A HOT NEW BIOPROCESS STRATEGY TO IMPROVE SMALL EV PRODUCTION

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As an emerging new therapeutic format, small extracellular vesicles (sEVs), are promising cell therapy substitutes and can serve as platform technology for engineered designer sEVs. Many studies lately demonstrated the huge potential of both approaches. For the future, economic productivity will be crucial for the applicational success of sEVs as new therapeutics. Just as in the early days of antibody production, current sEV production suffers from very low yields. Since the 1980s, monoclonal antibody yields were increased by several orders of magnitude from 0.1 to 10 g/L via media optimization, cell line- and process engineering. Therefore, the presented study aimed at boosting sEV production via new bioprocess strategies.
Initially, a screening DoE was performed to identify cultivation parameters influencing sEV productivity in HEK293 suspension cells, a prominently used cell type for designer sEVs. The evaluated parameters were oxygen supply, power input, redox milieu, and temperature. We found that short-term hyperthermia had a strong influence on sEV secretion, boosting the sEV yield 3-fold, while cells maintained >90 % viability with a slight decline in growth rate for up to 72 h at 40°C.
Subsequently, we applied next-gen transcriptome sequencing and quantitative SILAC-MS proteomics to analyze the effects of the temperature shift on cellular and sEV compositions, respectively. As expected, the response of the cellular transcriptome and proteome was primarily shaped by an upregulation of heat-shock proteins and other chaperones to cope with the increased temperature stress. However, among the upregulated transcripts were also 549 gene-ontology annotated “extracellular exosome” identified and the highest fold-change of upregulated transcripts was found for MOB4, encoding a protein involved in membrane trafficking. After manual curation, we overexpressed a selection of the emerged genes and found that some phenocopy the effect, e.g., Rab11. These genes might thus indeed be causative for the observed phenotype.
More surprisingly, the characteristic heat-shock response was not at all pronounced in the sEV composition. From the classical heat-shock proteins, only Dnaj1b and Hsp105 were enriched, while others like Hsp72 and Hspa4l were even depleted. Compared to the control sample, hyperthermic sEVs contained significantly less ribosomal and histone proteins, components mostly considered undesirable in this fraction, and were significantly enriched in exosome biogenesis and marker proteins, like Flotillin-1, Alix, CD81, Tsg101 and many more.
We conclude that a hyperthermic shift serves not only as a possible bioprocess strategy to boost EV production, but also does not harm EV quality in an obvious way. Quite the contrary, typical exosome markers were enriched, and common contaminants depleted.

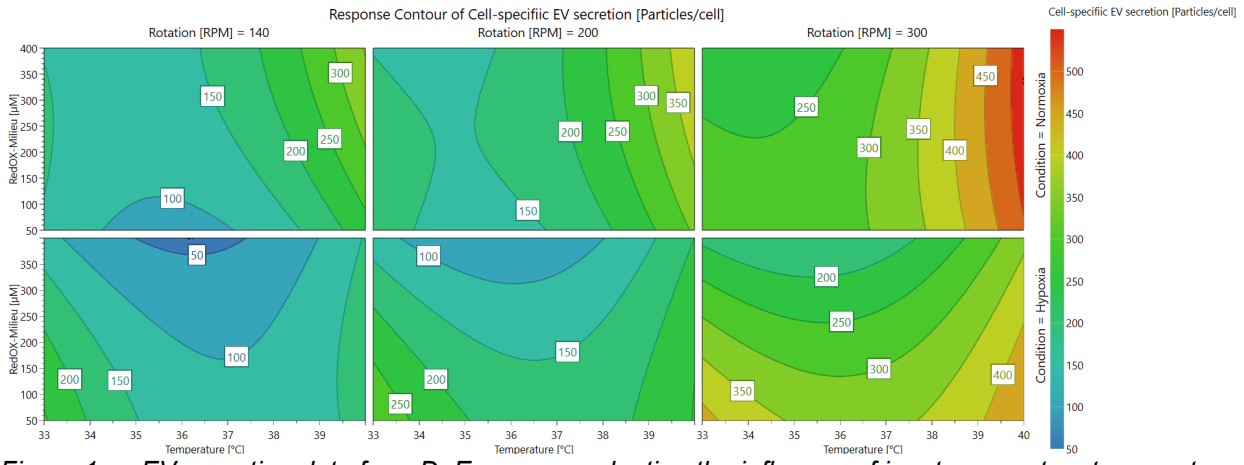

Figure 1 – sEV secretion data from DoE screen evaluating the influence of input parameters temperature, oxygen supply, rotation speed and redox milieu. Blue indicates low sEV secretion and red high sEV secretion.