Endothelial derivatives of human pluripotent stem cells show antiplatelet effects in 3D culture -steps towards vascular tissue engineering

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Background and purpose: Endothelial derivatives of human pluripotent stem cells may offer regenerative treatments in ischemic cardiovascular diseases. Here we studied differentiation conditions toward mature endothelial population as well as fate and function of these cells during 3D culturing.

Methods and results: To optimize endothelial development, human embryonic stem cells (hESC) were differentiated via embryoid body (EB) or monolayer method under normoxic and hypoxic conditions. CD31-positive endothelial cells (EC) were sorted by FACS and compared with human induced pluripotent stem cell-derived endothelial cells (hIPSC-EC) and human adult coronary arterial endothelial cells (HCAEC). Both hESC-EC and hIPSC-EC showed mature endothelial characteristics such as cobblestone pattern, ac-LDL uptake, and tube formation in vitro. Proteome profiling revealed high abundance of angiogenesis-related proteins in cell lysates and supernatant. As assessed by qPCR, angiopoietin2 mRNA levels increased in hESC-EC when differentiated via EB method (EB in normoxia 353.17±86.29; EB in hypoxia 323.89±86.63, monolayer 27.20±9.92 vs. hESC, $p<0.001$). Expressions of arterial (EphrinB2, Notch1–2) and venous (EphB4) endothelial markers were increased, suggesting the presence of mixed endothelial population in culture. However, no significant differences were found in ratio of arterial and venous subpopulations in the different developmental protocols. For engineering 3D vascular constructs decellularised human aortic slices (300μm) were repopulated with hESC-EC and hIPSC-EC and cells remained viable on engineered matrices in vitro. Engineered bioscaffold were incubated with platelet rich plasma from healthy adults. 3D culture conditions activated antiplatelet effects of the cells (as shown by secreted levels of chemokine Rantes (pg/ml): hESC-EC 229.6±37.9, hESC-EC on bioscaffold 83.6±52.1; hIPSC-EC 234.9±22.8, hIPSC-EC on bioscaffold 127.9±63.6, $p<0.01$, $n=6$).

Conclusions: Differentiation conditions modulate endothelial development and angiogenic gene expression. 3D culturing increased antiplatelet effects. Functionally active endothelial derivatives of human pluripotent stem cells promises vascular tissue engineering for therapeutic purposes.