Simplifying Progress

Purification of Newcastle Disease Virus Produced on a Novel Avian Cell Line CCX.E10

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Introduction

Newcastle disease virus (NDV) from the *Paramyxoviridae* family is a single-stranded, negative-sense, enveloped RNA virus of avian origin. There are many commercial vaccines based on live or inactivated virions that are used worldwide to protect against its high morbidity and mortality rates among infected poultry, thus minimizing the economic loss. Due to strong host-range restriction, NDV is highly attenuated in humans and other primates, therefore it has been evaluated as a vector for several vaccines and is also promising as an oncolytic vector. Whether for vaccines or viral-vector therapies, NDV particles must meet certain criteria for yield, purity, and concentration.

Having this in mind, the novel avian suspension cell line CCX.E10 is a significant advancement; the cell line is not a genetically modified organism (GMO), it comes in suspension culture or anchorage-dependent version and it grows in commercially available serum-free and animal-component–free media. When infected with NDV, CCX.E10 cells give high-titer harvests.

With a combination of production on a CCX.E10 cell line from Nuvonis, which enables drug manufacturers to operate independently of SPF chicken-egg supply, and CIMmultus SO3 monolith technology, we developed a highly effective purification process for NDV.

1. Materials and methods

- Production

NDV was produced using CCX.E10 suspension cells, which were cultivated at 37°C in a modified FreeStyle™ 293 expression medium (Thermo Fisher Scientific) in single-use spinner flasks ranging from 500 mL to 3 L (Corning) with population doublings of about 1/day. CCX.E10 cells were infected at a density of ~10^6 cells/mL with NDV La Sota strain and cultivated for 3 days. Infectious titer was analyzed by FFA (Focus-forming assay) in Vero cells. Harvest was clarified by centrifugation for 15 minutes at 4,400 g and subsequently treated for 2 hours at room temperature with Benzonase (20 U/mL, Millipore Sigma) and 2 mM $MgCl_2$.

- Purification

The load was prepared with 1:1 dilution of NDV HCB (clarified, benzonase-treated harvest) with equilibration buffer (50 mM HEPES, 200 mM saccharose, pH 7.0), and loaded on the CIMmultus SO3 column. After the sample application, column was washed with 50 mM HEPES, 200 mM saccharose, 75 mM NaCl, pH 7.5 folowing with linear elution 0 % to 100 % of 50 mM HEPES, 200 mM saccharose, 2 M NaCl, pH 7.5 for 50 CV. During chromatography ultraviolet (UV) absorbance measured at 260 nm and 280 nm was monitored as well as multi-angle light scattering (MALS, Wyatt), confirming the presence of large particles.

- Analytics

Collected fractions were analyzed for infectious viral titer with FFA, DNA concentration was measured using a Picogreen assay (Thermo Fisher), total protein concentration was measured with Bradford assay (BioRad), benzonase concentration was assessed by ELISA (SAFC), and size distribution was measured using Nanoparticle Tracking Analysis (NTA, NS300 Malvern Panalytical).

2. Results

Reducing the conductivity of the starting material enabled the binding of the virus to the CIMmultus SO3, furthermore promoting protein and DNA impurity removal in FT, which is indicated by high absorbance signals in FT. Low MALS signal in this fraction suggests the absence of large particles such as NDV (Fig. 1A). The main elution fraction resulted in 75 % recovery of infectious particles with 99.0 % depletion of proteins and 99.9 % removal of DNA impurities (Fig. 1B, 2A). Benzonase concentration in the eluate was below the instrument limit of quantitation (LoQ, 0.2 ng/mL). Thus, >99.8 % of the Benzonase reagent was depleted. The size distribution of eluted viral particles was more uniform compared with that of the load material, with mode particle sizes of 188.5 ± 1.9 nm and 168.9 ± 8.6 nm, respectively. No aggregates were observed (Fig. 2B, D).

Results from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showed that most protein impurities from the load material appeared in the FT fraction. A virus is eluted at high concentration and intense bands represent viral proteins (Fig. 2C). The protein impurity level is reduced in the eluted fraction, confirming protein impurity removal obtained with the Bradford assay (Fig. 2A, C). We determined the dynamic binding capacity (DBC) to be > 4.11 × 10^{10} FFU/mL of SO3 monolith (Fig. 2E). Transmission electron microscopy (TEM) confirmed the presence of intact NDV particles in the eluate.

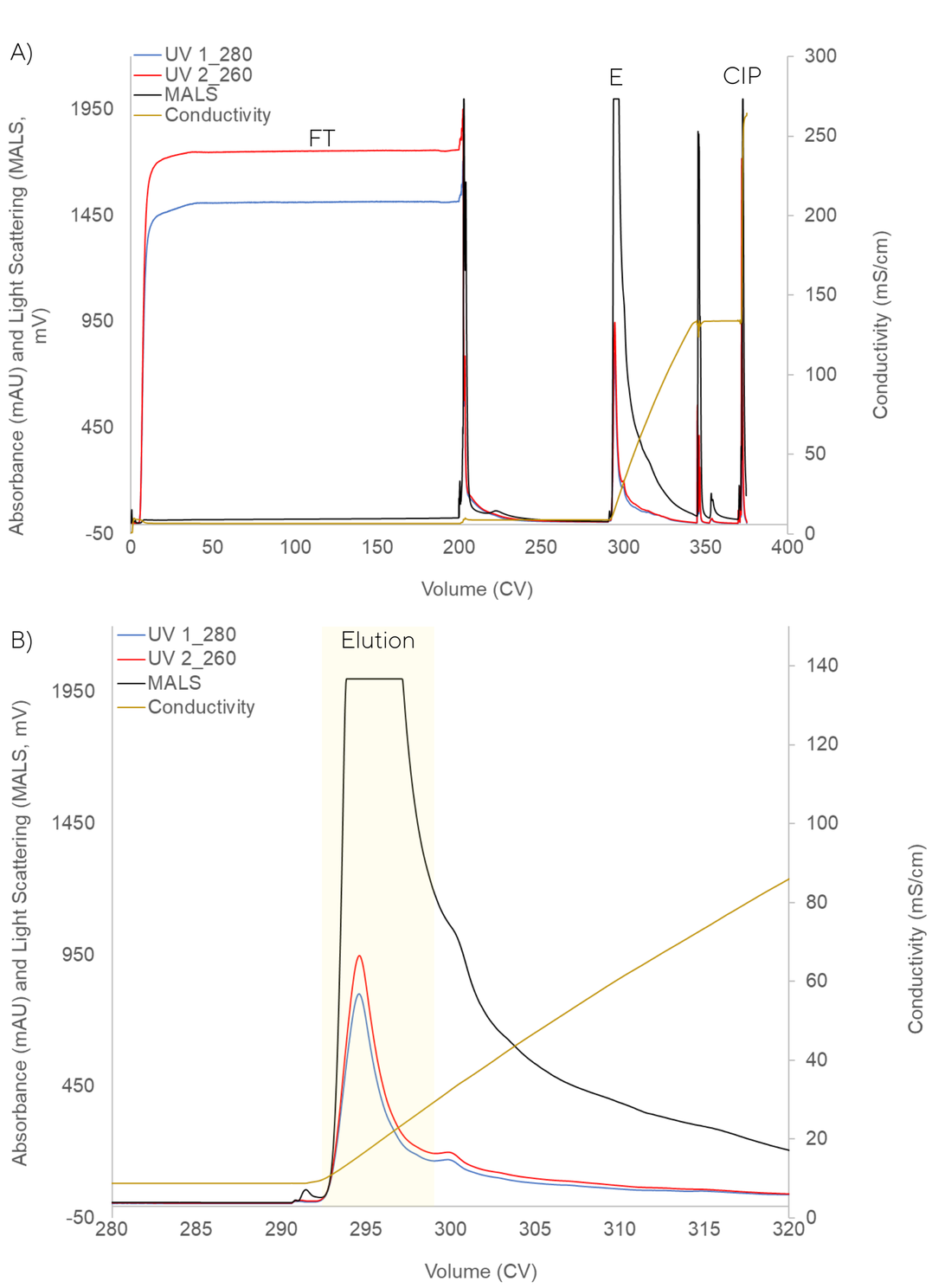
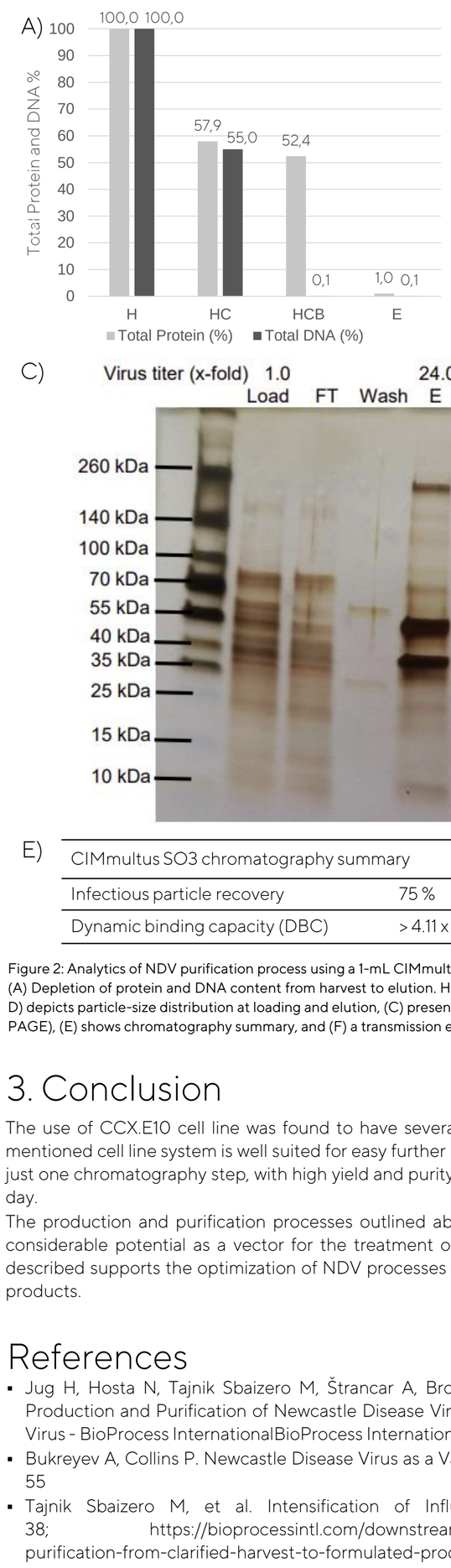


Figure 1: Purification of NDV using a CIMmultus SO3 1mL column; CIP = cleaning in place, E = elution fraction, FT = flowthrough, MALS = multi-angle light scattering, UV = ultraviolet, W = wash. (A) shows complete chromatogram, and (B) elution phase.



E)

CIMmultus SO3 chromatography summary	
Infectious particle recovery	75 %
Dynamic binding capacity (DBC)	> 4.11 x 10^{10} FFU/mL SO3

Figure 2: Analytics of NDV purification process using a 1-mL CIMmultus SO3 monolith;
(A) Depletion of protein and DNA content from harvest to elution. H = harvest, HC = clarified harvest, HCB = Benzonase-treated clarified harvest; (B, D) depicts particle-size distribution at loading and elution, (C) presents results from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), (E) shows chromatography summary, and (F) a transmission electron microscopy (TEM) scan of the elution fraction.

3. Conclusion

The use of CCX.E10 cell line was found to have several advantages over other avian cell lines. Virus produced on the mentioned cell line system is well suited for easy further processing. Downstream purification of NDV can be performed in just one chromatography step, with high yield and purity. The downstream process can provide a drug substance within a day.

The production and purification processes outlined above represent a robust manufacturing tool and show NDV has considerable potential as a vector for the treatment of infectious diseases and cancers in humans. The methodology described supports the optimization of NDV processes and enables biopharmaceutical companies to develop new NDV products.

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