Culture of Pulmonary Arterial Endothelial Cells from Pulmonary Artery Catheter Balloon Tips: Considerations for Use in Pulmonary Vascular Disease

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Online Supplementary Material

Supplementary Methods

Confirmation of Pulmonary Arterial Endothelial Cell Phenotype

All endothelial cells were used between passages 3 – 8. Information about negative controls, antibodies and dilution are included in Table E1 and Figure E1.

Apoptosis Assay

Endothelial cells were plated between 3 – 3.5 x 10^4 cells/ well in an eight well chamber slide that was coated with 0.2% gelatin (Fisher Scientific; Waltham, MA) or 30 μg/ml fibronectin (Gibco Life Technologies; Carlsbad, CA) in complete media (EndoGRO-MV complete media; Millipore Sigma; Billerica, MA) and incubated overnight. The next day, the media was removed, the cells washed 1X with EndoGRO-low serum (LS) and then incubated in EndoGRO complete media or LS with or without indicated concentration of TNF-α for 6 hours. For a positive control, cells were incubated with tert-Butyl hydroperoxide solution (Sigma-Aldrich; St. Louis, MO) (500 μM for 6 hours). Cells were fixed in 4% paraformaldehyde for 10 minutes, washed with phosphate-buffered saline (PBS) twice, and stored in PBS at 4ºC.

Apoptosis was measured via the indirect TUNEL method using ApopTag® Red In Situ Apoptosis Detection kits (Millipore Sigma; Burlington, MA). Cells were permeabilized with 0.1% Triton X-100 in PBS for 10 minutes at 25ºC. The cells were then washed with PBS twice and apoptosis staining was performed according to manufacturer's protocol. Cells were counterstained with VE-cadherin. Cells were blocked in 5% donkey serum in

PBS for 30 minutes and stained for VE-cadherin (Santa Cruz SC-9989; diluted 1:200 in 5% donkey serum in PBS) for 1 hour at 37^{o}C. Cells were washed with PBS twice and incubated with secondary antibody (mouse anti-donkey conjugated with FITC 488; diluted 1:350 in 5% donkey serum in PBS) for 1 hour at 37^{o}C. The cells were washed with PBS twice. The chamber slides were mounted in Prolong Gold with 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen; Carlsbad, CA).

Using ImageJ software, the total number and apoptotic positive nuclei were determined.

Migration Assay

Endothelial cells were plated at 7.5 x 10^4 cells/well in a 24 well dish. The confluent endothelial cell monolayers were scratched using a pipette tip and incubated in EndoGRO complete media, or LS EndoGRO with or without VEGF for 6 hours. Cell migration was monitored at 2-hour time intervals following the initial wound and images were captured at 10X magnification a Nikon Eclipse TE2000-U microscope. Cell migration was assessed using MiToBo analyzer software in Image J. An average from two to three wells was assessed to represent an n = 1.

Tube Formation Assay

Endothelial cells were plated at 5 x 10^4 cells/ well in a 24 well dish that was coated with Matrigel® (Corning, Inc.; Corning, NY) in EndoGRO complete media, or LS EndoGRO with or without vascular endothelial growth factor (VEGF) for 6 hours. Phase contrast

images were recorded in three non-overlapping regions of the well at two, four and six hours. Images were processed using AngioTool plugin for ImageJ.

Supplementary Figure Legend

Figure E1. Representative images, negative controls. Panel A) human pulmonary artery smooth muscle cells. α-smooth muscle cell actin staining (red); CD-31 staining (green). Panel B) human lung fibroblasts. Fibroblast surface protein (red); acetylated low-density lipoprotein uptake (green), images at 20X magnification. Scale bars = 50 μm.

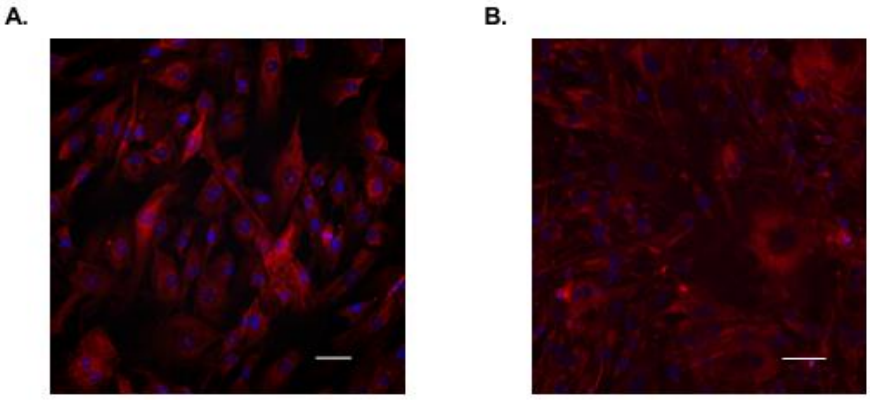

Table E1. Negative control staining to confirm of PAEC phenotype

Cell Type	Antibodies	Dilution
NHLF passage 9	vWF, VE-cadherin, α-SMA, fibroblast surface protein	1:200
NHLF passage 9	secondary	1:350
HPASMC passage 5	vWF, VE-cad, α-SMA	1:200
HPASMC passage 5	secondary	1:350

PAEC=pulmonary artery endothelial cell; NHLF=Lonza human lung fibroblasts; HPASMC=human pulmonary artery smooth muscle cells; vWF=von Willebrand factor VE=vascular endothelial; α-SMA=alpha-smooth muscle cell actin