreactor samples were also spun down using an Allegra™ X-12R swinging bucket benchtop centrifuge (Beckman Coulter) at 800 rpm to 2,000 rpm for 3 minutes. This range was selected to extend beyond the likely range of inlet flow rates and equivalent settling areas the DynaSpin centrifuge would cover to help determine the correction factor. A correction factor of 0.4 has been reported in the literature for stainless steel disk stack centrifuges [2].

The optical density (OD_{600}) of each test supernatant or centrate was measured after centrifugation, and the percentage of solids remaining was calculated using Equation 1.

Equation 1.

$$\frac{OD_{600}\text{ test sample} - OD_{600}\text{ solid-free sample} \times 100\%}{OD_{600}\text{ untreated sample} - OD_{600}\text{ solid-free sample}}$$

Each untreated sample was taken directly from the bioreactor, and each solid-free sample was generated by spinning down a bioreactor sample at 10,000 x *g* for 5 minutes.

Filter sizing study

Samples from each culture were collected in 50 mL conical tubes (40 mL working volume) and spun down on the benchtop swinging bucket centrifuge. The supernatants were pooled to perform a filter sizing (P_{max}) study along with the corresponding centrates from the DynaSpin centrifuge. The supernatants and centrates were run through 23 cm^2 single-stage Millistak+™ B1HC-grade depth filters (MilliporeSigma) at a flow rate of 100 $L/m^2/hr$ (LMH) until an inlet pressure of 15 psi was reached according to the manufacturer’s instructions. Samples were also taken from the DynaSpin centrates to evaluate cell lysis and clarification. A Cedex™ Bio HT Analyzer (Roche) was used to measure lactate dehydrogenase (LDH) in the samples, and turbidity was measured with a TL2360 LED Turbidimeter (Hach).

Flow rate evaluation

To simplify the scale-down model approach, an appropriate flow rate range was determined. A shear device is typically used to mimic the shear imparted by a centrifuge rotor. However, it has been reported that the high rotor speeds of a stainless steel centrifuge may cause additional cell lysis during harvest [3]. Cell lysis should thus be considered while developing a scale-down model using sigma theory. To that end, the LDH data from DynaSpin centrates obtained at the top recommended rotor speed (3,600 rpm) at flow rates from 4 LPM to 11 LPM were compared. The data indicate that the rotor itself does not cause shear-induced cell lysis, even at top speed. There was little to no increase in LDH with flow rate up to 8 LPM (Figure 1), although some increase was observed at higher flow rates depending on cell type and bioreactor condition. Due to the increase in LDH observed at higher flow rates, the methodology in subsequent steps was limited to mimicking a flow rate of 2–7 LPM on the DynaSpin centrifuge.

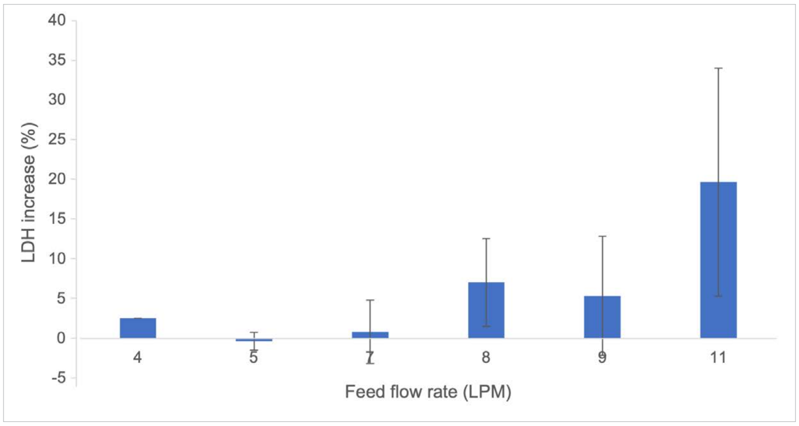

Figure 1. Increase in the LDH concentration measured after centrifugation with the DynaSpin Single-Use Centrifuge. The increase in LDH is shown as a function of inlet flow rate.

Method for benchtop centrifuge

To obtain supernatants of similar clarity using the swinging bucket benchtop centrifuge and ultimately determine the correction factor, the benchtop centrifuge first had to be characterized. The acceleration and deceleration times and their dependence on the selected rpm were determined, as were the distances from the axis of rotation to the top and bottom of the liquid while the centrifuge was operating. For a 50 mL conical tube with a 40 mL fill volume, the distance to the top of the liquid was 104 mm, and the distance to the bottom of the liquid was 188 mm. The ramp-up and ramp-down times were linearly correlated with the rpm setpoint (Figure 2).

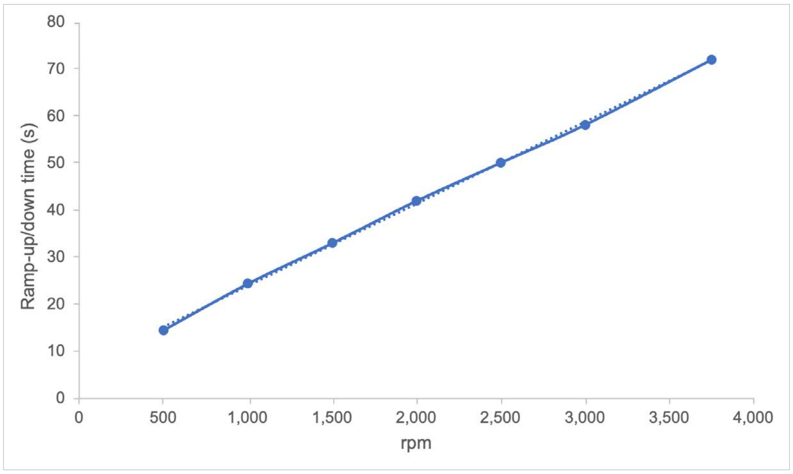

Figure 2. Acceleration/deceleration time on the swinging bucket centrifuge as a function of rotor speed.

Based on these characteristics, the equivalent settling area of the swinging bucket centrifuge ($\sum_{Lab}$) was calculated using Equation 2.

$$\Sigma_{Lab} = \frac{\omega^2(3 - 2u - 2d)V}{\ln\left(\frac{2r_2}{r_2 + r_1}\right)6g}$$

Equation 2. Equivalent settling area of the swinging bucket centrifuge, where ω is the angular velocity of the bowl; u is the fractional time for ramp-up; d is the fractional time for ramp-down; and r_1 and r_2 are the distances from the axis of rotation to the top and bottom of the liquid, respectively.

Similarly, the equivalent settling area of the DynaSpin centrifuge was calculated using Equation 3.

$$\Sigma_{DynaSpin} = \frac{2\pi n\omega^2(r_o^3 - r_i^3)}{3g\tan\theta}$$

Equation 3. Equivalent settling area of the DynaSpin centrifuge, where n is the number of disks; r_o and r_i are the outer and inner disk radii, respectively; θ is the half-disk angle; and g is the gravitational force. This can be simplified to Equation 4:

$$\Sigma_{DynaSpin} = 2.7252 \times 10^{-3} \times \omega^2$$

Equation 4. Equivalent settling area of the DynaSpin centrifuge as a function of rotor speed.

Once both centrifuges were characterized, a dataset covering a range of $Q/\sum_{DynaSpin}$ and $V/t\sum_{Lab}$ was generated. If the appropriate correction factor was determined, the DynaSpin continuous flow centrifuge could be equated to the swinging bucket centrifuge by Equation 5.

$$\frac{Q}{c\Sigma_{DynaSpin}} = \frac{V}{t\Sigma_{Lab}}$$

Equation 5. Q is the inlet flow rate of the DynaSpin centrifuge; c is the correction factor; V is the volume spun down in the swinging bucket centrifuge; and t is the spin time in the swinging bucket centrifuge.

Normalization

To use the different data sets from all of the cultures tested, some normalization was necessary. This was done by dividing the percentage of solids remaining under each condition tested for a given harvest by the percentage of solids remaining under the 5 LPM condition on the DynaSpin centrifuge run at 3,600 rpm. The normalized result for all tests conducted under the 5 LPM condition was thus 1. The normalized results for conditions that left a higher percentage of solids than the 5 LPM condition were above 1, and results for conditions that left a lower percentage than the 5 LPM condition were below 1. The actual percentage of solids remaining after centrifugation ranged from 1% to 27% (data not shown). Assuming the efficiency of the DynaSpin centrifuge was 100% (a correction factor of 1), normalization yielded the results shown in Figure 3. Although there was clearly a linear relationship for each centrifuge, the slopes were quite different.

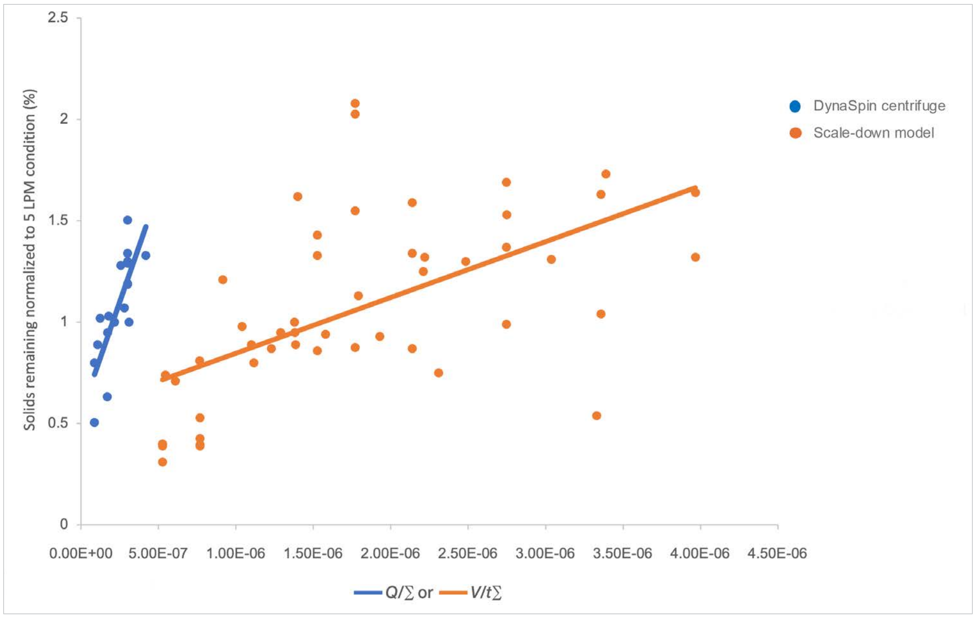

Figure 3. Solids remaining after centrifugation normalized to the 5 LPM condition with a correction factor of 1.

Correction factor determination

To determine the correction factor, a Monte Carlo simulation was performed to simulate conditions of a 10,000 scale-down model over a uniform distribution of the normalized remaining solid results over the range of $V/t\sum_{\text{Lab}}$ tested. Linear regression analysis was also performed with the normalized remaining solid results obtained for the DynaSpin centrifuge by plotting them against $Q/c\sum_{\text{DynaSpin}}$. Using a solver, the c term was allowed to change so that a new slope and intercept would be generated with each iteration. With the new slope and intercept, a paired result for the DynaSpin centrifuge was generated for each of the scale-down model conditions. The RMSE was calculated based on the difference between each predicted pair, and a minimum RMSE was found using a correction factor of 0.13. This correction factor was applied to the DynaSpin centrifuge data, and the values for each centrifuge were plotted as a function of $Q/c\sum_{\text{DynaSpin}}$ or $V/t\sum_{\text{Lab}}$ (Figure 4). Although variability was quite high, there was reasonable alignment between the two data sets.

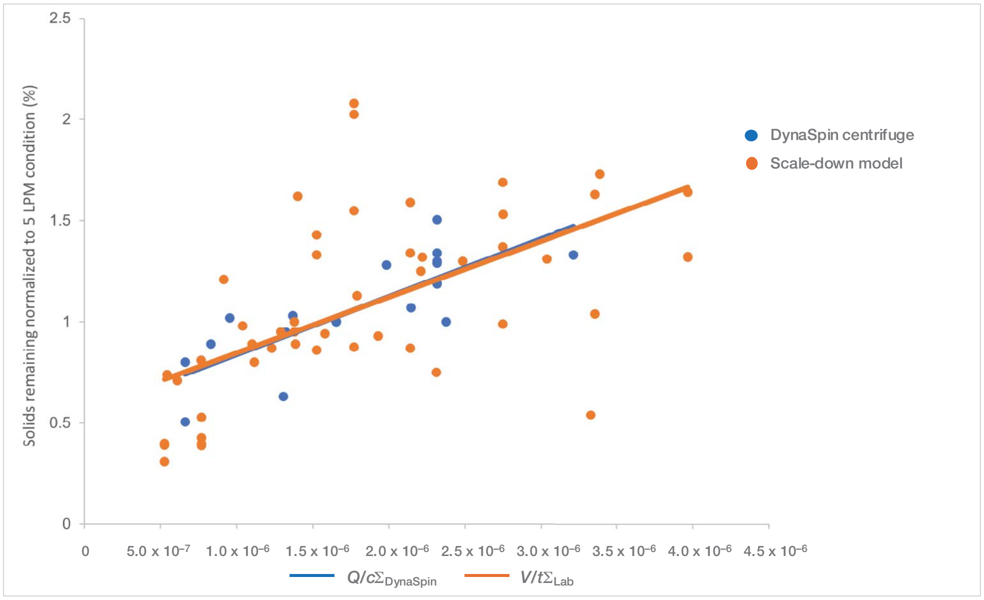

Figure 4. Solids remaining after centrifugation normalized to the 5 LPM condition with a correction factor of 0.13.

Depth filter loading

In addition to understanding and predicting the separation efficiency of the DynaSpin centrifuge for a given cell culture process, it is also important to understand the impact of separation on subsequent downstream processing. In this case, it would be the depth filter area required after centrifugation. By generating centrates under a range of bench-scale conditions ($V/t\Sigma_{\mathrm{Lab}}$) and performing P_{max} studies with the centrates, we could estimate what the filter loading would be at various flow rates and rotor speeds on the DynaSpin centrifuge ($Q/c\Sigma_{\mathrm{DynaSpin}}$). Cultures were spun down on the DynaSpin centrifuge, and simulations were run with the scale-down model at equivalent settling areas as summarized in Table 2.

Table 2. Scale-down model run summary.

		DynaSpin Single-Use Centrifuge		Scale-down model		
Trial	Cell line	Rotation speed (rpm)	Flow rate (LPM)	Spin-down time (s)	Rotor speed (rpm)	$Q/c\Sigma_{\mathrm{DynaSpin}}$ or $V/t\Sigma_{\mathrm{Lab}}$
1	ExpiCHO-S	3,600	7	180	998	2.32×10^{-6}
2	CHO-K1		5		1,175	1.66×10^{-6}
3	CHO-K1		7		998	2.32×10^{-6}
4	Cellca		5		1,175	1.66×10^{-6}
5	Cellca		7		998	2.32×10^{-6}
6	ExpiCHO-S		5		1,175	1.66×10^{-6}
7	ExpiCHO-S		7		998	2.32×10^{-6}

The filter loading results obtained with the scale-down model closely matched those obtained using the DynaSpin centrifuge (Figure 5), although the model slightly underpredicted loading by ~5% on average. With this method, process development scientists can determine the trade-offs of running the DynaSpin centrifuge at different flow rates or rpms without performing large-scale harvests.

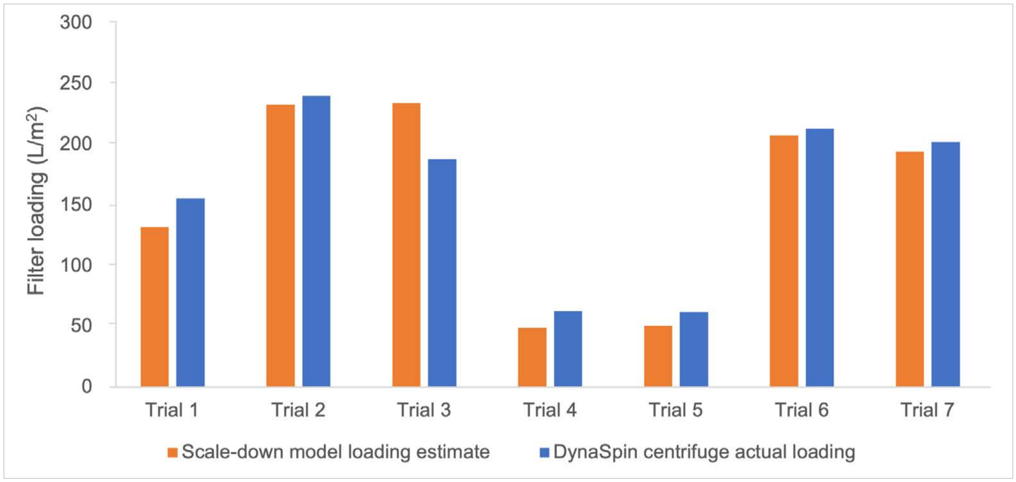

Figure 5. Filter loading predictions of the scale-down model vs. actual loading.

Practical application of the scale-down model

With these examples, a process development scientist can use the scale-down model to inform processing conditions at scale. It may seem intuitive to think that the faster the flow rate in the DynaSpin centrifuge, the faster harvest unit operation will be. However, when filter loading is considered, the trade-offs are not necessarily immediately obvious. Using the scale-down model, a process development scientist can spin down ~960 mL of cell culture (e.g., 24 x 40 mL in 50 mL conical tubes) using two different centrifuge conditions for a total of 1,920 mL of culture. In the example outlined in Table 2, the spin-down time was held constant at 180 seconds. The rotation rate of the swinging bucket centrifuge for Trial 1 was set at 998 rpm to mimic the 7 LPM flow rate at 3,600 rpm in the DynaSpin centrifuge, then set at 1,175 rpm for Trial 2 to mimic the 5 LPM flow rate at 3,600 rpm in the DynaSpin centrifuge.

By collecting supernatants and performing P_{max} studies, the number of depth filters needed for a large-scale harvest can also be estimated. Flow rates of 208 L/m^2 and 194 L/m^2 mimic the 5 LPM and 7 LPM conditions, respectively. This equates to 13 x 1.1 m^2 filters for the 5 LPM condition and 14 x 1.1 m^2 filters for the 7 LPM condition for a 2,000 L bioreactor harvest assuming a 50% safety factor. Due to the similar filter count and the fact that the same number of racks will be required to perform depth filtration, one may choose to scale up the process and run at the 7 LPM flow rate to make it more efficient and cost-effective. For a detailed analysis of the trade-offs between centrate clarity and processing speed, please refer to the application notes listed in the Reference section [4,5].

Conclusion

Using sigma theory, a benchtop batch centrifuge can be used as a scale-down model for the DynaSpin Single-Use Centrifuge. This model can be useful for generating representative simulated data for depth filter sizing studies without performing a large-scale bioreactor run. This method can be used to simulate flow rates below 8 LPM and rotor speeds of up to 3,600 rpm on the DynaSpin centrifuge without a capillary shear device. While each user will need to characterize their own bench-scale centrifuge, Equation 4 together with Equation 1 and Equation 3 can be used with a correction factor (*c*) of 0.13 to determine which settings to use for the bench-scale batch centrifuge to generate representative data for filter sizing with small-scale bioreactors.

References

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Thermo Fisher Scientific

Ordering information

Product	Cat. No.
DynaSpin Single-Use Centrifuge	DSPIN.9000
DynaSpin Single-Use Rotor	SUT00056

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