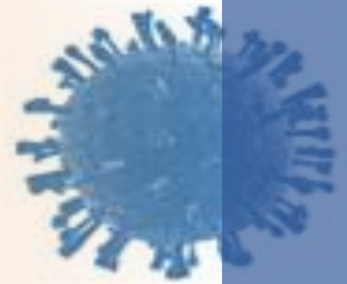

SINGLE-USE TECHNOLOGIES ENABLE MANUFACTURERS TO MEET PIVOTAL CHALLENGES OF VIRAL VECTOR MANUFACTURING

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Introduction

The compelling benefits of single-use technologies (SUT) have been discussed and demonstrated in bioprocessing for many years. Some examples explaining the popularity of SUT include low capital costs, fast change-over time between batches, and closed systems that reduce the chance of contamination and provide consistent batch-to-batch results. Each emerging biotherapeutic class comes with potentially unique process, regulatory, and commercial requirements that must be considered as the challenges and benefits of SUT are not a given. A thoughtful review of the process requirements needs to be considered. This paper will provide a discussion of the Viral Vector production process and highlight the difficulties and solutions in this growing segment of biopharmaceutical manufacturing. Finally, we shed some light on future challenges and what to watch for as these technologies mature and scale. This will enable you to make educated decisions for your process to aid you in safely and rapidly providing life-saving therapies to patients.

Background

Gene therapies have gained significant importance in recent years due to their therapeutic potential in treating serious diseases for which standard-of-care treatments are ineffective. Many of these products have durable therapeutic effects and can potentially be curative, eliminating the need for chronic administration of drugs. The approvals of Kymriah®, Yescarta®, Luxturna®, Tecartus®, Strimvelis®, Zolgensma®, Zynteglo™, Imlygic®, Breyanzi®, and the most recent, Abecma®, by the FDA or EMA over the past several years have boosted excitement, investment, and research in gene therapies globally. Cell and gene therapy (CGT) is a science that is becoming an industry, a “new era in medicine.” One of the main focuses of this technique is the optimization of delivery Viral Vector vehicles that are mostly nanostructures, or viruses. The viruses are more often utilized due to their efficient infection of cells and inserting their genetic material.

According to the Alliance for Regenerative Medicine first half of 2021 report [Ref. 1], there are 1,195 companies worldwide running cell and gene therapy as well as tissue engineering programs. There are 2,611 clinical trials in various phases of cell and gene therapy products. Of these, 1,299 programs are industry-sponsored and 1,312 are sponsored by academic, government or other institutions.

Gene therapy involves the transfer of genetic material (DNA or RNA) into the cells of a patient to treat a specific disease. The genetic material transferred to the target cell is known as a transgene. The expressed transgene mediates the therapeutic effect. Transgenes are transferred to target cells by vectors. The vector is the carrier, and the transgene is its payload.

This gene transfer step may occur *in vivo* or *ex vivo*, i.e., either inside or outside the patient’s body. In *in vivo* gene therapy, the vector containing the transgene is administered directly to the patient. The vector must first reach the target cells, then deliver the transgene payload to them. In *ex vivo* gene therapy, the target cells are removed from the patient, the vector is used to deliver the transgene, and the gene-modified cells are returned to the patient’s body. (See Figure 1.)

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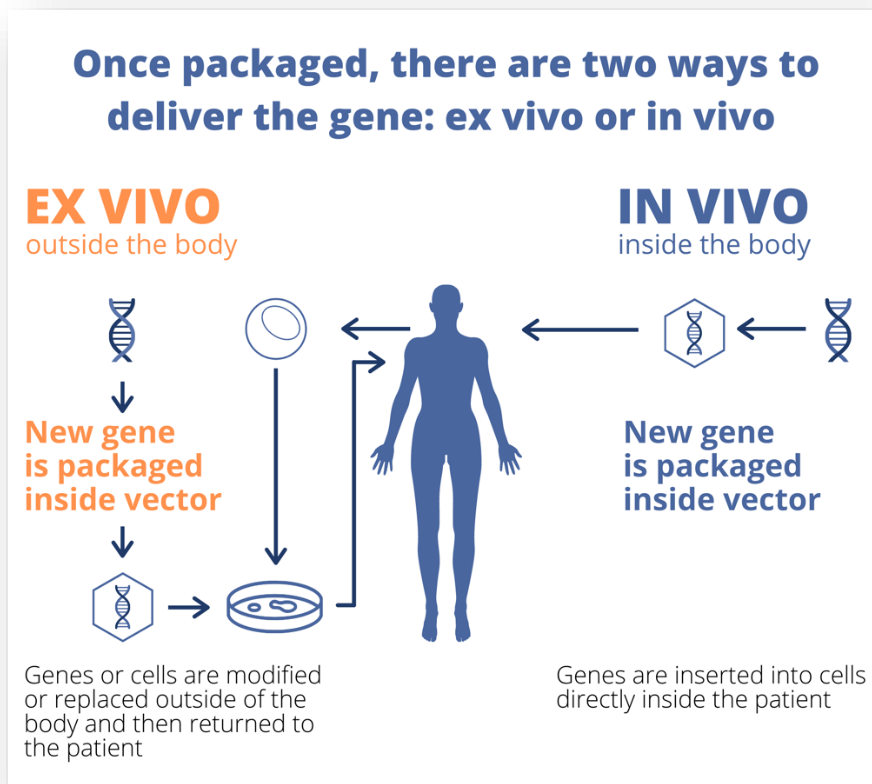
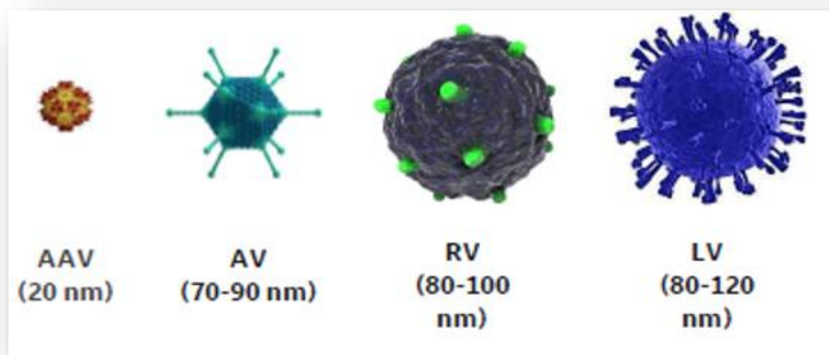
Figure 1. Illustration of In vivo and Ex vivo Gene Delivery



Many types of vectors, including viruses, plasmids, and transposons, may be used in gene therapy. Viral Vectors are the most commonly used, due to the inherent ability of viruses to deliver genetic material to cells. A variety of viruses can be used as vectors, but the most frequent are adenovirus (AV) (27.1%), retrovirus (RV) (25.4%), lentivirus (LV) (15.7%), adeno-associated virus (AAV) (12.4%), and vaccinia virus (VV) (9.3%). AAV is often preferred for *in vivo* gene therapy, and LV are frequently used for *ex vivo* gene therapy [Figure 2] [Ref 2]. The purpose of this paper is to look only at AAV.

Figure 2. Common Viral Vectors and Relative Particle Size



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Manufacturing and Processing of Viral Vectors

Manufacturing technologies for production of clinical and commercial-grade vectors have significantly improved over the last few years.

AAV vectors can be produced using either adherent or suspension-based cell culture platforms. However, there is a desire to move towards a suspension-based approach for ease of scale-up. The two-leading suspension-based scalable production platforms for AAV vectors are transient transfection of suspension adapted HEK293 cells and the infection of Sf9 insect cells using baculovirus expression vector system (BEVS).

Initial small-scale production of Viral Vectors primarily involves the use of adherent cells grown in rigid laminar culture using multilayer culture vessels, CellSTACK® or Cell Factory™. However, this approach has limited scalability, a major challenge when large amounts of Viral Vector are required. Fixed bed, disposable bioreactors are one option that can provide scale-up for adherent cell lines up to 500 m^2 area. However, large-scale production of common Viral Vectors using suspension cell culture in stirred tank bioreactors has been successfully demonstrated today. Viral Vectors produced using these methods are being evaluated in human clinical trials and some are in late clinical stages.

The manufacturing processes for both the mammalian and the insect cell culture-derived Viral Vectors are described in Figure 3. These processes are generally divided into upstream processing, where the Viral Vectors are produced in bioreactors by the relevant cell culture system, and downstream processing, where the harvested vectors are purified and formulated into either bulk drug substance or drug product for AAV, or as a process intermediate for LV vectors in most cases to be used in cellular immunotherapy. The process flow diagram in Figure 3 provides a general overview of the sequence of major unit operations for AAV Viral Vector manufacturing (VVM).

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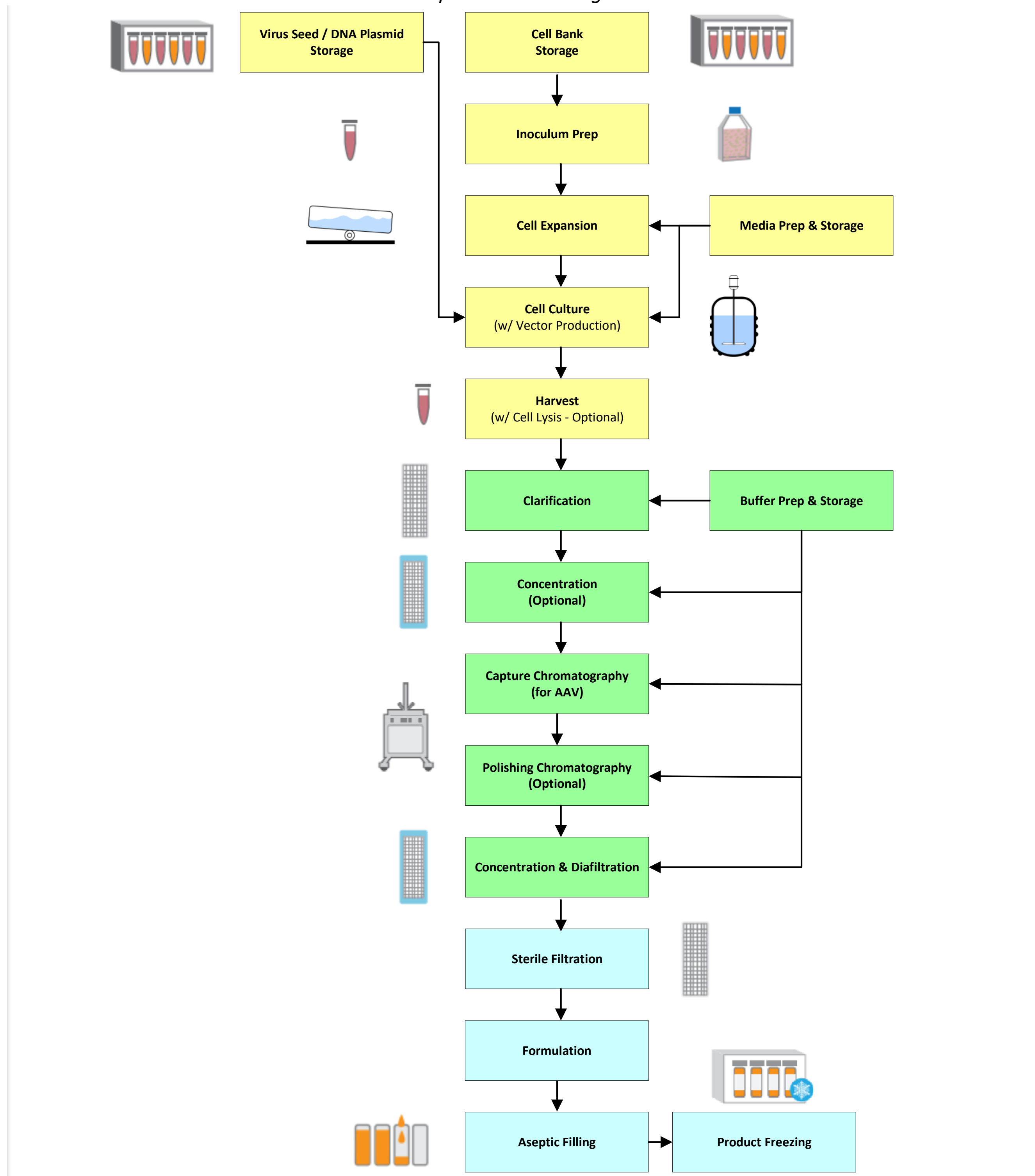
Figure 3. Typical Process Flow for Viral Vector Manufacturing

(yellow=upstream, green=downstream, blue=fill/finish)

Note: the steps shown in the Upstream Processing section above of virus seed storage for insect cell lines and infection of the production cell culture or Plasmid DNA storage for mammalian cell lines and transfection of the production cell culture are not required when using Producer cell lines.



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Upstream Production

The Viral Vector manufacturing processes begin with thawed cells from a validated working cell bank (WCB) that are expanded through a series of seeding bioreactors to a terminal production bioreactor. HEK 293 cells are transiently transfected with plasmid DNAs, while the Sf9 cells are infected with baculoviruses.

In the case of HEK 293 cell-based mammalian cell culture, a master and working cell bank is required for transient transfection along with large amounts of plasmid DNA. Chemical agents such as polyethyleneimine (PEI) are used to mediate transfection. The mixing must be carefully controlled. It is critical that you form a complex between the DNA and transfection reagent, otherwise the DNA will not be absorbed by the cell.

In contrast to this transfection process, the Sf9 cell infection does not require carefully controlled mixing. A small quantity of baculoviruses containing the necessary genes to generate the recombinant adreno-associated virus (rAAV) vector is introduced into the production bioreactor to infect the Sf9 cell culture. This system, however, requires significant upfront time investment of typically several months to clone and bank each of the two recombinant baculoviruses (rBV): one containing AAV helper genes, while the other containing genome of the rAAV. Master and working banks must be made for these baculoviruses seed stock as well as of the Sf9 cells themselves. [Ref 3]

Currently, clinical grade Viral Vectors are manufactured by transient transfection of plasmids encoding vector components into a commonly used cell line. Some manufacturing processes involve infection with virus(es) and transfection with a smaller number of plasmids. This manufacturing process entails high costs and long timelines, with challenges of sourcing the right plasmid DNA with large quantity and scaling up. Recent research focus has shed light on alternative stable producer cell lines, with all vector-encoding nucleic acid integrated into the host cell genome [Figure 3]. Adenovirus is often used to deliver the helper genes to the producer line.

Stable producer cell lines have the potential to achieve up to 1,000L scale of high consistency output, and offers high consistency options for continuous manufacturing and the ability to use chemically defined media without the need for plasmids. The drawback of this technology might be the increased timeline for cell line development. However, once a stable producer cell line is established for one product, especially with a large population of patients, large scale consistent manufacturing of Viral Vectors would drive down manufacturing costs.

After about 3-4 days post-infection using either type of cell line, the Viral Vectors are harvested (with or without cell lysis) from the bioreactor and are ready to be sent to downstream processing for purification to remove product and process-based impurities.

Downstream Purification

The AAV downstream process usually begins with clarification steps to remove cell debris because of lysis, conventional charged depth filters containing diatomaceous earth are typically used for this step with good yields. In the case of AAVs, some serotypes are expressed intracellularly, thus requiring cell lysis, however, some new serotypes are excreted and do not require cell lysis necessarily. Endonuclease treatment is usually necessary to remove/reduce the level of host cell DNA and plasmid DNA. This is accomplished via Benzonase® or DENARASE® treatment.

There are many challenges during purification of Viral Vectors, and many processes lose a significant amount of virus yield during downstream processing. A primary tangential flow filtration (TFF) step usually follows and pre-concentrates and diafilters the product for further chromatographic processing. Affinity chromatography is typically used on AAV vectors, although there are only a small number of different resins that could be used. A combination of Hydrophobic Interaction Chromatography (HIC) and strong Cation Exchange Chromatography (CEX)- based purification with ready-to-use, single-piece monoliths have shown promise as alternate low-cost approaches in AAV purification. The recovery across these chromatography steps could be low, necessitating significant optimization of process step. A polishing

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chromatography step is typically employed for AAVs to remove empty capsids from full capsid using AEX monoliths, membranes, or resins.

Size-based virus purification strategies such as density-gradient ultracentrifugation, two-phase extraction, and size exclusion chromatography have been used over the years prior to final formulation. However, some of these approaches are not easily scalable and the observed trend is to avoid such steps. It is common to use one additional TFF step for further concentration of the Viral Vectors before formulation and final filtration for bulk drug substance storage. However, there is hesitation in deviating from a 0.2 µm sterilizing grade filter due to parts of the downstream process such as chromatography not being fully closed. Significant efforts are usually spent in optimizing the drug substance sterile filtration step to achieve acceptable yields. It is a standard practice to use 0.2 µm sterilizing grade filters for final filtration of AAVs.

The production bioreactor scales for manufacturing these Viral Vectors typically tend to range from about 10 L through 1,000 – 2,000 L. Closed processing is highly desired by manufacturers and the use of various single-use technologies including seeding and production bioreactors, mixers and storage, aseptic connections/disconnections and transfer sets for fluid transfer and handling is quite prevalent.

Product Safety and Operator Safety

Due to the potential of infectivity, whether at small- or large-scale operations, Viral Vector manufacturing requires biocontainment for product safety and as well as operator safety. Biocontainment levels (i.e., biosafety levels or BSL) for Viral Vectors discussed in this paper have been established by the CDC and NIH where Adenovirus (AdV), herpes simplex virus (HSV), LV, and RV must be handled as BSL-2. AAV and BEVS can be handled as a BSL-1; however, these Viral Vectors are frequently handled as BSL-2 and are dependent upon the laws of the country of manufacture. Higher biocontainment (BSL-3 and BSL-4) is not foreseen based on the current Viral Vectors used in Viral Vector manufacturing.

Requirements for biocontainment in Viral Vector manufacturing are significantly different when compared to mAb commercial manufacturing where biocontainment is not typically required (good large-scale practice, or GLSP) may involve the lesser BSL-1, which only offers product protection from the operator and the processing environment. Regulations and recommendations for biosafety practices, equipment, and facility safeguards are documented in the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th edition as prepared by the USDHHS, CDC, and NIH [Ref. 4].

Processing in Closed Systems

Single-use technology (SUT) as a standalone bag, filter, or tubing, or as an integral assembly can be sourced as an engineered, integrity-tested, and closed system, which is an enabling technology in providing product safety and operator safety in Viral Vector manufacturing. Closed processing can be used in the unit operations of Viral Vector manufacturing including cell expansion, cell culture, transfection prep through harvest, purification, formulation, and finally in drug substance (DS) and drug product (DP) filling. Closed (or functionally closed) processing [Ref. 5] is essential to prevent operators from exposure through aerosols, leaks, or splashing.

Though not the focus of this paper, biosafety practices such as gowning, handwashing stations, PPE, equipment (e.g., biosafety cabinets or isolators), and facility safeguards such as architectural (e.g., airlocks), HVAC (e.g., HEPA filtration), and plumbing (e.g., dedicated biowaste drains) are simplified due to the closed and single-use nature of SUT.

SUT assemblies are tested to validate that the system functions as designed in providing a closed system. Tests may include mechanical and microbial ingress challenges. Mechanical tests may include burst testing, leak testing, and freeze-thaw testing (down to -80°C). Microbial ingress challenge tests (e.g., aerosol exposure and immersion exposure) are performed to demonstrate the ability of a connection to make and maintain a closed connection, which prevents

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leaks and potential operator exposure. (For additional microbial ingress challenge test guidance, reference the ASTM E3251-20 *Standard Test Method for Microbial Ingress Testing on Single-Use Systems*.

SUT assemblies can also be sourced already integrity tested by the manufacturer. However, due to the potential of damage during packaging, transit, storage, unpackaging, and connection to the process train, a pre-use leak test in the manufacturing area can be performed to confirm an integral system is in place to mitigate risk of operator exposure to infectious Viral Vectors. For storage of solutions, bags are manufactured by multiple films that provide strength, flexibility, and low gas permeability. (For additional integrity assurance guidelines, reference the BPSA paper *Design, Control, and Monitoring of Single-Use Systems for Integrity Assurance* [Ref. 6] and ASTM E3244-20 *Standard Practice for Integrity Assurance and Testing of Single-Use Systems*.)

SUT also provides the capability for quality control sampling using a closed system. Sample containers can be supplied as an integral system or can be adjoined by thermal welding or using an aseptic connector. Removal of sample containers can also be accomplished in a closed manner by thermal sealing, pinch and cut tools, or aseptic disconnectors. Where aliquoting of samples is necessary, this can also be done with a closed system (bags, bottles, vials) without the need for a biosafety cabinet or isolator. However, limitations associated to frequency of extraction of small volume samples (e.g., multiple samples of 1 mL in size) are better obtained by aliquoting in a biosafety cabinet or isolator, which could produce inconsistent results.

For through-wall transfers of solutions across a BSL boundary such as media transfer to a bioreactor, a single-use tubing pass through assembly supplied as a closed system can be utilized to isolate a non-BSL area from a BSL area. Throughout Viral Vector manufacturing unit operations and at the end of processing, SUT provides the means to safely disconnect containers, filters, tubing, etc. and prevent potential exposure of operators. This can be accomplished in the same manner sample containers are removed from a manifold by thermal sealing, pinch and cut tools, or aseptic disconnectors that include automatic shut-off valves to close off the flow path. These methods prevent exposure due to aerosols, leaks, or splashing.

Where it is not possible to break down equipment prior to changeover in a closed manner, it is recommended to inactivate Viral Vectors prior to opening the system. Common chemical agents for inactivation of Viral Vectors in 100% SUT systems include bleach or sodium hydroxide. An example of where a process is open or functionally closed is clarification and filtration steps where depth filters may be used. In this instance, multiple disposable filter membranes must be removed post-use. For inactivation of the Viral Vector, the chemical agent is flushed through the system. Following inactivation, a water source (e.g., water for injection) is flushed through the system to purge the chemical agent which is caustic and could present an exposure hazard to operators.

In hybrid systems where multi-use (e.g., stainless-steel) equipment is connected to single-use equipment (e.g., a bioreactor connected to media/supplement bags), the stainless-steel equipment must first be inactivated of Viral Vectors prior to removal of the single-use equipment and subsequent cleaning. SUT provides the means to isolate the multi-use equipment and single-use equipment with the utilization of steam-in-place (SIP) connectors. Other examples of multi-use equipment or single-use equipment where Viral Vectors must be inactivated prior to breakdown and changeover, post-use handling or cleaning is chromatography and tangential flow systems.

Following manufacturing, per CDC/NIH guidelines and local municipal codes, all potentially infectious (BSL-1 and BSL-2) liquid waste must be inactivated prior to discharge. With respect to single-use equipment, it is good practice to inactivate solid waste prior to exiting the manufacturing facility. Post-use handling and inactivation may be supported by in-house personnel and equipment or may be outsourced to a regulated hazardous waste hauler (i.e., "red bag" waste). (For additional information regarding post-use handling of SUT, reference the BPSA paper: *The Green Imperative: Life-Cycle Assessment and Sustainability for Single-Use Technologies in the Pharmaceutical Industry.* [Ref. 7].)

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Viral Vector Manufacturing Challenges

Viral Vectors are challenging to make reliably and robustly. Often manufacturers of gene therapies have to decide whether to manufacture in house or use a contract development manufacturing organization (CDMO), and in some cases, a parallel path will be chosen to use both in house and CDMO for risk mitigation purposes.

Some of the technical, economic, and safety challenges of Viral Vector production include:

- Ensuring consistency, quality, and performance of the processes
- In-line analytical capabilities to measure cell densities and metabolites (process analytical technologies (PAT)
- Rapid scale-up Viral Vector manufacturing capacity
- Obtaining cGMP-compliant raw materials
- High cost of goods
- Lower productivity (low percentage of full capsids)
- Slower growth rate of cells
- Achieving high cell densities
- Changes in cell expression patterns
- Analytical assays to measure product quality
- Lack of integrated, automated, and fully closed system
- Downstream purification challenges
- Stability of the virus (lentiviruses and retroviruses are fragile)
- Sterile filtration – losses up to 50%
- Exposure and effect of impurities and extractables/leachables (E&L) in the process
- Viral Vector safety concerns – BSL requirements
- Technology transfer issues when scaling up or out

Single-use technologies help address some of these challenges such as providing ease of scale-up from early clinical through commercial phases and providing connectivity in an aseptic manner within and between certain unit operations. We will focus on some of these benefits in the next section.

Single-Use Technology (SUT) Enables Cost-Effective and Scalable Manufacturing

Since the introduction of SUT in the late 1980s, biopharmaceutical manufacturers have aggressively adopted it in both clinical and commercial manufacturing.

The main drivers for adoption:

- Flexible production design – higher throughput
- Smaller equipment footprint
- Pre-sterilized, closed systems that minimize the likelihood of microbial contamination
- Lower labor and production costs
- Lower capital costs
- Reduction or elimination of sterilization and cleaning validation.

The cell and gene therapy market evolved from research hospitals and small start-up companies treating one patient at a time in small batches, which is not commercially scalable. Since bioprocessing is part of the expansion of cells required to create the drug product (DP), the biopharmaceutical industry has contributed to the development of the

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bioprocessing of engineered cells. SUT, manufactured from single-use components made from polymeric materials, sterilized, and ready to use are commercially available today. They are widely used for various purposes, ranging from classical biomanufacturing of proteins, monoclonal antibodies, vaccines in large scale, and are now being adopted for applications in the cell and gene therapy area and Viral Vector manufacturing [Ref. 8]. One recent discussion with a prominent CDMO that manufactures Viral Vector stated that almost all unit operations other than chromatography and TFF unit operations are single-use.

Single-Use Technology Shifts More Responsibility to the Supply Base

Along with the tremendous benefits of SUT, there have been questions regarding E&L, particulates, and durability. There has been continuous improvement in increasing the robustness and gaining better understanding of potential E&L and particulates [Ref 9, 10].

Reliability of data from suppliers is of high importance. The move from a stainless-steel process to single-use has put a lot of demand on the suppliers. For example, it is commonly expected that suppliers provide qualified extractables data for their SUT and the pharmaceutical industry is using these data for their risk assessment and the planning of their leachables studies. In consequence, this results in a shift of responsibility away from the end user of a final assembly to the SUT manufacturer, at least for providing useful extractables data. The SUT industry is monitoring and adopting the lessons learned from recombinant proteins manufacturing for Viral Vector manufacturing for gene therapies. Subject matter experts in SUT implementation from the mAb manufacturing companies have been employed by CGT companies and are adopting best practices in the Chemistry, Manufacturing and Control (CMC) for CGT processing. The FDA Center for Biologics Evaluation and Research (CBER) regulates CGT products in the US. Several CGT Guidance's for Industry have been published over the last few years for the producers to manage the regulatory approval process [Ref. 11].

Although the pharma laws in various countries demand that contact materials should not "... *alter a drug product, remove something from or add something into a drug product* ...", there is only one national guideline (USP 665) demanding explicitly E&L studies for SUT (e.g., in the frame of a process qualification). Other than having one official guideline on E&L for SUT, there are E&L papers related to SUT, which focus on analytical aspects, and the discussion, on suitable "simulation" conditions for a standardized extractables protocol [Ref. 9, 12, 13, 14].

Appropriate methods for the *assessment* of E&L for SUT do not exist currently. The common approach is to extrapolate the SUT extractables data to process equipment-related leachables (PERLs) and leachables. Such extrapolation is identical to the assessment of leachables from the container closure systems (CCS). However, a CCS is a closed system used under static conditions, where leachables can *accumulate* over years of storage. In contrast, SUTs are always used under dynamic conditions and often only for short durations. Leachables released from a CCS become a drug impurity with the risk of direct administration into patients. In contrast, PERLs released from a SUT can be efficiently removed from process liquids by certain unit operations in downstream processing such as chromatography and TFF, and consequently, are unlikely to become a drug product impurity [Ref. 15, 16, 17, 18, 19]. This is applicable for Viral Vectors manufacturing as well since it contains similar downstream unit operations as mAbs and recombinant proteins manufacturing. Furthermore, certain Viral Vectors such as LVs in most cases are drug substance intermediates for *ex vivo* cell therapies, and thus are further removed from DP.

A better understanding of the fate of PERLs in the different stages of a process is required irrespective of the type and modality of the biologic DP. [Ref. 20, 21]. Understanding the fate of PERLs in the dynamic environment of a production allows a reasonable estimation of the *potential exposure* of PERLs and leachables to host cells, Viral Vectors, and finally to patients; and only the knowledge of exposure values facilitates a comparison with effect concentrations, thresholds, and limits to establish safety margins. [Ref. 22].

Today, extractables data is available for a wide variety of SUT. Some of this data has been obtained with standardized extractables protocols, which are agreed upon by the users in the biopharma industry. Moreover, the suppliers provide

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methodologies to scale and combine extractables data and in silico methods are under development to calculate the PERL load in individual process steps or through the entire downstream process [Ref. 23, 24, 25].

Future Challenges for Single-Use Technologies in Viral Vector Manufacturing

Single-use technology continues to shape the progress and advancement of Viral Vector manufacturing, but future challenges remain for both suppliers and developers. Several key themes that will continue to emerge in the coming years are scalability and the need for standardization to drive compatibility and interchangeability of technology. Stronger collaboration and partnerships between suppliers and developers will drive more robust and reliable products to meet the intended applications. There continues to be a process step that remains open due to the regulatory uncertainty and the robustness of the process necessitating the use of BSC to be included in the manufacturing step. There is a need to design new closed-system solutions and automation to remove variability and lead to a more robust and consistent process.

Scalability is an important factor in developing cell and gene therapies and particularly the Viral Vector manufacturing processes. There continues to be an unmet need regarding commercially available components in building Viral Vector manufacturing processes. Many of the current cGMP commercially available single-use products were designed for large scale cGMP mAb processes. Viral Vector manufacturing starts in small scale, where cGMP ready single use products are not available; hence, laboratory components are used. The use of laboratory equipment which in most cases are used for R&D grade must now be validated for cGMP use. When development expands into cGMP scale operations, small scale, manual process of laboratory grade product will not meet future demands. A second round of validation process is required for larger volume process in cGMP operation.

Standardization of sizes for tubing, connectors, containers, and broader ranges of sizes for scaling the Viral Vector manufacturing process will be required as the industry continues to mature. Compatibility of components from multiple vendors is very complicated and often leads to higher complexity and risk when designing processes, understanding materials of construction, and conducting proper risk assessments will help mitigate this complication.

Change management: Collaboration between different suppliers with respect to extractables and leachables of SUT components data will drive flexibility for end users. Making this data more readily available will allow use of SUT components from multiple vendors interchangeably or the same vendor interchangeably. Sole source components can increase supply chain risk in producing life-saving drugs. Changing components in the process can be very costly if equivalency studies are required. The recently developed IT tools can help to evaluate the influence of component or material changes [Ref. 25].

Robustness and reliability data publications and case histories on SUT technology will advance the design of systems by ensuring that the components used will meet the intended application. Using components that are underspecified and fail integrity can cause major delays in DS or DP campaigns. Better availability of technical data on commercial components and a common repository of this data would move the industry in the right direction. [Ref. 6]

Quality: The manufacturing method for SUT systems and fluid pathways, which include components, tubing, bags, filters, etc., is a very manual process and has the potential to lead to high labor costs and potential variability in production. Introducing automation into the manufacturing process of making these fluid pathways will increase manufacturing throughput, reduce labor costs, and drive additional capacity needed to meet future demand for single-use products and systems.

Complete closure of the manufacturing process will allow developers to utilize a lower classification manufacturing environment. This can lead to a lower capital cost for a new production facility. Processes can now be designed to operate in ISO Class 7/8 environments, thereby lowering both capital and operating costs for the manufacturer.

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Conclusions

Viral Vector manufacturing is still in an early stage of development. This emerging segment in biopharmaceutical manufacturing is benefiting from the tremendous experience gained from the production of mAbs and vaccines especially now for quicker and large-scale processes with the outbreak of Covid 19. Viral Vector manufacturing has almost completely adopted SUT at the commercial scale. Many challenges exist in the scale-up of Viral Vector manufacturing process components, and the SUT supply base is responding to these needs. The future looks bright for patients who will require these lifesaving therapies. The supply base is collaboratively working together to meet technical and logistics challenges to create robust and reliable manufacturing.

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Glossary

Adeno-associated Virus (AAV)	Small viruses that infect humans and some other primate species. Not currently known to cause disease.
API	Active pharmaceutical ingredient.
AV	Adenovirus - Virus that replicates in the nucleus of the host cell.
Benzonase®	An endonuclease that cleaves all kinds of DNA and RNA into small fragments to aid in purification.
BEVS	Baculovirus expression vector system.
Biocontainment Levels	See biosafety level (BSL) definition.
BSL	A biosafety level (BSL), or pathogen/protection level, is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).
Closed System	A process system with equipment designed and operated such that the product is not exposed to the room environment. Materials may be introduced to a closed system, but the addition must be done in such a way to avoid exposure of the product to the room environment (e.g., by 0.2 µm filtration).
Container Closure System (CCS)	The sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection to the drug product. (www.fda.gov)
DENERASE®	An endonuclease that cleaves all kinds of DNA and RNA into small fragments to aid in purification.
DNA	Deoxyribonucleic acid is a molecule that carries genetic instructions.
Downstream	Clarifying and purifying biologic to produce API.
Drug Product	Formulated drug substance with excipients.
Drug Substance	Active pharmaceutical ingredient.
Extractable	Organic and inorganic chemical entities that are released from a pharmaceutical packaging/delivery system, packaging component, or packaging material of construction and into an extraction solvent under laboratory conditions. (www.usp.org)
HEK293	Cell line derived from human embryonic kidney.
HSV	Herpes simplex virus.

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ISO Class	ISO class refers to the number and size particles permitted per volume of air.
Leachable	Foreign organic and inorganic chemical entities that are present in a packaged drug product because they have leached into the packaged drug product from a packaging/delivery system, packaging component, or packaging material of construction under normal conditions of storage and use or during accelerated drug product stability studies. (www.usp.org)
LV	Lentivirus is a genus of retroviruses used as a delivery mechanism in CAR-T therapies.
Monoclonal Antibody	Laboratory-made antibodies that can be used as a therapeutic.
PEI	Polyethyleneimine
PERLs	Process equipment-related leachables. (www.usp.org)
QC	Quality control
RNA	Ribonucleic acid (RNA) is a molecule similar to DNA that transfers information from the genome into proteins by translation.
RV	Retrovirus - virus that inserts a copy of its RNA into the DNA of a host cell for replication.
Sf9	Insect-derived cell line expression system used for high-level protein expression.
SUT/SUS	Single-use technologies/single-use system.
TFF	Tangential flow filtration.
Upstream	General process of expanding and growing cells to produce a biologic and harvesting.
Viral Vectors	Vehicles for gene delivery usually derived from parental wild type viruses whose viral genes, which are necessary for replication and virulence, have been substituted with the genes intended for cell manipulation. These can be used to create gene-modified cell therapies such as chimeric antigen receptor cell therapy or directly used for in vivo gene therapy.
Virus-Vector Manufacturing (VVM)	Large scale manufacture of Viral Vectors for therapeutic use.
WCB	Working cell bank
WFI	Water for injection

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About BPSA

The Bio-Process Systems Alliance (BPSA) was formed in 2005 as an industry-led corporate member trade association dedicated to encouraging and accelerating the adoption of single-use manufacturing technologies used in the production of biopharmaceuticals and vaccines. BPSA facilitates education, sharing of best practices, development of consensus guides, and business-to-business networking opportunities among its member company employees.

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