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Research article

Dynamic expansion of mesenchymal stem/stromal cells in a stirred tank bioreactor promotes the release of potent extracellular vesicles

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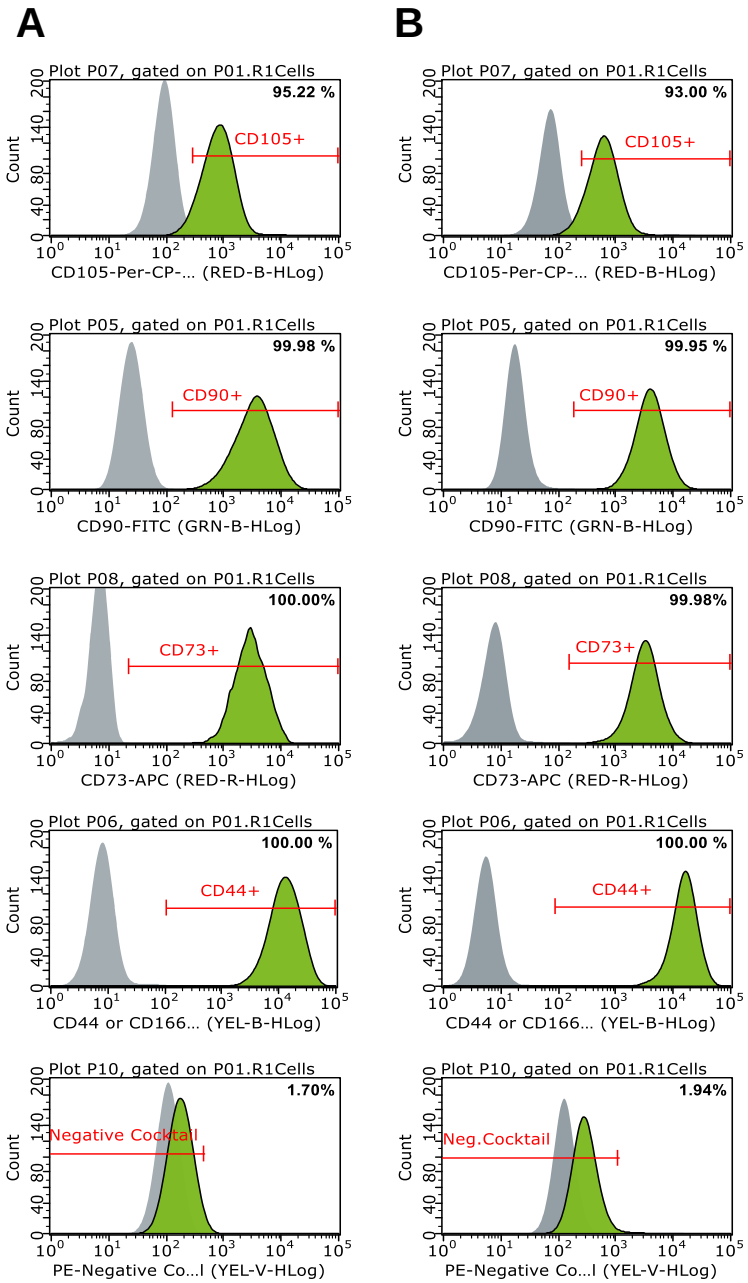
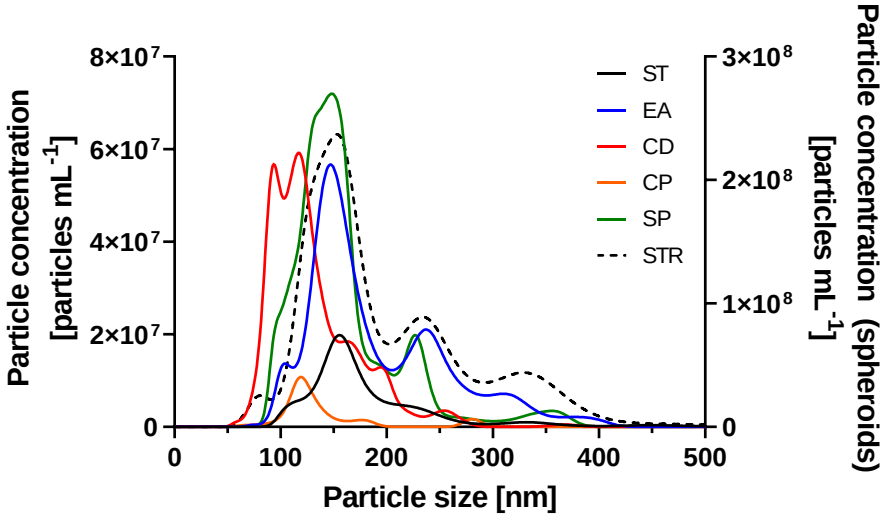
Abstract: Mesenchymal stem/stromal cell-derived extracellular vesicles (MSC-EVs) are considered a promising therapeutic tool in cell therapy due to their immunomodulatory, regenerative and angiogenic capabilities. However, there is a lack of process knowledge, particularly for a large-scale production of MSC-EV using fully controlled stirred tank bioreactor (STR) systems. For the establishment of a STR-based process, we investigated dynamic process set-ups in spinner flasks, using three different microcarriers, as well as in shaking flasks, using microcarrier-free spheroids. An immortalized cell line (hMSC-TERT) and a particle-free chemically defined medium was used for all approaches. Cell characteristics (e.g., growth, metabolism, cell-specific particle production rates), MSC-EV epitope markers and MSC-EV potency in migration assays were analyzed. We showed that the transfer to a dynamic system (non-porous microcarrier, spinner flask) significantly increased the cell-specific particle production rate (6-fold) and the expression of EV-specific markers. Moreover, MSC proliferation and, most importantly, the therapeutic potency of MSC-derived particles including EVs was maintained. We demonstrated that high cell-specific particle production rates were associated with an increased glucose consumption rate rather than cell growth, which can be utilized for future process development. Furthermore, we showed that dynamic conditions of a controlled 1 L STR significantly increased the cell-specific particle production rate (24-fold) as well as the final concentration (3-fold) of potent MSC-derived particles including EVs. This indicates that fully controlled STRs are an efficient production system for MSC-derived particles including EVs that may open and facilitate the path for clinical applications.

Keywords: potent EV production; microcarrier-based process; large-scale MSC-EV production; scratch assay; EV analysis

Supplementary



Supplementary Figure 1. Surface marker analysis of hMSC-TERT after 168 h in culture in column (A) ST control and column (B) spinner flasks using EA microcarriers. Positive markers were CD105, CD90, CD73 and CD44 and the negative cocktail consisted of CD45, CD34, CD19, CD11b and HLA-DR. Isotype controls are shown in gray and marker expression [%] in the top right corner.



Supplementary Figure 2. Analysis of mean particle size distribution of static (ST) cultures, dynamic cultures in spinner flasks using different microcarrier types (EA – Enhanced Attachment, CD – Cytodex 1, CP – Cytopore 2), microcarrier-free cultures as spheroids (SP) and cultures in STRs using EA microcarriers. Mean particle size distribution was assessed after 168 h for ST, EA, CD cultures, after 120 h for SP cultures and after 144 h in STRs. Data are presented as means of triplicates.