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A fast and efficient purification platform for cell-based influenza viruses by flow-through chromatography

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ABSTRACT

Since newly emerging influenza viruses with pandemic potentials occurred in recent years, the demand for producing pandemic influenza vaccines for human use is high. For the development of a quick and efficient vaccine production, we proposed an efficient purification platform from the harvest to the purified bulk for the cell-based influenza vaccine production. This platform based on flow-through chromatography and filtration steps and the process only involves a few purification steps, including depth filtration, inactivation by formaldehyde, microfiltration, ultrafiltration, anion-exchange and ligand-core chromatography and sterile filtration. In addition, in the proposed chromatography steps, no virus capture steps were employed, and the purification results were not affected by the virus strain variation, host cells and culturing systems. The results from different virus strains which produced by Vero or MDCK cells in different culturing systems also obtained 33–46% HA recovery yields by this platform. The overall removal rates of the protein and DNA concentration in the purified bulk were over 96.1% and 99.7%, respectively. The low residual cellular DNA concentrations were obtained ranged from 30 to 130 pg per human dose (15 μg/dose). All influenza H5N1 purified bulks met the regulatory requirements for human vaccine use.

1. Introduction

Since the outbreak of H5N1 avian influenza virus in 1997, the threat from newly emerging influenza viruses remains [1–3]. The vaccine industry is facing a difficult situation in that manufacturers have to deliver vaccines to the market quickly and allow for flexibility due to demand changes. Additionally, some countries, which did not have their own vaccine industries before, have to support the demand for pandemic influenza vaccines with self-production. Therefore, an efficient and economical manufacturing method for influenza vaccine production is urgently needed.

Cell-based vaccine production is considered to be an alternative to egg-based manufacturing due to its fast production in a short period [4,5]. In recent years, there have been several new downstream processes developed for virus purification instead of traditional sucrose gradient zonal ultracentrifugation (ZUC) [6,7]. Although purification by the ZUC method is efficient and has been used for decades, the drawbacks include high capital costs, extensive maintenance, and limited scalability [8–10].

A liquid chromatography method using a bind-and-elute mode has been extensively used for influenza virus purification. Lectin and cellulose sulfate ligands have been used for influenza virus adsorption [11–14]. Moreover, anion-exchange chromatography, which has membrane or monolith form supports, has been proven to efficiently purify viruses [15–18]. However, binding capacity variance [14] and virus yield [17,19] were high when purifying different virus strains and viruses from different host-cells. These variations caused by the binding affinity make it hard to adjust process parameters to provide robust production for the preparation of pandemic influenza vaccines.

In contrast to the bind-and-elute mode, liquid chromatography using a flow-through mode [20] shows potential for binding impurities and efficiently purifying monoclonal antibodies [21], viruses [22,23] and plasmid DNA [24]. This process has many advantages including easy operation, high productivity, and high and continuous purity [8]. Due to the size of influenza viruses (~120 nm), flow-through chromatography is a suitable purification method, and it can overcome the drawbacks of the bind-and-elute mode. In 2011, Lyer et al. reported a purification process for live influenza viruses using an anion-exchange chromatography (AEC) resin [8,25]. Impurities were adsorbed on the resin by diffusion into

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the bead's internal surface, and a high virus recovery was accomplished by using beads with reduced surface area. However, the virus yield was reciprocal to impurity removal, and the flow rate was limited by high resident times.

A ligand-activated core chromatography resin known as Capto-Core 700, which was designed for the flow-through mode, was launched by GE Healthcare and was used in a purification process for live influenza viruses [26,27]. In this process, cellular DNA was adsorbed by the AEC resins, and the cellular protein was entrapped by the Capto Core 700. The Capto Core 700 resin has an inactivated shell and a ligand-activated core, and large particles were prevented entry by shell pores with a cut-off size of 700 kDa. During the process, impurities are bound inside the core, while viruses pass through the pores. Additionally, the column can be used under various pH values and NaCl concentrations because of the multimodal ligands in the resin core [26]. The Capto Core 700 resin has been reported to efficiently eliminate impurities in the purification of adenovirus and respiratory syncytial virus (RSV) [28,29]. In the other applications, it has also substantially reduced ovalbumin concentrations for egg-derived influenza vaccines, and its removal rate was comparable to zonal centrifugation [30]. Several strains of cell-derived influenza A and B viruses were purified with the Capto Q and Capto Core 700 resins [31], and the purified results achieved the requirements set forth by the European Pharmacopeia [32]. This shows that the flow-through mode process is feasible and efficient for influenza virus purification. However, the above applications still require DNase treatment to remove the cellular DNA and have an extra cost for using Benzonase during the process.

In this study, a flow-through mode purification process combined with Capto Q and Capto Core 700 resins was established for inactivated whole-virion influenza vaccine production. A Benzonase treatment step was not used in this proposed process, and the purification was evaluated from the harvest to the bulk with a final sterile filtration. Different purification method strategies were also compared. The process performance was evaluated by purifying three different batches of H7N9 influenza virus harvests. Finally, evaluation of the flow-through purification process for purifying H7N9 and H5N1 influenza viruses cultured from different host cell lines and culture systems was studied.

2. Materials and methods

2.1. Virus production

Egg-derived influenza reassortant vaccine strains including H5N1 virus (IDCDC-RG6, which was derived from A/Anhui/1/2005, and provided by US CDC) and H7N9 virus (NIBRG-268, which was derived from A/Anhui/1/2013, and provided by the UK National Institute of Biological Standard and Control (NIBSC)) were used. Both viruses were adapted to grow in MDCK cells after several passages. An H5N1 influenza virus for culture in Vero cells was generated with modified HA and NA genes from the IDCDC-RG6 vaccine virus and six internal genes from the Vero-15 virus. Vero-15 is a high growth H5N1 vaccine donor strain that was adapted in Vero cells by the National Health Research Institutes (NHRI) [33]. An influenza H5N2 reassortant virus (E7-V15) was generated at the NHRI using the reverse genetics technology. The modified HA and NA genes were from a Taiwan local H5N2 virus (A/CK/CY/A2628/2012 E7) and six internal genes from the Vero-15 virus. Viruses produced by Vero cells were cultured in VP-SFM (Invitrogen, USA), while those produced by MDCK cells were cultured in Opti-Pro SFM (Invitrogen, USA). Cells grew on Cytodex 1 microcarriers at a concentration of 4 g/L in either spinner flasks (Coring, USA) or a 7.5-L bioreactor (NBS, USA). High-density cell culture systems, including a 500-mL BelloCell or a 20-L TideCell system (Cesco Bioengineering, Taiwan), were also used for cell growth and virus production.

2.2. Virus preparation from harvest

All of the virus solutions were first clarified with a 0.65 μm Sartopure PP2 depth filter (Sartorius, Germany) and then inactivated with 0.01% formaldehyde at 37 °C for 24 h. A further clarification was processed with a 0.45 μm Sartobran P filter (Sartorius, Germany). Then, the virus solution was concentrated five times via cross-flow ultrafiltration with a 300 kD PESU membrane (Sartorius, Germany). The feed flow rate was 170 mL/min, and the transmembrane pressure (TMP) was maintained at 0.1 bar. The filter area of the 0.65 μm depth and 0.45 μm micro filters were 500 cm^2, and the ultrafiltration cassette was 200 cm^2 for the 1 L virus harvest. The membrane area of filters and cassettes were proportional to the larger volumes used. Virus materials were stored at 4 °C until later liquid chromatography experiments.

2.3. Flow-through chromatography

An AKTA purifier 100 system (GE Healthcare, Sweden) was used for all the liquid chromatography experiments. The aim of this study was to evaluate how the overall process was affected by Capto Q and Capto Core 700 resins (GE Healthcare, Sweden). The experiment setup was adapted from application note 29-0435-49 AC from GE healthcare. The Capto Qresin was packed into a 16/40 column (GE Healthcare, Sweden) with a packing column length of 26 cm. The final column volume was 52 mL. The Capto Core 700resin was packed into a HiScale 16/20 column (GE Healthcare, Sweden) with a column length of 13 cm. The column volume for the Capto Core 700 resin was 26 mL. Before sample loading, the columns were equilibrated with 20 mM phosphate buffer with 0.5 M NaCl, and the pH value was adjusted to between 7.0 and 7.3. Virus samples were conditioned with 0.5 M NaCl and loaded with a flow rate of 250 cm/hr. After sample loading, two column volumes (CV) of buffer solution were used to wash out the remaining virus materials in the column. The flow-through and column wash solution fractions were both collected for measuring the virus recovery. In the flow-through mode, the virus sample was first loaded onto the Capto Q column, and the flow-through fractions were collected and then loaded onto the Capto Core 700 column. The Capto Q column was cleaned with 2 M NaCl and 1 M NaOH. The Capto Core 700 column was cleaned with 1 M NaOH with 30% isopropanol.

2.4. Dynamic binding capacity

A Vero-derived H5N2 influenza virus material was prepared and conditioned with 0.5 M NaCl before column loading. A 1 mL Hitrap Capto Q column (GE Healthcare, Sweden) was continuously loaded with 200 mL virus material, and the concentrations of cellular DNA were measured in the effluent. The 150 mL collected fractions from the Hitrap Capto Q column were then loaded onto a 1 mL Hitrap Capto Core column (GE Healthcare, Sweden). The concentrations of cellular DNA and proteins in the flow-through fraction were analyzed. The flow rate of the two columns was 1 mL/min, and the equilibration buffer was 20 mM phosphate with 0.5 M NaCl at pH 7.

The calculation of dynamic binding capacity (DBC) used the following equations:

$Q_{B,\ 10\%} = V_A \times C_0/V_C$

$Q_{B,\ 10\%}$ = Dynamic biding capacity at 10% level (μg/mL)

VA = Volume of feed applied up to the break point (mL)

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