Demonstration of Scalability for the Corning® Ascent® FBR Platform

Technical Brief

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Introduction

Corning has developed a novel fixed bed reactor (FBR) platform to address key challenges that continue to impact the cell and gene therapy industry. Existing technologies do not enable efficient scale up or adequate production of adherent cells to meet clinical demand for emerging cell and gene therapy applications. Corning Ascent technology was designed to address these challenges by enabling efficient scalability from process development to production-scale manufacturing. The Corning Ascent FBR System 5 (FBR System 5) supports reactor sizes from 1 to 5 m^2 and can be used for process development, seed train, or small-scale manufacturing applications. The Corning Ascent FBR System 100 (FBR System 100) supports reactor sizes from 20 to 100 m^2 and can be used for seed train or larger scale cell manufacturing applications, including clinical stage manufacturing. Key to the Ascent technology is the specially designed fixed bed reactor that contains a precisely oriented arrangement of polymer mesh disks which promotes uniform, low-shear fluid flow resulting in evenly distributed cell growth throughout the reactor.

In this brief, we present data demonstrating scalability of the Ascent technology using a standard viral vector producing cell line (HEK-293T). First, we demonstrate scalability of the platform by successfully passaging cells across three different reactors, with consistent performance observed at each stage. Cells were first expanded and harvested from an FBR System 5 reactor, and then used to directly inoculate an FBR System 100 reactor. The cells in the FBR System 100 reactor were then expanded, harvested, and used to inoculate a subsequent FBR System 5 reactor. The ability to efficiently harvest viable cells from the Ascent reactors is a key advantage of the technology and enables the systems to be used for seed train and scale up applications. We also demonstrate the ability to transfer optimized process parameters directly from the FBR System 5 to FBR System 100. This capability results in faster process transfer times without the need for significant optimization at the larger scale. Collectively, the results presented here, demonstrate the scalability of the Ascent platform and confirm that equivalent performance can be achieved on the FBR System 5 and FBR System 100. Importantly, the Ascent platform allows operators to use one technology, with a standardized workflow, for their process development, scale-up, and manufacturing processes.

Materials and Methods

Cell Culture and 2D Seed Train

HEK-293T cells (ATCC® CRL 3216™) were thawed in DMEM (Corning 15-018-CM) supplemented with 6 mM L-glutamine (Corning 25-005), 5% fetal bovine serum (FBS; Corning 35-010-CV) and 1X Penicillin/Streptomycin (Corning 30-002-CI) according to the manufacturer's recommendations. The cells were passaged twice in 175 cm^2 cell culture flasks with Corning CellBIND® surface (Corning 431328) and then expanded into Corning HYPERFlask® vessels (Corning 10020). Flasks were seeded at 10,000 - 15,000 cells/cm^2 and then cultured until they achieved 80-90% confluency (3 to 4 days). Cells were subcultured by dissociating the cells with 0.05% Trypsin (Corning 25-052-CI) or Accutase® (Corning 25-058-CI).

Corning Ascent FBR System 5 Conditions

Ascent fixed bed reactors (5 m^2) were used for the scalability experiments (Corning 6973). Prior to inoculation, the FBR System 5 was initialized, and the consumables were installed according to the published Corning Ascent FBR System 5 Main Process Protocol (CLS-AN-824). Next, the Media Fill/Prime phase was used to add 2,200 mL of media to the Media Conditioning Vessel (MCV). The FBR and associated tubing were then automatically primed. The Media Conditioning phase was started to stabilize the media and allow sensor calibration. Next, the Media Maintenance phase was started, enabling the system to automatically maintain the process parameters for the main culture process (MCV temp: 38°C, BRX temp: 37°C, MCV dissolved oxygen (DO): 100%, BRX DO: 20%, Min. CO_2%: 4%, pH: 7.2). To prevent oxygen depletion during cell expansion, each system passively monitors the dissolved oxygen concentration at the FBR outlet and automatically increases

the recirculation flow rate to prevent the oxygen concentration in the media from dropping below 20%. The Inoculation phase was initiated and the FBR was seeded at 15,000 cells/cm^2 (Attachment flow rate: 150 mL/min., Inoculation volume: 300 mL, Cell attachment duration: 180 min.). Following cell attachment, the system was controlled to the Media Maintenance set points. On the day after cell inoculation, 1,000 mL fresh media was added to the MCV to bring the system volume to 3,500 mL. Metabolite analysis was performed twice daily by removing a 1 mL sample from the MCV and measuring the metabolite concentrations on a BioProfile® FLEX2 analyzer (Nova Biomedical). A refeed step was performed when the glucose concentration in the media was between 1.0 and 1.5 g/L. During the refeed process, 2,000 mL spent media was removed from the MCV (200 mL/min.) and replaced with an equal volume of fresh media (20 mL/min.). A slower speed for media addition allows the system more time to adjust the temperature, pH, and DO of incoming media to the desired process parameters. On Day 5, the cells were harvested from the 5 m^2 FBR using the automated cell harvest process. Briefly, the FBR was first washed with 500 mL DPBS and then 500 mL Accutase® was added to flush the DPBS from the FBR. Next, an additional 800 mL Accutase was added to the system and recirculated for 40 min. at 150 mL/min. to release the cells. Following the cell release process, a series of cell removal steps was performed to collect the cells. A second cell dissociation step was performed by adding 800 mL Benzonase solution (DPBS, 2 mM $MgCl_2$, 25 units/mL Benzonase) to the system and recirculating for 15 min. at 150 mL/min. A series of cell removal steps was then performed to capture the residual cells. The cell stock was quantified on a Vi-CELL® XR analyzer (Beckman Coulter) and the final volume was measured to determine the overall cell yield.

Corning® Ascent® FBR System 100 Conditions

Following cell expansion at the 5 m^2 scale, a 100 m^2 Ascent FBR was used as the next step in the seed train (Corning 6673). On the day prior to inoculation, a new batch was initiated on the FBR System 100. During the Setup phase, a 100 m^2 reactor was installed on the system along with other consumables (Ascent System 100 50L DynaDrive™ bag (Corning 6683), Ascent FBR System 100 Media Harvest tubing set with glucose sensor (Corning 6674), BPC containing Sodium Bicarbonate (Corning 25-035-CI) and a BPC containing anti-foam reagent (Thermo Fisher A1036902)). Immediately following the Setup phase, Media Fill and Prime was used to add 48L fresh media to the MCV at 4L/min. Next, Media Conditioning was run overnight to stabilize the media (37°C, pH 7.2 and 100% DO saturation). The FBR was then inoculated at 15,000 cells/cm^2 (2L inoculation volume). Following inoculation, the cells were recirculated for 3.5 hours at 3L/min. to enable uniform cell attachment throughout the FBR. Cell attachment was monitored by collecting a 2 mL sample from the MCV every 30 minutes and quantifying the cell concentration on a Vi-CELL analyzer. Following cell attachment, the system automatically proceeded to the Media Maintenance phase. On Day 1 (1 day after inoculation), 10L fresh media was added to the MCV to bring the total system volume to 60L. Metabolite analysis was performed twice daily by removing a 2 mL sample from the MCV and measuring the metabolite concentrations on a FLEX2 analyzer. A refeed step was performed when the glucose concentration in the media was between 1.0 and 1.5 g/L. During the refeed process, 35L spent media was removed from the MCV at 4 L/min. and an equal volume of fresh media was added at 0.4L/min. On Day 5, the automated Cell Harvest phase was performed to dissociate and collect the cells from the FBR. During Cell Harvest, the FBR was first washed with 10L DPBS to remove the spent media and then 8L of Accutase was added to the FBR. Next, Accutase (20L total volume) was recirculated through the FBR for 40 min. at 5L/min. Two cell release steps were then performed to collect the cells. Next, 20L Benzonase solution (described previously) was recirculated through the FBR for 15 min. at 5L/min. and the residual cells were collected by performing a series of cell release steps. The cell stock was quantified on a Vi-CELL analyzer and the final volume was measured gravimetrically to determine the overall cell yield.

Results and Discussion

Demonstration of Corning Ascent Platform Scalability (Multi-passage Experiment)

Scalability is a critical requirement for any manufacturing platform. The Ascent platform enables true scalability from FBR System 5 to FBR System 100 with consistent performance across the different systems. A key advantage of the Ascent technology that enables scalability is the ability to harvest viable cells from the Ascent reactors. Operators can follow preprogrammed, automated cell harvest procedures on each system to release and collect high viability cells from the reactors. Another advantage of the Ascent platform is the ability to efficiently transfer parameters between the systems. Protocols are optimized on the FBR System 5 and the critical process parameters can be scaled and transferred directly to the larger systems. This results in reduced time required for optimization at the larger scales and faster process transfer times, resulting in significant cost savings. The Ascent platform also enables performance scalability. Comparable performance can be achieved on the FBR System 100 following process transfer from the FBR System 5.

We have demonstrated scalability of the Ascent platform by passaging cells across three different Ascent reactors (Figure 1). To demonstrate scalability, each reactor was inoculated at the same density (15,000 cells/cm^2) and then expanded for the same duration (5 days). Following the cell expansion phase, viable cells were collected using an automated cell harvest procedure on each system. The harvested cells were then used to directly inoculate the next Ascent reactor.

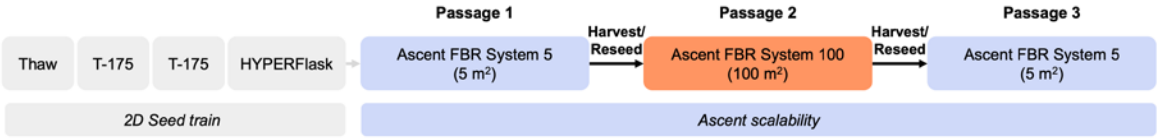

Figure 1. Experiment setup for demonstration of Ascent platform scalability.

During the seed train process, cells were passaged on T-175 flasks and then expanded into a Corning® HYPERFlask® vessel. A 5 m^2 FBR was then inoculated on the FBR System 5 and expanded for 5 days (Passage 1). Cells were then harvested from the FBR System 5 and used to directly inoculate a 100 m^2 FBR on the FBR System 100 (Passage 2). Following 5 days of expansion, the cells on the FBR System 100 were harvested and used to inoculate a subsequent 5 m^2 FBR (Passage 3). Table 1 summarizes the parameters and yields from the scalability experiment. Each reactor exhibited a cell yield of approximately 350,000 cells/cm^2 following the automated cell harvest procedure with cell viabilities exceeding 95%. Population doubling time (PDT) was calculated and served as a surrogate for cell functionality. Importantly, the cells harvested from each reactor exhibited a similar PDT (approx. 25 hrs.) indicating there was no decrease in performance during the harvest and reseed process.

Table 1. Experiment details and results for the multi-passage scalability experiment.

		Ascent FBR System 5 (Passage 1)	Ascent FBR System 100 (Passage 2)	Ascent FBR System 5 (Passage 3)
Details	Reactor size (m^2)	5	100	5
	Seeding density (cells/cm^2)	15,060	14,980	15,024
	Cell attachment flowrate (mL/min.)	150	3000	150
	Expansion time (hours)	120.4	116.5	118.3
Results	Viability (%)	95.3	95.6	96.6
	Cell yield (viable cells/cm^2)	347,130	371,735	364,086
	Total cells	17.4 million	372 million	18.2 million
	Fold-expansion	23.0	24.8	24.2
	PDT (hours)	26.6	25.2	25.7

As stated above, an important advantage of the Ascent platform is the ability to transfer critical process parameters (Process or Operational scalability). Protocols are optimized on the FBR System 5 and the critical process parameters can be scaled directly to the larger systems. For example, 'Cell attachment flow rate' is an important process parameter that needs to be optimized on the FBR System 5 to ensure uniform cell distribution throughout the FBR. To transfer this parameter, the FBR System 5 process parameter is multiplied by a scaling factor of 20 to determine the FBR System 100 flow rate. The ability to efficiently scale critical process parameters from FBR System 5 to FBR System 100 results in faster process transfer times and reduced optimization at the larger scales.

Demonstration of Corning Ascent® Platform Scalability (side-by-side comparison of FBR System 5 and FBR System 100)

To be considered scalable, a platform needs to demonstrate equivalent performance at small-scale and large-scale. This has been achieved for the Ascent platform by demonstrating comparable performance on FBR System 5 and FBR System 100 when the systems were run in parallel using the same conditions (Figure 2).

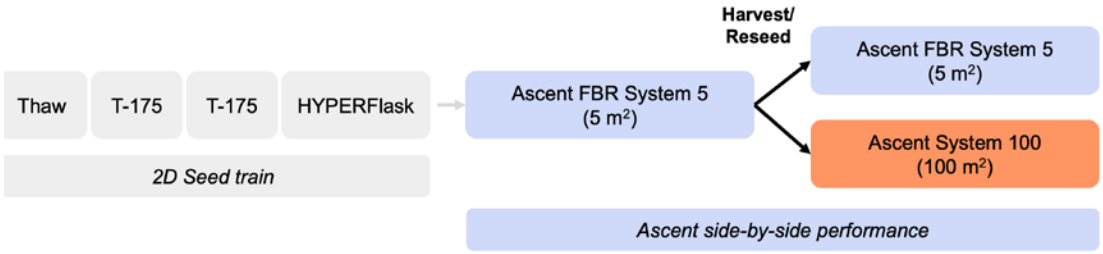

Figure 2. Experiment setup for demonstration of equivalent performance for Ascent FBR System 5 and Ascent FBR System 100 running in parallel.

Cells were harvested from an FBR System 5 reactor and used to inoculate another FBR System 5 reactor and an FBR System 100 reactor, in parallel, to demonstrate equivalent performance when both systems were inoculated with the same cell stock and run using the same conditions. Both reactors were inoculated at 15,000 cells/cm^2 and expanded for 5 days prior to automated cell harvest. The results in Table 2 illustrate equivalent performance for the FBR System 5 and FBR System 100. Both systems exhibited similar cell yields, cell viabilities, and PDTs. These results further reinforce the scalability of the Ascent platform by demonstrating similar performance when the FBR System 5 and FBR System 100 were run in parallel using the same conditions.

Table 2. Experiment details and results for Ascent FBR System 5 and Ascent FBR System 100 that were run in parallel (side-by-side system comparison).

		Ascent FBR System 5	Ascent FBR System 100
Details	Reactor size (m^2)	5	100
	Seeding density (cells/cm^2)	15,210	14,980
	Cell attachment flowrate (mL/min.)	150	3000
	Expansion time (hours)	116.1	116.5
Results	Viability (%)	96.2	95.6
	Cell yield (viable cells/cm^2)	356,008	371,735
	Total cells	17.8 million	372 million
	Fold-expansion	23.7	24.8
	PDT (hours)	25.4	25.2

Conclusions

- Scalability of the Corning Ascent platform was demonstrated by successfully passaging cells across three Ascent reactors with comparable performance observed for each reactor (5 m^2 → 100 m^2 → 5 m^2).
- High viability cells can be efficiently harvested from Ascent reactors and then used to directly inoculate a subsequent reactor with no degradation in performance. This capability allows the Ascent platform to be used for seed train and large-scale production applications.
- Equivalent performance was observed for the Ascent FBR System 5 and FBR System 100 that were run in parallel under the same conditions. Users can achieve comparable performance when transferring a process from Ascent FBR System 5 to Ascent FBR System 100.
- The Ascent platform allows operators to use one technology, with a standardized workflow, for their process development, scaleup, and production process. As a result, the Ascent platform provides an ideal option for emerging cell and gene therapy applications where large quantities of adherent cells are required.

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