

Supplementary data to Lund et al. ” Shear stress regulates inflammatory and thrombogenic gene transcripts in cultured human endothelial progenitor cells” (Thromb Haemost 2010; 104.3)

Materials and Methods

EPC Characterization

After 10 min incubation in 4% paraformaldehyd at room temperature, and blocking with 3% goat serum for 1 h, the cells were immunolabeled by incubation with mouse anti-human antibodies to CD31 (1:100; RD systems), or CD144 (1:100; RD systems) for 1 h. After washing, cells were incubated with the secondary antibody labeled with Alexa Fluor 488 (1:500, Molecular Probes) for 1 h. The nucleus was stained with Draq 5 (Biostatus Ltd, UK). Images were made in a confocal fluorescence microscope (LSM510 META, Carl Zeiss AG, Germany).

Table 1: Primer sequences of genes and probes evaluated in this study.

Gene Name	Gene bank Accession number	Primer Sequence (5→3)
CD31	NM_000442.3	FP AGA-GTA-CCA-GCT-GTT-GGT-GGA RP CAC-CTT-GGA-TGG-CCT-CTT-T P RocheProbeLibrary; # 47
CD45	NM_002838.3	FP CCA-ATG-CAA-AAC-TCA-ACC-CTA RP CCT-CTC-TCC-TGG-GAC-ATC-TG P RocheProbeLibrary; #27
CD144	NM_001795.2	FP AAG-CCT-CTG-ATT-GGC-ACA-GT RP CTG-GCC-CTT-GTC-ACT-GGT P RocheProbeLibrary; #58
CD146	NM_006500.2	FP GGG-TAC-CCC-ATT-CCT-CAA-GT RP CTG-GGA-CGA-CTG-AAT-GTG-G P RocheProbeLibrary; #63
COX2	NM_08059	FP GCT-CAA-ACA-TGA-TGT-TTG-CAT-TC RP GCT-GGC-CCT-CGC-TTA-TGA P TGC-CCA-GCA-CTT-CAC-GCA-TCA-GTT
HPRT1	NM_000194	FP GGA-CTG-ACA-CTG-GCA-AAA-CAA-TGC-A RP AGC-TTG-CGA-CCT-TGA-CCA-TCT P TTG-CTT-TCC-TTG-GTC-AGG-CAG-TAT-AAT-CCA
ICAM1	NM_000201.1	FP CCT-TCC-TCA-CCG-TGT-ACT-GG RP AGC-GTA-GGG-TAA-GGT-TCT-TGC P RocheProbeLibrary; #71
IL-6	NM_000600.2	FP CGG-GAA-CGA-AAG-AGA-AGC-TCT-A RP GGC-GCT-TGT-GGA-GAA-GGA-G P TCC-CCT-CCA-GGA-GCC-CAG-CTA-TGA
IL-8	NM_000584.2	FP GTT-TTT-GAA-GAG-GGC-TGA-GAA-TTC RP CAT-GAA-GTG-TTG-AAG-TAG-ATT-TGC-TTG

KDR	NM_002253.1	P	ATC-CAA-GAA-TCA-GTG-AAG-ATG-CCA-GTG-AAA-CT
		FP	GAA-CAT-TTG-GGA-AAT-CTC-TTG-C
		RP	CGG-AAG-AAC-AAT-GTA-GTC-TTT-GC
		P	RocheProbeLibrary; #18
MCP1 (CCL2)	NM_002982	FP	TTC-TGT-GCC-TGC-TGC-TCA-T
		RP	GGG-GCA-TTG-ATT-GCA-TCT
NOS3	NM_000603.3	FP	GAC-CCT-CAC-CGC-TAC-AAC-AT
		RP	CCG-GGT-ATC-CAG-GTC-CAT
		P	RocheProbeLibrary; #5
PPIA	NM_021130		Tataabiocenter (human endogenous control panel)
RPL13A	NM_012423	FP	CTG-GAC-CGT-CTC-AAG-GTG-TT
		RP	GCC-CCA-GAT-AGG-CAA-ACT-T
		P	RocheProbeLibrary; #18
Tissue Factor	NM_001993	FP	CCC-CAG-AGT-TCA-CAC-CTT-ACC-T
		RP	CAC-TTT-TGT-TCC-CAC-CTG-TTC-A
		P	AGA-CAA-ACC-TCG-GAC-AGC-CAA-CAA-TTC-A

FP: forward primer; RP: reverse primer; P: Probe

Results

Characterization of Human EPCs

After 9-21 days, colonies of outgrown EPCs appeared in the cultures. Morphologically these cells had a typical EC cobblestone structure (Fig 1A-C). Immunohistochemistry revealed that the isolated cells were positive for the endothelial cell marker CD31 and CD144 (Fig 1B and C). The endothelial phenotype of outgrown EPCs was further characterized by the expression of CD31, CD144, CD146, and KDR on the mRNA level (Table 2). The cells were negative for the expression of CD45 mRNA (Table 2).

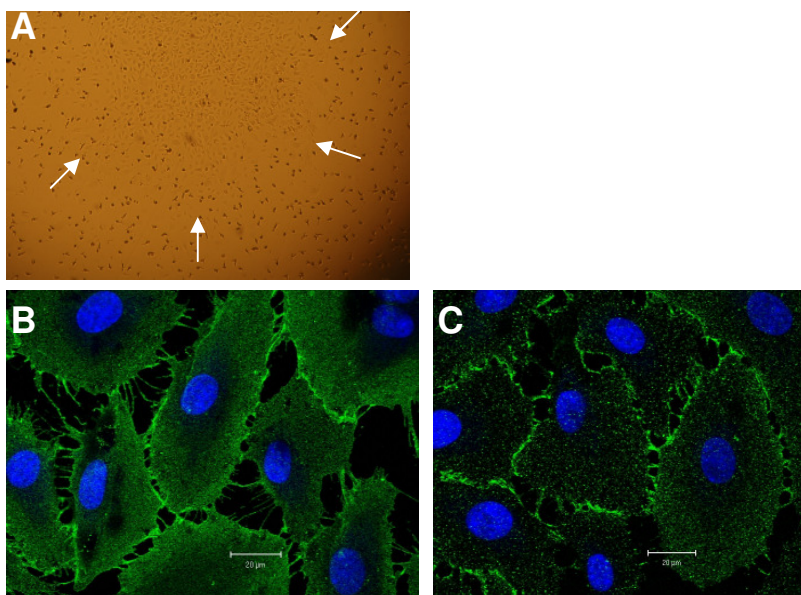


Figure 1: Characterization of EPC. A) Arrows outline a representative primary colony of EPCs. B) Immunofluorescence staining (green) of CD31 (PECAM1), and C) CD144 (Ve-cadherin). Cell nuclei are stained with DAPI (blue).

Table 2: Characteristic of Cell Populations on the mRNA Level.

Cell population	CD144 (Ve-cadherin)	CD45	KDR	CD31 (PECAM1)	CD146 (MCAM)
EPC (n=6)	15.64±4.09	No expression	3.14±0.69	5.0±1.52	4.93±1.09
HUVEC (n=1)	11.24	No expression	2.3	5.75	3.87
MNC (n=4)	0.01±0.0	8.94±1.46	No expression	5.79±3.38	No expression

mRNA levels of CD144 (Ve-Cadherin), CD45, KDR (VEGFR-2), CD31 (PECAM1) and CD146 (MCAM) were quantified by real-time PCR in EPC, HUVEC and MNC. Results are shown normalized to the stable expressed reference gene, HPRT1. Data are presented as mean with SEM. EPC, endothelial progenitor cells; HUVEC, human umbilical vein endothelial cells; MNC, mononuclear cells.