FALL 2013 • Volume 12 / Issue 3 • ISSN 1538-8786

BioProcessing JOURNAL

Trends & Developments in BioProcess Technology

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BioProcessing JOURNAL
TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

Expediting Clinical Batch Production of Viral-Vectored Vaccines and Gene Therapy Products

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Introduction

There are approximately 400 vaccines in commercial manufacture targeted at 24 infectious diseases. Of these vaccines, about 300 are viral-based. It is estimated that 1400 vaccines are in development, half of which are viral-based (~640 viral vaccines, ~200 viral vectors, ~60 virus-like particles). Another 240 gene therapy products are in development that utilize the same viral-based technology.[1]

A live vector vaccine is one that uses a weakened or harmless microorganism to transport antigenic viral pieces into a cell in order to stimulate an immune response. Vectored vaccines show promise in reliably inducing potent cell-mediated immunity, which is essential for complex diseases such as AIDS, malaria, and cancer.

Viruses and bacteria can both be used as vectors. Common bacterial vectors are attenuated *Salmonella typhi* (Ty21a) and *Lactobacillus acidophilus*. Bacterial vectors are only used as oral vaccines to offer mucosal immunity. Common viral vectors are adenovirus, canarypox, lentivirus, and alphavirus. Vector viruses infect cells with great specificity—they then transfect their own DNA into the host cell. This DNA uses the infected cell to produce new viral particles.

The Japanese encephalitis (JE) vaccine IMOJEV® (Sanofi Pasteur), using an attenuated yellow fever virus (YFV-17D) encoding the JE preM-env protein, is the first human viral-vectored vaccine on the market. There are also several viral vector vaccines currently in use for veterinary diseases. The viral vectors for approved vaccines involve the use of either adenovirus, fowlpox virus, attenuated yellow fever (YFV-17D), or vaccinia, all of which are relevant as potential viral vectors for human vaccines. Common viral vectors are listed in Table 1.

TABLE 1. Viral vectors in use for vaccine and gene therapy product development.

Vector	Size	Produced In	Advantages	Disadvantages
Adenovirus (Ad5)	70–90 nm	HEK293, PER.C6	• Easily produced in high titers • Large transgene capacity • Transduces resting and dividing cells	• Immunogenic • Pre-existing immunity to certain serotypes • CAR-dependant transduction • High doses needed to elicit immunity
Canarypox (ALVAC), Modified Vaccinia Virus Ankara (MVA)	200–300 nm	Chicken Embryo Fibroblasts	• Abortive infection • Accommodates multiple gene inserts • No prior immunity • Excellent immunogenicity	• Safety concerns in immune-compromised patients
Alphavirus (Sindbis, Semliki Forest, etc.)	70 nm	Chicken Embryo Fibroblasts	• Efficient transgene expression • No integration • Does not elicit anti-vector immunity • Targets dendritic cells	• Low transgene capacity • Short-term expression • Cytotoxic • Difficult to produce
Lentiviral Vector	80–100 nm	HEK293	• Low immunogenicity • Stable integration of resting cells	• Risk of insertional mutagenesis • Risk of recombinant pathogenic vector
Attenuated Yellow Fever Virus (YFV-17D)	50–90 nm	Embryonated Chicken Egg, Vero	• Easily manufactured • Non-pathogenic • Well-known safety profile	• Risk of insertional mutagenesis • Risk of recombinant pathogenic vector

Vectored vaccines elicit strong humoral and cell-mediated immune responses resulting in immunological memory. Vectors can be targeted by viral tropisms for particular cells (*e.g.*, intestines, brain, etc.) inducing the desired immunity. Vectored vaccines can encode for several antigens from different pathogens, introducing the possibility of a single vaccine for several diseases, which are relatively inexpensive.

However, since the live virus being used is an attenuated form of an infectious pathogen, there is always a risk of reversion to virulence. Some of the vectors under consideration, such as adenovirus, have the capability of transforming cells to a cancerous phenotype. Many large vectors (canarypox and vaccinia) can't be sterile filtered since they are too large to pass through a 0.22 µm membrane. Therefore, completely closed or aseptic manufacturing practices are followed when processing large vectors.

This article will focus on the manufacturing process of viral-vectored vaccines and introduce a method using adenoviruses that accelerates production of virus-vectored vaccines and gene therapy products.

Adenoviruses are non-enveloped, double-stranded DNA viruses that range in size from 70–90 nm. They are very efficient at transducing target cells and can be produced at high titers (>10^{11}/mL). Wild-type adenoviruses are pathogens that cause respiratory diseases and the common cold. They are also capable of infecting neurons, damaging cells, and invoking strong immune responses. The adenoviruses used as vectors are genetically modified and specifically designed not to cause any disease. They are generally regarded as safe.

The manufacturing process for an adenovirus vector is straightforward and fairly templated. A general outline of the process is presented in Figure 1. The typical pilot-scale bioreactor size used in adenovirus production for clinical trial material is small (20–50 L). There tends to be 4–5 times the volume reduction after the initial ultrafiltration step followed by further volume reduction up to 20–30 times after chromatography and final sterile filtration. Full-scale bioreactor process volumes may range from 100–200 L and overall process yields are typically 60–70%.

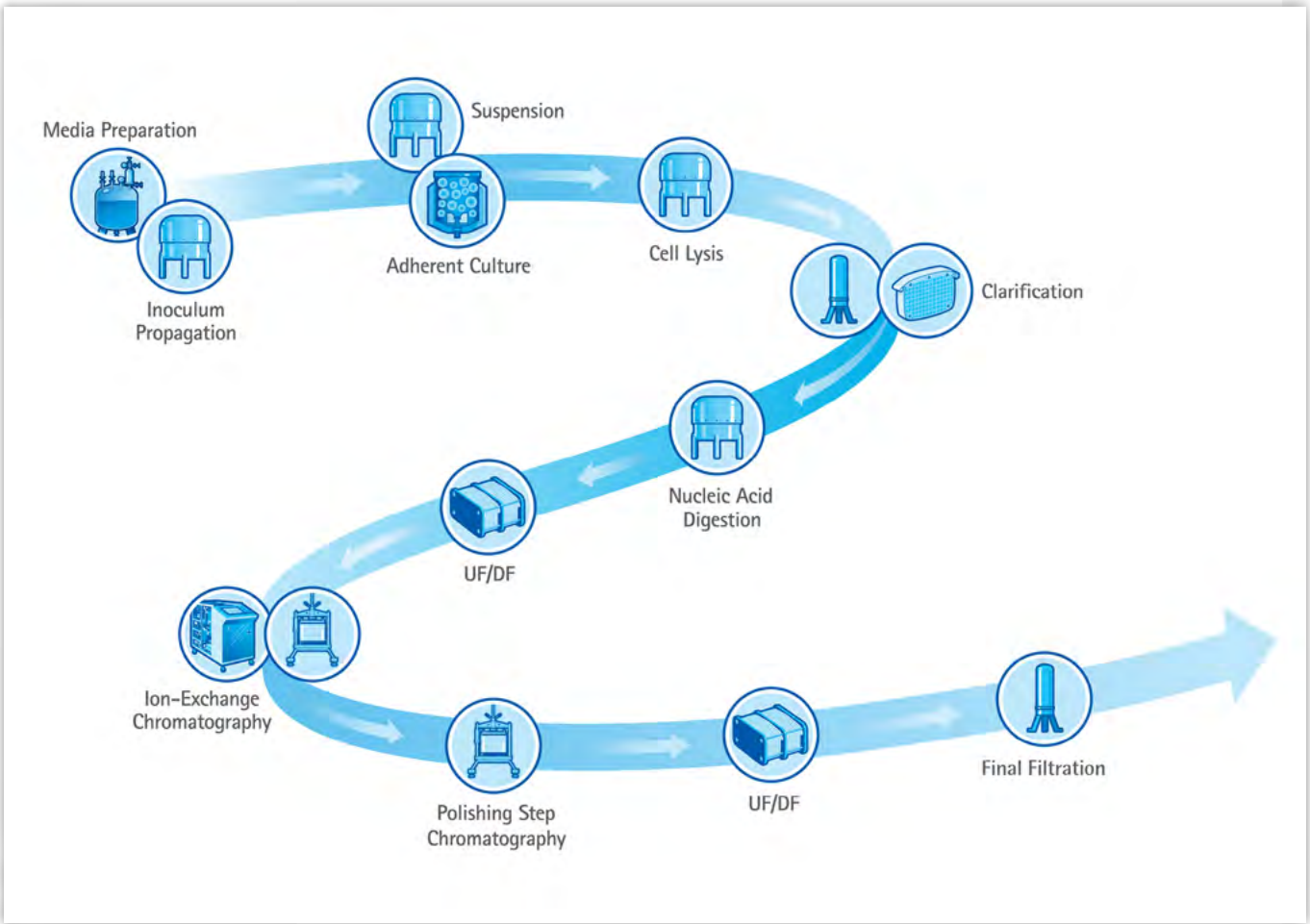

FIGURE 1. Generic adenovirus-based vectored vaccine process.

Upstream Processing

Cell culture media is used for expansion of cells. During the growth phase, most of the cell culture undergoes media exchange by employing either a continuous or batch-mode approach. The exchange replaces the cell growth media with virus propagation-specific media. All media is pre-filtered, and we recommend using EMD Millipore's depth filters (Millistak+® and Polysep® II 1.0/0.5 µ) followed by filter sterilization (Millipore Express® SHC).

Efficient manufacture of adenovirus vectors can be accomplished using genetically engineered human cell lines that complement the deleted adenoviral genes required for replication (*e.g.*, 293-ORF6, HEK293, PER.C6 cells). These cells can be adapted for growth in serum-free suspension culture and have a well-characterized safety profile. Production cells are grown in stirred tank bioreactors using serum-free culture medium.

During the adenovirus infection phase, the metabolic processes of the production cell line are significantly increased to support vector manufacture. In order to facilitate successful vector production, a medium exchange step is performed to remove spent growth medium containing metabolites such as lactic acid, which can be detrimental to virus production. The virus yield goes down significantly when the media pH drops below 7.0. A 50–100% medium exchange is commonly performed at this time. For the media exchange, 0.22 µm microfiltration can be employed (EMD Millipore Prostak® with a two-pump system). After this step has been completed, cells are concentrated and followed up by resuspension in fresh medium. Typical cell concentrations during adenovirus infection are 0.5 to 9.0×10^7 cells/mL and the adenovirus titer during harvest generally ranges from 10^9 to 10^{11} pfu/mL. Some manufacturers also use a stainless steel mesh-type filter for cell removal.

Downstream Processing

Cells are lysed either mechanically or by a chemical lysis agent (*e.g.*, non-ionic detergents such as Triton X-100) to harvest the viruses. For clarification, depth filtration is typically used, performed at room temperature to remove the cells or cell debris and harvest adenoviruses. Microfiltration (tangential flow filtration (TFF)] can also be used, allowing for low shear conditions. Capacities of filters used at the clarification step depend heavily upon cell density at harvest, the degree of lysis, and the particle size distribution. Typical lysate turbidity is >200 nephelometric turbidity units (NTU). A two-stage depth filtration step comprised of 3.0 µm Clarigard® polypropylene cartridge filters (EMD Millipore) for large particle removal is followed by a 0.5 µm glass and cellulose depth filter (CGW6 Polysep® II) to remove small particles and colloids. This can successfully clarify the lysate. The 0.1 or 0.22 µm open-screen TFF microfiltration (Pellicon® 2 V screen/Prostak) may also be an option in addition to the normal-flow depth filtration options. Centrifugation is also an alternative for primary clarification. In some instances, filtrate from secondary clarification has been passed through a 0.45 µm filter for bioburden reduction (~250–500 L/m^2). Post-clarification turbidity ranges from 5–10 NTU.

DNA/RNA Clearance

Carryover nucleic acid from lysed cells is a key contaminant in adenovirus vaccine processes. Viruses propagated in cells such as HEK293 and PER.C6 pose a greater risk of nucleic acid carry-over. According to regulatory requirements, carryover host cell nucleic acid content in the final dose of attenuated viral vaccine should be below 10 ng/dose.[2] Nucleic acids are negatively charged, large molecular components that interfere with virus purification. Benzonase® (EMD Millipore), a special recombinant, animal-component-free endonuclease of ~60 kDa (dimer), is commonly used to degrade nucleic acids (RNA, DNA, etc.) to as small as 3–8 base pairs (<6 kDa). Virus harvest treated with about 0.9–1.1 U/mL of Benzonase at 30–34°C for 4–8 hours is effective in digesting host cell DNA.

Ultrafiltration/Diafiltration

After DNA/RNA clearance, the harvest is diafiltered using TFF (100–300 kDa UF devices) to remove cleaved DNA fragments and spent nuclease (Benzonase). There is a 4–10 times concentration and 5–8 N diafiltration resulting in more than 99% retention of adenoviruses, typically. Vector concentration might be required to reduce overall volume before the Benzonase treatment step and then diafiltration is conducted to facilitate buffer exchange for further processing such as the downstream chromatographic step. Sometimes an overnight hold step is employed prior to downstream purification. The filtration step is performed to reduce the risk of bioburden and to protect the downstream chromatography columns.

Chromatography

Small-scale clinical lots are usually purified by using cesium chloride-based density gradient ultracentrifugation. However, for large-scale production, chromatography is employed. A two or three-step chromatographic purification is normally done for viral vector production. Ion exchange and size-exclusion chromatography can be used for purification of vectors. Size-exclusion or anion exchange (EMD Millipore Fractogel® DEAE 650 [M] resin) chromatography is used for trace contaminant removal (host cell proteins, DNA, RNA, etc.). Generally, adenovirus

is loaded to the Fractogel column in 50 mM Tris HCl, 100 mM NaCl, 2 mM $MgCl_2$, and 2% sucrose (pH 8.0). The column is washed at 0.2 M NaCl and bound virus elutes at 0.35 M NaCl with >92% purity and 70–80% yield. The binding capacity of Fractogel DEAE (M) exceeds 5×10^{12} viral particles per mL of resin.[3] Next, the chromatography elute is pooled and diafiltered using 100–300 kDa TFF (Pellicon 2). Typically 10 times concentration and 8–10 N diafiltration are performed at this step. The final purified product is filter-sterilized.

TABLE 2. Sampling and testing of viral vectors for manufacturing process control.

Process Steps	Analytical Testing
Virus Seed and Cell Banks	Identity, Purity, Stability
Crude Harvest	Adventitious Agents
Purified Bulk	Purity, Stability
Final Product	Identity, Potency, Stability, Physical Parameters

Quality Control

During manufacturing of viral vectors, samples are collected at multiple points for quality control (QC) testing. Standard sampling points and tests performed are shown in Table 2. The manufacturing process described in this paper can take 5–6 weeks; 4–5 weeks for upstream processing, depending on the cell concentration; and another week beyond that for downstream processing. Virus inoculation through harvest can take up to two days; lysis and purification, up to three days; and then one day for sterile filtration and the final filling operation. When the viral material is intended for clinical trials, a successful outcome is not a guarantee. Therefore, capital investments for large-scale manufacturing infrastructures are not warranted for early phase development. Single-use technology can be implemented to significantly lower the financial risks involved in the manufacture of clinical-grade trial materials. Commercially available, off-the-shelf systems and single-use assemblies are commonly employed. Figure 2 illustrates how such components can fit seamlessly into the manufacturing template for viral vectors.

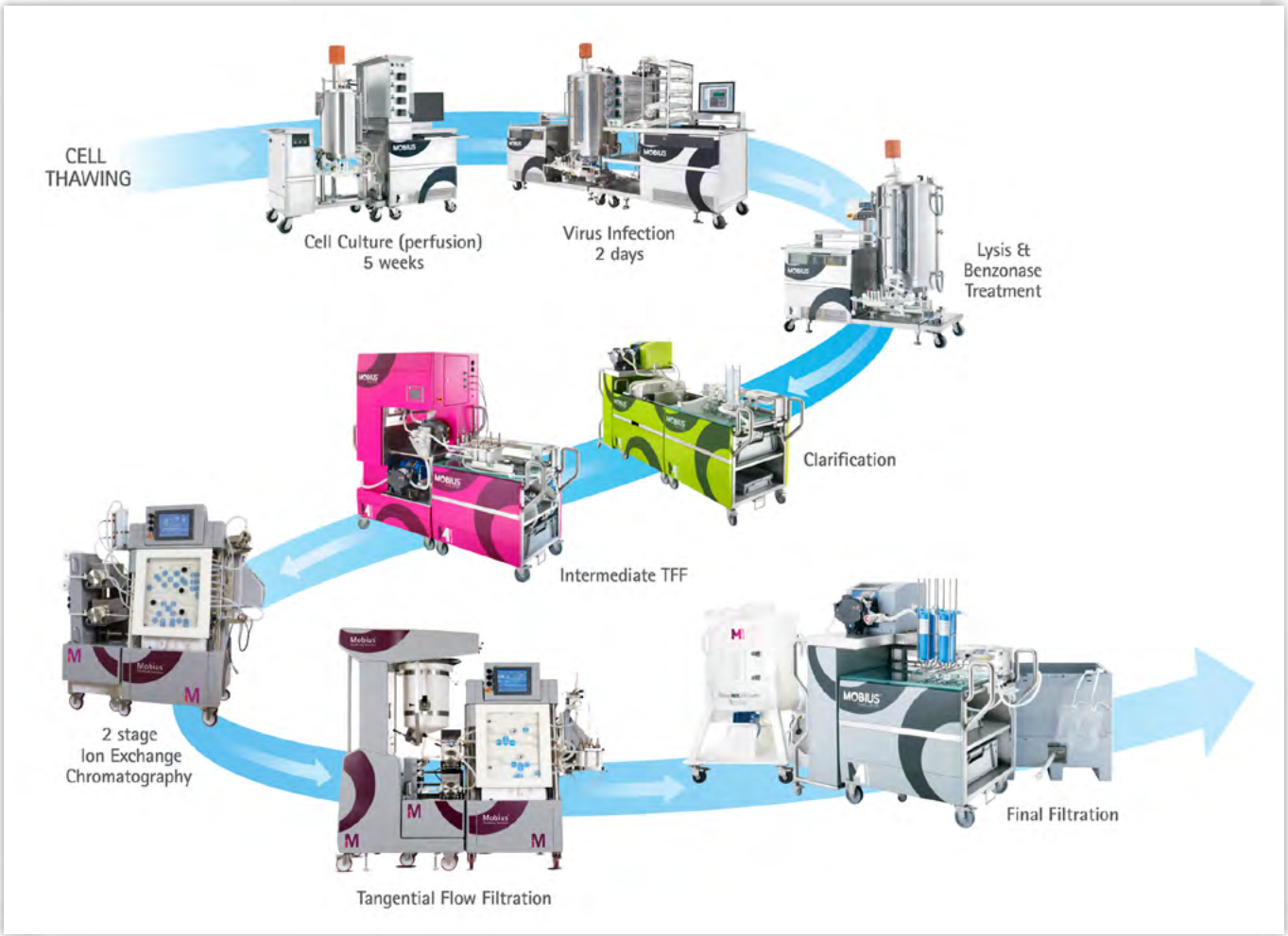

FIGURE 2. Integrated assembly of commercially available, off-the-shelf systems and single-use components for viral vector manufacturing.

Note

Content from this article was presented by the first author at Vaccine World Summit, Pune, India. 5-7 March 2013.

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