ASK THE EXPERT

Enabling Commercial-Ready LVV and EV Manufacturing Through Process Intensification

with Jae Jung and Keen Chung

RoosterBio and Repligen collaborated to develop a mesenchymal stem cell (MSC) derived extracellular vesicle (EV) production process that uses tangential-flow depth filtration (TFDF) for clarification. A separate Repligen collaboration with SK pharmteco applied the same TFDF technology in perfusion mode for lentiviral vector (LVV) production. In the LVV process, TFDF perfuses the bioreactor and harvests continuously. In the MSC-EV process, TFDF clarifies conditioned collection medium in a single downstream step. The perfusion approach achieved high viable cell densities (VCDs) at bench and commercial scales, demonstrating that intensified viral production scales linearly across different volumes. Integration of clarification into the harvest eliminated conventional depth filtration steps, streamlining downstream workflows and significantly increasing overall yield at commercial scale. Jae Jung (process development scientist at RoosterBio) and Keen Chung (associate director at Repligen) share their experiences in process development for both modalities in a June 2026 Ask the Expert webinar.

For LVV production, the transformation from traditional batch production to a TFDF-based perfusion workflow provides three critical advantages. First, the system enables high VCDs at transfection, establishing optimal conditions for viral production. Second, continuous perfusion after transfection enhances cell-specific productivity. Third, continuous harvest preserves viral-vector potency by minimizing residence time and preventing degradation that typically occurs in traditional batch processes.

For MSC-EV production, TFDF operates only as a clarification step, so the advantages take a different form. A single TFDF operation replaces the conventional multistage depth filtration train while delivering EV recoveries above 85%. The step scales directly from bench to pilot volumes at constant permeate flux, so clarification capacity keeps pace with upstream scale-up. EV critical quality attributes also survive the process, with identity and function confirmed in clarified material.

EV Platform Development

RoosterBio's MSC-EV manufacturing platform pairs MSCs with expansion medium and dedicated EV collection medium operating as a single integrated system. The 10-day bioreactor process includes an *N* – 1 seed chain and is designed for reproducibility at commercial scale rather than just bench feasibility. The fed-batch process incorporates a midprocess medium exchange to EV collection medium that contains minimal proteins and particles. That exchange ensures removal of growth-medium proteins and particles from the MSC-derived material and EV collection medium.

TFDF technology addresses clarification challenges by running conditioned collection medium tangentially across depth-filter hollow fibers, allowing permeate to pass through. Meanwhile, the tangential motion sweeps the fibers' surfaces to delay fouling. This geometry allows EVs to pass through into permeate while tangential sweep maintains flux without dead-end flux decay from build-up.

Key process parameters scaled excellently from 2.6 L at bench to 50 L at pilot scale, representing a roughly 20-fold volume increase. Filter area scaled proportionally from 30 cm^2 to 750 cm^2. Permeate flux remained constant at 650 $L/m^2/h$ across both scales, with diafiltration held at 0.49 diavolumes (DVs) of Dulbecco's phosphate-buffered saline (MilliporeSigma).

EV recovery through TFDF clarification consistently exceeded 85%, with upstream titers reaching >10 billion EVs/mL. Identity confirmation through tetraspanin marker expression and MemGlow-488 lipid-binding dye demonstrated that EV characteristics remained unchanged through processing. Functional assays such as anti-inflammatory activity and CD73 enzymatic activity confirmed that clarified EVs maintained their therapeutic properties, establishing TFDF as a scalable, productive, and characterizable EV clarification process suitable for clinical advancement.

Ultimately, the RoosterBio platform leveraged a high-productivity conditioned medium that yielded robust EV. Repligen's TFDF technology clarified the medium at both 3-L bench scale and 50-L pilot scale with a total particle recovery between 86% and 100% across runs.

LVV Process Development and Scale-Up Results

This portion of the collaboration began with adapting an existing SK pharmteco batch process into a TFDF-based perfusion workflow, starting with proof-of-concept studies at ≈2-L scale. Following feasibility confirmation, the process was scaled up to ≈50 L using identical parameters to validate performance for commercial LVV production. The harvest incorporated an integrated clarification step that simplified conventional workflows while increasing overall yield.

Studies comparing batch production and TFDF perfusion at 2-L scale yielded remarkable results across two independent runs. The first perfusion run demonstrated an 18-fold increase in LVV production compared to the controlled batch process, whereas the second run achieved an 11-fold improvement. The second run involved a different cell line. Although the batch process resulted in comparatively better titers than it did initially, the TFDF perfusion process maintained substantial advantages, confirming the robustness and reproducibility of the perfusion approach.

Scale-up from bioreactors of ≈1.6 L to 35 L represented a 20-fold volumetric increase, translating to 5 L to 120 L of total harvest volume. That change in scale resulted in a 23-fold increase in LVV production, demonstrating that outputs scaled proportionally and even slightly exceeded the values achieved at the initial scale. That scalability validates the TFDF perfusion process for commercial applications.

From a commercial perspective, a 50-L TFDF-intensified culture could yield >31,000 doses for in vivo chimeric antigen receptor T-cell (CAR-T) applications, assuming a dose of 2×10^8 transfection units (TU) per dose and a process yield of 20% recovery. To achieve equivalent output using traditional batch processes would require a 500-L bioreactor, representing significantly higher operational footprint and costs.

Questions and Answers

What are the key criteria for choosing TFDF over alternating tangential flow (ATF) and other such perfusion or clarification technologies? The selection between TFDF and ATF is primarily driven by the nature of a product and desired harvest strategy. Both TFDF and ATF are effective technologies for perfusion cell culture and to achieve high VCDs. However, taking product size into consideration, ATF filters use 0.2- or 0.5-µm pores. Such sizes are optimal for monoclonal antibody (mAb) processes but will retain larger particles such as LVVs. By contrast, TFDF filters have pores of 2–5 µm. Those filters allow viral vectors to pass through to harvest. Additionally, LVVs are unstable and thus must be transferred to cold storage immediately after harvest.

What impurities are most significantly reduced by continuous clarification and batch clarification? There are three impurities to account for: host cell proteins (HCPs), host cell DNA (hcDNA), and debris (e.g., from cells, dissolved proteins, and particles). In batch processing, impurities can accumulate in the bioreactor until harvest. This will result in much higher HCP and hcDNA burdens compared with the perfusion mode of culture. Although perfusion involves a higher VCD, it does not necessarily create high levels of impurities because of the continuous removal of metabolic waste. Results show that perfusion results in lower ratios of HCPs and hcDNA per virus particle.

What is the single biggest lever to unlock scalable cell and gene therapy (CGT) manufacturing in the next two years? We need to drive the cost down through process intensification. Using intensification technologies such as TFDF could produce large amounts of product in a short period of time, and we can reduce the time to deliver product to patients while reducing costs and minimizing footprints. For EVs specifically, the best way to prepare us for scalable CGT manufacturing is being able to provide media and cells at good manufacturing practice (GMP) grade at the scales needed to support commercialization.

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