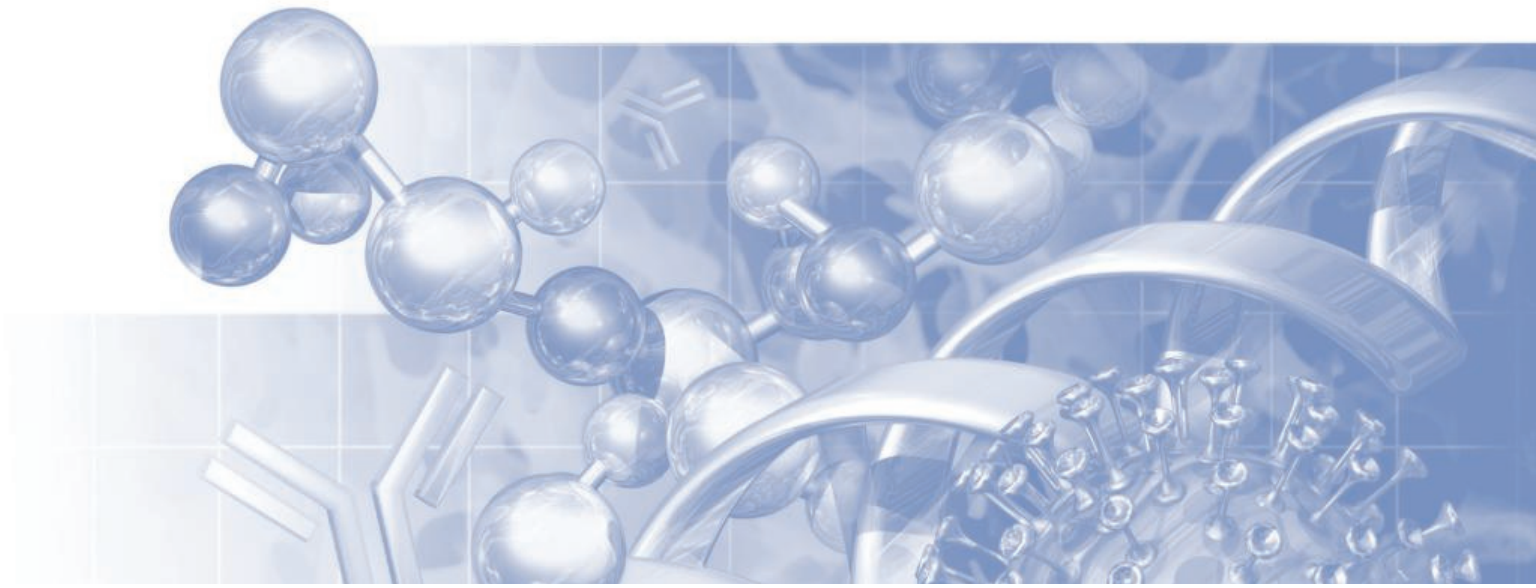
PALL Life Sciences

White Paper

USD3244

Choice of Upstream Bioreactor Technologies for Industrial Scale Viral Manufacturing



Introduction

Gene therapy, including oncolytic viruses, where new genetic material is introduced into the body, holds promise to cure or alleviate symptoms of many different diseases. In the last few years, the field has seen rapid growth with at least 11 drugs approved globally. A ClinicalTrials.gov search on the term 'gene therapy' returns 734 industry sponsored clinical trials worldwide with 464 in the US. The promise of the field has led to large amounts of investment and it is expected that the value of the gene therapy market will exceed $10B in 2025[1].

Viruses are a popular choice for gene delivery due to their target cell specificity, relatively high target cell infectivity and low toxicity upon infection. Table 1 shows typically used viruses and their suitability for various applications. Adeno-associated virus (AAV) vectors are the most widely used system in current clinical development.

Gene therapy can be broadly broken down into two categories depending on whether the genetic modification takes place *in-* or *ex-vivo*. This, along with the length of genetic material being introduced, the cell type being targeted, and whether the therapies' efficacy requires integration of the genetic material into the patient's genome determines which vector is best suited.

Table 1

Characteristics of virus used for gene therapy

Parameter	Retrovirus	Lentivirus	AAV	Adenovirus
Coat	Enveloped	Enveloped	Naked	Naked
Packaging capacity (kilobase)	8	8	4.5	7.5
Tropism/infection	Dividing cells	Broad	Broad but not hematopoietic stem cells	Broad
Inflammatory potential	Low	Low	Low	High
Host genome interaction	Integrating	Integrating	Integrating/ non-integrating	Non-integrating
Transgene expression	Long lasting	Long lasting	Potentially long-lasting	Transient or long-lasting depending on immunogenicity

Clinical Success

Current clinical interest in gene therapy is recent. The field had been under a cloud since the death in 1999 of a young patient brought most clinical trials to a halt[2]. Two events are often cited as catalysts for the revitalization of the field. The first was the publication of clinical trial results of a successful gene therapy trial in hemophilia B in which, of the six patients treated, 4 were able to discontinue clotting medications while the other two were able to reduce their doses, with no significant adverse events being reported[3]. The second was the approval by the European Medicines Agency of UniQure's Glybera♦ to treat lipoprotein lipase deficiency in 2012[4]. More recently the approval of Kymriah♦ in August 2017 and Yescarta♦ in October 2017, the world's first two marketed chimeric antigen receptor (CAR)-T cell therapies[5] for the treatment of various leukemias and lymphomas, is a significant milestone in this very promising new class of therapies that could be adapted to a wide variety of diseases. The approval of Luxturna♦ in the USA shows the continued momentum in the field. Table 2 shows the gene therapies with marketing approval.

Much of the initial development of these therapies takes place at academic centers but as the therapies progress into clinical development, there is a requirement to increase quantity and quality of the virus produced. This requires investment and therapies generally get licensed out to established companies or spun-off into separate companies in order to raise the capital required. As the industry matures, companies will require higher quality products at an earlier stage in development and manufacturing systems that can scale to meet projected commercial demand.

Table 2

Currently approved gene therapies

Drug Name	Indication	Viral Vector Used	Jurisdiction	Year Approved
Gendicine	Head and neck squamous cell carcinoma	Adenovirus	China	2003
Oncorine	Late-stage refractory nasopharyngeal carcinoma	Adenovirus	China	2006
Rexin-G	Multiple cancers	Retrovirus	Philippines	2007
Glybera	Lipoprotein lipase deficiency	AAV	Europe	2012
Neovasculagen	Peripheral arterial disease	Plasmid DNA	Russia	2012
Imlygic♦	Melanoma	HSV	USA	2015
Zalmoxis♦+	Adjunctive treatment in haploidentical hematopoietic stem cell transplantation	Retrovirus	Europe	2016
Strimvelis♦	ADA-SCID	Retrovirus	Europe	2016
Kymriah	Paediatric relapsed/refractory acute lymphocytic leukaemia	Retrovirus	USA	2017
Yescarta	Diffuse large B cell lymphoma, primary mediastinal B cell lymphoma, and transformed follicular lymphoma	Retrovirus	USA	2017
Luxturna	RPE65-mediated inherited retinal dystrophy	AAV	USA	2017

+Conditional approval

Culture Systems for Viral Vector Manufacturing

A major decision in viral vector product development for gene therapy is the choice of expression system. This can be either adherent or suspension-adapted cells. Adherent cells are cells which must be attached to a surface in order to grow and produce the intended virus. Suspension cells can be grown in traditional suspension bioreactors. Typically, most suspension cells were originally adherent and have been adapted to work in suspension culture.

In addition to the type of cell culture another consideration is the means by which the material to assemble the virus is introduced into the production cell. Transient transfection, infection and creation of producer cells lines are all utilized. Transient transfection uses plasmid DNA, usually two or three plasmids, each with part of the genetic material required to assemble the virus within the cell. An infection process uses recombinant viruses containing vector genetic information to infect the target cell and cause it to create the viral vector. Producer cells are cells into which the genetic material to create the viral vector is stably integrated into the cell.

Early on in therapeutic development, viral vectors are manufactured using transient transfection in adherent cells on 2-dimensional systems such as cell factories or roller bottles. These processes are sufficient for Phase I/II trials which are typically only a few patients. Scale-up to Phase III/commercial-scale, requires a change in manufacturing to achieve the amount of virus required to treat even a small patient population. As gene therapy evolves, it will move towards indications requiring high amounts of virus either because of the tissue targeted (e.g. muscular dystrophy) or the large patient populations (e.g. hemophilia, cystic fibrosis).

Scale is also a consideration as therapies move into Phase III and beyond. Batch size can vary widely depending on the number of patients and the dose required per patient. For some rare diseases, one batch may only produce enough virus for one patient. However, for some of the more prevalent indications, batches must be able to produce enough virus for hundreds of patients. As a result, batch sizes could vary from 10^{12} viral genomes (vg)/batch up to 10^{18} vg/batch or even higher. Cell factory and roller bottle processes quickly run into scaling issues where the number of vessels required leads to very long manufacturing times, high labor costs and high risk due to the number of manipulations required.

The alternatives are to scale-up an adherent cell process in a fixed-bed bioreactor or move to suspension culture using stirred tanks. Suspension culture requires either adapting the adherent cells to suspension, or moving to a different system such as producer cell lines or insect cells. These changes are not trivial and can have an effect on product quality, yield, cost and acceptance by regulatory agencies all of which can have a serious impact on project timelines and ultimately time to market.

Hemophilia A is an example of the manufacturing challenge of a high dose therapy for a large patient population. Hemophilia A affects an estimated 16,000 males in the U.S. and 320,000 worldwide. During clinical trials, patients have been given 6 x 10^{13} vg per kilogram of patient body weight[3]. If this becomes the approved dose and assuming the average patient weighs 35 kg (the target patient population is generally under 12 years of age), then a typical dose is 2.1 x 10^{15} vg/patients. Using a suspension culture, baculovirus-based process to produce AAV and assuming a 25% final yield after downstream processing including empty capsid removal means that the upstream process has to produce 1.68 x 10^{16} vg/patient. Treating 1000 patients/year would require 1.68 x 10^{19} vg coming from the upstream process. Assuming 2 x 10^{14} vg/liter, 42,000 liters of culture material would need to be produced annually.

Single-Use, Fixed-Bed Bioreactors

Fixed-bed bioreactors have emerged as an ideal technology to support the large-scale (10^{16} - 10^{17} vg per batch) production of viral vectors. Primary advantages of employing a fixed-bed bioreactor technology include direct transfer of the 2D reference process which minimizes risk and saves time; larger surface areas to which adherent cells can attach, expand and then be infected or transfected for viral vector production; the ability to achieve higher densities of cells/mL; real-time control of parameters such as pH, dissolved oxygen (DO), temperature, perfusion, and agitation; and low shear-stress on shear-sensitive cells[6].

The iCELLis® bioreactor (Figure 1) is a fully-integrated, high-cell density bioreactor designed to simplify processes by combining the advantages of single-use technologies with the benefits of a fixed-bed system. The compact system – designed for quick implementation and ease-of-use – represents a new generation of single-use bioreactors.

Figure 1
The iCELLis bioreactor

The fixed-bed in the iCELLis bioreactor system is comprised of 0.62 x 2.3 cm macrocarriers, each with a total surface area of ~13.9 cm^2 made from hydrophylized, non-woven, USP class VI compliant polyester (PET) fabric (Figure 2). The macrocarriers are contained in a housing through which media flows from the bottom to the top and remain fixed in place. The iCELLis bioreactor system is available in two formats: the benchtop iCELLis Nano bioreactor system and the large-scale iCELLis 500 bioreactor. The height of the fixed-bed can vary as can the density of carrier packing. Available configurations for the iCELLis Nano and iCELLis 500 bioreactors are shown in Table 3 and their comparison to existing flatware technology is shown in Table 4.

Figure 2

The macrocarriers within the iCELLis bioreactor

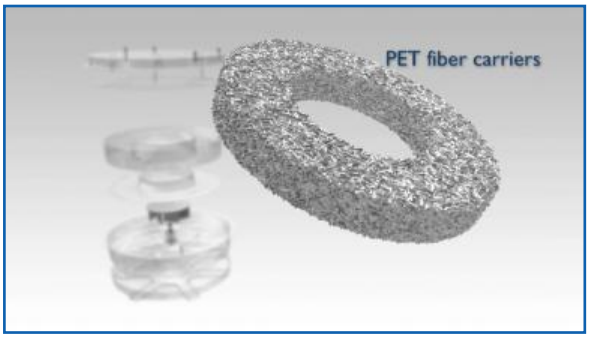


Table 3

Physical specifications of the iCELLis bioreactor

					Surface Area/m^2	
Bioreactor	Diameter (mm)	Height (mm)	Fixed-Bed Volume (L)	Bioreactor Volume (L)	Low Compaction	High Compaction
iCELLis Nano	110	20	0.04	1	0.53	0.8
iCELLis Nano	110	40	0.08	1	1.06	1.6
iCELLis Nano	110	100	0.2	1	2.65	4
iCELLis 500/100	860	20	5	75	66	100
iCELLis 500/200	860	40	10	75	133	200
iCELLis 500/500	860	100	25	75	333	500

Table 4

Comparison between iCELLis bioreactors and other adherent technologies

	iCELLis Nano Bioreactor				iCELLis 500 Bioreactor			
Bed Height/ Compaction	Surface Area (cm^2)	850 cm^2 Roller Bottles	10 Layer Stacks (cs/cf10) (6,360 cm^2/unit)	High Capacity Stacks (18,000 cm^2/unit)	Surface Area (cm^2)	850 cm^2 Roller Bottles	10 Layer Stacks (cs/cf10) (6,360 cm^2/unit)	High Capacity Stacks (18,000 cm^2/unit)
2 cm/C1	5,300	6.2	0.8	0.3	660,000	776	104	37
2 cm/C1.5	8,000	9.4	1.3	0.4	1,000,000	1176	157	56
4 cm/C1	10,600	12.4	1.7	0.6	1,330,000	1565	209	74
4 cm/C1.5	16,000	18.8	2.5	0.9	2,000,000	2353	314	111
10 cm/C1	27,000	31.8	4.3	1.5	3,330,000	3918	524	185
10 cm/C1.5	40,000	47.1	6.3	2.2	5,000,000	5882	786	278

The iCELLis bioreactor technology is being adopted worldwide with almost 200 benchtop iCELLis Nano bioreactor systems in use. Adoption of the commercial-scale iCELLis 500 bioreactor is gaining momentum as candidates move into late-stage development. Material made in the iCELLis 500 bioreactor is in Phase III clinical use under an investigational new drug application (IND) and filings for marketing approval are forthcoming.

A body of scientific publications and presentations has been established over the past few years reporting on experimental results in the iCELLis bioreactor system with the major virus types in use – AAV, adeno-virus, lentivirus and retrovirus. Some examples:

AAV – St Jude Children's Research Hospital, Memphis, TN, has reported results using HEK-293T/17 cells in a two plasmid process to produce an AAV human Factor IX (a blood clotting factor) viral vector[7]. In a 0.53 m^2 iCELLis Nano bioreactor, they achieved an average yield of 6.78 x 10^{11} viral particles (vp)/cm^2 which extrapolates to 3.39 x 10^{17} vp in a 500 m^2 iCELLis 500 bioreactor. St Jude concluded, "The results from this work provide a foundation for continued process development, scale-up, and the manufacturing of AAV gene therapy vectors using iCELLis 500 at St. Jude. Studies are ongoing to evaluate feeding strategies, bed compaction, and bed height in preparation for transfer to the iCELLis 500 for cGMP manufacturing. Studies will also examine the similarities and differences in production of other AAV serotypes and gene products."

Adenovirus – FinVector has reported results using HEK-293 cells for producing an Ad5 viral vector in the iCELLis 500 bioreactor at 100 m^2[8]. They achieved 6.1 x 10^9 vp/cm^2 for a total yield of 6.1 x 10^{15} vp concluding that "iCELLis equipment has provided us an efficient way to manufacture large batches of gene therapy products suitable for large preclinical animal models and potentially beyond to through to Phase III clinical trials and commercial supply." In a subsequent presentation, they have presented data from their consistency runs in the iCELLis 500 system which supported a regulatory filing to allow use of material from the iCELLis 500 bioreactor process to be used in a Phase III clinical trial under an IND which is currently underway.

Lentivirus – MolMed presented their results with the iCELLis Nano bioreactor for lentivirus production at the 2016 Cell Therapy Manufacturing and Gene Therapy Congress in Amsterdam. They reported achieving 5.1 x 10^6 TU per cm^2 in the iCELLis Nano bioreactor and projected that scaling to an iCELLis 500 66 m^2 bioreactor would yield 3.4 x 10^{12} TU/batch. They also note that they are implementing the iCELLis 500 bioreactor at their facility.

Retrovirus – Memorial Sloan Kettering Cancer Center has reported results using stable packaging HEK-293Vec and PG-13 cells comparing the iCELLis Nano bioreactor (2.65 m^2) with their 2D process[9]. Using the iCELLis Nano bioreactor they were able to extend the harvest window from 3 days to 10 days. Overall, the iCELLis Nano bioreactor produced 10.4 x more vector in PG-13 cells and 18.6 x more vector in HEK-293Vec cells versus the 2D reference process. Total yields were 1.93 x 10^{11}/TCIU (tissue culture infectious units) and 2.53 x 10^{12}/TCIU respectively. The vectors produced from the fixed-bed bioreactors passed the release test assays for clinical applications. The authors concluded, "Therefore, a single vector lot derived from the 293Vec is suitable to transduce up to 500 patients cell doses in the context of large clinical trials using chimeric antigen receptors or T-cell receptors. These findings demonstrate for the first time that a robust fixed-bed bioreactor process can be used to produce y retroviral vector stocks scalable up to the commercialization Phase."

The iCELLis bioreactor platform is evolving, both from the design and operation of the system and from process development and optimization through actual use. For instance, a joint Genethon-Pall poster — Development of a Scalable Viral Vector Upstream Process for Gene Therapy: rAAV-8 Production by Transient Transfection of HEK-293 Cells in iCELLis Bioreactor — presented at the 2017 European Society of Animal Cell Technology (ESACT), reported significant improvements in specific productivity (the amount of virus produced per cell) as result of media exchange at transfection and optimization of transfection efficiency (reducing the amount of plasmid DNA required per batch) in serum free conditions. Yields of up to 4 x 10^{13} viral genomes/m^2 were achieved which would equal 2 x 10^{16} in a 500 m^2 iCELLis 500 bioreactor.

Stirred-Tank Bioreactors

Stirred-tank bioreactors are by far the most prevalent bioreactor used for the commercial manufacturing of monoclonal antibodies and recombinant proteins. As a result, the technology is very well characterized and both industry and regulatory authorities are very familiar with their operation. They are the most efficient means of scaling up to large volumes. While they can be used to grow adherent cells with the use of microcarriers, they are best adapted to the growth of suspension cells. Pall's Allegro™ STR single-use bioreactor is available in 50, 200, 1000 and 2000 L scales (Figure 3). The Allegro STR bioreactor is distinguished by its compact footprint owing to its cubical design and its ease of use.

Figure 3
Pall's Allegro STR jacketed 200 L, 1000 L and 2000 L bioreactors

Two studies were undertaken in Pall's Allegro STR bioreactors. In the first study, lentivirus was produced in a 200 L Allegro STR bioreactor using HEK293 cells and titers reached >1 x 10^7 TU/mL or 2 x 10^{12} TU total. The second study was done to produce AAV. In this study, titers reached 2.3 x 10^{14} vg/L for a total of 4.6 x 10^{16} vg in 1 run (Table 5).

Table 5
Viral yields from the Allegro STR bioreactor

Viral Type	Unit	Titer	Total
Lentivirus	Transduction units	1.1 x 10^{10} TU/L	2.2 x 10^{12} TU
AAV	Viral genome	2.3 x 10^{14} vg/L	4.6 x 10^{16} vg

Downstream Processing

At present, there can be large loss of product (> 50%) during downstream processing and this has implications for the scale of the upstream process required to produce the required quantity of virus. Furthermore, the type of technology chosen for upstream can impact both the cost and performance of downstream processing technologies. For example, viral concentrations coming from fixed-bed bioreactor systems can be considerably higher than from suspension bioreactor systems thereby requiring different scales of downstream unit operations. For viruses that require cell lysis, fixed-bed bioreactors can also provide some initial product clarification by trapping some of the cell lysate before putting the product through standard depth filtration.

Conclusion

Gene therapy is a rapidly emerging market for biologic drug development because of its potential to address many currently incurable genetic diseases. Manufacturing of viral material at sufficient cost and scale remains a bottleneck to the further development of the industry.

Manufacturing of material for orphan diseases with small patient populations can be addressed through current technologies although there are efficiencies to be gained from moving to more closed, automated systems. Conditions with larger patient populations or high dose requirements will require development of other approaches to meet market demand. The fixed-bed iCELLis bioreactor is an example of a system that can grow adherent cells to large scale and therefore reduce the risk during process development as cells do not need to be adapted to suspension culture. Titers also remain high and there can be further efficiencies gained in downstream processing due to the high viral concentration produced in the reactor. Suspension producer cell lines which don't require transfection would be the closest comparison to traditional antibody production but at present producer cell lines suffer from low productivity and, in some instances, lack of a regulatory acceptable parent cell line. Infection based processes such as the baculovirus/insect cell system for AAV vector production hold the promise of high level specific productivity, high cell densities and resultant large-scale viral vector production that will be required as this field matures.

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PALL Life Sciences

Corporate Headquarters
Port Washington, NY, USA
+1.800.717.7255 toll free (USA)
+1.516.484.5400 phone
biopharm@pall.com e-mail

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone
LifeSciences.EU@pall.com e-mail

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone
sgcustomerservice@pall.com e-mail

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