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**Human pluripotent stem cell-derived endothelial cells are vasoactive in vitro and capable of engineering 3D vascular grafts**

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Human pluripotent stem cell-derived endothelial cells (hPSC-ECs) may be suitable for engineering three-dimensional vascular endothelial constructs. Here we studied the role and characteristics of 3D culture in vitro, in vivo and ex vivo on the arterial and venous fate of human embryonic and induced pluripotent stem cell-derived endothelial cells (hESC-EC and hiPSC-EC).

We found that differentiated hPSC-EC could not only adhere to decellularised extracellular biomatrices but remained viable and functional during recellularization and underwent further maturation. The 3D culture of hiPSC-EC on acellular biomatrices increased expression of endothelial marker genes in vitro. In vivo conditioning of hPSC-EC in athymic nude rats also induced expression of arterial and venous endothelial marker genes. Levels of secreted angiogenesis (e.g. angiopoietins, endoglin, FGFs) and cell-matrix adhesion-related proteins (e.g. collagen XVIII, MMP8 and 9, TIMP1) were markedly higher when hiPSC-ECs were cultured on 3D vascular biomatrix compared to 2D cultures. Factors involved in the regulation of platelet aggregation or fibrinolytic pathway were also activated. Functional characterization of hESC-EC and hiPSC-EC revealed antiplatelet effects in engineered 3D vascular constructs and direct myogenic vasoactive effects in isolated vessel platform on aortic rings. In conclusion, the hPSC-ECs show mature endothelial characteristics and functional behaviour in 3D cultures. Our results improve understanding endothelial specification in 3D under in vitro and in vivo conditions may be critical in vascular tissue engineering.