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RESEARCH ARTICLE

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Mechanistic insights into viral clearance during the chromatography steps in antibody processes by using virus surrogates

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Abstract

Viral safety is required for biological products to treat human diseases, and the burden of inactivation and or virus removal lies on the downstream purification process. Minute virus of mice (MVM) is a nonenveloped parvovirus commonly used as the worst-case model virus in validation studies because of its small size and high chemical stability. In this study, we investigated the use of MVM-mock virus particle (MVP) and bacteriophage ΦX174 as surrogates for MVM to mimic viral clearance studies, with a focus on chromatography operations. Based on structural models and comparison of log reduction value among MVM, MVP, and ΦX174, it was demonstrated that MVP can be used as a noninfectious surrogate to assess viral clearance during process development in multiple chromatography systems in a biosafety level one (BSL-1) laboratory. Protein A (ProA) chromatography was investigated to strategically assess the impact of the resin, impurities, and the monoclonal antibody product on virus removal.

KEYWORDS

ΦX174, chromatography, minute virus of mice (MVM), MVM-mock virus particle (MVP), viral clearance

1 | INTRODUCTION

Biologic drugs produced by mammalian cell culture processes could potentially be contaminated by endogenous and/or pathogenic adventitious viruses, mainly from animal-based raw materials, or from the manufacturing process.[1] Demonstration of viral clearance, including virus removal and virus inactivation, is critical for any biopharmaceutical manufacturing process to ensure patient safety. Currently, viral clearance is assessed by spiking infectious model viruses during good lab practice (GLP) compliant viral validation studies using a scaled-down model of a downstream purification process. Since the handling of infectious viruses requires special safety facilities (minimally, biosafety level 2), these validation studies are usually performed at a contract research organization (CRO), which are costly, time-consuming, and logistically challenging. Noninfectious and robust virus surrogates are, therefore, highly desired for process development to gain an early in-house assessment of the viral clearance capability of the manufacturing process before the GLP viral clearance validation.

Adequate virus safety for enveloped viruses can be achieved by viral filtration and viral inactivation methods, such as low pH, detergent, or heat.[2,3] However, since inactivation procedures are ineffective for nonenveloped virus, virus removal by filtration must be supplemented by other orthogonal methods, such as chromatography, in order to provide adequate virus clearance across the overall downstream process. Minute virus of mice (MVM), a nonenveloped parvovirus, has been used often as a worst-case virus in validation studies because it is more challenging to remove in the downstream process due to its small size and chemical stability.[4,5]

Rachel Dyer and Yuanli Song contributed equally to this study.

[Correction added on September 3 2020, after first online publication: URL for peer review history has been corrected.]

Over the years, there have been numerous efforts to use virus surrogates to assess viral clearance during bioprocessing. The use of virus surrogates for assessing viral filtration, which is a size-based virus removal unit operation, has been very well established using model virus surrogates, such as bacteriophages PP7, PR772, and ΦX174.[6-10] Virus surrogates have also been utilized to define the virus particle binding mechanism to various chromatography resins, such as anion exchange (AEX), hydrophobic interaction (HIC), and multimodal (MMC) resins.[11] Hydrophobicity and isoelectric point (pI) of viruses have been reported to guide the development of chromatography steps for optimal virus removal, but the impact of the product protein and resin properties, as well as impurity levels, all have an effect on the retention of viruses, are not as easily predicted.[12-15] However, in some studies, bacteriophages have been shown to be different from MVM in charge and/or hydrophobicity.[12] Due to these differences, the interactions to chromatography resins, protein of interest, and impurities may not be representative, and, therefore, bacteriophages may be limited in chromatographic applications to consistently trend with MVM removal. Recently, a new noninfectious MVM-mock virus particle (MVP), with similar size, hydrophobicity, and pI as MVM, has been developed and showed promise as a virus surrogate to evaluate viral clearance by filtration and AEX.[16-18]

In this work, we compared the performance of ΦX174 (a well-studied bacteriophage) and MVP (the new noninfectious recombinant virus particle) as virus surrogates to MVM to perform preliminary, in-house viral clearance risk assessments for chromatography steps, with the ultimate goal of being able to assess the impact of process changes during development activities. In addition to experimental results, a molecular structural model was implemented to illustrate the surface properties of MVM, MVP, and ΦX174 in order to better understand the results we observed. Furthermore, we elucidate the mechanistic behaviors of viral clearance during Protein A (ProA) chromatography. We investigate the interactions between virus particles, monoclonal antibody (mAb), impurities, and chromatography resin as well as the pH impact on viral removal during ProA chromatography. The understanding of the interactions of virus-resin, virus-protein, and virus-impurity is helpful to guide process development and improve process robustness.

2 | MATERIALS AND METHODS

2.1 | Materials

All chemicals used in chromatography buffers were purchased from the following vendors: J.T. Baker, Amresco, Avantor, Macron, Croda, EMD Millipore, Ajinomoto, and Thermo Fisher Scientific. Resins including MabSelect™ Sure LX, Capto™ Phenyl (high sub), Capto™ MMC, and cyanogen bromide (CNBr)-activated Sepharose® were purchased from GE Healthcare. Other resins including POROS® 50 HQ and POROS® 50 XS were purchased from Thermo Fisher Scientific. Resins were packed into Omnifit columns (ID 0.66cm). The virus surrogates ΦX174 and MVP were purchased from Carolina Biological Supply Company (Burlington, NC) and MockV Solutions (Rockville, MD), respectively. All mAbs were produced at Bristol-Myers Squibb using a Chinese Hamster Ovary cell expression system. ΦX174 quantitative polymerase chain reaction (qPCR) reagents including Invitrogen™ PureLink™ Pro 96 Viral RNA/DNA Purification Kit and Real-Time Quantitative PCR (RT-qPCR) reagents were purchased from Thermo Fisher Scientific.

2.2 | Methods

2.2.1 | Virus and virus surrogate quantification

To quantify ΦX174, DNA was extracted using the Invitrogen™ PureLink™ Pro 96 Viral RNA/DNA Purification Kit following the vendor's protocol. An internal quantitative PCR (qPCR) method was developed to quantitate ΦX174 genomic DNA. The method was performed using a fluorogenic probe and flanking forward and reverse primers designed to bind to a repetitive sequence within the ΦX174 genome.[19] The forward primer sequence was 5′-CGCCATTAA TAATGTTTTCCGTAA-3′, the reverse primer sequence was 5′-CAT-CCCGTCAACATTCAAACG-3′, and the probe sequence was 6FAM-5′-CGCCTTCCATGATGAGA-3′-MGBNFQ. QPCR experiments for ΦX174 were performed using a QuantStudio™ 7 Flex Real Time PCR system (Thermo Fisher Scientific). Results were calculated using the QuantStudio Analysis Software.

To quantify MVP, Immuno-qPCR using the MVM-MVP kit from MockV Solutions was conducted. First, the samples were applied to the wells of a 96-well plate that was pre-coated with a capture antibody. After incubation, the wells were washed and a DNA conjugated detector antibody was added. After another incubation step, the wells were washed and a buffer was added to recover the DNA. The samples were then transferred to a nonbinding deep well plate. qPCR was performed according to vendor's instructions using a ViiA 7 Real-Time qPCR System (Thermo Fisher Scientific).

MVM data were generated at CROs using quantitative PCR or plaque assays following viral clearance validation study protocols.

2.2.2 | Virus surrogate comparability assessment

Several monoclonal antibodies with validated MVM clearance data were used in this study, and these mAbs cover a broad range of pI and hydrophobicity. For experiments using ΦX174, the load material was spiked to 1×10^7 phage/mL using a ΦX174 stock with a titer of 2×10^{10} phage/mL. For experiments using MVP, the load material was spiked to 1×10^9 particles/mL using an MVP stock with a titer of 1×10^{12} particles/mL. All chromatography experiments were performed in duplicate on an ÄKTA Avant 150 system (GE Healthcare) at room temperature. The operating conditions were the same as the validated MVM viral clearance studies conducted at CROs, including, but not limited to, column loading, pH, conductivity, flow rate, and column bed height (details not listed).

The virus removal capability of multiple modalities, including ProA, AEX, CEX, HIC, and MMC, were evaluated using different mAbs spiked with the virus surrogates (Table 1). ProA and CEX chromatography were performed in bind-elute mode, AEX and HIC chromatography were performed in flow-through mode, and MMC chromatography was operated in both flow-through and bind-elute modes. Load and eluate or flow-through samples were collected for ΦX174 or MVP quantification. For select processes, fractions from other steps, including washes and strips, were also collected for virus particle quantification.

2.2.3 | Virus particle structural modeling

Structural models of MVM, ΦX174, and MVP were generated using deposited structures with PDB codes 1MVM, 2BPA, and 4ZPY, respectively. Size and surface properties, such as charge and hydrophobicity, were analyzed using PyMol.[20-22] The virus particles were color coded according to the distance from the center of the particle ranging from 110 Å (blue) to 160 Å (red). The hydrophobicity of the virus particles was analyzed with normalized consensus hydrophobicity scale and displayed in PyMol with the most hydrophilic residue (Arg) in white and the most hydrophobic residue (Ile) in red.[23] The electrostatic potential of virus particles was calculated using Adaptive Poisson-Boltzmann Solver (APBS) with the Poisson-Boltzmann model.[24] The charged surface of each individual capsid protein in the virus particle was displayed in PyMol with positive charges in blue and negative charges in red.

2.2.4 | Investigation of viral clearance in protein A chromatography

In order to investigate the mechanism of virus removal in ProA chromatography, we evaluated the interaction of MVP with mAbs, the Protein A resin, and impurities.

First, we conducted a study to assess the interaction between MVP and different mAbs in a simple system. Several mAbs were conjugated to CNBr-activated Sepharose® resin following the vendor's instructions, which is optimized for the conjugation of mAbs to the resin base matrix. Briefly, lyophilized resin was resuspended and conjugated with 10 g mAb/L of resin. Conjugated resin was washed with Tris to remove any unconjugated mAb and block any unreacted CNBr sites, and the washes were analyzed for protein content to ensure that the resin was conjugated with an equivalent amount of mAb. The conjugated and washed resin was packed in a 0.66 cm ID Omifit column. A volume of 100 μL of the MVP stock (containing 1×10^{12} particles/mL) was injected into the column using an ÄKTA Avant 150 chromatography system (GE Healthcare), and the retention time of the MVP was measured using the UV absorbance profile at 280 nm. A column packed with unconjugated resin (no mAb) was also run as control condition.

Furthermore, we studied the impact of impurity content in the feed stream and impact of the impurity wash pH to deconvolute the interactions among MVP, mAb, Protein A resin, and impurities. The ProA chromatography experiments were performed on an ÄKTA Avant 150 chromatography system (GE Healthcare) at room temperature. The ProA experiments involve equilibration, load, wash 1 (chase, PBS buffer), wash 2 (impurity reduction, acetate, Tris, or carbonate buffer ranging from pH 5 to pH 10), wash 3 (bridging, Tris buffer at pH 7.5), elution (sodium acetate buffer at pH 3.2–4.0), strip, clean in place, and storage phase. The load, flow-through, wash, elution, and strip pools were collected and analyzed for MVP content. Each feed stream, containing different impurity contents, was spiked with 1×10^{9} particles/mL using an MVP stock with a titer of 1×10^{12} particles/mL. To evaluate the interaction of MVP and the Protein A resin (base matrix and ligand), we used phosphate-buffered saline (PBS) as ProA load. To investigate the interaction of MVP and impurities, we used null cell culture material. To investigate the interaction between MVP and the product protein, we used purified mAb buffer exchanged into PBS at a concentration of 4.9 g/L. As a process control, we concentrated the purified mAb load to 68 g/L and spiked into the null cell culture material to a concentration of 4.9 g/L, in order to investigate the clearance in a more representative feed stream. ProA chromatography were performed using either a pH 9 or pH 10 impurity clearing wash.

TABLE 1 Monoclonal antibody properties and experiment in this work

Molecule	pI	Hydrophobicity[a]	Subtype	Unit operations	Surrogate
mAb1	8.8	Mid	IgG1/IgG2	Protein A, HIC	MVP
mAb2	7.9	Mid	IgG4	Protein A, AEX	MVP, ΦX174
mAb3	6.9	High	IgG4	Protein A, CEX, AEX	MVP
mAb4	7.0	High	IgG4	Protein A, CEX	MVP, ΦX174
mAb5	6.8	Mid	IgG4	MMC	MVP
mAb6	7.7	Low	IgG4	HIC	MVP, ΦX174
mAb7	5.9	Mid	IgG4	N/A	MVP
mAb8	8.2	High	IgG1	N/A	MVP

Abbreviation: MVP, MVM-mock virus particle.
[a]mAb relative hydrophobicity was determined by measuring elution salt concentration during a HIC linear salt gradient experiment, data not shown.

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of ΦX174 and MVP as virus surrogates for MVM

In order to evaluate noninfectious virus surrogates to be used for preliminary assessment of viral clearance risk during process development, two virus surrogates, ΦX174 and MVP, were selected based on their similar physicochemical properties to MVM including size, pI, and hydrophobicity (Table 2). Following the vendor's suggestion, preliminary spike recovery studies were performed to screen buffers to ensure accurate quantification of the virus surrogates. We also demonstrated the virus surrogates can undergo up to two freeze thaw cycles (data not shown). The mAbs used in this study cover a wide range of pI and hydrophobicity, and different isotypes (Table 1). To compare ΦX174 and MVP as virus surrogates for MVM, we conducted the spiking study using the same operating conditions as the validated viral clearance studies performed at the CRO. Load, elution, or flow-through pools were analyzed by qPCR method to quantify ΦX174 titer, or by immuno-qPCR method to quantify MVP titer. Log reduction value (LRV) was calculated based on the following equation to align with the CRO on MVM LRV calculation.

$$\mathrm{LRV} = \log 10\left(\frac{\text{Load virus concentration} \times \text{Load volume}}{\text{Elution or flow through pool virus concentraiton} \times \text{Pool volume}}\right)$$

Figure 1 shows a comparison of LRV results between the surrogate data and data generated at the CRO using MVM. Several chromatography steps were evaluated including ProA, AEX, CEX, HIC, and MMC. In Figure 1a, we observed a correlation in LRVs between the ΦX174 surrogate results and the results using MVM for ProA chromatography. However, for HIC and AEX chromatography, ΦX174 was not capable of reflecting the MVM performance. In contrast, we observed better correlation in LRVs between the MVP surrogate results and the results using MVM over a broader range of chromatography operations, including ProA, AEX, CEX, and MMC chromatography (Figure 1b). Interestingly, a trend of slightly higher LRV for the MVP compared to the MVM was observed in ProA chromatography. One reason could be that the MVP is not real virus and lacks of the same post-translational modification, such as glycosylation and phosphorylation, as MVM. Thus, such difference could cause MVP relatively easier to be washed off in ProA chromatography.

TABLE 2 Comparison of MVM and viral surrogate properties

Attribute	MVM	ΦX174	MVP
Type	Parvovirus	Bacteriophage	Virus-like particle
Enveloped	No	No	No
Genome	ssDNA	ssDNA	No DNA
Capsid shape	Icosahedral	Icosahedral	Icosahedral
Size (nm) [10,17]	21–28	26–32	22–28
pI [17]	5.99	6.6	5.81
Hydrophobicity [17]	Mild	Low	Mild
Expression system	Mammalian	E. Coli	Insect

Abbreviations: MVM, minute virus of mice; MVP, MVM-mock virus particle; ssDNA, single stranded DNA.

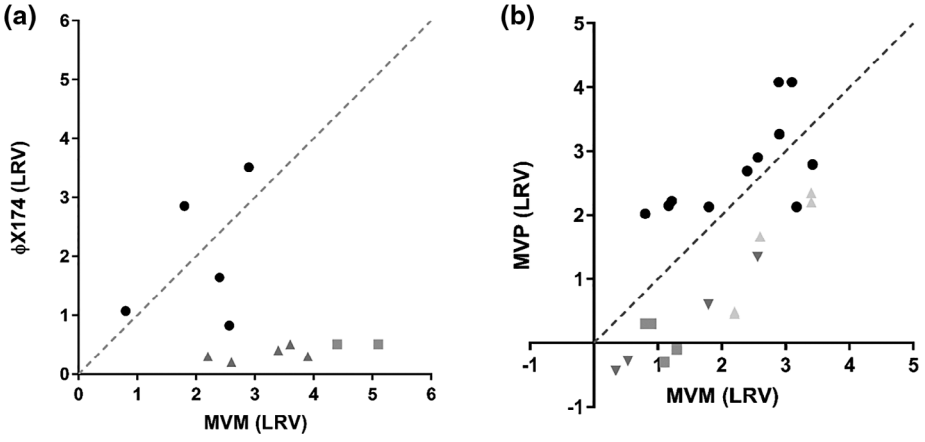

FIGURE 1 Viral clearance correlation between surrogates and MVM. (a) Correlation between ΦX174 and MVM clearance for Protein A chromatography (black circle) and for polishing chromatography steps HIC (gray square) and AEX (gray triangle). (b) Correlation between MVP and MVM for capture step Protein A (black circle) and polishing chromatography steps CEX (gray square), MMC (gray inverted triangle), and AEX (gray triangle). MVM, minute virus of mice; MVP, MVM-mock virus particle

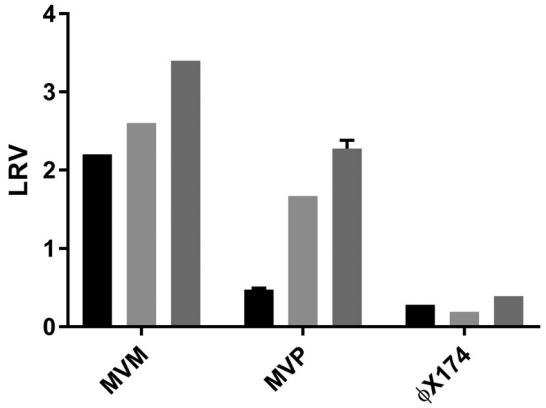

FIGURE 2 Virus clearance comparison of MVM, MVP, and ΦX174 in mAb3 process at the AEX step under AEX load conductivity of 2 mS/cm (black bar), 5 mS/cm (light gray bar), and 8 mS/cm (dark gray bar). Error bars indicate variation from duplicate experiments. MVM, minute virus of mice; MVP, MVM-mock virus particle

In order to evaluate predictive capability of the two virus surrogates for MVM clearance in a polishing step, their clearance in anion exchange chromatography was performed. AEX is one of the most commonly used polishing steps during bioprocessing, serving an important function to remove impurities such as host cell proteins (HCPs), DNA, as well as viral contaminants.[5,25] In this study, we tested the impact of load conductivity on viral clearance at 2, 5, and 8 mS/cm at a constant pH of 7.2, and compared these values to that from a validated MVM clearance study. The MVM, MVP, and ΦX174 LRV results are compared in Figure 2. The results show that the MVP clearance was improved with increasing conductivity from 2 to 8 mS/cm, and that this trend was also consistent with the result from the validated MVM clearance study. ΦX174 clearance data, however, do not follow the same trend as MVM. In an AEX system, virus binds to the resin, primarily, due to electrostatic interactions and it has also been reported that virus particles behave similarly to proteins.[13,15,25,26] Consequently, it is expected that viruses will bind to AEX resin more strongly than mAbs, and this interaction should be disrupted by increasing the salt concentration in the mobile phase. Previous literature has shown that under a low conductivity operating range (<9 mS/cm), the LRV could increase slightly with increasing conductivity.[25,27] In this study, MVP trended qualitatively with MVM clearance data, further demonstrating that MVP is a good virus surrogate to MVM, especially in chromatographic systems. To further explain this phenomenon, we used modeling to illustrate the differences in the structure of these two virus surrogates.

3.2 | Structural analysis of MVM, MVP, and ΦX174

It is known that MVM particle is comprised of 60 subunits including three viral proteins, designated VP1, VP2, and VP3 with size of 83.3 kDa, 64.3 kDa, and 61.4 kDa, respectively.[20] These three viral proteins start from different residue number at the N-terminus and share an identical sequence at their C-terminus. VP2 is the dominant variant in both full and empty viral particles. We selected 1MVM as the structural model for full MVM particle. MVP is assembled using recombinant VP2 without nucleic acids. We selected empty MVM structure 4ZPY as model for MVP. We selected ΦX174 atomic structure 2BPA as the model structure. The ΦX174 particle contains 60 copies of the J, F, and G proteins, as well as 12 copies of the H protein, per virion. Particle size, accessible exposed hydrophobic surface area, and accessible exposed net negative surface area were analyzed based on the selected model protein structure using PyMol, as shown in Table 3. The full MVM model (Figure 3a) is slightly larger than the MVP model (Figure 3c). The ΦX174 model has a larger radius up to 160 Å, including the surface protrusions, as shown in Figure 3b. The MVM model (Figure 3d) demonstrates similar accessible surface hydrophobicity to the MVP model (Figure 3f), but with higher accessible surface hydrophobicity than the ΦX174 model (Figure 3e). The accessible exposed net negative charge surface area of three models is not significantly different (Figure 3g–i). Our results are consistent with previous research that ΦX174 is less hydrophobic than MVM, and also slightly different in pI.[11] ΦX174 has large protrusions, which may reduce the virus particle contact to the resin (either AEX or HIC), and less surface contact will result in weaker interactions. Accessible exposed hydrophobic surface areas of MVM and MVP are similar, while that of ΦX174 is less than that of both MVM and MVP. Very subtle difference among accessible exposed net negative surface areas of MVM, ΦX174, and MVP is observed. The similarity in physicochemical properties between MVP and MVM support why we observed that MVP is a better surrogate for MVM in chromatographic systems than the bacteriophage ΦX174, as shown in Figure 1. In addition, the structural model can be utilized to explain the different performance between MVP and MVM. There are slightly less accessible hydrophobic exposed area and accessible exposed net negative surface area of MVP compared to MVM (Table 3), which could cause MVP bind to POROS HQ resin slightly weaker than MVM, and a lower LRV as a result (Figure 2).

Above all, it is clear that MVP is more similar to MVM from molecular structure level, which makes MVP a better virus surrogate

TABLE 3 Summary of MVM, ΦX174, and MVP structural analysis

Attribute	MVM	ΦX174	MVP
Distance of center to surface (Å)	110–128	115–160	108–125
Accessible exposed hydrophobic surface area (Å^2)	35,974	25,694	34,656
Accessible exposed net negative surface area (Å^2)	14,967	12,973	13,789

Abbreviations: MVM, minute virus of mice; MVP, MVM-mock virus particle.

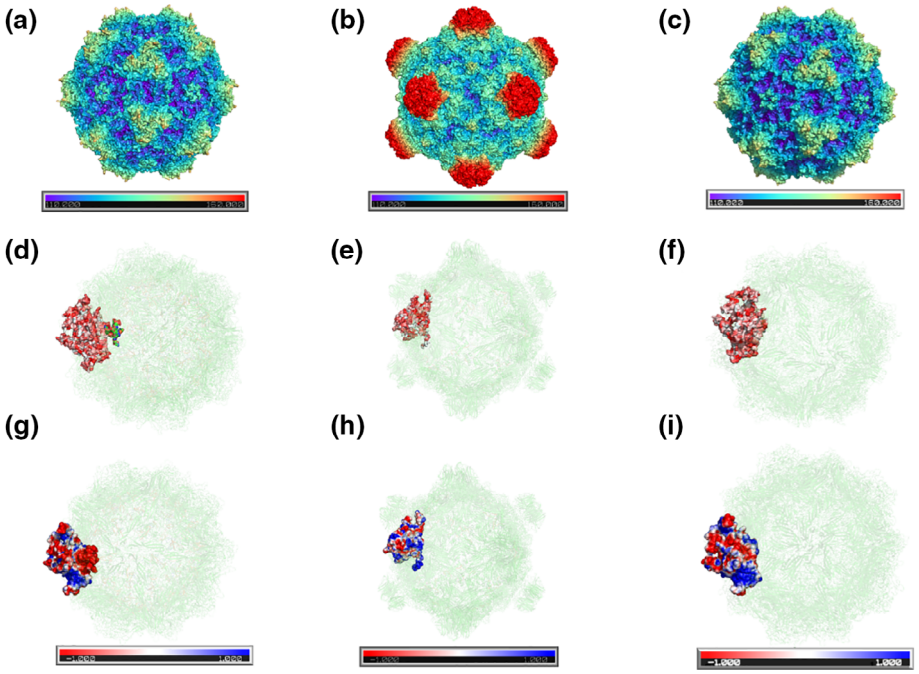

FIGURE 3 Size, hydrophobicity, and electrostatic potential of MVM, ΦX174, and MVP particles. (a–c) Models were colored according to the distance to the center of particles from 110 Å as blue color to 160 Å as red color. (d–f) Hydrophobicity surface of one protein unit of MVM, ΦX174, and MVP, respectively. Surfaces are shown in red as hydrophobic and in white as hydrophilic. Single protein unit is shown in the whole virus particle which is shown in transparent green cartoon mode. (g–i) Charge surfaces of MVM, ΦX174, and MVP. Negative charge surface is colored in red and positive charge surface is colored in blue. The whole virus particle is shown in transparent green cartoon mode. MVM, minute virus of mice; MVP, MVM-mock virus particle

to MVM. Hence, all subsequent studies have used MVP as the virus surrogate.

3.3 | Investigation of viral clearance during protein A chromatography

ProA chromatography is the most commonly used unit operation to capture monoclonal antibody and Fc-Fusion proteins during bio-processing.[28] It has also been reported that ProA can provide decent parvovirus clearance (1-4 LRV).[29-31] A better understanding of the virus removal mechanism during this step is important to design a robust Protein A step.

3.3.1 | Virus surrogate MVP interactions with mAbs

Cross-interaction chromatography has been successfully used to investigate protein and HCP interactions.[32] We applied a similar approach to investigate mAb and virus surrogate interactions. In this work, we immobilized several purified mAbs onto CNBr-activated chromatography resin via cross-linking, then injected the MVP onto the immobilized mAb chromatography columns. The control in this experiment is resin that was not conjugated with any mAb, but taken through the same conjugation process. Data show that the MVP has different retention profiles on these mAb-conjugated columns (Figure 4), which implies that viruses interact differently with mAbs and this can contribute to differences in viral clearance observed amongst different mAbs under the same chromatographic conditions. Unfortunately, we were not able to correlate the differences in

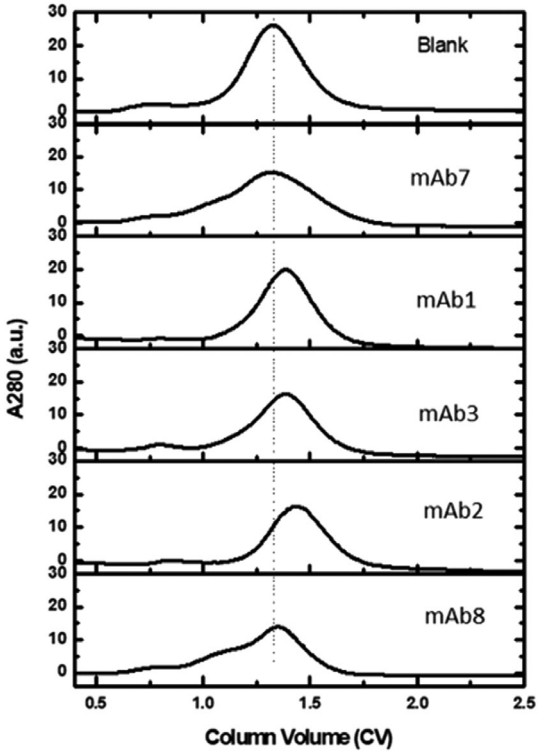

FIGURE 4 Chromatography profiles of MVP eluted from mAb conjugated columns using isocratic PBS buffer at pH 7.4. MVP, MVM-mock virus particle; PBS, phosphate-buffered saline

retention time to either the hydrophobicity, or the pI of these mAbs. This is likely due to the fact that the interaction between MVP and mAbs is a combination of hydrophobic, electrostatic, and many other interactions.

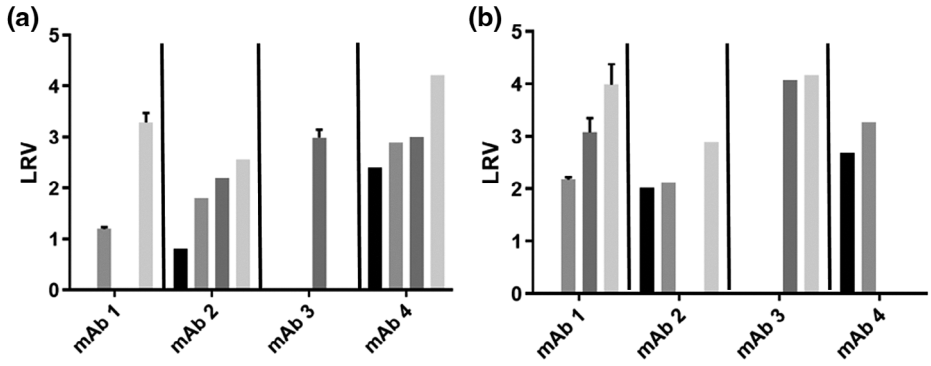

FIGURE 5 Protein A wash 2 buffer pH impact showing pH 5 (black bar), pH 7 (medium gray bar), pH 9 (dark gray bar), and pH 10 (light gray bar). (a) LRV data for MVM. (b) LRV data for MVP. Error bars indicate variation from duplicate experiments. LRV, log reduction value; MVM, minute virus of mice; MVP, MVM-mock virus particle

3.3.2 | Impact of protein A wash pH on virus clearance

Literature has also shown an impact of impurity levels on virus removal in chromatographic separations.[5,15,26] Given this information, we hypothesize that virus clearance can also be impacted by the interactions with both mAb and impurities in the feed stock. In an attempt to better understand this more complex interaction with the "dirty" feed stocks of ProA, we investigated the impact of wash conditions and different feed stocks on virus clearance in the ProA step.

We first performed a comprehensive study using four different mAbs and varying pH (5, 7, 9, 10, and) of the intermediate wash buffer (referred to as wash 2 buffer). Both MVP and MVM were used in this study and the summary of the LRV data is shown in Figure 5. As can be seen from the figure, for all four mAbs, virus clearance improved with increasing pH of the wash 2 buffer. More importantly, both MVM (Figure 5a) and MVP (Figure 5b) showed similar qualitative trends (if not exact LRVs) further building confidence in our virus surrogate model. A high pH wash in ProA chromatography has been reported to enhance the removal of impurities such as HCP and DNA.[33,34] It is expected that, similar to impurities, interactions with the virus may also be impacted by pH (pH > 8), hence the improvement in virus clearance observed at high pH conditions in these studies.

To further evaluate the impact of the wash 2 pH on ProA virus clearance, we conducted a set of experiments using two different pHs (pH 9 and pH 10). During ProA chromatography, the load sample as well as flow through, wash 1, wash 2, wash 3, elution, and strip fractions were collected. From these fractions, MVP levels were quantified and LRV was calculated for each individual fraction as shown in Figure 6. The strip sample MVP content was below the limit of quantification, so the data are not shown. From the data in Figure 6, it is clear that the lowest LRV, which is high MVP content, was found in the flow-through fraction, indicating that MVP flowed through the column while the antibody was retained on the resin. This is consistent with a previously discovered virus removal mechanism in ProA.[31] Interestingly, the wash 2 and wash 3 fractions show lower LRV when using the pH 10 wash and subsequent higher LRV in the elution pools indicating more MVP is removed with the higher pH wash. This result suggests that we can modify wash 2 conditions in order to optimize virus clearance during ProA. Furthermore, it shows that we can use the MVP as a virus surrogate to optimize the ProA step for viral clearance during process development.

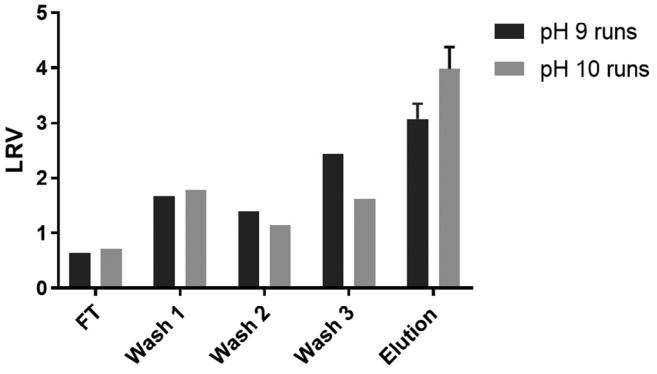

FIGURE 6 LRV of MVP in Protein A chromatography fractions with mAb1. During Protein A chromatography, the load sample as well as flow through, wash 1, wash 2, wash 3, elution, and strip pools were collected and LRV values were calculated. Strip data are not shown (all < LOQ). The molecule underwent experiments with impurity clearing wash (wash 2) at pH 9 (black) and pH 10 (gray). Error bars indicate variation from duplicate experiments. LOQ, limit of quantitation; LRV, log reduction value; MVP, MVM-mock virus particle

3.3.3 | Impact of protein A load material on virus clearance

In order to further explore the mechanism of removal and the complex relationship between MVP, resin, mAbs, and impurities in the ProA load, four sets of experiments were performed to de-convolute these interactions. We conducted each experiment with different feed streams with wash 2 buffer at pH 9 or pH 10. MVP levels of the elution pools were quantified and LRV was calculated (Table 4). We observed different interactions with each feed stream. MVP clearance from the PBS and null cell harvest material were very high (>4 LRV), out of the quantitation range, indicating the MVP is not being retained strongly on the column without the presence of the mAb. The MVP removal from feed streams containing the purified mAb, however, shows lower LRV, indicating a strong interaction with the

TABLE 4 Protein A chromatography MVP clearance with different feed streams and wash pH

Feed stream	Elution pool LRV	
	pH 9 wash	pH 10 wash
PBS	≥4.21	≥4.46
Null cell harvest	≥4.01	≥4.39
Purified mAb	3.11	2.64
Null cell harvest + purified mAb	3.21	≥4.65

Abbreviations: LRV, log reduction value; MVP, MVM-mock virus particle; PBS, phosphate-buffered saline.

mAb. In addition, it showed MVP clearance was not improved with pH 10 wash as expected from the previous studies. However, in the last set of the experiments, with the more representative feed stream containing the mAb with the impurities, it shows an increase in LRV from 3.21 to ≥4.65 with the pH 10 wash. Given this result, as well as previous studies demonstrating improved impurity removal with increased wash pH,[33,34] the data suggest that MVP clearance improvement is due to the increased clearance of impurities. This set of studies also suggests that the pH 9 wash is not strong enough to overcome the mAb interaction that we observed, hence the lower LRV value in the pH 9 experiments with feed streams containing the mAb with or without impurities. From this study, we observed that the MVP displays a low-level interaction with the resin and stronger interactions with the impurities as well as the mAb product. With the increase in impurity clearance with this molecule (data not shown) using the pH 10 wash, MVP appears to be removed along with the impurities, leading to improved clearance. In this study, the product aggregate level in the load material was <0.5%, which poses minimal risk of interacting with the virus surrogate. In future studies, the impact of aggregate levels could be explored further.

4 | CONCLUSIONS

In this work, we identified MVP, a noninfectious virus-like particle, as a predictive surrogate to MVM in a wide variety of chromatographic applications, including ProA, CEX, AEX, HIC, and MMC. This virus surrogate can be utilized for preliminary viral clearance assessments, as well as for research studies to enhance process understanding and process robustness. It could be widely utilized as it does not carry the risk that bacteriophages pose to bacterial cell lines. The quantifiable range of the MVP assay is currently the primary limitation for broader applications, as it does not allow for an accurate determination of LRV larger than 4. Due to this assay limitation, most viral clearance studies using AEX and VF are not quantifiable. However, with MVP as a surrogate, and a focus on unit operations and process conditions that result in virus reduction within the quantifiable assay range, we were able to demonstrate the use of the MVP in process development applications. Our structure analysis demonstrated that MVP had higher similarity to MVM with respect to size, topology, surface hydrophobicity, and surface charge. This provides the confidence that the data generated using this surrogate can predict the similar trend to that of the true virus removal. With this confidence in the surrogate, we investigated the mechanism of MVP removal at the ProA chromatography step. Our data suggest that the product (mAb)-MVP interaction plays a role on MVP removal in ProA chromatography, however, impurities (such as HCPs)-MVP interaction dictates the MVP removal, and the measures that improve impurity removal could also enhance MVP removal as a result. The impact of the product (mAb)-MVP interaction will likely play a significant role in polishing chromatography where impurity levels are much lower, this will be investigated further.

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