Viral Vector Production in the Integrity® iCELLis® Single-Use Fixed-Bed Bioreactor, From Bench Scale to Industrial Scale

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Introduction

Wild-type or recombinant viruses used as vaccines and human gene therapy vectors are an important development tool in modern medicine. Some have demonstrated high potential such as lentivirus, paramyxovirus and adeno-associated-virus (AAV). These vectors are produced in adherent and suspension cell cultures (e.g. HEK293T, A549, VERO, PER.C6, Sf9) using either transient transfection (e.g PEI, calcium phosphate precipitation) or infection (e.g. modified or recombinant viruses) strategies. Most of these processes are currently achieved in static mode on 2-D systems (Roller Bottles, Cell Factories, etc.) or on suspended microcarriers (porous or non-porous). However, these two systems are time-consuming (large numbers of manipulations, preparation of equipment, etc.) and hardly scalable. In regards to process simplification and traceability, Integrity® iCELLis® bioreactors offer a new solution for scalability and monitoring of adherent cell cultures.

The Integrity iCELLis Bioreactor

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, designed for adherent cell culture applications such as recombinant protein, viral vaccine and gene therapy vector production. Using medical grade polyester microfiber carriers trapped into a fixed-bed, cells grow in a 3-D environment with temperature, pH and dissolved oxygen controls. This regulation maintains excellent cell physiology and can lead to higher specific productivity compared to classical vessels (e.g. Roller Bottle or Cell Factories). The iCELLis technology can be used at small-scale (iCELLis nano from 0.5 to 4m², *Figure 1*) and manufacturing scale (iCELLis 500 from 66 to 500m², *Figure 2*) which eases process scale-up and its overall utilization.

Figure 1: *The iCELLis nano bioreactor system (from 0.53 to 4m²).*

Materials and Methods

All the experiments described here have been performed in the bench-scale and pilot-scale iCELLis bioreactors containing iPack carriers made of 100% pure non-woven PET fibers. Crystal violet was used for cell nuclei counts from carriers.

Figure 2: *Integrity iCELLis 500 bioreactor in operation mode.*

Virus production

The transfer of virus production from static or stirred macro beads systems to the iCELLis bioreactors is mainly based on the existing process. In general, after a few days of cell growth inside the fixed-bed, viruses are injected into the bioreactor and infect cells while passing through the fixed bed. During the culture, cell and metabolite concentrations are assessed which determine the appropriate times of infection and harvest. Examples of viruses produced in iCELLis are listed below.

Viruses produced with the iCELLis system:

- VERO cells – Influenza virus – serum free conditions
- VERO cells – Undisclosed lytic virus and medium
- HEK293 cells – Adenoviruses
- MDBK cells – Bovine Herpes Virus (BHV) – MEM with 5% FBS and 1% NE AA
- CEF – MVA

Recombinant adeno-associated virus vector production

Similarly to the viral vaccines, some recombinant viruses are produced in the iCELLis bioreactors using hybrid vectors. For example, A549-stable packaging cell line, maintained in Optipro medium + 1% FBS, can deliver recombinant AAV vectors frequently used in gene transfer applications (Inserm UMR649, Institut de Recherche Thérapeutique.).

Alternatively, others rAAV vectors are obtained by transient transfection. In this case, HEK293-T cells are regularly found to be sensitive to the viral DNA and transfection reagent complex (generally polyethylenimine - PEI or phosphate calcium precipitate). The transfer of the transfection process from static or dynamic systems to the iCELLis bioreactors requires some adaptation in order to fully benefit from both technologies. Using a fluorescent protein marker, the transfected cells can be observed during the culture and the viral vectors can be quantified after the harvest (*Figure 3*).

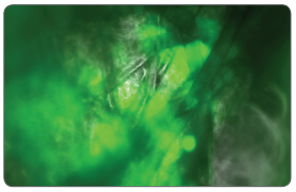

Figure 3: *GFP-expressing HEK293-T cells attached on iPack carriers 48 hours post-transfection.*

Transfection method using the PEI/DNA complexes is frequently found in cell suspension processes due to its high efficiency and adaptability to high-throughput systems. The circulation pattern of the medium through the fixed-bed of the iCELLis system allows a good contact between cells and transfection complexes (*Figure 4*).

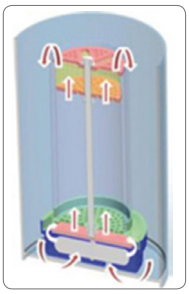

Figure 4: *Liquid circulation pattern into the iCELLis nano.*

The transfection by phosphate precipitation is a static method where the DNA precipitate settles on the cells. For this reason, it is difficult to apply this in dynamic conditions. To be able to implement it in the iCELLis bioreactor, the agitation speed has to be minimal to get a slow medium circulation through the fixed-bed. This maintains the precipitate in suspension while giving the longest contact time possible between the precipitate and the cells. The iCELLis system with its pH regulation and low-shear circulation is well adapted for this method which is sensitive to small pH changes and consistency of the reagent mix.

Results

Bovine Herpes Virus and Influenza Production

Virus production from small to intermediate and large-scale iCELLis bioreactors has been successful for several processes using VERO and MDBK cells. The scale-up of these processes was eased by the configuration of the bioreactors (constant fixed-bed height and compaction rate at small and large scales). Comparisons of processes in the different bioreactor scales were possible by keeping cell culture parameters constant (inoculation density, medium volume ratios, culture duration, pH, DO and Temp. set points).

Total virus production of the Bovine Herpes Virus (BHV) by MDBK cells in the iCELLis showed good scalability from 4 to 200m² fixed-bed (*Table 1*). Moreover, viral productivity was about 1.9x10⁷ PFU/cm² in the iCELLis compared to 1.325x10⁷ PFU/cm² in the stirred tank using Cytodex 1 as cell growth support.

Scalability in the iCELLis bioreactors from 4 to 20m² was also tested with VERO cells producing flu vaccine in a serum-free medium. Cell concentrations reached in the iCELLis systems and stirred tank were comparable whereas viral productivity was significantly higher in the iCELLis bioreactors.

Cell Lines and Viruses	iCELLis System	Total Surface (m²)	Cell Density of Infection (10⁶ cells/ml)	Total Biomass of Infection (10⁹ cells)	Total Virus Production	Equivalent Number of CF-40
MDBK – BHV Vaccine	0.1L iCELLis	4	46	4.6	870x10⁶ PFU	2.0
	0.5L iCELLis	20	57	28	3440x10⁶ PFU	8.0
	5L iCELLis	200	44	220	NA	NA
	75L stirred tank, Cytodex-1 (6g/L)	20	5.8	43	2650x10⁶ PFU	6.0
VERO Cells – Flu Vaccine	0.1L iCELLis	4	~40	4	~13x10⁶ HA ~150x10⁶ TCID50	0.6 for TCID50 2.9 for HA
	0.5L iCELLis	20	~30	15	~50x10⁶ HA ~500x10⁶ TCID50	2.0 for TCID50 9.7 for HA
	10L stirred tank, Cytodex-1 (6g/L)	26	2.5	25	~350x10⁶ TCID50	1.4 for TCID50
VERO Cells – Undisclosed human vaccine	0.5L iCELLis	20	~60	30	Undisclosed	Undisclosed
	1L iCELLis	40	~60	60		
	3.3L iCELLis	130	~60	~ 200		
	25L iCELLis	660	~60	~ 1500		

Table 1: *Production of viruses with MDBK and VERO cells in iCELLis fixed-bed bioreactor.*

Recombinant adeno-associated virus vector production

Recombinant AAV vectors were produced in an A549-based stable packaging cell line containing the AAV2 rep and cap genes from various AAV serotypes. Using a dual adenovirus infection (wild-type Ad5 followed by hybrid Ad/AAV) in the iCELLis nano bioreactor under perfusion mode, recombinant particles were harvested up to 96 hours post-infection. The expression levels of the AAV2 rep and cap genes from various AAV serotypes were assessed by western-blot and qPCR. This 8-days process demonstrated higher vector particles production in the iCELLis bioreactor compared to CS-5 control (4.5x10⁸ vs 3.1x10⁸ vg/cm², 72h after the first infection, *Figure 5*) (Inserm UMR649, Institut de Recherche Thérapeutique.).

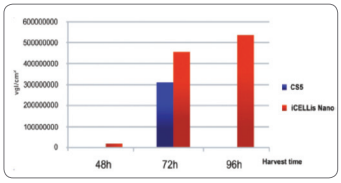

Figure 5: *Production of rAAV2/8 vector particles (Vg/cm² measured by qPCR) in iCELLis and CellSTACK (CSs) control.*

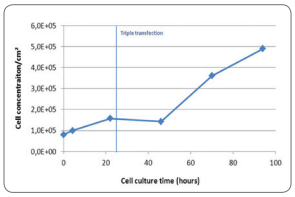

Figure 6: *Cell growth curve in the iCELLis 0.53m² bioreactor before and after triple transfection.*

Triple transient transfection using PEI was performed in the iCELLis nano system (0.53m², 40mL fixed-bed) for the production of serotype 5 AAV in HEK 293T cells. Cells were seeded at 80,000 cells/cm² in the CS10 and the iCELLis bioreactor. Twenty-four hours post-inoculation, the DNA-PEI mix containing the GFP gene was added to fresh medium inside the bioreactor. Cells were still growing on the carriers after the transfection (*Figure 6*). The expression of GFP by cells demonstrated that the transfection had a high efficiency rate in both vessels (FACS analysis on sampled carriers for the iCELLis bioreactor) (*Figure 3* and *Figure 7*). Green Fluorescent Units (GFU) and Viral Genomes (VG) were measured for the CS10 control and the iCELLis nano bioreactor. Viral particles were harvested using a freeze/thaw method, which is suboptimal in the case of the iCELLis system. The GFU and VG titers/cm² in the iCELLis bioreactor were about 53% of the control (*Figure 8*). (Dept. of Biochemical Eng. - UCL.)

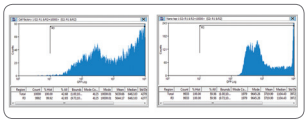

Figure 7: *FACS analysis of transfected cells expressing GFP from CellSTACK control and iCELLis nano 0.53m².*

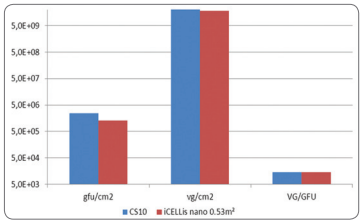

Figure 8: *Comparison of Green Fluorescent Units and Viral Genome/cm² and VG/GFU ratio in the CS10 and iCELLis nano 0.53m².*

Conclusions

We demonstrated that the iCELLi system could be useful for production of viral vaccine and gene therapy vectors. The iCELLis platform facilitates handling and scale-up, high biomass amplification and sterile containment within a closed system. Moreover, in many cases, the culture environment enhances virus production yields.

Specifically, after some optimization of the culture parameters, it was demonstrated that rAAV vectors were produced by modified A549 cells in high viral level in the 0.53m² iCELLis bioreactor. The maximum viral yield achieved in the bioreactor was 4.5x10⁸ vg/cm², which was higher than the yield per cm² obtained in a CellSTACK vessel (3.1 x10⁸ vg/cm²).

Finally, the preliminary results of transfection demonstrated that the method using PEI is applicable in the iCELLis bioreactors, with optimization of the virus recovery at harvest yet to be performed. This also demonstrated that the iCELLis can be considered as a solution for transient transfection processes at large scales.