

Cord Blood Derived Endothelial Progenitor Cells for Vascular Tissue Engineering: Enhanced Knowledge of Functional Properties

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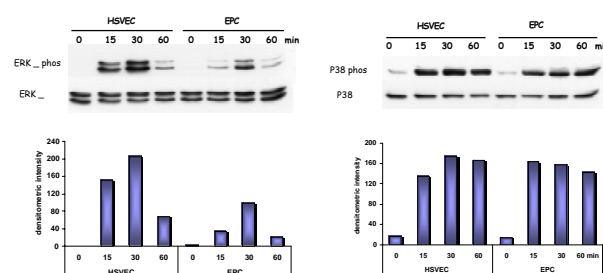
INTRODUCTION: Tissue engineering of small diameter blood vessel substitute for use in femoro-popliteal and coronary bypasses surgery is still a challenge today. Because autologous grafts are not always available, a shift in the focus of research towards reconstructing the endothelial cell (EC) living of synthetic vascular prosthesis wall was an innovation designed to improve patency rates. However, the procedure of obtaining ECs from the patient has disadvantages and limits. Thus, alternative sources of ECs, such as endothelial progenitor cells (EPCs) derived from umbilical cord blood have perspectives for therapeutic applications among which cardiovascular tissue engineering. Before envisaging the use of EPCs in such a way, it is first essential to investigate their in vitro behavior compared with mature vessel wall cells. The aim of our study was thus to explore features of EPCs : tissue factor (TF) biological activity and mitogen – activated protein kinase (MAPK) phosphorylation after activation.

METHODS: CD34+ mononuclear cells were isolated from cord blood by a magnetic beads separation, plated onto gelatin-coated wells and cultured under endothelial conditions [1]. Their EC characterization was assessed by CD31, von Willebrand factor (vWF), VE-cadherin, KDR and Flt1, dil-Ac-LDL, using immunocytochemistry and flow cytometry. For the comparison primary human saphenous vein endothelial cells (HSVEC) were isolated from vein remnants provided by a cardiovascular surgery department, harvested [2] and grown in a complete culture medium. The ECs were activated i) in static conditions with IL-1 α for measuring MAPK phosphorylation (western blot analysis of cell lysates according to [3]) and TF activity (EC-associated procoagulant activity (PCA) was determined in a one-stage clotting assay by the acceleration of clotting time of recalcified normal citrated platelet – poor plasma); ii) by fluid shear stress (parallel flow chamber, 16 dynes/cm²) followed by MAPK phosphorylation.

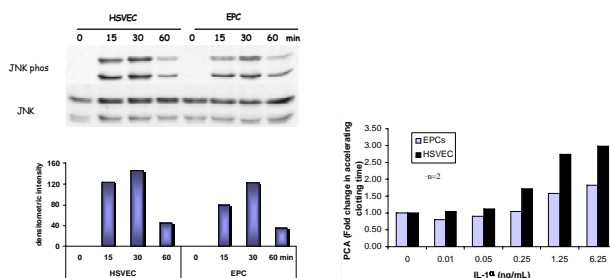
RESULTS: CD34+ mononuclear cells differentiate into cells with a typical endothelial

phenotype: ECs derived from CD34+ cells are positive for all endothelial markers. IL-1 α

provoked on both cell types MAPK phosphorylation (Fig1-A, B, C). Stimulation with IL-1 α generated a dose-dependent PCA response as compared to unstimulated cells (Fold change in accelerating clotting time): fig 2. Shear stress activated p38 phosphorylation after 10 min of



stimulus in both cell types, followed by a decrease



at 30 min.

Fig. 1-A

Fig. 1-B

Fig. 1-C

Fig. 2

DISCUSSION & CONCLUSIONS: This study is the first to check PCA on EPCs as well as MAPK participation in transmitting hemodynamic forces to cytoplasmic pathways. Whether shear stress attenuates IL-1 α - induced TF expression on EPC surface is in progress. Cord blood derived EPCs are known for exceptional growth characteristics [1,4] and demonstrate phenotype of ECs. EPCs seem to be a promising cell source with regard to vascular tissue engineering.

REFERENCES: ¹H. Bompais, J. Chagraoui, X. Canon et al (2004) *Blood* **103**:2577-84. ²J. Golledge, R.J. Tumer, S.L Harley et al. (1997) *Eur J Vasc Endovasc Surg* **13(6)**:605-12. ³P. Fernandez, R. Daculsi, M. Remy-Zolghadri et al (2005) *Tissue Eng* in press. ⁴D. Schmidt, C. Breyman, A. Weber et al (2004) *Ann Thorac Surg* **78**:2094-8.