3D culture conditioning modulates arterial specification of human pluripotent stem cells-derived endothelial cells. Foldes G, Gara E, Husveth-Toth M, Nemes A, Merkely B, Harding SE.

Introduction. Endothelial derivatives of human embryonic and induced pluripotent stem cells (hESC-EC, hiPSC-EC) have emerged as one of the potential sources of new vascular cells in the therapy of ischemic cardiovascular diseases. Our aim was to study how 3D culturing may influence endothelial differentiation of hPSC, their arterial-venous specification as well as endothelial function.

Methods. Human ESC-ECs (H7 line) and hiPSC-ECs (IMR90-4 line) were grown in 2D cultures in endothelial growth medium or seeded on fibronectin-coated 300 μm thick decellularised aortic segments. To quantify the extent of cell seeding, confocal imaging algorithm have been developed. Expressions of endothelial and angiogenesis-related genes and proteins were assessed by qPCR and proteome profiling. Human ESC-EC and hiPSC-EC were subcutaneously transplanted in Matrigel plugs into athymic nude rats to study in vivo conditioning.

Results. Proteome profiles of hESC-EC and hiPSC-EC in 2D cultures showed similar pattern, suggesting that cells derived from different hPSC lines may have similar functional characteristics. Levels of several angiogenesis-related proteins (e.g. angiopoietins1 and 2) were markedly higher when cells were cultured on 3D biomatrix. Cell-matrix adhesion-related proteins (collagen XVIII, MMP8, MMP9, TIMP1, MCP-1) showed robust increase in 3D culture, thereby increasing adhesive capacity of cells upon reseeding of matrices. Expressions of arterial (EphrinB2, Notch1-2) endothelial markers were also increased, both in 3D cultures and in vivo (all $p<0.01$, ANOVA, $n=3$).

Conclusions. Our results suggest that 3D culture conditions are prerequisite for full maturation of hPSC-EC. Beside transcriptional regulation, optimised in vitro and in vivo conditions can also modify endothelial specification.