Human pluripotent stem cells-derived endothelial cells for vascular tissue engineering

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Human embryonic stem cells-derived endothelial cells (hESC-EC) and human induced pluripotent stem cells-derived endothelial cells (hiPSC-EC) can serve as suitable sources of vascular constructs. The aims of this study were to culture hESC-EC and hiPSC-EC in 2D and 3D cell cultures, analyse gene and protein expression profiles in these cultures and test functional properties of the cells.

In this study we have used human aortic segments to decellularize those in 2% sodium dodecyl-sulphate (SDS) and 0.05% sodium azide-containing solution after a washing period in phosphate buffered saline (PBS). At the end of the process samples were purified in antibiotics-containing PBS in order to remove the remaining detergent and cell debris. For tissue engineering hESC-ECs (H7 hESC line, WiCell, USA) and hiPSC-ECs (ReproCELL, Japan and IMR-90-4) were grown in 2D cultures n endothelial cell growth medium (EGM2) or on fibronectin-coated 300 μm thick 3D aortic segments. For the 4-day recellularisation process 10^6 cells per sample were used in a 96-well cell culture plate. Recellularisation process was followed by anti-human CD31 endothelial cell marker staining step. In order to quantify the extent of recellularisation and to standardize tissue engineering an ImageJ based algorithm was developed. Before and after the reendothelisation process, samples were homogenised and proteomics and polymerase chain reaction (PCR) were used to assess the expression of arterial, venous and common endothelial markers (EphrinB2, Notch1, Notch2, Dll4; EphB4, FLT4; and C31, VE-Cadherin, respectively).

Protein expression from the 3D tissue engineered endothelial cells is altered and secretion of the angiogenesis associated proteins (VEGF, Angiopoietin-2) was increased. Significant increase was observed in the expression of endothelial markers due to 3D cell culturing (hiPSC-EC Dll4 138.76 ± 60.91; hESC-EC Notch2 4211.73 ± 2303.39; EphrinB2 9306.16 ± 199.29-fold changes; n=3; $p<0.001$). During the recellularisation there is a marked increased specification and maturation in stem cells-derived endothelial cells.

This suggests a direct regulatory role of the extracellular matrix on endothelial specification. These results also show that the recellularisation protocol developed here enables the generation of 3D vascular endothelial structures.