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Tangential flow filtration for virus purification

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ABSTRACT

Purification of virus particles and viral vectors for viral vaccines and gene therapy applications is a major large-scale separations challenge. Purification of parvovirus particles such as adeno-associated virus, the leading candidate for gene therapy applications, is particularly challenging given their small size, typically 18–26 nm. We have investigated the use of ultrafiltration for purification of *Aedes aegypti* densonucleosisvirus, a mosquito parvovirus.

Four ultrafiltration membranes with molecular weight cut offs of 30, 50, 100 and 300 kDa have been tested in tangential flow mode. Experiments have also been conducted where part of the permeate is pumped co-current to the feed. This results in a more constant transmembrane pressure drop along the entire membrane. Maintaining a more constant transmembrane pressure results in improved passage of host cell proteins and smaller virus particles.

Currently chromatographic separations such as ion exchange are used to remove DNA and host cell proteins. Our results indicate that by carefully selecting the molecular weight cut off of the membrane and maintaining a constant transmembrane pressure along the membrane, virus particles may be purified and concentrated by ultrafiltration. Reducing the impurity load to subsequent chromatographic steps could result in the use of smaller columns leading to cost savings.

1. Introduction

Production of virus particles and viral vectors for gene therapy applications and viral vaccines requires the development of robust purification operations. Today large-scale production of virus vectors for gene therapy applications and viral vaccines is a major challenge. For example, it is estimated that between 10^{11} and 10^{14} viral vectors will be required to satisfy gene therapy regimens [1]. Thus efficient and scalable processes for producing viruses and virus vectors are needed. Since the downstream purification steps are directly affected by changes in the cell culture methods, it is essential that both cell culture and purification steps be developed in parallel. This is particularly significant given that the purification steps could account for up to 70% of the production costs [1].

Virus particles vary in size from 18 nm (parvovirus) to more than 300 nm. However most viruses of interest to the biotechnology industry are less than 140 nm. For example, human influenza virus is an enveloped virus with an average size of 100 nm. On the other hand parvovirus particles such as adeno-associated virus (AAV) are only 18–26 nm in size and nonenveloped. Today AAV particles are being extensively investigated as gene therapy vectors since they do not induce an immune response towards viral components; they can integrate into human chromosome 19 and they do not require actively dividing cells for transduction and are non-pathogenic [1].

While there are many studies in the literature on the application of tangential flow filtration for protein purification, far fewer published studies focus on the use of tangential flow filtration for feed streams containing virus particles. Though the focus of this work is the purification of virus particles, validation of virus clearance in the manufacture of protein-based biopharmaceuticals is a major concern [2]. During virus filtration for validation of virus clearance, the virus particles are rejected by the membrane. Consequently reuse of the membrane is not practical as one would need to validate removal of the virus particles from the membrane. Thus virus filtration for validation of virus clearance is usually conducted using the simpler dead end or normal flow mode of operation [3]. While tangential flow virus filters designed for the validation of virus clearance are available commercially, e.g. Viresolve® 70 and 180, Millipore Corporation, Bedford, MA, their application is limited to cases where the product of interest and virus particles to be removed are less than an order of magnitude different in size leading to significant product rejection during normal flow filtration. Recently Grzenia et al. [4] summarized studies on the use of

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tangential flow microfiltration and ultrafiltration for purification of virus particles.

This work focuses on the use of ultrafiltration for purification of parvovirus particles for a number of reasons. Parvovirus particles such as AAV have been purified from cell lysates using density gradient centrifugation. However these vector preparations have been found to be contaminated with impurities that can cause local inflammation *in vivo* [5,6]. Consequently significant effort has been spent developing chromatographic procedures, especially ion exchange and affinity chromatography, for purification of AAV. Development of an ultrafiltration step may provide an efficient method to purify and concentrate adeno-associated and other parvovirus vectors. For example, including an ultrafiltration step to purify and concentrate the virus particles, upstream of a chromatographic separation, could lead to a reduction in the impurity load to be removed by the chromatographic step thus leading to smaller columns and a cheaper process.

Here we have investigated the use of ultrafiltration for purification of *Aedes aegypti* densonucleosisvirus (AeDNV). AeDNV is highly pathogenic to *A. aegypti* and a number of other Culicine mosquito species. The *A. aegypti* mosquito is a vector of the viruses that cause dengue fever and yellow fever, and AeDNV shows promise as a biological control agent with important applications in integrated vector control programs against mosquito-borne diseases. This parvovirus is similar in size to AAV. Since ultrafiltration is a size exclusion based separation process, AeDNV is a good model for AAV and other similar sized virus particles. The virus particles are easy to grow using a cell culture based system. A PCR based assay is available which allows easy measurement of the virus titre [7,8]. Consequently we use AeDNV as a model for investigating the use of ultrafiltration for purification of parvovirus particles.

Tangential flow ultrafiltration is frequently used in the biotechnology industry for protein concentration and buffer exchange by diafiltration [9]. It has generally been assumed that separation of solutes which differ in size by less than an order of magnitude is difficult by ultrafiltration [10–12]. However recent studies have indicated that by carefully controlling the operating conditions, efficient separation of solutes which differ in size by less than an order of magnitude is possible [9,13,14]. Pujar and Zydney [15] have shown that electrostatic and electrokinetic interactions can lead to changes in the sieving coefficient of proteins of up to 100-fold. Saksena and Zydney [16], van Eijndhoven et al. [17], Iritani et al. [18] and Yang and Tong [19] have indicated the feasibility of fractionation of proteins by carefully selecting the ionic strength and pH of the suspending buffer.

van Reis et al. [20] have described an emerging technology for fractionation of proteins whose size is within an order of magnitude of each other. Known as high performance tangential flow filtration (HPTFF), the method combines optimization of the ionic strength and pH of the suspending buffer with membrane charge and transmembrane pressure (TMP) during ultrafiltration. In conventional tangential flow ultrafiltration only a feed pump is generally used (see Fig. 1). The feed pressure varies along the feed flow path from the inlet pressure (P_i) to the outlet pressure (P_o). Typically P_o is close to atmospheric pressure. The average TMP is given by $\mathrm{TMP} = (P_i + P_o)/2 - P_p$, where P_p is the permeate side pressure. However since the feed side pressure varies from P_i to P_o the TMP will also vary along the feed flow path. This variation in TMP along the feed channel will reduce the resolving power of conventional tangential flow filtration systems [17]. In HPTFF, part of the permeate is returned to the permeate side of the module (see Fig. 2) such that the permeate flows co-current to the feed. By creating an axial pressure drop along the permeate flow channel, the TMP is more nearly constant throughout the module [21]. HPTFF has been used to separate monomers from oligomers [20], proteins varying by only one amino acid [22] and antigen binding fragments from a similar impurity [9].

Here we focus on the use of ultrafiltration for recovery and purification of virus particles. An ideal ultrafiltration step will not only concentrate virus particle but also purify them by removing contaminants such as host cell proteins and DNA. We have investigated retention of virus particle and passage of host cell proteins using conventional tangential flow ultrafiltration and the HPTFF mode of operation.

2. Materials and methods

2.1. Cell culture and virus production

AeDNV particles were produced using the *Aedes albopictus* cell line C6/36 (ATCC, Manassas, VA) in a serum free medium as described by Suchman and Carlson [23]. Cells were grown in T-75 flasks containing 14 mL serum and protein free medium (SFPFM) (SF-900 II SFM, Invitrogen Corporation, Grand Island, NY) at 28 °C. When the cells reached more than 30% confluency (about 2–3 days), they were transfected with pUCA using a Qaigen effectine kit as described by Afanasiev et al. [24]. The media was changed 8–18 h post transfection to remove the pUCA plasmid. Transfected C6/36 cells were then transferred from the T-75 flask to a 100 mL spin flask (stirred bioreactor) (Wheaton Science Products, Millville, NJ)

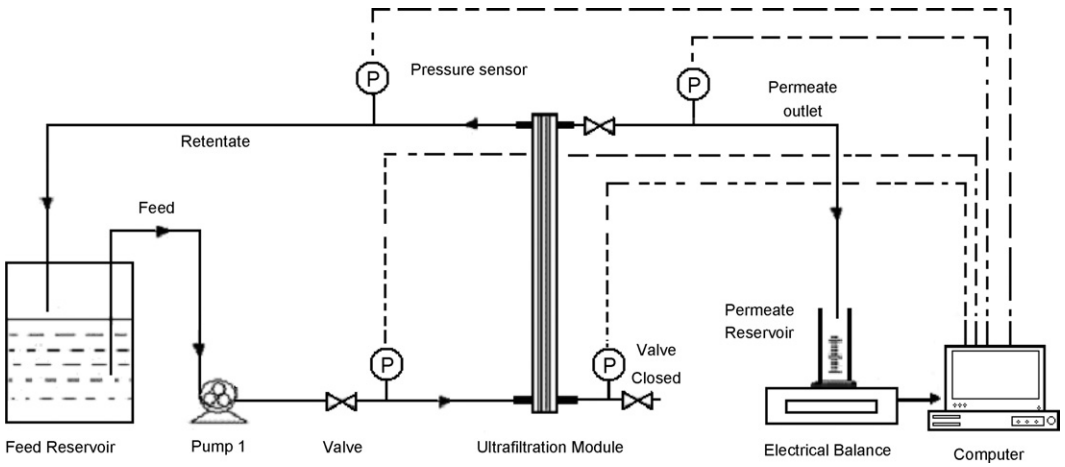

Fig. 1. Experimental set-up for tangential flow filtration. Only a feed pump is used.