

Filtration Processes Applied in Viral Vector Based Gene Therapy Production

Introduction

Gene therapy involves the transfer of genetic material into a patient's cells in order to correct a disease state. The objective is to provide the necessary genetic material to correct a genetic deficiency and allow a particular therapeutic protein to be temporarily or permanently expressed. Candidates for gene therapy are often missing a particular gene or have deficiencies in their genome preventing them from producing fully functional, therapeutic proteins. Examples can include hemophiliacs (missing blood clotting factors) and diabetics (missing or producing insufficient quantities of insulin). With the increased understanding of the genetic basis of disease and the Human Genome Project, the potential for new gene therapy treatments is rapidly increasing.

As referred to above, gene therapy requires inserting genetic material into a patient's genome. Beyond obtaining the proper sequence of DNA, the insertion process requires a mechanism for allowing the donor DNA to enter the patient's target cells. There are a number of vehicles for transferring DNA, the most common methods being Adenovirus, Adeno-Associated Virus, synthetic vectors (liposomes), and DNA directly.

This 3M Purification Inc. Application Brief addresses filtration applications employed in the production and purification of Adenoviral vectors used for gene therapy processes.

The Process

Adenovirus and Adeno-Associated Virus are two of the more common viral vectors used for transfer and insertion of genetic material into a patient's targeted cells. The Adenovirus or Adeno-Associated Virus is first modified in the laboratory by genetic engineering techniques. Genetic engineering involves inserting the genes to be transferred to the patient into the viral genome and inactivation of any viral genes that could harm the patient. Production of these viral vectors begins with cell culture fermentation.

Genetically engineered Adenovirus or Adeno-Associated Virus is used to infect logarithmically growing mammalian host cells. At the optimum point, the fermentation is stopped and the host cells are harvested. The harvested cells are then suspended in an appropriate buffer and lysed to release the internal viral particles. At this point, filtration is employed to separate the viral particles from the debris-laden solution. The appropriate filters allow the viral particles to pass, while removing cell debris. The clarified suspension of viral particles is then further purified using a combination of filtration and chromatographic steps. An overview of the virus production and purification process is illustrated in Figure 1.

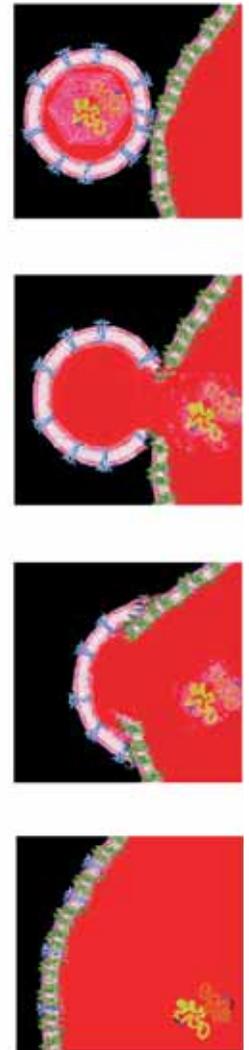


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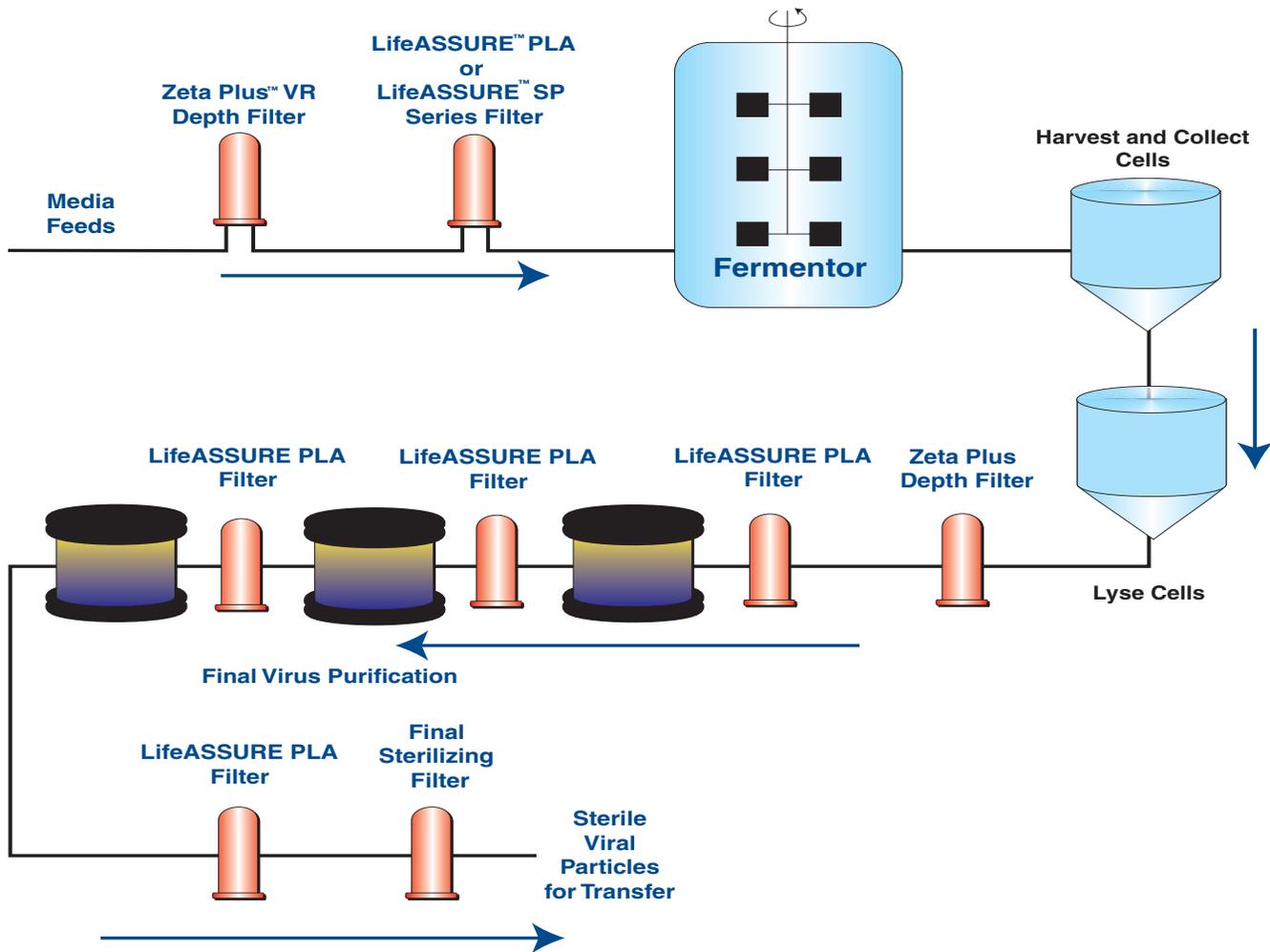


Figure 1 — Production and Purification of Gene Therapy Virus

The Problem

As described above, in gene therapy production processes using viral vectors, the virus grown in cell culture is the end product. It is therefore desirable to obtain as high a yield as possible of the virus particles. One of the key steps in virus purification involves separating the viral particles from the lysed cellular debris. The challenge for this separation is to produce a clarified filtrate *without* retaining the virus particles.

Depth filters offer an effective and economical means to separate cellular debris from viral particles. In some cases viral particles may be observed to pass directly through depth filters while in other systems, the yield of viral particles following depth filtration may be greatly reduced. The reasons for reduced viral transmission through depth filters are complex. Viral particles may bind to cellular debris or to the depth filter medium itself. In these cases, manipulation of solvent conditions such as pH or ionic strength may be required to achieve acceptable viral vector yield following depth filtration.

In addition to achieving acceptable viral yield, the cell culture clarification step must produce clarified fluid able to be filtered through downstream 0.2 micron rated filters. The clarified filtrate must also be able to pass freely through downstream chromatography columns. If a sufficient degree of separation is not achieved, it may be impossible to reliably remove bacterial and other particulate contaminants by filtration. Further, insufficient clarification can lead to plugging or channeling of chromatography columns.

The 3M Purification Inc. Solution

Separation of cellular debris from soluble protein and virus requires a filter designed to retain large amounts of gelatinous cellular debris without plugging prematurely, yet is able to allow virus particles to pass. Zeta Plus™ depth filters are designed for this purpose and have performed reliably in such applications. ZetaPlus depth filters consist of a cellulose matrix, filter aid and a resin system to provide a filtration media with a series of rigid, interconnected pore path ways. Each of the filter media components can be varied to produce a filter media with the desired pore structure and with an appropriate surface chemistry. An electron micrograph of Zeta Plus filter medium is shown in Figure 2.

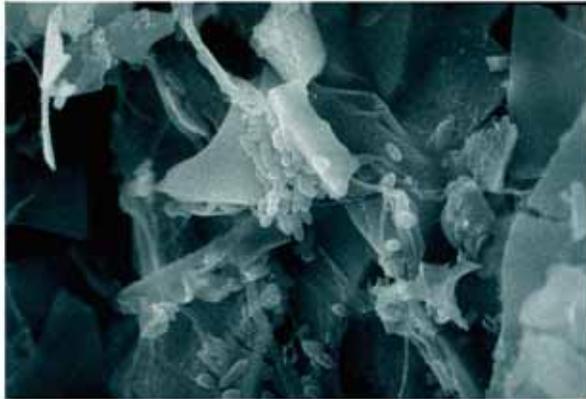


Figure 2 — Zeta Plus Filter Medium

most particles and biological species are negatively charged and are thus more readily retained by positively charged filter surfaces. Conversely, retention of negatively charged virus can be a detriment in gene therapy purification. For this reason, 3M Purification Inc. produces a variety of Zeta Plus chemistries and porosities such that the optimum Zeta Plus grade for virus purification can be selected. Figure 3 shows the charge capacity of three different Zeta Plus resin systems: Z, S, and C. As can be seen in this figure, Zeta Plus S and C Series resin systems have a lower charge capacity at neutral pH. Zeta Plus Series C and S would be the best first choice for virus separation and are available in porosities ranging from approximately 0.8 micron to 0.2 micron.

The results of trials with several different resin systems and grades of Zeta Plus media and with a 0.65 micron rated membrane filter used to filter a cell lysate containing Adenovirus are presented in Table 1. One objective of these trials was to determine yield (transmission) of Adenovirus with the various filters.

The filter aid component used in Zeta Plus depth filters is primarily diatomaceous earth (DE). The effective filter pore size can be controlled by varying the amount of cellulose and by controlling the porosity of the DE used. In addition, the surface chemistry of the media can be affected by the resin system used to bind the DE and cellulose fibers. 3M Purification Inc. has developed a number of resin systems that vary the charge imparted to the filter media. These resin systems produce Zeta Plus depth filters with strong to weak positive surface charge. The filter charge capacity is also affected by the pH and ionic strength of the solution in which the filter is used. A positive charge is a benefit in many separations as

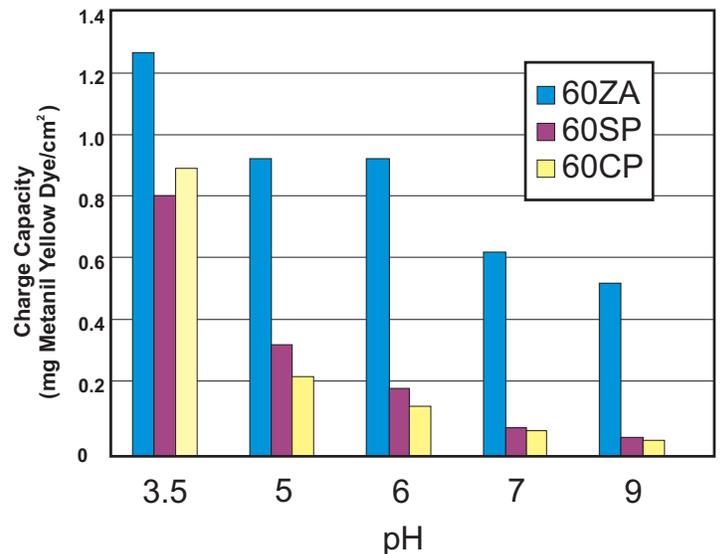


Figure 3 — Zeta Plus Charge Capacity and Resin Type

Table 1 — Purification of Adenovirus Cell Lysate

Filter Type	% Adenovirus Recovery
10 SP	82-85
10M02	82-85
10CP	82-85
50CP	82-85
0.65 micron rated membrane filter	65

The results in Table 1 represent (4) different Zeta Plus filter formulations and one membrane filter. Zeta Plus™ filters 10 SP and 10M02 are constructed using the S resin system. Both filter types have essentially equal porosity of approximately 0.8 micron. The 10M02 Zeta Plus filter has dual zone porosity. Zeta Plus filters 10CP and 50CP contain the C resin system. The charge capacities of the S and C resin systems are shown in Figure 3. The S-resin system has a slightly higher charge capacity at neutral pH than the C-resin system. The 10CP and 50CP Zeta Plus filters have porosities of approximately 0.8 micron and 0.5 micron, respectively. In these trials, the percent recovery of Adenovirus was equivalent with each of the Zeta Plus filters. The membrane filter tested was a dual layer composite media with a rating of 0.65 micron. As can be seen in Table 1, the percent Adenovirus recovery with the membrane filter was significantly lower than with the Zeta Plus depth filters. As stated earlier, depth filters are designed for difficult cell lysate separations requiring retention of cell debris and high transmission of soluble protein and virus.

In some cell culture systems, significant retention of virus may occur. The reason for this may be electrostatic interaction between the viral particles and the depth filter surface charge. In order to reduce or eliminate this effect, and increase viral transmission, it may be necessary to rinse filters with higher ionic strength buffer, rinse with higher pH buffer or both. Higher pH and higher ionic strength conditions reduce the electrostatic interaction allowing recovery of trapped viruses or reduce virus retention initially. Table 2 summarizes the effects of pH and ionic strength on Zeta Plus C and S resin system filters.

Table 2 — Effect of pH and Ionic Strength on Zeta Plus Filters

Solvent Condition	Charge Capacity of C and S Resin System Zeta Plus Filters
Low pH (3-5)	Strong
Neutral pH (6-8)	Moderate
High pH (9-12)	Weak
Low ionic strength (< 0.1M)	Strong
High ionic strength (> 0.1 M)	Weak

The conditions in Table 2 can be used to design solvent systems optimal for transmitting or retaining viruses in purification processes.

Conclusion and Summary

This 3M Purification Inc. Application Brief has presented Zeta Plus depth filters used in purification of virus for gene therapy. A typical process for production of genetically engineered Adenovirus involves virus propagation by fermentation followed by virus purification. Adenovirus is grown in mammalian cell culture and then separated from lysed mammalian cells. One of the difficult purification steps involves clarification of the lysate solution such that virus can be obtained in high yield and the solution can be readily processed by further downstream unit operations. 3M Purification Inc. produces a number of Zeta Plus filter media that can meet the objectives of adequate lysate clarification and virus transmission.

Additional 3M Purification Inc. Literature

Title	Old Literature Identification	New Literature Identification
3M Purification Inc. Filter Systems for Bioprocess and Biological Separations	LITCATBP	70-0201-8680-8
Zeta Plus™ S Series Filter Cartridges	LITZPS01	70-0201-8869-7
Zeta Plus C Series Filter Cartridges	LITZPC01	70-0201-8853-1
Zeta Plus LA Series Filter Media	LITZPLA2	70-0201-8860-6
Zeta Plus EXT Series Filter Cartridges	LITZPMEXT	70-0201-8862-2
LifeASSURE™ PLA Capsule and Cartridge Filters	LITCLAPB1	70-0201-8713-7
LifeASSURE SP Membrane Filters	LITCZR020SP	70-0201-8738-4
ZPB Model Zeta Plus Filter Housings	LITHSZPBC	70-0201-8762-4
ZWB Model LifeASSURE Filter Housings	LITZRH106	70-0201-8884-6
3M Purification Inc. Application Brief - Chromatography Column Protection with 3M Purification Inc. LifeASSURE PLA Membrane Filters	LITCABLA2	70-0201-8632-9
3M Purification Inc. Application Brief - Filtration of Cell Culture Growth Media and Process Buffers	LITCABLA3	70-0201-8633-7
3M Purification Inc. Application Brief - Zeta Plus Depth Filtration and Alternative Technologies for Cell Culture Clarification	LITCABZPS1	70-0201-8667-5
LifeASSURE SP Validation Guide	LITVGSP20	70-0201-8844-0
LifeASSURE PLA Filter Regulatory Support File	LITDRSFLA	70-0201-8821-8

3M Purification Inc. Literature Descriptions

3M Purification Inc. Filter Systems for Bioprocess and Biological Separations — 3M Purification Inc. is a leader in advanced depth filter systems and membrane-based separations, offering a range of products for all stages of biopharmaceutical and biological processing from bench top to pilot-scale to manufacturing scale operations.

Zeta Plus™ S Series Filter Media are depth filters composed of inorganic filter aids and cellulose. They are completely free of asbestos and glass microfibers and are ideally suited for pharmaceutical, food & beverage and cosmetic industry applications. The strong positive charge exhibited by Zeta Plus filters results in increased filter efficiency and cost effectiveness when compared to conventional un-charged depth filters and pleated prefilters. The high contaminant holding capacity of Zeta Plus filters prolongs final filter life when used as a prefilter. Stringent quality controls ensure maximum product performance for each production lot.

Zeta Plus™ C Series Filter Media are depth filters composed of inorganic filter aids and cellulose. They are completely free of asbestos and glass microfibers and are ideally suited for pharmaceutical, food and beverage, and cosmetic industry applications. The strong positive charge exhibited by Zeta Plus filters results in increased filter efficiency and cost effectiveness when compared to conventional uncharged depth filters and pleated prefilters.

Zeta Plus™ LA Series Filter Media development was prompted by the growing concern for metallic extractables, especially aluminum in parenteral solutions and infant food products. This premium grade media has been specifically designed for processes that require a high degree of filtration and low aluminum extractables. It is ideally suited for parenteral, blood fraction, dextrose, dianeal solutions and infant food/formula applications. The Zeta Plus LA Series are completely free of glass microfibers and asbestos, and are constructed from materials listed in CFR 21 “Food and Drugs.” Stringent quality controls ensure maximum product performance for each production lot.

Zeta Plus™ EXT Series Filter Media — Now added to this well-established family of Zeta Plus filters is the ground-breaking Zeta Plus EXT Series. Featuring a new dual zone construction, Zeta Plus EXT Series filters significantly increase throughput per unit area of filter media, while optimizing the desired effluent clarity. Zeta Plus EXT filter media consists of two distinct layers, or “zones” of filter media with the upstream zone more open than the downstream zone. This structure enhances the contaminant holding capacity of the filter media, since larger particles are trapped in the upper zone of the filter media and smaller particles are trapped in the lower zone, reducing premature plugging and extending service life. These two layers can be selected independently of each other to optimize performance. Standard combinations determined to have the optimum throughputs are included in the ordering guide on the back, although the user can select custom arrangements if required.

LifeASSURE™ PLA Cartridge and Capsule Filters — LifeASSURE PLA filter cartridges and capsules are 3M Purification Inc.’s latest advance in membrane filter technology. Encompassing two leading-edge processes, FlexN membrane manufacture and MaxMedia pleating construction, the LifeASSURE PLA series of filters offers unmatched protection of final membrane filters, as well as exceptionally long service life. Designed with pleated Nylon 6,6 membrane in an all- polypropylene cartridge construction, LifeASSURE PLA filters are ideally suited for a wide range of prefiltration and clarification applications in the pharmaceutical, biological, and bioprocess industries.

LifeASSURE™ SP Series Sterilizing Grade Cartridge and Capsule Membrane Filters — 3M Purification Inc. pioneered the development of charge modified Nylon 6,6 filters for the pharmaceutical industry. LifeASSURE SP series sterilizing grade filters and capsules are validated for absolute bacteria retention and provide reliable sterile filtration performance. In addition to a fixed bacteria retentive pore structure, LifeASSURE SP series membrane is charge modified to provide enhanced removal of negatively charged biological contaminants such as endotoxin, virus and nucleic acid fragments. The combination of a validated bacteria retentive membrane, together with enhanced removal of negatively charged contaminants, make LifeASSURE SP membrane an ideal choice for pharmaceutical and biopharmaceutical sterilizing applications.

Zeta Plus™ ZPC & ZPB sanitary filter housings provide the ultimate standard for totally-enclosed Zeta Plus filter cartridge systems. Constructed from 400 grit, mirror finish, 316L stainless steel, Zeta Plus ZPC & ZPB housings meet the exacting sanitary quality standards of the pharmaceutical, food and beverage, fine chemical, and microelectronics industries. Both housing styles accommodate from one to four 8”, 12”, or 16” diameter Zeta Plus filter cartridges to offer a wide choice of filter media area and flow rates.

LifeASSURE™ ZWB sanitary design cartridge housings provide the ultimate for critical clarification and sterile filtration applications. Manufactured to the strictest of standards and from high quality 316L stainless steel, ZWB mirror polished filter housings are designed to meet the exacting quality standards of the food & beverage industry. The ZWB series are multi-cartridge housings designed to accommodate three, five, seven, or twelve sanitary style filter cartridges up to 40 inches long.

Scientific Applications Support Services

The cornerstone of our philosophy is service to customers, not only in product quality and prompt service, but also in problem solving, application support and in the sharing of scientific information.

The **Scientific Applications Support Services (SASS)** group is a market-oriented group of scientists and engineers who work closely with customers to solve difficult separation problems and aid in the selection of the most effective and economical filtration systems. 3M Purification Inc. offers specialized support to the pharmaceutical and biotechnology industry through our **Validation Support Services Program**. SASS routinely provides end-users with:

- Validation And Regulatory Support
- Extractable And Compatibility Analysis
- Filter System Optimization Studies
- CUNOCheck™ 2 Integrity Tester Validation.

For more information regarding our Validation Support Services, please contact 3M Purification Inc. Technical Services or your local Distributor.



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