ENHANCED PROCESS CONTROL OF AN INTEGRATED AND SCALABLE BIOPROCESS FOR PRODUCTION AND ISOLATION OF MSC-DERIVED EXTRACELLULAR VESICLES FOR CARDIAC REPAIR

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Several cell types, including mesenchymal stem/stromal cells (MSC), have been explored in clinical settings addressing cardiac regeneration. The MSC role in tissue repair predominantly relies on paracrine mechanisms, including extracellular vesicles (EV)-mediated responses in the damaged tissue. Particularly, hypoxic preconditioned MSC have been shown to promote growth of blood vessels and stimulate endogenous repair pathways in the myocardium and could therefore constitute a relevant cell source for an augmented production of EV with cardiac regenerative properties. Since the clinical translation of MSC-derived EV is currently limited by their scalability, we propose the use of a bioreactor-based platform to maximize the yields and potency of the generated EV while implementing scalable and standardized downstream processing protocols that could be integrated in a continuous bioprocess.

To establish scalable manufacturing of MSC-derived EV, we have developed a baseline process using microcarrier technology combined with stirred-tank bioreactor for MSC expansion and EV production, and downstream processing for EV isolation and concentration by Tangential Flow Filtration followed by Size Exclusion Chromatography (TFF-SEC). The potential of the applied strategy is highlighted by direct comparison with cell culture in static systems and isolation of MSC-derived EV by the gold standard, although less scalable, density gradient ultracentrifugation-based protocols (DG-UC).

The use of a chemically-defined medium to support continuous manufacturing of both MSC and derived EV under cGMP-compatible and reproducible conditions has contributed to improve EV production and purification yields without compromising their quality attributes, with the TFF-SEC protocol yielding 6.5 times higher number of EV comparatively to the conditioned medium processed by DG-UC. Additionally, the manufacture of cell and cell-based products can benefit from a tight control of critical process parameters (CPP) supported by Process Analytical Technology (PAT) tools such as Raman Spectroscopy during cell culture in stirred-tank bioreactors. Herein, we have shown that Raman Spectroscopy tools can be employed to accurately track total viable cell numbers and metabolites concentration, whose control is essential to maximize EV production. We therefore propose an approach where continuous process monitoring can contribute to manage process risks and help advancing scalable manufacturing bioprocesses of MSC and their derived EVs with clinical potential.

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