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Rational development of two flowthrough purification strategies for adenovirus type 5 and retro virus-like particles

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ABSTRACT

We report on the rational design and implementation of flowthrough (FT) platforms for purification of virus vectors (VVs) and virus-like particles (VLPs), combining anion-exchange polyallylamine membranes (Sartobind STIC) and core–shell octylamine resins (CaptoCore 700). In one configuration, the VV bulk is concentrated and conditioned with appropriate buffer in a ultra/diafiltration (UF/DF) unit prior to injection into the STIC chromatography membrane. The FT pool and an intermediate cut of the elution pool of the STIC membrane are admixed and directed to a second UF/DF. Finally, the retentate is injected into a CC700 packed bed adsorber where the purified VVs are collected in the FT pool, whereas the residual amount of DNA and host cell protein (HCP) are discarded in the eluate. The experimental recovery achieved with this downstream processing (DSP) platform is close to 100%, the DNA clearance is roughly a 4-log reduction, and the HCP level is reduced by 5 logs. The platform developed for VLP purification is simpler than the previous one, as the STIC membrane adsorber and CC700 bed are connected in series with no UF/DF unit in between. Experimentally, the FT scheme for VLP purification gave a recovery yield of 45% in the chromatography train; the experimental log reduction of DNA and HCP were 2.0 and 3.5, respectively. These results are in line with other purification strategies in the specific field of enveloped VLPs. Both DSP platforms were successfully developed from an initial design space of the binding of the major contaminant (DNA) to the two ligands, determined by surface plasmon resonance, which was subsequently scaled up and confirmed experimentally.

1. Introduction

Viral vectors are playing an increasingly important role in the vaccine and gene therapy fields. Adenoviruses (AdVs), in particular, are considered one of the most suitable platforms for the production of viral vaccines and gene therapy vectors [1–3]. AdVs are medium-sized (90–100 nm), nonenveloped, icosahedral viruses composed of a nucleocapsid and a linear, non-segmented double stranded DNA genome that is about 36 kb long. Their broad tissue tropism and large transgene packing capacity make Ads attractive candidates for innovative virotherapies [4]. They can be produced in a complementary cell line in both adherent and suspension culture systems, such as HEK-293 or PER-C6 cells, or A549 for oncolytic therapies [5,6].

Recently, the development of virus-like particles (VLPs) for vaccine applications has been receiving more attention [7,8]. VLPs are non-infective and non-replicating, as they are essentially devoid of infectious genetic material. They display antigenic epitopes in the correct conformation and in a highly repetitive manner, leading to cross-linking of B-cell immunoglobulin receptors and B-cell activation. Because VLPs lack DNA, they are in general safer than AdVs. Nevertheless, these two types of biopharmaceuticals are currently very important platforms for vaccine development [9,10].

Chromatography is a well-established purification tool for recombinant AdVs used for vaccine and gene therapy. Indeed,

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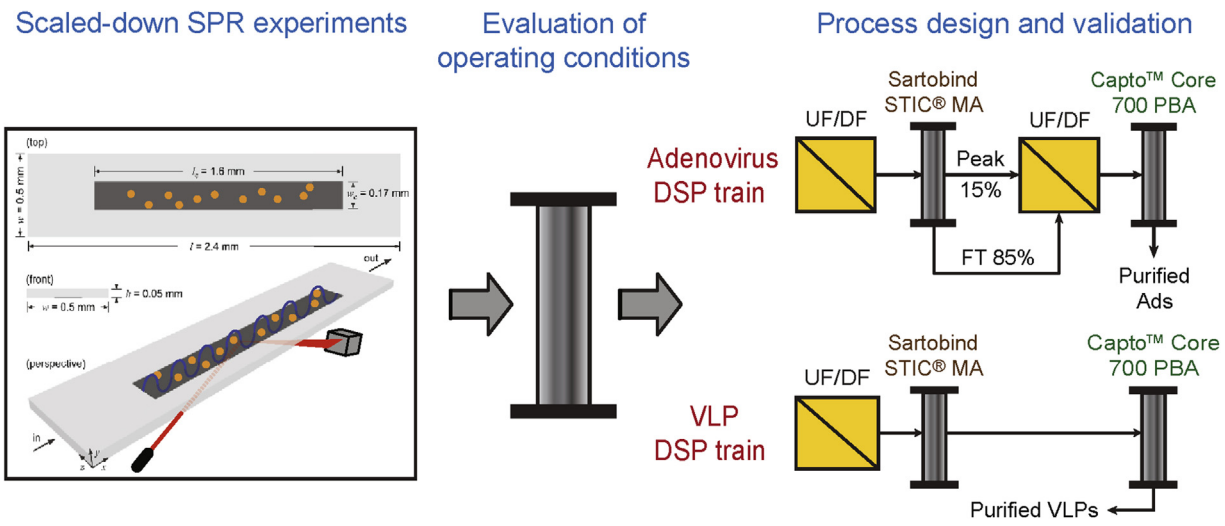

Fig. 1. Design strategy for optimization of FT platforms for Adv and VLP purification. The ability of polyallylamine (PAA) and octylamine (OA) ligands to bind DNA is evaluated by surface plasmon resonance (SPR). The SPR results are confirmed and the operating conditions are tested on a Sartobind STIC® membrane adsorber (MA) and on a Capto™ Core 700 packed bed adsorber. Once the constrains are identified, the FT processes are designed and validated experimentally for AdV5 and retro-VLP purification.

ion-exchange chromatography (IEC) is the most used technique for the purification of complex biopharmaceuticals [11–13].

Currently, the purification of viruses and VLPs by IEC is typically operated in positive (bind-and-elute) mode: most of the impurities are collected in the flowthrough pool, whereas the virus particles and some of the impurities are retained in the resin. Due to the differences in charge of the different biomolecules, it is possible to use IEC with high-resolution elution gradients for separating the biomolecules into fractionated cuts, even though the molecules are closely related.

Lately, however, new chromatography resins have also been developed aimed at intermediate purification and polishing in negative or flowthrough (FT) mode, which hereafter will be referred to as FT mode. For example, GE Healthcare Life Sciences recently launched CaptoCore™ 700 (CC700), a resin composed of a ligand-activated core and an inactive SE shell [14]. Small contaminant molecules enter into the beads where they are captured; viruses and other entities larger than a given cut-off molecular mass are excluded and are collected in the FT pool. Moreover, the multimodal character of the CC700's hydrophobic and positively charged octylamine ligands allows the resin to operate over a wide range of pH and salt concentration.

Iyer et al. [15,16] proposed a method for FT purification of viruses and VLPs using a combination of binding and size-exclusion chromatography (SEC). The technique relies on minimizing the external surface area per unit volume available for virus binding by increasing the mean diameter of the beads used in the column. At the same time the impurity binding capacity of the column is maximized by utilizing beads with multiple functionalities of the optimum size. These authors showed that the purification of different types of viruses and VLPs can be achieved using this technique.

Negative mode purification with membrane adsorbers has been used almost exclusively in the monoclonal antibody (mAb) industry [17–19]. Anion-exchange (AEX) membranes bind impurities such as viruses, host cell proteins, and DNA, while allowing the mAbs to flow through. If the membrane contains quaternary amine ligands (Q), the feed pH is usually greater than 7.0 and the ionic strength is low, as a high ionic strength can inhibit the electrostatic interactions and reduce the removal of contaminants. However, new primary-amine-based ligands are being investigated to circumvent the drawbacks of the Q ligand, whose binding strength is compromised at high salt concentrations. Sartobind STIC® (Sartorius Stedim Biotech) and ChromaSorb™ (Merck Millipore) are two examples of commercially available AEX membranes based on primary amine ligands [20]. A few studies of the performance of these novel chromatographic membranes have already been disclosed [21–23]. However, attempts to use membrane chromatography in FT mode for virus purification have not yet been reported in the open literature.

The present work reports on the rational design and implementation of FT platforms for AdV and VLP purification combining STIC membrane chromatography and CC700 packed bead bed technology; Fig. 1 shows a schematic of the proposed strategy.

As a preliminary design step, the intrinsic binding of DNA to the ligands of the STIC membrane and to those of the CC700 resin were evaluated by Surface Plasmon Resonance (SPR). This analytical technique is a powerful tool for real-time measurement of molecular interactions in a label-free environment [24,25] that can be used for downstream bioprocess development as a scale-down model of a chromatographic adsorber [26,27].

The design spaces for the STIC membrane and CC700 resin predicted by the SPR experiments reported in the present work were confirmed in a Sartorius Vivapure® Mini Ion Exchange (MIEX) spin column and in a XK 16/20 packed column, respectively, under various buffer conditions. Virus recovery yield and DNA and Host cell protein (HCP) clearances were assessed. Furthermore, the dynamic binding capacity for DNA under various ionic strengths was determined for both systems. Lastly, the data from the batch experiments were used in the design of platform FT processes for serotype-5 AdVs and retro-VLPs.

2. Material and methods

2.1. Cell line and medium

The AdVs were produced from 293 cells purchased from ATCC (ATCC-CRL-1573), which 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), in a humidified atmosphere of 8% CO_2 in air at 37 °C using shake flasks (Corning, USA). The cells were routinely propagated twice a week using an inoculum of 0.5×10^6 cells/mL. The cell concentration and viability were determined by counting cells on a Fuchs-Rosenthal