THE ROLE OF EXTRACELLULAR VESICLES IN THE MODULATION OF THE CELL DENSITY EFFECT

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One of the current challenges in the animal cell bioprocessing field is the so-called cell density effect (CDE). It refers to the reduction in cell-specific productivity when transient expression is carried out at increasing densities, as a result of reduction in transfection efficiency [1]. Although it has perplexed the cell bioprocessing field for many years, the molecular causes of the CDE remain unknown to date. When producing HIV-1 Gag virus-like particle (VLP) in HEK293 cells by transient transfection, cultures above ~3-4·10^6 cells/mL are not successfully transfected and VLP cell-specific productivity drops. Studying the cell culture media at high cell density (HCD), we observed that changes in extracellular vesicle (EV) concentration impacted transfection efficiency. In this work, the role of EVs in the modulation of the CDE was studied with the aim of restoring transfection efficiency at HCD, improving scalability of the VLP production process.

Our results suggest that EVs influence transfection efficiency of cell cultures via both physical and physiological effects. EVs physically affect the uptake ability of the cells to incorporate DNA:PEI polyplexes as their interaction triggered polyplex break down. The reduced quality of the resulting polyplexes upon interaction with EVs was characterized by cryo-TEM, DLS and NTA. To test this effect, cells at 2·10^6 cells/mL were transfected after replacing the medium with spent medium coming from a 12·10^6 cells/mL with high EV concentration, resulting in complete inhibition of transfection. After depleting this spent medium from all EVs, transfection was restored to standard values. Moreover, VLP production improved 2-fold compared to a standard transfection at 2·10^6 cells/mL.

Likewise, EVs also act as vehicles for cell-to-cell communication. EVs coming from HCD cultures triggered physiological changes in low cell density (LCD) cultures emulating the CDE at LCD. These physiological changes were studied with confocal microcopy and high throughput proteomics. After EV fractionation, all proteins carried in the EV fraction responsible for blocking transfection were identified, characterizing the signaling molecules triggering this physiological shift.

These findings could shed some light on the molecular mechanism behind the CDE and allow the implementation of new bioprocessing strategies to overcome it.

[1] J. Lavado-García, P. Pérez-Rubio, L. Cervera, and F. Gòdia, “The cell density effect in animal cell-based bioprocessing: Questions, insights and perspectives,” *Biotechnol Adv*, vol. 60, p. 108017, Nov. 2022, doi: 10.1016/J.BIOTECHADV.2022.108017.