Poster Content as Presented at ASGCT 2020 Virtual

Single-Use Platform for Scalable Purification of a VSV-G Lentiviral Vector

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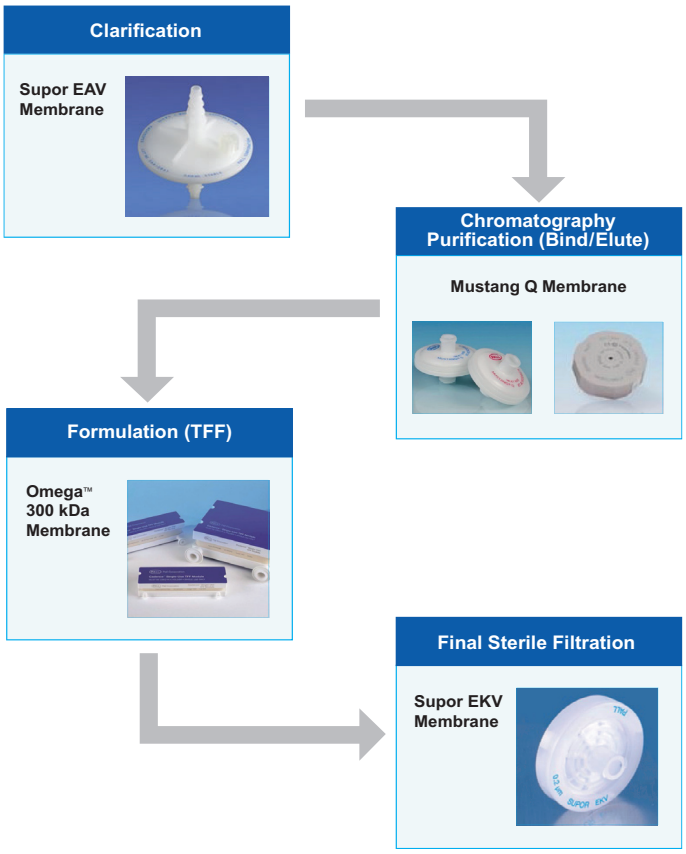
INTRODUCTION

Recent advances in viral vector based gene therapies have opened the door to life-saving, curative treatments. Many of these treatments are based on recombinant lentivirus (LV) for gene transfer. Purification of these vectors is complicated by their large size (120 nm) and fragile lipid envelope. Efficient purification of these vectors can be achieved using existing scalable, single-use technologies.

PURIFICATION STRATEGY USING PALL SINGLE-USE TECHNOLOGIES

The purification strategy achieves harvest clarification using bioburden reduction filters (0.45 µm), vector purification by membrane-based, anion exchange chromatography using Mustang® Q membrane, formulation concentration and diafiltration by flat-sheet tangential flow filtration (TFF), and final sterile filtration (0.2 µm sterilizing grade). Here, we demonstrate a single-use platform for purification of a VSV-G lentiviral vector using all downstream materials from Pall Biotech.



CLARIFICATION

Materials and Methods - Lentivirus Production and Quantitation

- HEK-293T cells (ATCC, Manassas, VA) grown in 10-layer CellSTACK♦ flasks
- 4-plasmid lentivirus packaging system with green fluorescent protein (GFP) transgene (iBET internal plasmids)
- Transient transfection with PEIpro transfection reagent
- LV harvested 48 and 72 hours post-transfection with full media exchange
- Cultures were treated with Benzonase♦ for 30 minutes before clarification (50 U/mL and 2 mg/mL $MgCl_2$)
- Infectious particle (IP) concentration determined by flow cytometry (GFP transduction)
- Total particles determined by P24 ELISA, INNOTEST♦ HIV Antigen monoclonal antibody (mAb)

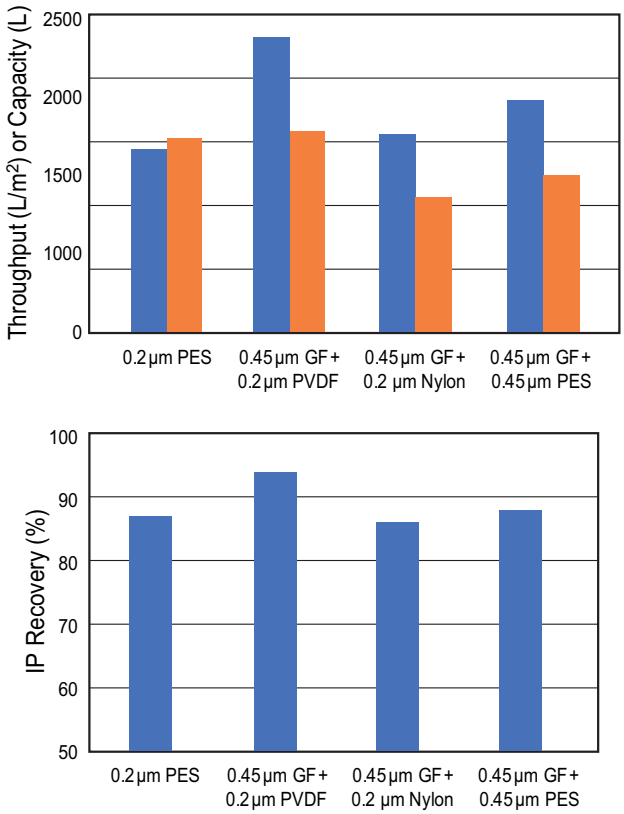
Materials and Methods

- 47 mm membrane disc filters (3.7 cm^2): 0.45 µm polyethersulfone (PES) (Supor® filter), 0.45 µm polyvinylidene fluoride (PVDF) (Fluorodyne® II DBL filter), 0.45 µm Nylon (Ultipor® N66 membrane), 0.45 µm PreFlow® UB capsules (glass fiber), 0.2 µm PES (Supor machV membrane)
- Supor machV EAV 20 cm^2 mini Kleenpak™ 20 capsule - KM5EAVP2S
- Constant pressure mode (0.5 and 1.0 barg)
- Equilibration buffer (EB) (10 mM histidine,150 mM NaCl, pH 7.5)
- Filters washed with 18 MΩ water and preconditioned with EB before clarification (20 L/m^2 each)

Results

- Bioburden reduction filters were evaluated for clarification performance under constant pressure mode at 0.5 barg and 1.0 barg. PVDF, Nylon and PES filters showed volumetric throughputs between 200-800 L/m^2 and recovery >70%. Much higher throughput and recovery were seen with the glass fiber (GF) filter reaching 1800 L/m^2 throughput and near 100% IP recovery. This GF filter (PreFlow UB capsule) is a nominal rated 0.45 µm filter. An absolute rated 0.45 µm is required (data not shown).
- We evaluated the performance of these absolute rated 0.45 µm filters with addition of PreFlow UB capsule as the first stage in a 2-stage configuration. A 0.2 µm PES filter (Supor EAV) was also included. Clarification was performed at 0.5 barg. Results are shown in Figure 1.

Figure 1
Clarification of lentivirus harvest



- 0.2 µm PES (Supor EAV filter) and 0.45 µm GF + 0.45 µm PVDF (PreFlow UB + Fluorodyne II DBL filters) showed the best performance when normalized to capacity of a 10 in. filter capsule, ~1500 L/m^2
- The 0.45 µm GF + 0.45 µm PVDF configuration showed the highest IP recovery, ~94%
- 0.2 µm PES is available as a single filter option and resulted in 87% IP recovery.
- 0.45 µm GF prefilter improved the volumetric throughput of all 3 absolute rated filters to >1500 L/m^2 (5–10X increase) with very good functional LV recovery (>85%).
- Generally higher LV recoveries are observed with higher operating pressure (1 barg compared to 0.5 barg) (data not shown).

MUSTANG Q MEMBRANE PURIFICATION

Materials and Methods

- Mustang Q Acrodisc® XT capsules (0.86 mL, MSTGXT25Q16) and Mustang Q XT capsules (5 mL, XT5MSTGQPM6)
- Loading flow rate 10 membrane volumes (MV) /min and pH 7.0, 60 MV wash with EB
- Elution performed as linear gradient (0.15 to 2 M NaCl in 25 MV) or 3-step elution (0.4, 1.0, 1.5 M NaCl)
- Elution buffer contained 0%, 3% or 17% sucrose
- Eluted LV fractions immediately diluted in low salt buffer

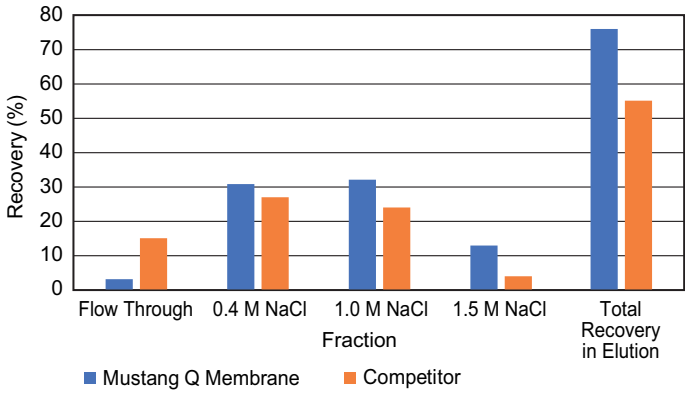
Results

Ion exchange purification is the next step in this purification process. Mustang Q 0.86 mL Acrodisc capsules were evaluated for LV binding and elution using a linear salt gradient up to 2.0 M NaCl. Functional LV was recovered in elution fractions up to approximately 1.0–1.2 M NaCl. Binding capacity of Mustang Q membrane was estimated to 1.5 x 10^{10} total LV particles/mL membrane (data not shown).

The effect of 0%, 3% and 17% sucrose in elution buffer was evaluated at 0.86 mL scale. Sucrose did not have a significant effect on elution performance (data not shown). 3% sucrose was used in elution buffer for all presented data.

Mustang Q XT capsules were evaluated for binding and elution performance using a 3-step elution strategy (0.4, 1.0, 1.5 M NaCl). Performance was compared to a similar sized competitor product. Functional LV recovery is shown in Figure 2.

Figure 2
LV binding and step elution in 0.86 mL Mustang Q capsules



- Mustang Q capsule showed lower LV detected in the flow through than the competitor product.
- Mustang Q capsule showed slightly higher recovery than the competitor product at each step resulting in ~20% higher global recovery.
- Data generally consistent between functional titer (TU assay) and total particles (P24 ELISA, data not shown)

FORMULATION

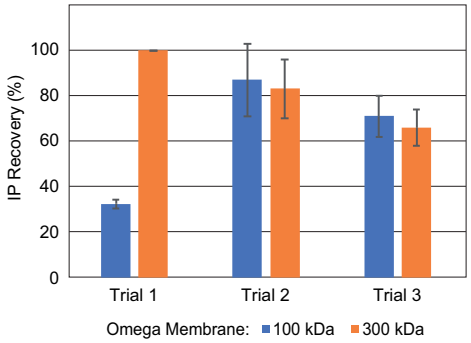
Materials and Methods

- 100 kDa Omega™ TFF cassette (T01 – 93 cm^2, (CSUM100T001) and 300 kDa Omega TFF cassette (T02 – 200 cm^2, OS300T02)
- Feed volume 20 L/m^2, constant feed flux of 5 $L/min/m^2$ (LMM), transmembrane pressure (TMP) of approximately 0.6 barg
- 5X volumetric concentration followed by diafiltration with 3 volumes of equilibration buffer
- Final system washed with 1 system volume of equilibration buffer, 10 mM histidine, 150 mM NaCl, pH 7.5

Results

Three trials were performed to evaluate to concentrate and diafilter LV into final formulation buffer (EB) using 100 kDa and 300 kDa Omega TFF cassettes. LV material was purified through Mustang Q bind/elute purification at 5 mL capsule scale. 20 cm^2 EAV clarification filters used. (Note that a different clarification filter was used in 1 trial). Highest conductivity elution fractions were directly eluted into low conductivity buffer. Pooled LV material was concentrated and diafiltered. LV recovery is shown in Figure 3.

Figure 3
LV after final formulation



- IP recoveries > 80% are possible with both 100 kDa and 300 kDa Omega membrane cassettes
- Poor recovery of virus with 100 kDa in the first trial
- Concentration limited by available material, final titer ~10^7 TU/mL

FINAL STERILE FILTRATION

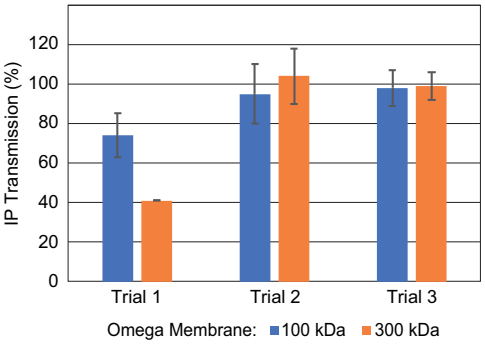
Materials and Methods

- Pall EKV Mini Kleenpak syringe filters (KM2EKVS)
- Filter preconditioned by flushing with water followed by EB, >20 L/m^2 each
- Material filtered manually

Results

The final step in this manufacturing process is sterile filtration. LV was sterile filtered with Supor EKV syringe filters. LV material from 100 kDa and 300 kDa TFF were kept as separate process fluids. IP recovery is shown in Figure 4.

Figure 4
LV final sterile filtration with 0.2 µm Supor EKV membrane

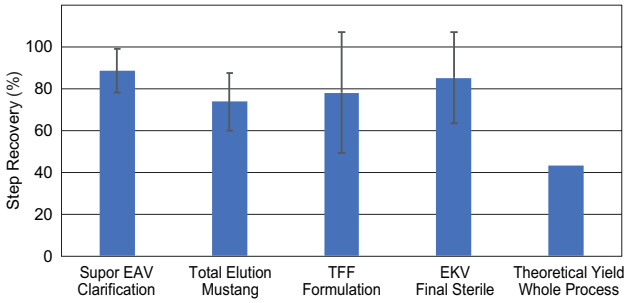


- Very good transmission of LV in Supor EKV filters in 2 of 3 trials. Note the LV titer of these fluids were generally 10^7 TU/mL. Further evaluation of Pall sterile filters for lentivirus transmission at significantly higher concentration are underway
- No apparent differences in LV recovery between 100 kDa or 300 kDa TFF cassettes used in the previous process step

FULL DOWNSTREAM PROCESS

Each unit operation was optimized separately. Figure 5 shows the average recovery for each unit operation during the 3 trials at 5 mL Mustang Q scale. The average theoretical full process yield was 43%.

Figure 5
Process yields by unit operation



Unit Operation	Trials Averaged
Clarification (20 cm^2)	2
Mustang Q purification (5 mL)	3
Formulation	6
Final sterile filtration	6

SUMMARY

- This data establishes feasibility of a full lentivirus vector purification process using all single-use materials from Pall Biotech.
- Lentivirus feedstock can be clarified with high volumetric throughput and IP recovery using either Supor EAV filter or 2 stage PreFlow UB + Fluorodyne II DBL filters.
- Mustang Q capsule shows higher global IP recovery than a competitor product. This was observed in both 0.86 mL and 5 mL capsules.
- Flat sheet TFF can be used for lentivirus final formulation with IP recoveries > 80% possible.
- Supor EKV filters can be used for final sterile filtration with near 100% LV transmission in 2 of 3 trials.
- Higher recoveries and system performance are expected with further process optimization.
- Further work with higher LV challenges is ongoing.

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