Human iPS-derived pre-epicardial cells direct cardiomyocyte aggregation expansion and organization in vitro

ABSTRACT

Epicardial formation is necessary for normal myocardial morphogenesis. Here, we show that differentiating hiPSC-derived lateral plate mesoderm with BMP4, RA and VEGF (BVR) can generate a premature form of epicardial cells (termed pre-epicardial cells, PECs) expressing WT1, TBX18, SEMA3D, and SCX within 7 days. BVR stimulation after Wnt inhibition of LPM demonstrates co-differentiation and spatial organization of PECs and cardiomyocytes (CMs) in a single 2D culture. Co-culture consolidates CMs into dense aggregates, which then form a connected beating syncytium with enhanced contractility and calcium handling; while PECs become more mature with significant upregulation of UPK1B, ITGA4, and ALDH1A2 expressions. Our study also demonstrates that PECs secrete IGF2 and stimulate CM proliferation in co-culture. Three-dimensional PEC-CM spheroid co-cultures form outer smooth muscle cell layers on cardiac micro-tissues with organized internal luminal structures. These characteristics suggest PECs could play a key role in enhancing tissue organization within engineered cardiac constructs in vitro.