Progenitor and differentiation potential of human fetal and adult endothelial cells

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Objectives: Vasculogenesis and angiogenesis are the processes responsible for the formation of blood vessels. It has long been assumed that vasculogenesis (*de novo* building of blood vessels from stem/progenitor cells) occurs only in the early embryogenesis, whereas angiogenesis (vessel sprouting from pre-existing vessels) additionally proceeds during the adult life. However, recent findings suggest that endothelial progenitor cells (EPC) derived from the bone marrow enable postnatal vasculogenesis [1]. Furthermore, a complete hierarchy of EPC has been identified in endothelial cell cultures derived from vessel walls [2], and especially venous fetal endothelial cells were shown to have a juvenile phenotype with differentiation potential [3].

Here we hypothesized that venous endothelial cells represent a constant source of progenitor cells at later stages of embryonic development and in the adult. Therefore, we investigated the progenitor and differentiation potential of adult arterial (a-AEC) and venous endothelial cells (a-VEC) from different human vascular beds (ilia, mesenteries) and compared it under different culture conditions with the pheno- and genotype of fetal arterial (f-AEC) and venous endothelial cells (f-VEC) from the human term placenta.

Material and Methods: Fetal and adult endothelial cells were isolated by enzymatic perfusion of corresponding blood vessels and cultured under standard (20% O_2), low oxygen (2% O_2), adipogenic and osteogenic conditions. Structural differences of the phenotype and morphological changes were examined by phase contrast microscopy and scanning electron microscopy. The proliferative activity was determined using standard procedures. The progenitor and stem cell potential was investigated by immunocytochemistry and reverse transcriptase polymerase chain reaction. Adipogenic and osteogenic differentiation potential of endothelial cells were demonstrated by Oil Red O and Alizarin Red S staining, respectively.

Results: f-AEC and a-AEC are polygonal shaped and grow in loose arrangements forming classical cobblestone monolayers under standard culture conditions, whereas f-VEC and a-VEC are more spindle-shaped cells that grow closely apposed to each other (Fig 1). Both f-VEC and a-VEC show a higher proliferation potential and a stronger gene expression of Oct-4, Sox-2, and Nanog (transcription factors in undifferentiated cells) and the EPC markers CD34 and CD14 than their arterial counterparts. Furthermore, these genes are higher expressed in fetal endothelial cells than in adult endothelial cells. Under low oxygen conditions, all cell types increase in their cell size and become more polygonal shaped, whereas the examined genes are lower expressed.

More than 50% of the f-VEC show lipid droplets and calcium deposits after adipogenic and osteogenic conditions, respectively. In contrast, only limited or no induction could be observed in the other cell types.

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Conclusion: These data provide evidence for the heterogeneity and plasticity of endothelial cells derived from human fetal and adult tissues. Low oxygen results in morphological and functional changes on all cell types studied. The higher expression of stem/progenitor cell markers on VEC and in particular the adipogenic and osteogenic differentiation potential of f-VEC may indicate the role of VEC as tissue-resident endothelial progenitors. We suggest that endothelial cells should not be regarded as a homogenous cell population, but include several subpopulations with different properties of progenitor and differentiation potential. Thus, they may represent a potential cell source for regenerative cell therapy.

References:
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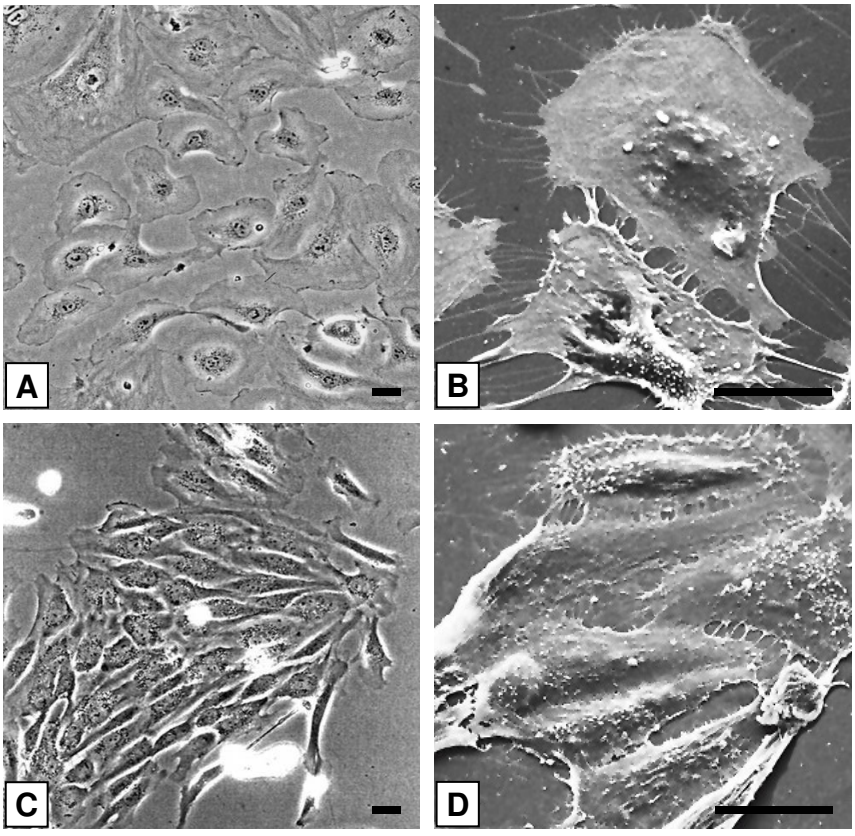

Figure 1. Phase-contrast micrographs of arterial (A) and venous (C) endothelial cells. The polygonal-shaped AEC grow in loose arrangements. In contrast, VEC are more spindle-shaped and grow closely apposed to each other.
Scanning electron micrographs of arterial (B) and venous (D) endothelial cells. AEC show a smooth cell surface with few microvilli. They are linked to each other by pseudopodial-like contact zones and thin cytoplasmic protrusions. The exalted zone contains the nucleus and most of the organelles. The peripheral margins show a low cytoplasmic density. VEC exhibit numerous microvilli at their cell surface and extrude thin cytoplasmic projections connecting them to their neighbouring cells. Scale bar 25µm.