hiPSC AND hiPSC-CARDIOMYOCYTES ARE ALTERNATIVE EV BIOFACTORIES FOR CARDIAC REGENERATION

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In cardiac regenerative medicine, there is a growing interest in using EV as cell-mimetic therapeutics due to their potential superior efficacy and overall advantages over cell transplantation: i) absence of oncogenic risk, ii) low immunogenicity [1], iii) easier large-scale manufacturing and iv) consistent product profile. However, most studies have focused particularly on the potential of either mesenchymal or cardiac progenitor cell-derived EV to promote cardiac repair [2].

Here, we study the potential of human induced pluripotent stem cells (hiPSC) and hiPSC-derived cardiomyocytes (hiPSC-CM) as alternative cell factories for the production of a high yield of therapeutic EV for cardiac regeneration. Due to their high self-renewal ability and capacity to differentiate into functional cardiomyocytes, these cells can provide an unlimited source of EV for application in cardiac regeneration.

We generated and characterized EV derived from key stages of hiPSC-CM differentiation and maturation, i.e. from hiPSC (hiPSC-EV), cardiac progenitors (CPC-EV), immature (CMi-EV) and mature (CMm-EV) cardiomyocytes, with the goal of studying their potential role as therapeutics, and whether their yield and function was influenced by the state of their parent cell. Two hiPSC lines were differentiated into hiPSC-CM and cultured as 3D spheroids in a fatty acid supplemented medium to improve CM maturation [3,4]. EV isolation was performed based on density separation on an iodixanol discontinuous gradient, and EV were characterized in terms of particle size and particle size distribution, presence of EV-specific markers, and imaging through transmission electron microscopy. Functional studies were performed using human umbilical vein endothelial cells (HUVECs) to evaluate EV-uptake, migration and angiogenesis.

EV yield varied along CM differentiation stages, with a minimum for CPC, for both cell lines. Bioactivity assays with HUVECs showed that uptake of PKH26-labelled EV could be blocked by dynasore, an inhibitor of dynamin-2, a GTPase that plays a crucial role in clathrin and caveolin-dependent endocytosis. Increased migration was observed in HUVECs treated with hiPSC, CPC and CM-derived EV (92.25 ± 14.69% wound closure at 24h for hiPSC, 77.13 ± 13.64 % for CPC, 74.71 ± 19.86% for CMi, 69.2 ± 19.12% for CMm versus 45.65 ± 7.26% for control), but angiogenic properties were found only for hiPSC-EV (fold change of 11.2 ± 4.59 in total segment length vs. control, $p<0.001$).
Current efforts towards the characterization of EV small RNA cargo aim at understanding the correlation between cargo composition and *in vitro* activity, to identify the optimal cell factory for scalable therapeutic EV production.

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