Small-scale Bioreactor Cultivation of HEK293-based Suspension Cells Increases Extracellular Vesicle Yield

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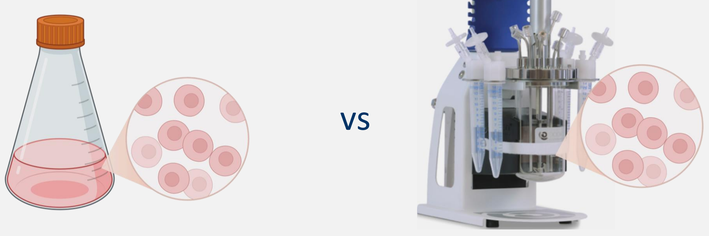
Background

Extracellular vesicles (EVs) are increasingly explored as natural vehicles for drug delivery and gene therapy approaches. However, **reproducible yield and scalability of EV production still pose major challenges** in the clinical translation of EV-based therapies. Currently, therapeutic EVs are commonly obtained from human embryonic kidney (HEK293) cells. At **ExoVectory**, we explore HEK293-based derivatives such as the suspension-grown Expi293F™ and investigate their potential to produce (therapeutic) EVs in a controlled scalable manner.

Objective

The objective of this work is to quantify and characterize EVs released by suspension-cultured **HEK293 cells (Expi293F cells) grown in shaker flasks or small-scale bioreactors** with the aim to investigate how the culturing environment affects EV production yield.



Methodology

Expi293F cells were cultivated (n=3) in either shaker flasks or a multi-use bioreactor system (miniBio 500 mL) and **total cell density, viability,** and **size** were monitored. Supernatants were drawn daily post-cell seeding and were analyzed for **EV quantity & size, morphology, and CD63 expression**.

Countess NTA EVQuant TEM WB

Results

1. Comparison of Expi293F **cell growth** in shaker flasks and bioreactors

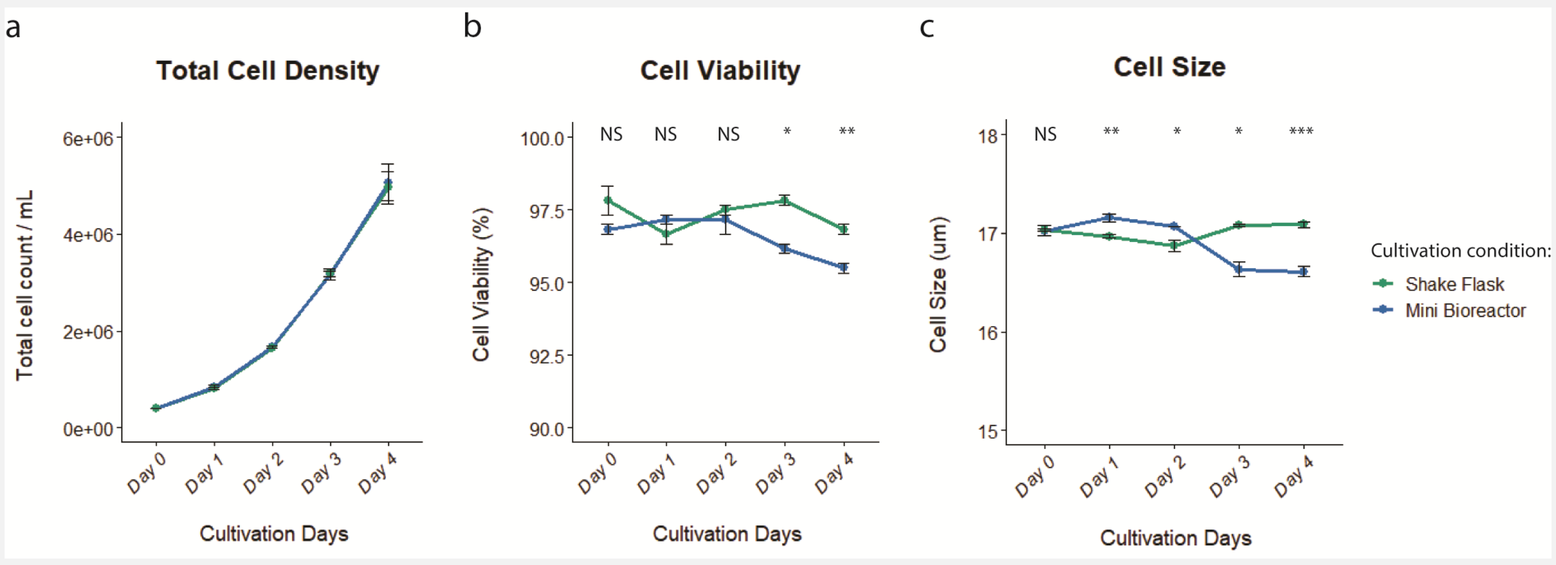


Figure 1 - Three independent runs (n=3) were performed for each cultivation method and **cell growth parameters** were analyzed on clarified supernatants collected daily during the cultivation period. **a)** Analysis of total cell density indicated no differences between the cultivation settings. **b)** Analysis of cell viability and **c)** cell size indicated modest but statistically significant decreases for Expi293F cells cultivated in the bioreactors at the end of the cultivation period compared to cells cultivated in shaker flask conditions. Data shown as Mean ± SD for the three independent runs.

2. Comparison of Expi293F **EV production** in shaker flasks and bioreactors

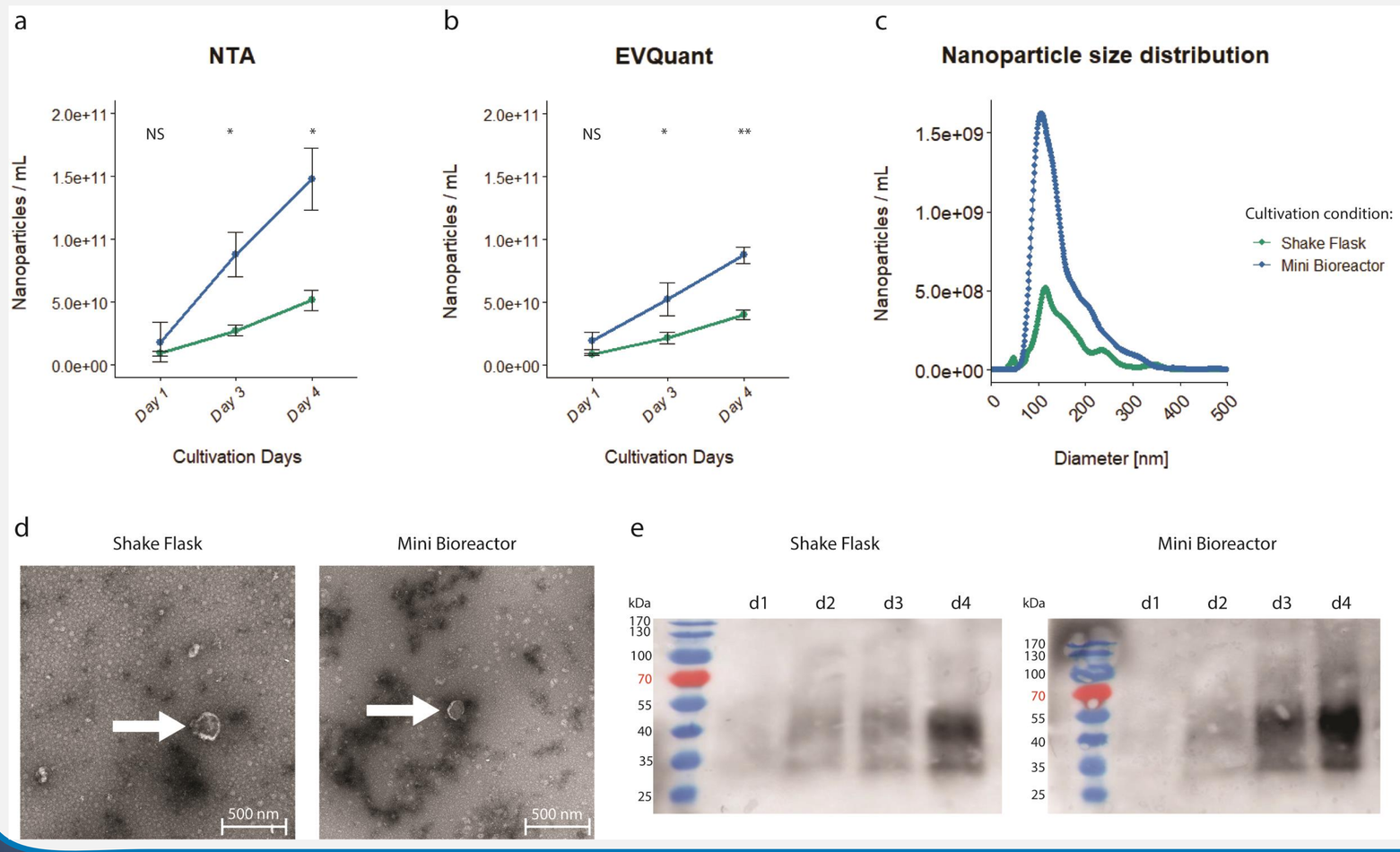


Figure 2 - Three independent runs (n=3) were performed for each cultivation method and **EV production characteristics** were analyzed on clarified supernatants collected at day 1, 3 and 4 during the cultivation period. Nanoparticle quantification by **a)** nanoparticle tracking analysis (NTA) and **b)** EVQuant demonstrated significantly increased nanoparticle yields by Expi293F™ cells cultivated in bioreactors compared to the shake flask environment at the end of the cultivation period (NS: non-significant, *: $p < 0.05$, **: $p < 0.01$). **c)** Size analysis by NTA demonstrated similar profiles in samples drawn from either cultivation condition. Data shown represents measurements from one shaker flask (green) and one bioreactor (blue) sample. **d)** Morphological assessment of the nanoparticles revealed cup-shaped structures, indicative of EVs. **e)** Western blot directed against the putative exosome marker CD63 demonstrated increasing signal over the course of the cultivation period, with the strongest signals observed in the clarified supernatant samples drawn from the bioreactors. Data shown as Mean ± SD for the three independent runs.

Conclusion

- Both cultivation conditions yield comparable production cell numbers
- Significantly increased EV production yield in the bioreactor system
- No differences in terms of EV characteristics
- The multi-use bioreactor system (miniBio 500 mL) is highly useful for the controlled production of EVs

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