Literature review of applications and processes in iCELLis™ bioreactor

Background and introduction

Adherent cell culture processes involve the use of substrate surfaces that allow cell attachment, growth, and proliferation in a controlled, artificial environment. Cell culture in adherent conditions represents a valuable platform for viral vector biomanufacturing for therapeutic purposes. Lentiviruses (LV), adenoviruses, and adeno-associated viruses (AAV) are key therapeutic agents employed for genetic material delivery in cell and gene therapies and are typically produced with adherent-based cell culture technologies. Traditional cell culture approaches are based on the use of plastic flatware like T-flasks or multitray systems. However, in the last decade, we have experienced a significant increase in demand for cell and gene therapy products that has fostered the advancement of better-suited means of production. Fixed-bed bioreactors enable efficient production of viral vectors. However, establishing a new process in fixed-bed bioreactors poses different challenges with regards to the identification of operating spaces and process scale-up. Determining the optimal cell culture parameters and conditions often demands intensive process development effort.
The iCELLis bioreactors are single-use, scalable fixed-bed bioreactors for adherent cell culture processes.As of 2023, four of the six approved gene therapy products in the US use iCELLis technology; including one adenovirus, one oncolytic virus, and two AAV processes. In this review, we evaluated recent publications with diverse applications of the iCELLis™ bioreactor family to provide an overview and guidelines for researchers developing new processes. In the first section, features of the technology are presented. In following, common process conditions for AAV and LV, the main viral vectors for gene therapy, are described, such as cell lines, cell density, media composition, feed strategies, transfection methods, and operational parameters (pH, dissolved oxygen, temperature). Finally, overviews of oncolytic therapies, vaccines, recombinant proteins, and extracellular vesicles production are provided.

(A)

(B)

Fig 1. iCELLis bioreactors: (A) iCELLis Nano Bioreactor; (B) iCELLis 500+ Bioreactor.

Technology overview

The iCELLis bioreactors are single-use fixed-bed bioreactors that allow for scalable adherent cell culture to manufacture products that do not require cell recovery, such as viral vectors, recombinant proteins, and extracellular vesicles. By providing automated, accurate monitoring and control of pH, dissolved oxygen (DO), temperature, and biomass, these bioreactors provide optimal growth conditions for adherent cells. The fixed-bed represents a key feature of this line of bioreactors that allows for high cell densities and high viral particles productivity. The iCELLis bioreactor family comprises two systems: the iCELLis Nano Bioreactor and the iCELLis 500+ Bioreactor (Fig 1). Together, they provide a scalable platform from bench scale to commercial manufacturing scale.

Depending on the height and compaction of the carriers in the fixed-bed, different configurations are available. The iCELLis Nano Bioreactor (bench scale bioreactor) provides a range of cell growth surfaces from a minimum of 0.53 m^2 to a maximum of 4 m^2 whereas the iCELLis 500+ Bioreactor (manufacturing-scale bioreactor) supports cell growth surfaces ranging from 66 m^2 up to 500 m^2. Both bioreactors come with three fixed-bed heights: 2cm, 4cm and 10cm. The diameter is increased from 11cm in the iCELLis Nano bioreactor to 86cm in the iCELLis 500+ bioreactor thus allowing, thanks to the geometrical similarity, relatively simple scale-up strategy. The working volume in the iCELLis Nano Bioreactor ranges from 600 mL to a maximum of 900 mL whereas the iCELLis 500+ Bioreactor supports working volumes up to approximately 70 L. For production processes with increased media and nutrients needs, recirculation and perfusion methods can be performed in the iCELLis Bioreactors.

A summary of the 12 configurations of iCELLis Bioreactors is shown in Table 1.

Table 1. Configurations of iCELLis Nano Bioreactor and iCELLis 500+ Bioreactor

Bioreactor	Bioreactor diameter (cm)	Fixed-bed height (cm)	Bioreactor working volume	Surface area (m^2) low compaction	Surface area (m^2) high compaction
iCELLis Nano	11	2	600 mL– 900 mL	0.53	0.8
iCELLis Nano	11	4	600 mL– 900 mL	1.06	1.6
iCELLis Nano	11	10	600 mL– 900 mL	2.65	4
iCELLis 500+	86	2	~ 70 L	66	100
iCELLis 500+	86	4	~ 70 L	133	200
iCELLis 500+	86	10	~ 70L	333	500

A magnetic impeller ensures cell culture medium circulation inside the iCELLis bioreactors from the bottom to the top of the fixed-bed (Fig 2). Falling from the top as a thin fluid film, medium gets oxygenated thus allowing high gas-liquid mass transfer in the bioreactor while maintaining low shear forces.

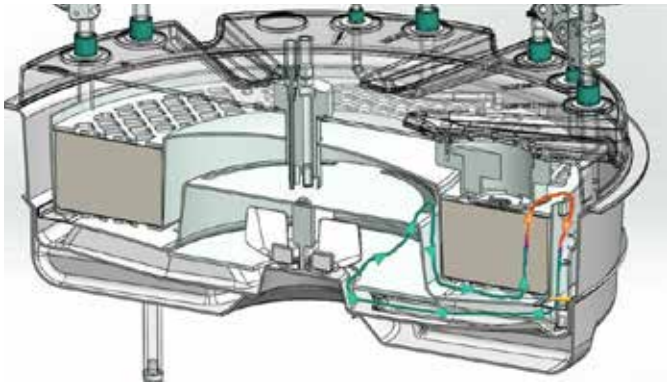

Fig 2. Media circulation flow in the iCELLis 500+ Bioreactor.

Thanks to the fixed-bed design, low shear agitation, optimal media circulation, and accurate cell culture parameter regulation, the iCELLis bioreactors can achieve high viable cell densities and high viral particles titers. In this application note, we illustrate and summarize various processes including viral vectors, vaccines, proteins and exosomes that involve the use of the iCELLis bioreactors as a scalable platform from the bench to large-scale biomanufacturing.

AAV processes

In previous years, AAVs have gained a critical role in gene therapies due to their safety, efficacy, and ability to infect different cells (1). Currently, there are many clinical studies with this vector and of the first two AAV-based therapies approved, Zolgensma is produced in the iCELLis Bioreactor (2). Although some therapies have reached market approval, there are still challenges in the manufacturing of these viral vectors, mainly, for scaling up. Most common production methods are in 2D flasks with limited process control. In this context, recent studies in the iCELLis Bioreactor showed that it is a suitable system for AAV production scaling up by providing a controlled environment designed for achieving high cell densities, increased viral yields, and lot-to-lot consistency from R&D to manufacturing scales.

Triple transient transfection with polyethyleneimine (PEI) in adherent cell cultures of HEK293 is the main AAV production method conducted in the iCELLis bioreactor. Literature has demonstrated that the successful technology transfer of this process from 2D flatware to the iCELLis Bioreactor depends on cell line adaption, efficiency of the transfection method, and maintenance of the quality attributes of the viral vectors (3, 4, 5, 6, 7). Aiming at these features, many process parameters were extensively reviewed and desirable ranges for process control were obtained. Some of them are presented in Table 2. These findings can be used as guidelines for the development of AAV production, although each process may have its particularities, and further optimization studies are necessary.

Table 2. Summary of most common parameters used in literature for AAV production in iCELLis Bioreactors

Process parameter	Range	Units
Seeding density	6000–100 000	cells/cm^2
Transfection density	150 000–250 000	cells/cm^2
DNA to cell ratio	0.1 -2.0	µg total DNA/ million cells
PEI to DNA ratio	3:1 and 2:1	mg/mg
DO setpoint	40–50	% of air saturation
pH setpoint	7.1–7.4	
Temperature setpoint	37	°C
Linear velocity	0.75–2.5	cm/s
Harvest time post-transfection	72–96	hours

The parameters presented in the table summarize currently available literature at the point of writing and do not suggest recommended conditions. Contact your Cytiva upstream applications scientist for recommendations.

Although stable-packing cell lines are promising to achieve higher yields (8), the cell strains originating from HEK293 are the most common host used in the iCELLis bioreactor. In general, seed densities vary from 6000 to 100 000 cells/cm^2, with 10 000 cells/cm^2 being the most frequent (3, 4, 9, 6, 10). An extensive range of cell densities from 70 000 to 250 000 cells/cm^2 was tested at transfection (6, 11). Alfano *et al*. (2021) (11) obtained significantly higher titer with 70 000 cells/cm^2 than 200 000 cells/cm^2 in serum-free media. In addition, Illingworth *et al*. (2014) (12) and Pegel *et al.* (2013) (13) obtained lower yields than reference flasks with transfection densities above 200 000 cells/cm^2. Therefore, the data suggest that lower transfection densities in this range can benefit AAV production.

Different media compositions were chosen for growth and production, with fetal bovine serum (FBS) content ranging from 0% to 10% (3, 9, 6, 10). In addition to media composition, the feed method was evaluated. Studies were performed with media recirculation during the growth phase, media exchange before and after transfection, and perfusion during the production phase. Nass *et al*. (2019) (10) observed that there is a direct correlation of vector yield and working volume after transfection. Also, Lefebvre *et al.* (2017) (6) achieved up to 8-fold higher productivity by performing post-transfection media exchange. Furthermore, they observed that stopping the recirculation of media benefited transfection efficiency by increasing cell-vector contact.

It was reported that some parameters were critical to the efficiency of the transfection process, such as the DNA to cell ratio (0.1–2.0 µg DNA/million cells) (9), PEI to DNA ratio (3:1 and 2:1 mg/mg) (4, 9, 10), incubation time of the complex (10), contact time of the complex with cells (10), medium exchanges after transfection (9), and the harvest time (72 to 96 hours after transfection) (9). Another critical variable identified for the harvest is the AAV serotype since it will determine the ideal harvest day and the recovery method of intra- or extracellular vectors (3).

Regardless of the serotype, high-yield production of AAV serotypes 5, 8, 9, and recombinant vectors were obtained in the iCELLis bioreactor. In these studies, different fixed-bed heights (0.53 to 4 m^2) and compaction (1× and 1.5×) were evaluated, and the main process parameters were: dissolved oxygen (DO) kept at 40% to 50% of air saturation; pH setpoint between 7.1 to 7.4 with one-side control (CO_2 only), 7.2 being the most common; and temperature at 37°C during the entirety of the culture. Another critical parameter is the linear velocity with which media circulates through the fixed-bed, since it is crucial for gas exchange, contact time between the vectors and the cells, and obtaining a low-shear stress level. Linear velocities from 0.75 to 2.5 cm/s were studied and altered during the process to keep ideal conditions, such as maintaining cell growth and reducing product degradation (3, 4, 6, 9, 10).

Chemical lysis is the most reported method with the application of different buffers (Tris and HEPES), salts (NaCl and $MgCl_2$), and the addition of nucleases (6, 7, 9, 10). After harvest, rinse can increase yield by recovering additional AAV (7). Many studies obtained from 10^8 to 10^{10} viral genomes per cm^2. In some of them, the yields in the iCELLis bioreactor were comparable to the multi-tray flasks (CellSTACK (CS) and Cell Factory (CF)). However, there are some limitations, and further process optimization may be required to obtain comparative productivity to reference systems, as shown in Table 3. In this review we summarized only studies in the iCELLis Nano bioreactor. The basic scaling-up strategy to the iCELLis 500+ Bioreactor will be keeping the bed characteristics (height and compaction), and the volumetric mass transfer coefficient of oxygen by using the scale-up tool from Cytiva. Please contact your FAS — field application scientist representive if you are interested in process development and scale-up.

Table 3. AAV productivities published in the literature

Product	Cell line	Bioreactor size (m^2)	iCELLis yield	Control flask yield	Source
rAAV2	A549 (stable packing cell line)	0.53	4.5×10^8 vg/cm^2	3.1×10^8 vg/cm^2 (CS5)	Lennaertz *et al.* (2013) (3)
AAV-5	HEK293T	0.53	5.5×10^9 vg/cm^2	5.6×10^9 vg/cm^2 (CS10)	Lennaertz *et al.* (2013) (3)
rAAV-8	HEK293	0.8 4	4.5×10^9 vg/cm^2 3.4×10^9 vg/cm^2	N/A	Lefebvre *et al.* (2017) (6)
AAV	HEK293	4	4.0×10^9 vg/cm^2	1.9×10^9 vg/cm^2 (CS10)	Nass *et al.* (2019) (10)
AAV human factor IX	HEK293T/17	0.53	6.9×10^{10} vp/cm^2	2.5×10^{11} vp/cm^2 (CF10)	Powers *et al*. (2016) (5)
rrAAV-2	HEK293T	0.53	2.2×10^{10} vg/cm^2	4.7×10^{10} vg/cm^2 (CS10)	Pegel *et al*. (2013) (13)
rAAV5	HEK293T	0.53	4.4×10^9 vg/cm^2	6.3×10^9 vg/cm^2 (CS10)	Illingworth *et al.* (2014) (12)
rAAV	HEK293T	0.53	2.2×10^{10} vg/cm^2	4.7×10^{10} vg/cm^2 (CS10)	Emmerling *et al.* (2016) (4)
AAV	HEK293	4	6.8×10^{10} vg/cm^2	N/A	Kaspar *et al.* (2019) (7)

Note: CS5 = CellSTACK 5-layer culture chamber, CS10 = CellSTACK 10-layer culture chamber, CF10 = Cell Factory 10-layer culture chambe

Lentivirus processes

LV vectors are the second most utilized viral vector after AAVs, being used in 30% of gene therapies that are in the pipeline (14). This is due to inherent characteristics — they can transduce both dividing and non-dividing cells, hence delivering genes into a broad range of mammalian cell lines. They also have the capacity to permanently integrate into the host cell genome (15), and they elicit a relatively low immune response in target cells (14). Based on available literature, the range of iCELLis process parameters currently being used is summarized in Table 4.

Table 4. Summary of most common parameters used in literature for LV production in iCELLis Bioreactors

Process parameter	Range	Units
Seeding density	7000–15 000	cells/cm^2
Transfection cell density	60 000–250 000	cells/cm^2
Plasmid concentration	200–400	ng (total DNA) /cm^2
DNA to PEI ratio	1:1 and 1:2	mg/mg
DO setpoint	40–55	% of air saturation
pH setpoint	7.0–7.25 pre-transfection 6.8–7.0 post-transfection	
Temperature setpoint	37	°C
Linear velocity	1–3	cm/s
Harvest time post-transfection	24–96	hours

The parameters presented in the table summarize currently available literature at the point of writing and do not suggest recommended conditions. Contact your Cytiva bioreactor applications scientist for recommendations.

The main LV production platform involves transient transfection. However, the transient transfection step is not easily reproducible, resulting in heterogeneity between batches. Also, the use of clinical-grade plasmids and transfection reagents is costly. Hence there is a need to develop stable producer cell lines. Powers *et al*. (2020) (5) developed their GPRTG-EF1α-hγc stable packaging cell line that produced comparable LV titer and transduction efficiency of the vector to that produced from 10-layer multitray systems. However, it will take some time before more stable cell lines are available, therefore the HEK293 cell line is still the most commonly used. Generally, cell seeding density varies from 7000 to 15 000 cells/cm^2, and the target cell density at transfection ranges from 60 000 to 250 000 cells/cm^2, which may be achieved from Day 2 to Day 4 (5, 11, 16, 17, 18, 19, 20, 21, 22).

In terms of media composition, 10% FBS is commonly supplemented to high-glucose growth media as it provides the nutrients for efficient HEK293 cell growth. Additionally, the presence of fatty acids and lipids in FBS helps to protect the stability of LV vectors (17). However, there is concern over the stability and consistency of FBS. Moreover, the presence of FBS in media presents challenges to downstream processing, and there will be an availability issue of FBS in the long run.

In current practice, there are two types of plasmid systems: 2nd-generation (using three separate plasmids) and 3rd-generation systems (using four separate plasmids). The 3rd-generation system is more frequently used as it is safer with the elimination of the HIV protein Tat from the system (23). The transfection reagent commonly used is PEI as it is less sensitive to pH variations and substantially less toxic than calcium phosphate, and it is relatively inexpensive (15). Critical parameters influencing the transfection efficiency include the DNA to PEI ratio (1:1 or 1:2) and the concentration of plasmids, which can range from 200–400 ng/cm^2 (16, 17, 18, 19, 21, 22, 24). Recent research also showed that transferrin-mediated transfection can improve transfection efficiency (20, 21).

Different fixed-bed heights (0.53 to 333 m^2) and compaction (1× and 1.5×) have been evaluated. It was found that the low-compaction fixed-bed demonstrated higher productivity per surface area (16). The main process parameters were as follows: DO maintained at 40% to 55% of air saturation; temperature of 37°C during the culture batch; and pH controlled at 7.0–7.25 before transfection and at 6.8–7.0 after transfection (5, 11, 16, 17, 19, 20, 21, 22). It has been shown that lower pH increases LV production (16). The linear speed was varied between 1–2 cm/s during cell growth, with higher stirring speeds of 2–3 cm/s during inoculation and transfection to ensure homogeneity in the fixed-bed (5, 11, 16, 17, 19, 20, 21, 22).

Due to the low stability of LVs at room temperature, perfusion can help maintain the biological activity of the vector by reducing the holding time before downstream processing, decreasing the volume handled at one time, and collecting product efficiently. Yoganathan *et al.* (2020) (19) reported that the concentration of LV vector particles measured in the iCELLis Nano bioreactor vessel decreased over time, while consistently increasing inside the external perfusion bottle. Fiol *et al.* (2023) (25) reported the LV yield with perfusion process was twice as high as batch process. In the perfusion mode, several strategies are possible in terms of the target glucose concentration or the perfusion rate to achieve it. Different research groups have experimented to target between 0.5 to 2 g/L of glucose in the bioreactor. Valkama *et al.* (2018) (16) found that when targeting a lower glucose concentration of 0.5 g/L, the cells were able to proliferate well with less glucose. Overall, this led to a decrease in the required perfusion medium and total product volume. LV collection can usually begin 2 days post-transfection as the transfection reagents should have been completely removed (16).

Table 5. LV productivities published in literature

Cell line	Bioreactor size (m^2)	Product yield	Control flatware	Source
HEK293T	2.67 4	2.2×10^5–3.7×10^5 TU/cm^2 5.2×10^4 – 2.9×10^5 TU/cm^2	7.12×10^5 TU/cm^2	Valkama *et al* (2018) (16)
HEK293T	2.67 333	5.4×10^4 – 6.7×10^5 TU/cm^2 6.1×10^4 TU/cm2	–	Leinonen *et al*. (2019) (17)
HEK293T	2.67	4.9×10^5–6.4×10^5 TU/cm^2	9.8×10^5 TU/cm^2 (Macrocarrier)	Leinonen *et al*. (2020) (18)
HEK293T	0.53	6×10^4 TU/cm^2	4.3×10^4 TU/cm^2	Yoganathan *et al*. (2020)
HEK293T	100 333	1.3×10^6 TU/mL 2.0×10^9 TU/mL	–	Valkama *et al*. (2020)
HEK293T	0.53	1.1×10^7 IFU/mL	6.3×10^7 IFU/mL	Alfano *et al*. (2021)
HEK293T	0.53 66	1.0×10^8 GC/cm^2 3.5×10^8 GC/cm^2	–	Pelletier *et al*. (2021) (25)
HEK293T	0.53	1.2×10^6 TU/cm^2	2.7×10^7 TU/cm^2	Fiol *et al*. (2023) (25)
CD34+	2.6	1.7×10^7 TU/cm^2	1.6×10^7 TU/mL	Powers *et al*. (2020) (5)

After harvest, the iCELLis bioreactor yields 5.2×10^4 to 1.7×10^7 transducing units (TU)/cm^2 of LV vectors (5, 16, 17, 18), as shown in Table 5. Although the lower productivity per surface area than flatware has been reported (16), even in that case the total transducing units produced in a 2.67 m^2 iCELLis Nano bioreactor run was found to be approximately 30 times higher than in a 500 cm^2 TripleFlask, and at best equivalent to over 100 TripleFlask units (16). For further improvements to productivity, researchers need to consider the following: development of stable producer cell lines, optimization of harvest conditions by perfusion, and design of downstream processes to efficiently process the large product volumes, while maintaining vector functionality and minimizing product losses.

Adenovirus, oncolytic virus, and other viral vectors

Adenovirus is a non-enveloped viral vector and has traditionally been used for vaccine development as in some COVID-19 vaccines (26). Unlike AAV and LV, the production of adenoviruses requires the amplification of the virus by infection rather than by transfection. Both suspension and adherent systems can be used for adenovirus production, but the ability of iCELLis bioreactor to rapidly scale up from small-scale adherent systems is also applicable to adenovirus production. As summarized in Table 6, Adenovirus yields were in the range of 7.0×10^7–1.36×10^{10} IFU/cm^2 with iCELLis Nano bioreactor; however, in optimized and scale-up process with 66 m^2 fixed-bed of iCELLis 500 bioreactor, 1.57×10^{10} IFU/cm2 has been reported. These results showed that the iCELLis bioreactor produced scalable and consistent results in adenovirus production.

Recombinant retrovirus vectors other than LV have also been studied as viral vectors. Typical retrovirus vector production is performed with a stable producer cell line. Wang et al. (2013) showed that two producer cell lines, 293GP and PG13, grew normally in an iCELLis Bioreactor, and virus yields were 6–10 times higher than control flatware.

Oncolytic virus can be considered a similar mechanism to viral vectors in terms of gene transfer, and as with gene therapy vectors there are several examples of production in iCELLis bioreactors as shown in Table 6. These publications (Correia et al., 2019; Sable et al., 2020; Wohlfarth et al., 2021) (25, 26, 27) showed the oncolytic virus production in the iCELLis bioreactor is comparable or greater than in flatware processes.

Table 6. Productivities of other gene therapy vectors and oncolytic viruses published in literature

Product	Cell line	Bioreactor size (m^2)	Product yield	Control	Source
Adenovirus	A549	2.65	1.1×10^{10} TCID50/cm^2	–	Knowles *et al.* 2013 (27)
Adenovirus	HEK293	0.8, 2.67, 4, 100, 500	6.1×10^9 IU/cm^2	4.0×10^9 IU/cm^2 (T-flask)	Lesch *et al.* 2015 (28)
Adenovirus vector	HEK293	2.65, 66	1.6×10^{10} IFU/cm^2	1.6×10^9 IFU/cm^2 (Multilayer vessel)	Legmann *et al.* 2017 (29)
Adenovirus	HEK293	0.53	7.0×10^7 vp/cm^2 8.1×10^5 IFU/cm2^2	3.2×10^7 vp/cm^2 3.2×10^5 IFU/cm^2 (Multilayer vessel)	Yakshe *et al.* 2018 (30)
Adenovirus	293T, HEK293	–	8.53×10^{10} vp/mL	–	Leinonen *et al.* 2019 (18)
Adenovirus (Ad5)	HEK293	0.53–4, 66	9.91×10^9 IFU/cm^2	1.6×10^9 IFU/cm^2	Cytiva and Orgenesis 2019 (31)
Retrovirus	293GP*	2.65	4×10^6 TU/mL	1.8×10^6 TU/mL (Multilayer vessel)	Wang *et al.* 2013 (32)
Retrovirus	PG13*	2.65	1.83×10^6 TU/mL	0.57×10^6 TU/mL (Multilayer vessel)	Wang *et al.* 2013 (32)
Oncolytic viral vector	Vero	0.53–4	(Expression only)	–	Correia *et al.* 2019 (33)
Oncolytic virus	MRC-5	2.65, 4	200%–400% (of flatware)	100% (Multilayer vessel)	Sable *et al.* 2020 (34)
H-1 protoparvovirus	NB-324 K	0.53, 4	5.7×10^6 PFU/cm^2	2.0×10^7 PFU/cm^2 (T-flask)	Wohlfarth *et al.* 2021 (35)

*Producer cell lines.

Vaccines, recombinant protein, and exosomes

Since iCELLis bioreactor technology is applicable to adherent cell culture process with good scalability, there are various examples of applications other than gene therapy vectors. Here we summarize other applications including vaccines, recombinant protein, and exosome production.

Viral vaccine production

Vaccine production has a long history in the manufacturing of biological medicines and has well-established process in conventional flatware. However, even in these processes, the iCELLis bioreactor can provide a more controllable and space-efficient environment for modern production processes. As shown in Table 7, these various viral vaccine products with established adherent cell lines such as Vero, showed comparable cell growth and virus production with conventional flatware processes. These results indicate that the iCELLis bioreactor can be an alternative option for producing various vaccines using adherent cell culture processes.

Table 7. Viral vaccine productivities published in literature

Product	Cell line	Bioreactor size (m^2)	Product yield	Control	Source
Poxvirus (Modified Vaccinia Ankara virus)	CEF	0.7*	3.5×10^6 PFU/cm^2	8.5×10^6 PFU/cm^2 (Multilayer vessel)	Havelange *et al.* 2009 (36)
Paramyxovirus	Vero	0.7*	6.4×10^5 TCID50	–	Knowles *et al.* 2013 (27)
Bovine Herpes Virus	MDBK	4	1.9×10^7 PFU/cm^2	–	Lennaertz *et al.* 2013 (3)
Influenza	Vero	4	–	–	Lennaertz *et al.* 2013 (3)
Influenza B	MDCK	0.53	1.7 × 109 PFU/cm^2 (Estimated by HA assay)	1.5×10^9 PFU/cm^2 (Multilayer vessel)	Yakshe *et al.* 2018 (30)
Chikungunya virus	MRC-5	4	2×10^6–3.2×10^7 PFU/mL	2.8×10^4–2×10^6 PFU/mL (Roller bottle)	Rajendran *et al.* 2014 (37)
Hepatitis-A virus	MRC-5	4	–	–	Rajendran *et al.* 2014 (37)
Rabies virus	Vero	4	1.8×10^9 TCID50/mL	1.2×10^9 TCID50/mL (Roller bottle)	Rajendran *et al.* 2014 (37)
Rabies virus	Vero	0.53	6×10^7 LD50/mL	2.7×10^7 LD50/mL (Roller bottle)	Becheau *et al.* 2020 (38)
Rotavirus	Vero	–	$7.0 \pm 0.2 \times 10^{10}$ FFU/mL	$7.3 \pm 0.3 \times 10^{10}$ FFU/mL (Multilayer vessel)	Hamidi *et al.* 2021 (39)

*Pilot version of iCELLis bioreactor.

Protein and exosome production

The iCELLis technology has also shown its usefulness for other cell-based processes. As listed in Table 8, these include CHO and insect cell lines for protein production (40, 41), which is usually performed in suspension cell cultures. These results showed that the iCELLis technology is applicable for various types of cells, which typically includes cells that require high oxygen supply and suspension environment in stirred-tank bioreactors.

Another interesting application is exosome production. Exosomes are small extracellular vesicles secreted by cells that are being studied for bioactivities and potency of drug delivery systems (42). Exosome production by stem cells is typically performed in an adherent environment because of the sensitivity to environmental changes. The iCELLis Bioreactor can provide a gentle adherent environment and large surface area for production-scale processes, and recently Haylock et al. (2022) (43) reported a comparable yield of exosome production in the iCELLis Bioreactor as expected. (Exosome applications with iCELLis technology are still limited in number. Please contact your FAS representive if you are interested in process development)

Table 8. Protein and exosome productivities published in literature

Product	Cell line	Bioreactor size (m^2)	Product yield	Control	Source
mAb	rCHO		4 g/L/day		Drugmand *et al.* 2009 (29)
rVGP*	S2	4	2.5 µg/10^7 cells (0.14 µg/cm2)	1.9 µg/10^7 cells (Stirred tank)	Ventini-Monteiro *et al.* 2015 (41)
rVGP*	SF9	4	–	–	Ventini-Monteiro *et al.* 2015 (41)
Exosome	GT1-7	0.53	2.4–5.0 × 10^5 particles/mL	–	Haylock *et al.* 2022 (43)
Exosome	iMSC	0.53	7 × 10^5 particles/cells	1 × 10^4 particles/cells	Vakil *et al.* 2022 (44)

*rVGP:Rabies virus glycoprotein.

Conclusion

Here we summarized various examples of processes in the iCELLis bioreactor. One of the main applications is the production of gene therapy vectors. In AAV processes, this bioreactor has shown high production efficiency regardless of serotype and is already used in the commercial production of a gene therapy drug. For LV production, the inherent perfusion capability of the iCELLis Bioreactor minimizes the exposure of LV vectors to shear or high temperature conditions, consequently increasing yields compared with fed-batch. In addition to viral vector production, the iCELLis bioreactor has an increasing variety of applications including vaccine production, oncolytic virus production, and has recently expanded to exosome production. The wide range of products and ease of process transfer from existing adherent cell culture processes shows versatility and further potential of the iCELLis bioreactor.

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