Single-use technologies

Growth and scale-up of HEK293F derivatives in DynaDrive Single-Use Bioreactors

Author

Paula Decaria, Senior Engineer, Research and Development, Thermo Fisher Scientific

Introduction

With the tremendous growth of gene therapy and viral vector vaccine markets, and many of these therapies approaching commercial launch, companies are now facing the decision on how to scale their manufacturing processes. While traditional single-use processes have been limited to either smaller or multiplexed systems, recent advances in single-use technologies have enabled scale-up abilities from 50 L to 5,000 L sizes while offering improved mixing and gassing capabilities. In this study, performance and scalability of Thermo Scientific™ DynaDrive™ Single-Use Bioreactors (S.U.B.s) for gene therapy and viral vector production applications are demonstrated using industry-relevant HEK293F derivatives.

Keywords

HEK293 cells, gene therapy, DynaDrive, transfection, viral vectors, viral production, single-use bioreactors

Goal

These studies evaluated the performance of DynaDrive S.U.B.s for growth and scalability from 50 L to 3,000 L using two different HEK293F cells lines (Table 1). These experiments were designed to demonstrate that DynaDrive S.U.B.s can be successfully implemented for use with HEK293 cell lines at increasing volumes with standard scale-up criteria. The first cell line was tested at multiple volumes within the 50 L S.U.B. to demonstrate in-vessel scale-up that would be typical for a transient transfection process. The second cell line was first tested at multiple volumes within the 50 L vessel, and then scaled up from our 50 L to our 3,000 L vessels to demonstrate scalability of the systems.

Table 1. Evaluated Gibco™ cell lines and media in DynaDrive S.U.B.s.

Parameter	Cell line 1	Cell line 2
Cell line	Gibco™ Viral Production Cells (VPC)	Gibco™ Viral Production Cells 2.0 (VPC 2.0)
Production medium	Gibco™ LV-MAX™ Production Medium	Gibco™ Viral Production Medium

Case study 1

Evaluation of Gibco Viral Production Cells at various volumes in the 50 L DynaDrive S.U.B.

Methods

Gibco VPC were expanded in shake flasks prior to scaling up to the 50 L DynaDrive S.U.B. at an N-1 stage of a 10 L working volume, or 5:1 turndown ratio. Fresh medium was added to the reactor after 4 days to bring the volume up to 25 L with a starting cell density of >1.0 x 10^6 viable cells/mL. Once the cells reached a concentration of 5.5 x 10^6–6.5 x 10^6 viable cells/mL, the culture was brought up to full volume to mimic a transfection step where the cells would be diluted with fresh medium on the day of transfection. The cells were then allowed to grow 3 more days before the study was ended. The study was repeated for a total of two runs to show reproducibility. Cell counts, viability, dissolved gasses, nutrients, and metabolites were measured offline daily throughout the culture. Table 2 shows the operating conditions that were used.

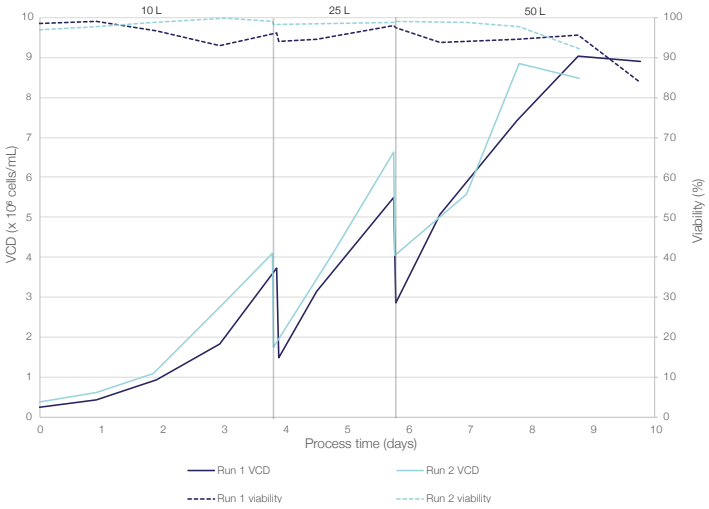

Figure 1. Viable cell density (VCD) and viability comparison of Gibco VPC in the 50 L DynaDrive S.U.B.s with in-vessel scale-up from 10 L to 25 L to 50 L working volumes.

Table 2. Operating parameters for evaluation of Gibco VPC at different volumes in the 50 L DynaDrive S.U.B.

	50 L DynaDrive S.U.B.		
Target starting volume	10 L	25 L	50 L
Seeding density	0.3 x 10^6–0.4 x 10^6 cells/mL	>1.0 x 10^6 cells/mL	3.0 x 10^6–4.0 x 10^6 cells/mL
Temperature	37°C		
pH	≤7.25		
pH control	Acid control: sparged CO_2 through drilled-holed sparger (DHS); base control: none		
Agitation	90 rpm	95 rpm	95 rpm
Dissolved oxygen (DO)	40%		
Air headspace	10% (v/v), 1.0 slpm	10% (v/v), 2.5 slpm	10% (v/v), 5.0 slpm
DO cascade	Air supplemented with O_2 through DHS		

Results

Figure 1 shows the viable cell density (VCD) and viability for the cultures. The cell counts were consistent between the two studies and reached expected cell densities while maintaining high cell viabilities. Metabolite data collected offline indicated that cultures were healthy with typical glucose consumption and metabolic byproduct production; lactate production peaked at just over 2.0 g/L and dropped as the glucose levels were depleted.

Multiple gassing strategies were tested to optimize growth, and it was found that air supplemented with O_2 provided sufficient growth while reducing sparging stress and controlling DO within desired concentrations (Figure 2). Using these strategies, DO was maintained at 40% with no major deviations during the duration of the run.

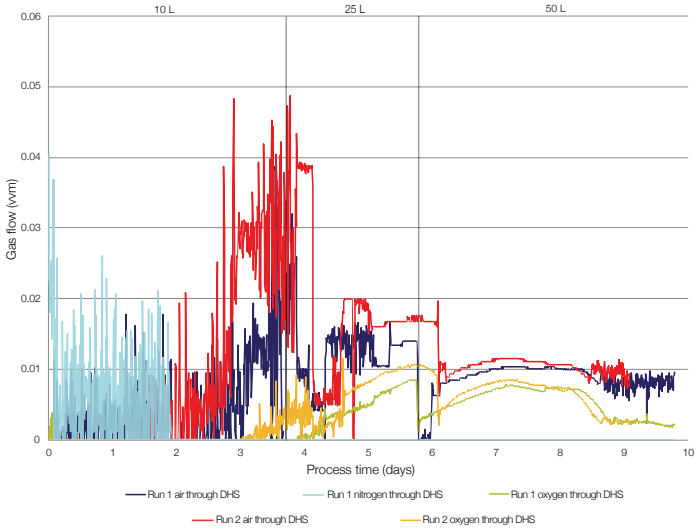

Figure 2. DHS gas flow rates for Gibco VPC in the 50 L DynaDrive S.U.B.s with in-vessel scale-up from 10 L to 25 L to 50 L working volumes.

Case study 2

Evaluation of Gibco VPC 2.0 in the 50 L, 500 L, and 3,000 L DynaDrive S.U.B.s, utilizing the 50 L DynaDrive S.U.B. as the seed train vessel

Methods

Gibco VPC 2.0 were expanded in shake flasks prior to scaling up to the 50 L DynaDrive S.U.B. for each study. The first study focused on testing varying low-turndown volumes of 5 L, 10 L, and 15 L within the 50 L S.U.B., before bringing the culture up to full volume with a seed density of 1.5 x 10^6 viable cells/mL and allowing the cells to grow for 4 days before harvesting. Cells in an 125 mL Erlenmeyer shake flask were grown in parallel with the S.U.B. in this study to show comparability. At each volume change in the 50 L S.U.B., the flask was reseeded to match the inoculation concentration of the bioreactor. Cell counts, viability, dissolved gasses, nutrients, and metabolites were measured offline daily throughout the culture. Table 3 shows the operating conditions used for the 50 L DynaDrive S.U.B.

In the second study, the Gibco VPC 2.0 were expanded into the 50 L DynaDrive S.U.B. at an N-3 stage of a 10 L working volume, or 5:1 turndown ratio. The 500 L DynaDrive S.U.B. was seeded from this culture at a turndown ratio of >15:1 of 30 L for an N-2 stage, and the 50 L DynaDrive S.U.B. was reseeded at 10 L to run in parallel with the 500 L vessel. Fresh medium was added to the reactors after 3 days to bring the volumes up to 150 L and 15 L, respectively, for the 500 L and 50 L vessels for an N-1 culture, and ultimately to a full volume after another 3 days with a starting cell density of >1.5 x 10^6 viable cells/mL. Cell counts, viability, dissolved gasses, nutrients, and metabolites were measured offline daily throughout the culture.

Table 3. Operating parameters for evaluation of Gibco VPC 2.0 at different volumes in the 50 L DynaDrive S.U.B.

	50 L DynaDrive S.U.B.			
Target starting volume	5 L	10 L	15 L	45 L
Seeding density	0.6 x 10^6 cells/mL	0.6 x 10^6 cells/mL	0.6 x 10^6 cells/mL	>1.5 x 10^6 cells/mL
Temperature	37°C			
pH	≤7.25			
pH control	CO_2 added through headspace at 8%	Acid control: sparged CO_2 through DHS; base control: none		
Agitation	90 rpm	90 rpm	90 rpm	95 rpm
DO	40%			
Air headspace	50% (v/v), 2.5 slpm total gas flow, air + 8% CO_2	10% (v/v), 1.0 slpm	10% (v/v), 1.5 slpm	10% (v/v), 4.5 slpm
DO cascade	Air supplemented with O_2 through the DHS			

Table 4 shows the operating conditions used for the 500 L DynaDrive S.U.B. Operating conditions used for the 50 L were the same as shown in Table 3.

For the final study, Gibco VPC 2.0 were once again expanded into the 50 L DynaDrive S.U.B., at an N-4 stage of a 10 L working volume, followed by an N-3 stage of a 30 L working volume. The 3,000 L DynaDrive S.U.B. was seeded from this culture at 275 L at an N-2 stage and the 50 L DynaDrive S.U.B. was reseeded at a 10 L working volume to run in parallel with the 3,000 L vessel. Fresh medium was added to the reactors after 4 days to bring the volume up to 1,000 L and 15 L, respectively, for the 3,000 L and 50 L vessels for an N-1 culture. After 3 days, the cultures were brought up to full volume with a starting cell density of >1.5 x 10^6 viable cells/mL and allowed to grow 5 days prior to ending the study. A bolus addition of glucose at 3 g/L was added on day 2 of the production run for both the 3,000 L and the 50 L vessels. Cell counts, viability, dissolved gasses, nutrients, and metabolites were measured offline daily throughout the culture. Table 5 shows the operating conditions used for the 3,000 L DynaDrive S.U.B., and operating conditions used for the 50 L were the same as shown in Table 3.

Table 4. Operating parameters for evaluation of Gibco VPC 2.0 at different volumes in the 500 L DynaDrive S.U.B.

	500 L DynaDrive S.U.B.		
Target starting volume	30 L	150 L	500 L
Seeding density	0.4 x 10^6 cells/mL	0.6 x 10^6 cells/mL	>1.5 x 10^6 cells/mL
Temperature	37°C		
pH	≤7.25		
pH control	CO_2 added through headspace at 8%	Acid control: sparged CO_2 through DHS; base control: none	
Agitation	65 rpm	70 rpm	70 rpm
DO	40%		
Air headspace	5.8 slpm total gas flow, air + 8% CO_2	7.0 slpm	7.0 slpm
DO cascade	Air supplemented with O_2 through the DHS		

Table 5. Operating parameters for evaluation of Gibco VPC 2.0 at different volumes in the 3,000 L DynaDrive S.U.B.

	3,000 L DynaDrive S.U.B.		
Target starting volume	275 L	1,000 L	3,000 L
Seeding density	0.55 x 10^6 cells/mL	0.55 x 10^6 cells/mL	>1.5 x 10^6 cells/mL
Temperature	37°C		
pH	≤7.25		
pH control	CO_2 added through headspace at 8%	Acid control: sparged CO_2 through DHS; base control: none	
Agitation	35 rpm	40 rpm	40 rpm
DO	40%		
Air headspace	45 slpm total gas flow, air + 8% CO_2	36.2 slpm	36.2 slpm
DO cascade	Air supplemented with O_2 through the micro DHS	Air supplemented with O_2 through the micro DHS	Air through the macro DHS, O_2 supplemented through the micro DHS
Feeding strategy	–	–	Bolus feed of 3 g/L glucose on day 2*

* Same bolus feed was done for 50 L vessel.

Results

Figures 3–5 show the VCD and viability for each study. The cells were consistent with the study control and reached expected cell densities while maintaining high viabilities. Metabolite data collected offline in each study indicated that cultures were healthy with typical glucose consumption and metabolic byproduct production; lactate production peaked between 1.5–2 g/L on average and dropped as the glucose levels were depleted.

The gas flow rates for each of the studies were controlled based on culture oxygen demand (Figures 6–8). For the 50 L and 500 L S.U.B.s, the DO was maintained by sparging air first, then supplemented with O_2 through the DHS. The DO for the 3,000 L vessel was maintained by sparging air through the micro DHS first, then supplemented with O_2 through the micro DHS. As the culture was scaled up to the 3,000 L working volume, adjustments to the gassing strategy were made on day 2 of the culture. The air was switched from the micro DHS to the macro DHS and was added at a steady rate of 20 slpm, while the oxygen stayed on the DO cascade going through the micro DHS. This allowed for improved CO_2 stripping within the vessel, as well as minimizing gas flow rates to maintain the DO at setpoint. Using these strategies, DO was maintained in each S.U.B. at 40% with no major deviations during the duration of the run.

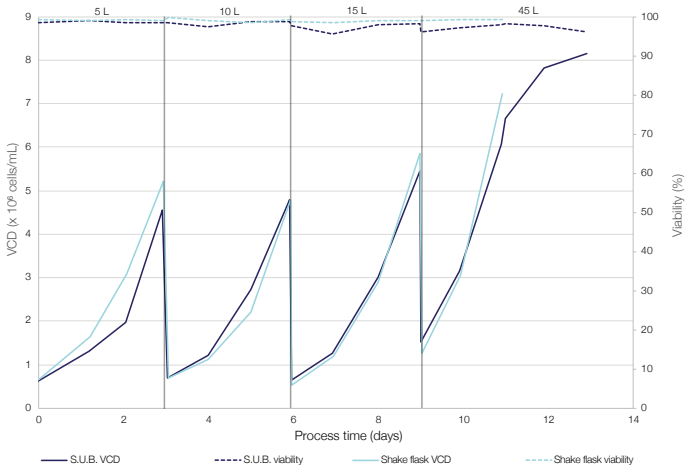

Figure 3. VCD and viability comparison of Gibco VPC 2.0 in the 50 L DynaDrive S.U.B. and shake flasks with in-vessel scale-up in the S.U.B. at 5 L, 10 L, 15 L, and 45 L working volumes.

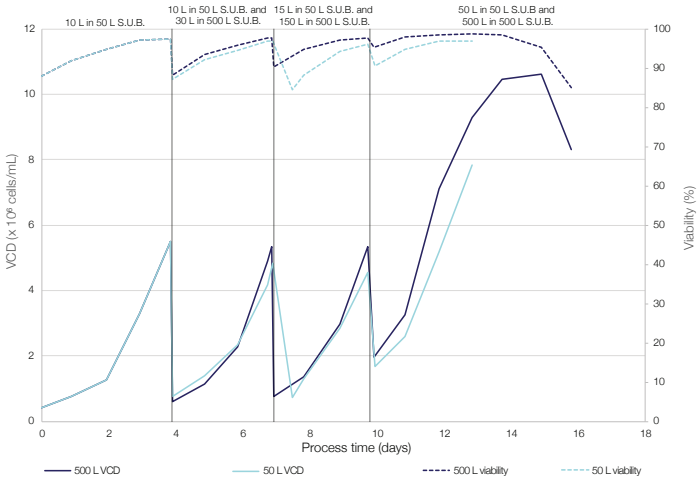

Figure 4. VCD and viability comparison of Gibco VPC 2.0 in the 50 L and 500 L DynaDrive S.U.B.s, utilizing the 50 L S.U.B. as a seed train for the 500 L S.U.B., in addition to in-vessel scale-up to reach full working volumes.

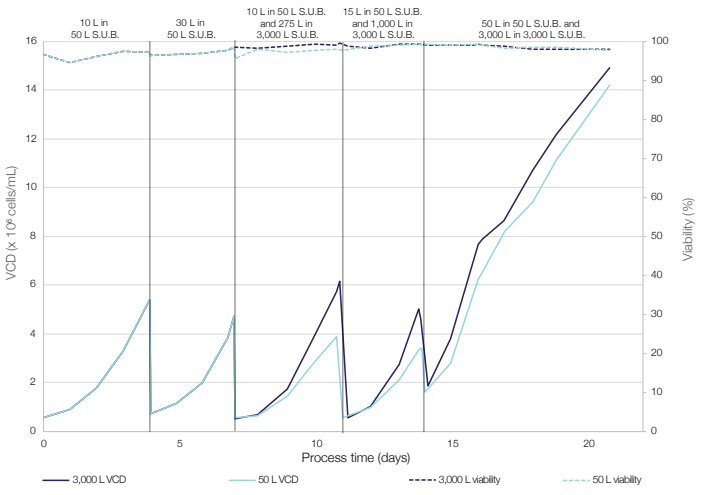

Figure 5. VCD and viability comparison of Gibco VPC 2.0 in the 50 L and 3,000 L DynaDrive S.U.B.s, utilizing the 50 L S.U.B. as a seed train for the 3,000 L S.U.B., in addition to in-vessel scale-up to reach full working volumes.

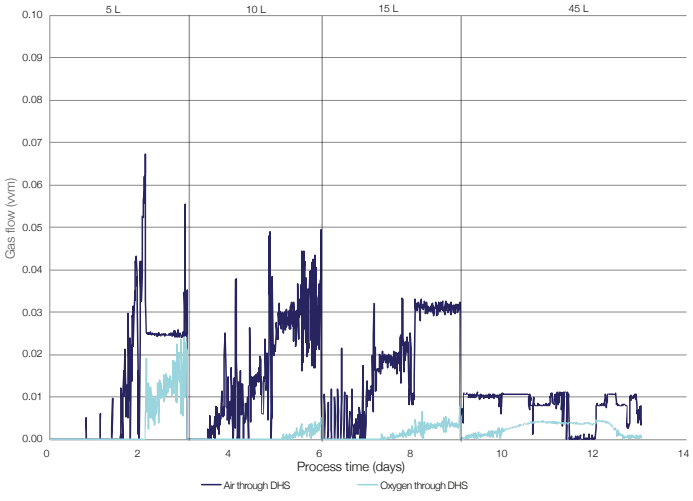

Figure 6. DHS gas flow rates for Gibco VPC 2.0 in the 50 L DynaDrive S.U.B. with in-vessel scale-up at 5 L, 10 L, 15 L, and 45 L working volumes.

Conclusions

The DynaDrive S.U.B.s were able to support HEK293F-derived clones and provide controlled conditions to achieve target VCDs while maintaining high viability. This was achieved in the 50 L scale by choosing simple scale-up parameters from past work done in 3 L glass bioreactors. Those same target VCDs and viabilities were maintained as the cultures were scaled into the 500 L and 3,000 L S.U.B.s, utilizing the same scale-up parameters from the 50 L scale, including consistent power input and gas flow rates. The gas flow rates and agitation rates tested provided sufficient mass transfer to maintain the required DO setpoints, while remaining low enough to not cause shear damage to the cells.

Additionally, each reactor was seeded at low working volume: >5:1 turndown ratio in the 50 L S.U.B., >15:1 turndown ratio in the 500 L S.U.B., and >11:1 turndown ratio in the 3,000 L S.U.B. This allowed for reduction in the complexity of seed train requirements by eliminating intermediate vessels, consumables, and operation space, while increasing the efficiency of the seed train itself through reduced connections and transfer losses.

Overall, the demonstrated scale-up processes show the versatility of the DynaDrive S.U.B.s to maintain culture setpoints at each scale through simple scale-up criteria. The modified drive train and sparging did not have adverse effects on the cell culture, as demonstrated by the VCD and viabilities achieved. With the demonstrated consistency of each scaled step, the DynaDrive S.U.B. is ideal for gene therapy and viral vector platforms, enabling users to stay within S.U.B.s with minimal process changes as they scale up their process to meet commercial therapeutic demand.

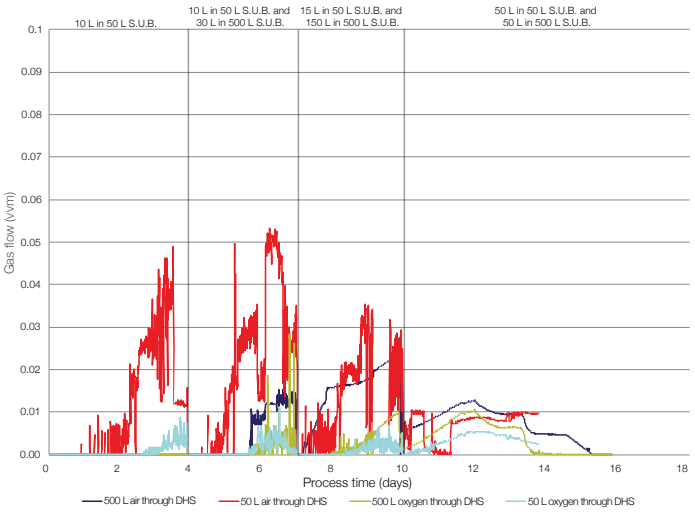

Figure 7. DHS gas flow rates for Gibco VPC 2.0 in the 50 L and 500 L DynaDrive S.U.B.s, utilizing the 50 L S.U.B. as a seed train for the 500 L S.U.B., in addition to in-vessel scale-up to reach full working volumes.

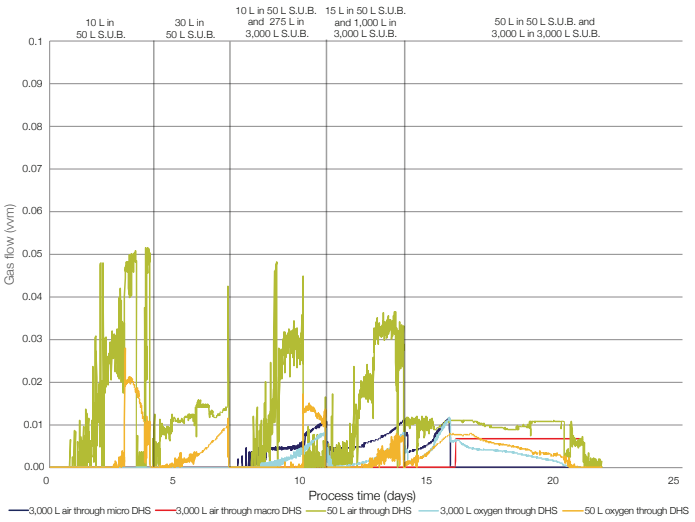

Figure 8. DHS gas flow rates for Gibco VPC 2.0 in the 50 L and 3,000 L DynaDrive S.U.B.s, utilizing the 50 L S.U.B. as a seed train for the 3,000 L S.U.B., in addition to in-vessel scaling to reach full working volumes.

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