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Non-infectious virus-like particles for the validation of membrane integrity and column performance in bioprocessing

*Hui Wang**, *Anton P.J. Middelberg*

Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane 4072, QLD, Australia

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ABSTRACT

Effective viral clearance is critical in both water treatment and biopharmaceutical manufacture to protect public health. In this study, virus-like particle (VLP) was used as a surrogate for membrane and column performance assessments. A reliable ELISA method was developed to quantify VLPs with high sensitivity and specificity. Log_{10} reduction value (LRV) was measured in a scaled-down manner by mimicking the unit operation using VLPs. This study provides a potential to utilise VLPs in the real-time validation on membrane and column performance in water-treatment and biopharmaceutical manufacturing industries.

1. Introduction

Viral contamination can be a serious safety issue for water treatment and in biopharmaceutical manufacturing. Physical separation processes, such as membrane filters and chromatographic resins in columns, are widely applied to achieve fractional separation but also to remove potentially-harmful viruses. It is critical to establish steps to validate virus clearance efficiency by monitoring membrane integrity and column performance in real-time.

Membrane processing has become a major technology for treatment and desalination of water (Alkhudhiri et al., 2012; Ebro et al., 2013). System performance and the quality and indeed safety of the water product can only be assured if the membrane is intact. Performance monitoring currently relies on surrogates for membrane integrity, which was considered to be an underperforming surrogate by the industry (Zydney and Reis, 2007; Antonya et al., 2012). For example, mixture of dextran was used as model molecule to monitor the integrity of membrane, which covers a large molecular weight range (Zydney and Reis, 2007). However, due to significantly different properties in terms of charge, structure or hydrodynamic permeability, it is inadequate to estimate the efficiency of a membrane with respect to another for removing viral particles (Rotureau et al., 2007; Langlet et al., 2008). An ideal test for performance monitoring would be an actual virus; however the injection of live virus into process streams is not viable from a cost or safety perspective. An acceptable surrogate that suitably mimics an actual virus and, critically, can be produced at low cost, remains an unmet yet important need.

In biopharmaceutical manufacture, the expression of therapeutic proteins in host cells will potentially be accompanied

* *Corresponding author. Postal address:* Patheon Biologics Brisbane, 37 Kent Street, Woolloongabba, Brisbane 4102, QLD, Australia. Tel.: +61 7 38962835.
E-mail address: h.wang2007@gmail.com (H. Wang).
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by the generation of viral impurities and potential viral contaminants, which are required to be removed or inactivated before formulation (FDA, US, 1997; Therapeutic Goods Administration, Australian, 2009). Ion exchange chromatography (IEC) is an efficient polishing step to remove impurities, including viral contaminants (Falconer et al., 2001; Cipriano et al., 2012). However, multiple re-use of IEC media will involve fouling, molecular build-up, ligand loss or degradation of the matrix support (Levison et al., 1999; Brorson et al., 2003). All those factors will conceivably lead to declining performance of resins and diminished virus removal. Thus it is crucial to establish efficient and robust methods to validate virus removal performance via IEC.

The virus $\log_{10}$ reduction value (LRV) is widely utilised for treatment efficacy measurement. LRVs are calculated by taking the $\log_{10}$ of the ratio of the influent virus concentration and the concentration in effluent material, with 1 LRV equalling a 10-fold reduction (Eq. (1)).

$$\mathrm{LRV} = \log_{10}\frac{C_i}{C_e} \quad (1)$$

where C_i is influent pathogen concentration and C_e is effluent pathogen concentration. The criterion of environmental agencies, such as USEPA in United States, DWI in United Kingdom, etc., for efficient reduction of viruses is to achieve an LRV greater than 4. Traditionally infectivity assay (Mattison and Bibawid, 2009) and real time quantitative PCR (Haramoto et al., 2010) are conducted to assess virus removal or inactivation. However there are several challenges facing those techniques, as they are time- and cost-consuming, and lack sensitivity to bacteria and viruses at low concentrations.

A virus-like particle (VLP) is an assembly of proteins (capsomeres) further organised into a nanoparticle (capsid) (Pattenden et al., 2005; Lua et al., 2014). From this definition it is clear that a VLP, unlike the parent virus it is modelling, is incapable of replication and is therefore inherently non-infectious. This singular characteristic makes VLPs interesting as a surrogate for membrane integrity and column performance testing; they resemble the virus yet cannot cause infection. They are also easier to manufacture at scale as cultivation of the live virus is avoided. Indeed, VLPs can be made very cost-effectively using bacterial cell factories coupled with self-assembly processing in reactors (Chuan et al., 2008; Ding et al., 2010). These characteristics make VLP an ideal tool with which to test membrane integrity and column performance within an operating process.

In this work, a 50 nm murine polyomavirus (MPV) VLPs assembled from VP1 protein was used for methodology development to assess the virus removal efficiency of membrane and chromatography, respectively. A sensitive and specific ELISA method was developed to quantify VLPs and determine LRV. An efficient method for membrane integrity monitoring has been established in a scaled-down manner by utilising centrifugal filters. Virus removal efficiency of IEC was also demonstrated by comparing various elution conditions.

2. Material and methods

2.1. Expression and purification of VP1 capsomeres

Recombinant wild-type MPV VP1 were expressed in *Escherichia coli* (*E. coli*) Rosetta (DE3) pLysS cells (Novagen, CA, USA) and purified as previously described (Chuan et al., 2008; Lipin et al., 2008). Briefly VP1 was expressed as a glutathione-S-transferase (GST) fusion protein that formed capsomeres in vivo. GST capsomeres were captured by affinity chromatography, after which the GST tag was removed by digestion with thrombin and subsequently gel filtration.

2.2. Assembling and analysis of VP1 VLP

Purified VP1 capsomeres were assembled into VLPs by dialysis against Assembly Buffer (0.5 M $(NH_4)_2SO_4$, 20 mM Tris, pH 7.4, 5% (v/v) glycerol, 1 mM $CaCl_2$) for 15 h at 4 °C. VLPs were analysed by transmission electron microscopy (TEM) as described previously (Lipin et al., 2008). VLP size distribution was analysed by Asymmetric Flow Field Flow Fractionation (AF4) coupled to multi angle light scattering (MALS) as previously reported (Chuan et al., 2008).

2.3. ELISA

Diluted VLP samples were absorbed to high-binding 96-well ELISA plates (Costar 3590; Corning Inc., NY) at 100 μL per well. After overnight incubation at 4 °C, the plate was saturated with PBST (137 mM NaCl, 2.7 mM KCl, 10.15 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4, 0.05% (v/v) Tween 20) containing 5% skim milk (23 °C, 90 min). After three times wash with PBST, plate was incubated with mouse sera, which were obtained from wild type VP1 VLP immunised mice on day 34 (Middelberg et al., 2011). Mouse sera were loaded initially at 5000-fold dilution followed by two-fold serial dilutions (23 °C, 90 min). After washing 4 times with PBST, HRP-conjugated goat anti-mouse IgG (Sigma-Aldrich, USA) was added at 2500-fold dilution (23 °C, 90 min). After 4 times washing with PBST, 100 μL tetramethyl benzidine chromogen solution (Invitrogen, Australia) was added to each well and incubated in the dark for 10 min. The reaction was stopped with 50 μL of 1 M sulfuric acid. Spectrophotometric analysis was performed at 450 nm using an ELISA plate reader (SpectraMAX 340 Microplate spectrophotometer; Molecular Devices, Sunnyvale, CA). End point titers were obtained from ELISA and determined as the highest dilution of serum for which the OD was 3 standard deviations above the mean OD of blank wells.

2.4. Virus removal via centrifugal membrane

Two kinds of centrifugal filters, Vivaspin® 2 (50 kDa COMW, GE Healthcare, Australia) and Ultrafree-MC (100 nm pore size, Millipore, USA), were used in this work. To mimic a compromised filter membrane, holes were made in the Ultrafree-MC centrifugal membrane using needles. One hundred microliter VLPs at 1 mg mL^{-1} was loaded onto filter membrane and centrifuged at 15,000 rpm, 5 min at 4 °C. VLPs in flow through were quantified via ELISA.

2.5. Virus removal via IEC

VLPs in Assembly Buffer were dialysed (4 °C, 15 h) against the Equilibration Buffer (25 mM Tris, pH 8.0, 50 mM NaCl) in a custom-made dialysis unit fitted with a 10 kDa Snake-Skin membrane (Thermo Scientific, Rockford, IL, USA). An AKTÄ Explorer 10 liquid chromatography system equipped with HiTrap Q FF anion exchange column (1 mL, GE Healthcare, Australia) was equilibrated with Equilibration Buffer. One hundred microliter of 220 μg mL^{-1} VLP sample in Equilibration Buffer was injected. A flow rate of 1 mL min^{-1} was applied.