# Separation and functional characterization of Stem Cell-derived Exosomes from Scalable Manufacturing Processes

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Stem cells and stem cell-derived products, such as extracellular vesicles (EVs), provide an outstanding therapeutic perspective for multiple diseases. To ensure translation into viable products, established lab-scale cell proliferation procedures have to be evolved into scalable manufacturing processes. Consistent and scalable cultivation of sensitive adherent cell lines, such as multipotent Mesenchymal Stromal Cells (MSCs), is challenging. Phoenestra has developed a stable process that uses telomerized MSC (MSC/TERT) lines and cell carriers inside an agitated packed bed bioreactor system under serum-free conditions. With this system, we are able to produce clinically meaningful EV quantities in small bioreactor volumes and in a fairly short amount of time. The downstream process results in high yields of EVs, determined and characterized by a combination of analytical methods. The PATfix™ analytical system was used to evaluate the composition of the samples and track several surface markers of EVs. Produced EVs show relevant biological functionality e.g., in cell-based anti-inflammatory or anti-fibrotic bioassays. In addition, bioassay results are put into the perspective of protein and miRNA profiles analyzed from EV preparations harvested from different MSC/TERT. In this presentation, we will show results of in-depth analytical characterization of samples processed from EV production runs, with the goal of getting closer to identifying mediators of biological activity. In our view, these insights will help product definition and accelerate clinical translation in the future.