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Impact of grafting on the design of new membrane adsorbers for adenovirus purification

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ABSTRACT

The impacts of quaternary amine ligand density and matrix structure, namely hydrogel grafted and directly grafted, on state-of-the-art chromatographic membranes operated in bind-and-elute mode were evaluated for the purification of adenovirus serotype 5. The experiments were performed on a 96-well plate membrane holder, which is a convenient high-throughput screening tool for obtaining the best operating conditions for a process yield optimization. The results show that the hydrogel-grafted membranes are more suitable for virus purification than the directly grafted ones. By reducing the number of grafted ligands to low (1.7 μmol/cm^2) or medium (2.4 μmol/cm^2) density, it is possible to increase the recovery of purified virus by 60% compared to a highly charged membrane (3.3 μmol/cm^2) that yielded a recovery rate lower than 30%. In the reported experiments, Sartobind® Q, chosen as benchmark comparison, provides a better compromise between high recovery and large dynamic binding capacity. Overall, this work contributes to the understanding and development of new membrane adsorbers specifically designed for virus purification.

1. Introduction

Adenoviruses are considered to be one of the most suitable platforms for producing viral vaccines and gene therapy vectors. These viruses can be produced using complementary cell lines in both adherent and suspension culture systems, such as HEK-293 or PER-C6 cells, or A549 for oncolytic adenovirus (Segura et al., 2008). Despite the recognized interest in these viral vectors, the development of an integrated downstream purification platform for adenoviruses is not yet mature. The complexity of this biopharmaceutical product is partly related to the large batch-to-batch variability of the bio-reaction bulk, which is a challenge for the development of reliable and robust purification processes (Morenweiser 2005). Moreover, it is still a hurdle to achieve a high-purity grade while keeping host cell protein (HCP) and deoxyribonucleic acid (DNA) within the limits set by regulatory authorities.

Chromatography is a well-established purification tool of recombinant adenovirus used for vaccine and gene therapy. Indeed, ion exchange (IEX) chromatography is the most used technique for the purification of complex biopharmaceuticals. However, packed-bed chromatography suffers from a number of disadvantages: the pressure drop across the bed is usually high and may increase during operation due to media deformation or blockage, and pore diffusion is slow and often leads to degradation of the protein product. In addition, packed beds display a relatively small dynamic binding capacity for virus particles at common process-scale linear velocities of 150–450 cm/h, because binding is restricted to the surface of conventional resin particles (Weaver et al., 2012).

Convective chromatography media, such as membranes and monoliths, offer substantial improvements in capacity, recovery, and reduction of process time (Hahn et al., 2007). To date, monoliths have generally offered larger capacity and higher resolution than membranes (Gerster et al., 2013; Whitfield et al., 2009), but the latter have proven to be less prone to clogging, and their relatively low cost has made it practical to discard them after a single

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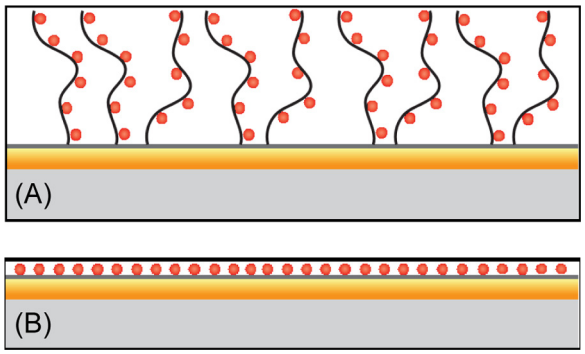

Fig. 1. Schematic representation of the two membrane structures: (A) hydrogel-grafted membrane in which the Q ligand is immobilized onto the tentacle-like structure, and (B) directly-grafted membrane in which the ligand is directly immobilized onto the membrane surface. The red dots represent the Q ligands. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

use rather than to invest additional resources on developing and validating regeneration and sanitization procedures.

Membrane adsorbers are used in the biopharmaceutical industry almost exclusively in flowthrough mode for mAb purification (Boi, 2007; Knudsen et al., 2001; Zhou and Tressel, 2006). More recently, membrane adsorption chromatography has been applied to the purification of viral vectors using a bind-and-elute mode, yielding good overall recovery rates (Lee et al., 2009; Peixoto et al., 2006, 2008). However, two major challenges still remain: on one hand, the adequate removal of contaminants (DNA and HCP) that are closely related with the viral product, and on the other hand, the low binding capacity for highly concentrated feeds. There is, therefore, a need for developing membrane chromatography specifically designed for virus purification (Riordan et al., 2009; Vicente et al., 2011).

The best virus recovery yields reported to date have been obtained with ligands containing quaternary ammonium ions grafted onto the surface of a macroporous membrane to produce a strong anion-exchange adsorber; examples of such membrane adsorbers include Sartobind® Q (Sartorius Stedim Biotech, Germany) and Mustang Q® (PALL, Life Sciences, USA) (Wang et al., 2009; Weaver et al., 2013). The design of new matrices with appropriate ligand density and degree of grafting is key for further improving the performance of the bind-and-elute purification mode for viruses.

This paper studies the effects of ligand density and membrane structure on the binding and elution of adenovirus serotype 5 (Ad5) feed stock. Ad5 was chosen as a model virus given its wide utilization and robustness. Two different membrane platforms manufactured by Sartorius Stedim Biotech (SSB), respectively hydrogel grafted and directly grafted, were evaluated using a 96-well plate membrane holder. In the hydrogel-grafted membrane the ligand is immobilized via a hydrogel support (Zhong et al., 2011), creating a tentacle-like structure; this structure is currently used in Sartobind® Q. In the directly grafted membrane the ligand is directly coupled to the cellulose membrane backbone (Fig. 1).

In the first part of this study the membrane binding and virus recovery are assessed under various operating conditions, namely, pH, buffer type, and ionic strength. In the second part of this work, the dynamic binding capacities of the hydrogel-grafted membranes for the target product (Ad5) and its related impurities (DNA and HCP) are determined using a 96-well plate holder with a very small membrane volume. An attempt is made to elucidate the shapes of the breakthrough curves by matching the frontal analysis experiments to a simple equilibrium-dispersive adsorption breakthrough model.

2. Materials and methods

2.1. Cell line and medium

The 293 cells purchased from ATCC (ATCC-CRL-1573) were adapted to suspension and grown in a commercial serum-free medium, Ex-Cell 293 (SAFC Biosciences, USA), supplemented with 4 mM of glutamine (Invitrogen, UK), and cultured in a humidified atmosphere of 8% CO_2 in air at 37 °C using shake flasks (Corning, USA).

2.2. Virus production

A replication-defective adenovirus, derived from adenovirus serotype 5 (Ad5) expressing GFP protein, was used. The virus particles were produced in a 2 L bioreactor with 1 L working volume (Sartorius Stedim Biotech, Germany). The agitation rate started at 70 rpm and the dissolved oxygen was controlled at 50% air saturation by gas mixture and stirred cascade control with an airflow of 0.01 L/min; pH was controlled at 7.2 by aeration with a CO_2 gas mixture and 1 M $NaHCO_3$; all infections were done at a cell concentration (CCI) of 10^6 cells/mL using a multiplicity of infection (MOI) of 5; the adenoviruses were harvested at 46 hpi.

2.3. Virus clarification and concentration

The bioreactor was harvested and treated with 0.1% Triton X100 (Sigma Aldrich, MO, USA) and incubated for 2 h at 37 °C with 50 U/mL of Benzonase (Merck Millipore, Germany). Subsequently, the bulk was microfiltrated using 0.8 μm + 0.45 μm Sartopore 2 (Sartorius Stedim Biotech, Germany) and concentrated/diafiltered with Vivaflow 100 (Sartorius Stedim Biotech, Germany) with a cut-off of 100 kDa. The concentration/diafiltration step was performed with both HEPES 50 mM and Tris 20 mM to produce two clarified bulks conditioned into two different buffer types. The bulk was stored in aliquots at −80 °C until further use.

2.4. Quantification of total particles

The protocol for the quantification of total particles was a two-step procedure: DNA extraction according to the instructions on the "High Pure Viral Nucleic Acid Kit" (Roche) manual and real-time PCR. The determination of the number of viral DNA copies was performed by real-time PCR and LightCycler system (Roche Diagnostic) using Fast Start DNA master SYBR Green I kit to track a specific adenovirus 5 sequence (Roche Diagnostics). Internal plasmid of 2.07×10^9 copies/μL was used as reference standard. The total particle concentration was also measured using Nanosight NS500 (NanoSight Ltd., UK), which also quantifies the particle size distribution.

2.5. Protein analysis

The protein profile analysis was done in 4–12% NuPage gradient pre-cast gels (Invitrogen). The gels were stained by Coomassie Instant Blue (Expedeon Ltd., UK) and the protein concentration was determined using BCA kits (Pierce, Rockford, USA) taking bovine serum albumin (BSA) as the standard protein.

2.6. DNA quantification

The DNA was quantified by QuanT-iT Picogreen kit (Invitrogen). After 5 min of incubation, the fluorescence was measured by Luminometery (Turner BioSystems). Purified DNA was used as standard.