Highlighting a Manufacturing Process for Stem-Cell–Derived Exosomes

with Elie Zakhem, Lauren Torres, and Jeremy Neidert

Demand continues to grow for supply of extracellular vesicles (EVs) such as exosomes that play an important role as intracellular messengers. However, EV manufacturing comes with challenges because of the material's complexity and fragility. Elie Zakhem (senior manager process development at RoosterBio), Lauren Torres (field application scientist from Repligen), and Jeremy Neidert (bioprocessing account manager from Repligen) presented an Ask the Expert webinar about scaling up EV manufacturing, which necessitates using well-designed upstream and downstream platforms to ensure reproducible yields.

The Presentation

RoosterBio partnered with Repligen to develop robust, scalable manufacturing processes for EV-based therapies that would be affordable and accessible to patients. The companies used RoosterBio's industrialized EV production platform and Repligen's scalable and low-shear filtration technologies. EVs were produced using RoosterBio's high-quality mesenchymal stem cells (MSCs), proprietary growth media and collection systems, and specialized analytical tools. Processing leveraged Repligen's clarification, storage, and disposal technologies, as well as its KrosFlo KTF filtration system.

Cell Therapy Innovation: Zakhem began by explaining industry interest in MSC-derived EVs. Such EVs have similar regenerative capabilities to MSCs but are easier to deliver than living cells. EVs are secreted molecules with better stability, more straightforward engineering, and a better safety profile than cells have. RoosterBio standardizes and industrializes its EV-manufacturing supply chain by producing high-volume cell banks paired with media system for cell expansion and EV collection.

Zakhem presented an EV-generation process diagram. RoosterBio used XenoFree human bone-marrow–derived MSCs to initiate a seed train for inoculation of a 3-L stirred–tank bioreactor. Cells were grown in RoosterNourish medium for five days, followed by media exchange for EV collection. After five days of collection, the medium was harvested for further processing.

Downstream Processing: Torres described Repligen's KrosFlo tangential–flow depth filtration (TFDF) technology. Repligen scientists split material from a 3-L bioreactor into three aliquots to screen filtration conditions using a 3-cm^2 flow path before scaling up to 30 cm^2. The new process reduced turbidity by 60.3%, enabling a recovery yield of 81%. The technology also reduces processing time and can scale up for use with 240-L bioreactors.

Neidert discussed how Repligen and RoosterBio sought to optimize the ultrafiltration and diafiltration (UF/DF) process to filter out the greatest amount of impurities at the highest flux possible. They tested membranes with different molecular-weight cutoffs (MWCOs). Ultimately, a membrane with a 750-kDa MWCO retained the most product while providing the greatest purification capability at the highest flux. During a reproducibility study, material from the process had limited product in the permeate, with >99% of product appearing in the retentate. Such results confirmed the utility of the 750-kDa MWCO.

The team also performed a study with applied parameters from the initial screening experiments to 600 mL of material. Processing for <3 hours gave typical flux-decay and pressure profiles and confirmed total recovery of >90%, with <1% of EVs in the permeate. The team then processed an entire 4-L batch through three reproducibility runs with similar flux, pressure, and processing time. Results showed favorable recovery.

Critical Quality Attributes (CQAs): Zakhem concluded by discussing different CQAs of the EVs. One such attribute is EV identity, determined by staining to show whether filtered material retained needed surface markers. Compared with material from the cell-culture harvest, samples that underwent TFDF or TFF showed similar percentages of positive staining. The team also evaluated EV bioactivity using an in vitro wound-closure assay. EVs generated in this process closed >80% of the wounds. Those results confirmed that the EVs maintained their identity and biofunctionality after being processed.

Questions and Answers

What buffers and temperatures are needed for long-term EV storage? Both literature and internal testing recommend –80 °C storage for purified EVs in phosphate-buffered saline (PBS).

Why is 750 kDa more effective than 500 kDa in EV recovery? We saw higher flux and filter rates when using 750 kDa compared with 500 kDa.

Where do you source your MSCs from? RoosterBio sources such cells from human bone marrow, adipose tissue, and umbilical cords.

View the full webcast at https://bioprocessintl.com/sponsored-content/advanced-technology-for-vector-based-therapies-highlighting-stem-cell-derived-exosomes-manufacturing-process.

Find the full webinar online at www.bioprocessintl.com/category/webinars.