Linear Scalability of Virus Production in Integrity® iCELLis® Single-Use Fixed-Bed Bioreactors, From Bench Scale to Industrial Scale

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Introduction

Over the past decade, single-use bioreactors have been developed and accepted for cGMP use in the biomanufacturing industry. Among them is the Integrity® iCELLis® series of bioreactors from ATMI LifeSciences, were designed for adherent cell culture.

Adherent cell processes for production of biomolecules and viruses often involve 2-D vessels (e.g. roller bottles, Cell Stacks/Cell Factories, etc.) requiring many manual operations and large areas of cleanroom space, or microcarrier cultures which require complex cell transfer steps and a very high level of expertise. Some cell culture processes allowing continuous production by medium perfusion also use adherent cells retained in a bioreactor while culture media is continuously perfused.

In order to maximize surface area within a compact space and retain cells for easy medium exchange, the iCELLis bioreactors contain macro-carriers trapped in a fixed-bed providing a 3-D matrix for the cells to grow. These bioreactors also enable precise temperature, pH and dissolved oxygen control which cannot be done in 2-D cultures.

The iCELLis technology can be used at small and large scales with straightforward process scale-up, easy single-use operations and minimal space requirement.

Here we present a summary of adherent cell process developments in iCELLis bioreactors, including:

- HEK 293 cell expansion for production of adenovirus
- MVA virus production in CEF cells
- Bovine Herpes Virus production in MDBK cells
- Recombinant Adeno-Associated Virus in A549 cells
- Adenovirus production in A549 cells
- Influenza virus production in Vero cells
- Paramyxovirus production in Vero cells
- Undisclosed lytic virus in Vero cells

The Integrity iCELLis Bioreactor

Figure 1: *The iCELLis 500 control system and bioreactor.*

The single-use iCELLis 500 bioreactor (*Figure 1*) and scale-down version iCELLis nano (*Figure 2*) each contain a compact fixed-bed with carriers made of medical grade polyester. This matrix provides a large growth surface area in a relatively small volume: the iCELLis design provides up to a 500m² in only 25 liters of fixed-bed. Commercially available iCELLis bioreactors range in size from 0.53 to 500m² with two compaction densities of carriers (*Table 1*).

Evenly distributed medium circulation is powered by a built-in centrifugal-based flow impeller, ensuring low shear stress. The cell culture medium flows through the fixed-bed from the bottom to the top. From the top, the medium flows as a thin film down the outer wall where it efficiently takes up oxygen, resulting in a high mass transfer co-efficient (kLa).

Figure 2: *The iCELLis nano bioreactor system (from 0.53 to 4m²).*

Fixed-Bed (FB) Compaction Density	FB Height	iCELLis nano (Process Development)		iCELLis 500 (Large-Scale Production)	
		FB Volume (L)	FB Surface Area (m²)	FB Volume (L)	FB Surface Area (m²)
96g/L	2	0.04	0.53	5	66
(C 1)	4	0.08	1.06	10	133
13 m²/L	10	0.2	2.67	25	333
144 g/L	2	0.04	0.8	5	100
(C 1.5)	4	0.08	1.6	10	200
19.5 m²/L	10	0.2	4.0	25	500

Table 1: *Commercially available iCELLis bioreactor size specifications.*

In the iCELLis bioreactors, the following parameters can be measured and controlled: pH, DO, biomass (using a biomass probe), temperature, gas flow rate, agitation, pressure and medium recirculation/perfusion rate.

The unique waterfall oxygenation together with gentle agitation, biomass immobilization and process control provides a favored growth environment that can increase the cell specific productivity of the desired protein or virus. The ability to retain cells also allows for efficient medium exchange without the need for an external cell separation device.

Transfer and Scale-Up of a HEK293 Cell Culture Process for Production of Adenovirus

Hardware

- The iCELLis nano bioreactor (from 0.53 to 4m²)
- The iCELLis 500 bioreactor (from 66 to 500m² - 660m² prototype not commercially available)
- Metabolites were analyzed with a Bioprofile-100 bio-analyzer (Nova Biomedical, MA, USA)

Biological Material

- HEK293 cells were grown in serum-supplemented medium

Cell Culture Parameters

An existing process using HEK293 cells for the production of adenovirus was first transferred from multi-tray systems to an iCELLis nano bioreactor (0.53m², 40ml of fixed-bed) by keeping equivalent cell culture parameters:

- Temperature, pH and DO (% saturation with air)µ
- Cell seeding density (cells/cm² and cells/mL)
- Multiplicity of infection (pfu/cell)
- Culture duration
- Time of infection

Additional experiments were performed with lower cell densities at inoculation in order to reduce the number of pre-culture steps at large scale. The following parameters were also optimized for cell growth and virus productivity:

- Compaction of carriers inside the fixed-bed (96g/L or 144g/L)
- Fixed-bed height (2, 4 or 10cm)
- Linear velocity of medium through the fixed-bed (cm/s)

Linear Velocity Through the Fixed Bed

The linear velocities through the fixed-bed have been characterized relative to the fixed-bed height, compaction of carriers and agitation speed in the iCELLis nano bioreactors (*Figure 3*). It has been demonstrated with HEK293 cells in a 2- to 4-cm high fixed bed that a linear velocity of 2 to 3cm/s at inoculation with a single-cell suspension results in a uniform distribution of cells.

In order to keep the linear flow rate constant at large scale, this parameter was likewise characterized in iCELLis 500 bioreactors with 133m² and 500m² surface areas using medium volumes corresponding to different falling film (FF) heights (*Figure 4*).

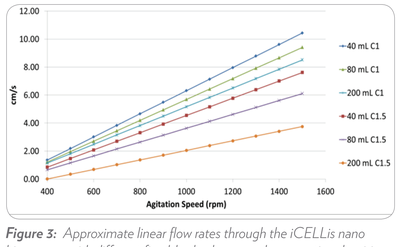
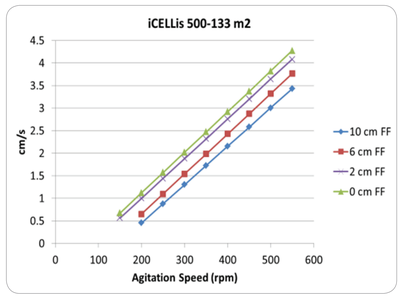


Figure 3: *Approximate linear flow rates through the iCELLis nano bioreactors with different fixed-bed volumes and compaction densities (C1 vs C1.5), with 900 mL working volume.*

Industrial Scale-Up

The scale-up of the iCELLis technology is similar to that of chromatography columns. The difference in fixed bed geometry from small to large scale is that the cross-sectional area increases, while the fixed bed (FB) height remains constant. Therefore, cell seeding, nutrient and oxygen delivery throughout the fixed bed are comparable at small and large scale.



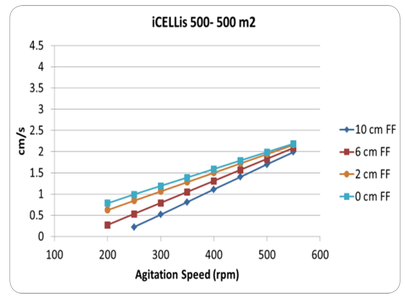


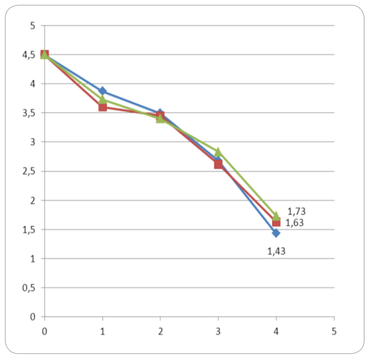
Figure 4: *Approximate linear flow rates through industrial scale bioreactors with different falling film (FF) heights.*

After determining optimal parameters at small scale, HEK293 cell culture runs were performed in duplicate with small and large scale bioreactors. Inoculation density, medium volume ratios, culture duration, pH, DO and temperature set points were kept identical at small and large scale. Cell density results are shown in *Table 2*. Cell densities were determined by sampling carriers, cell lysis, crystal violet staining and cell counting by hemocytometer. The results indicate that cell growth was consistent and comparable at both small and large scale.

Analysis of glucose and lactate (*Figure 5*) at both scales in comparison to a 5-tray Cell Factory control indicated that cell metabolism was comparable between small and large scale iCELLis bioreactors and the standard 2-D process.

Bioreactor Scale	Fixed Bed Surface Area (m²)	Fixed Bed Height (cm)	Fixed Bed Volume (L)	Average Cell Density Reached (cells/cm²)	Average Total Cells per Bioreactor
iCELLis nano	1.06	4	0.08	2.8 to 3.8 x 10^5	3.5 x 10^9
iCELLis 500	133	4	10	2.7 to 3.4 x 10^5	4.1 x 10^{11}

Table 2: *Results of HEK293 cell culture four days after inoculation in small- and large-scale iCELLis bioreactors.*



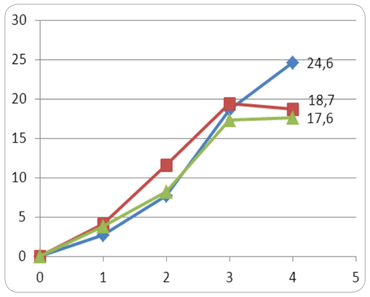


Figure 5: *Comparability of Glucose (Top Panel) and Lactate (Bottom Panel) Profiles of HEK293 culture in the iCELLis 133m² (Blue), iCELLis nano 1.06m² (Green) and 5-tray Cell Factory (Red).*

Additional Virus Production Process Development

Hardware

- Artefix bioreactor - iCELLis nano predecessor (0.07m²)
- The iCELLis nano bioreactor controller and single use bioreactors (0.53 – 4m²)
- Pilot-scale iCELLis (prototype not commercially available, 20m²)
- The iCELLis500 bioreactor skid and single use bioreactor (660m² prototype)

Biological Material

- Embryonated eggs were used to generate CEF cells and produce MVA virus in serum-free medium
- MDBK cells were used to produce Bovine Herpes Virus (BHV) in serum-supplmented medium
- A549 cells were used to produce recombinant adeno-associated virus (rAAV) and adenovirus in serum-supplemented medium
- Vero cells were used to produce human influenza virus and paramyxovirus in serum-free medium
- Vero cells were used to produce an undisclosed lytic virus in serum-supplemented medium

Cells	Virus	Bioreactor	Surface Area (m²)	Average Cell Density at TOI (cells/cm²)	Specific Virus Productivity	Total Virus
CEF	MVA	Artefix	0.07	3.9E+05	3.5E+06 pfu/cm²	1.7E+13 pfu
MDBK	BHV	iCELLis nano	4	1.2E+05	2.2E+07 pfu/cm²	8.7E+11 pfu
		iCELLis pilot	20	1.4E+05	1.7E+07 pfu/cm²	3.4E+12 pfu
		iCELLis 500	66	3.3E+05	3.3E+07pfu/cm²	2.2E+13 pfu
A549	rAAV	iCELLis nano	0.53	6.0E+04	5.3E+08 vg/cm²	2.8E+12 vg
	Adenovirus	iCELLis nano	2.67	2.3E+05	1.1E+10 TCID50/cm²	3.0E+14 TCID50
Vero	Influenza	iCELLis nano	4	1.0E+05	3.8E+06 TCID50/cm²	1.5E+11 TCID50
		iCELLis pilot	20	7.5E+04	2.5E+06 TCID50/cm²	5.0E+11 TCID50
	Paramyxovirus	iCELLis nano	2.67	2.7E+05	6.4E+05 TCID50/cm²	1.7E+10 pfu
Vero	Undisclosed Lytic Virus	iCELLis pilot	40	1.5E+05	Confidential	Confidential
		iCELLis 500	133	1.5E+05		
		iCELLis 1000	660	2.3E+05		

Table 3: *Results for different viruses from various cell lines at different bioreactor scales.*

Results of experiments performed for production of several viruses in various cell lines at various bioreactor scales are shown in *Table 3*. Bench scale bioreactors were used for each process to determine what conditions and feeding strategies sustained the highest growth rates and cell densities For chicken embryonic fibroblasts (CEF) and production of Modified Vaccinia Ankara (MVA), a prototype "Artefix" bioreactor (the predecessor of iCELLis) with a 0.07m² fixed-bed surface area was tested. Intermediate "pilot" scale prototype iCELLis bioreactors with surface areas of 20 or 40m² were used to test Vero and MDBK cell processes. The Vero cell process was scaled up to a 660m² bioreactor. In this case, cells were inoculated at only 3200 cells/cm² using two 40-tray Cell Factories (2.5m² each), equivalent to fifteen roller bottles (1700cm² each). With such a low seeding density the seed train required for inoculation is simplified extensively compared to standard 2-D cell culture processes (*Figure 6*). The Vero cell density reached 2.3 x 10^5 cells/cm² for a total biomass of 1.5 x10^{12} cells in 11 days. A complete medium exchange was then performed, followed by virus infection. Continuous perfusion of medium was used during the production phase. While the virus type and productivity data is confidential, the results indicated that virus output was equivalent or better than expected based on the standard 2-D process.

Figure 6: *The iCELLis 500 seed train. As few as twenty roller bottles (1700 cm2) could be used to seed a 500m².*

Conclusion

This summary of experiments demonstrates that the fixed-bed design of the Integrity iCELLis bioreactor enables high cell densities to be achieved and maintained in both small and large bioreactor volumes. Different processes have been easily scaled up by keeping cell culture conditions and process parameters identical to the standard 2-D cell culture process. The iCELLis bioreactor can be inoculated at a very low cell density, leading to a dramatic simplification of seed train operations and a significant reduction of development timelines.

In conclusion, large biomass amplification and excellent virus productivities, combined with the advantages of a fully closed disposable system with low shear stress, make the iCELLis fixed-bed bioreactor a simple and straightforward solution for industrial production of viruses.

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