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An affordable method to obtain cultured endothelial cells from peripheral blood

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Reunión Científica Heracles 2014

MADRID. SPAIN, MAY 21st – 22nd 2014

PROCELL PROJECT

3-year coordinated project (Dr. Magda Heras): Circulating endothelial cells and endothelial progenitor cells in acute cardiovascular disease. Correlation with endothelial function and clinical outcomes

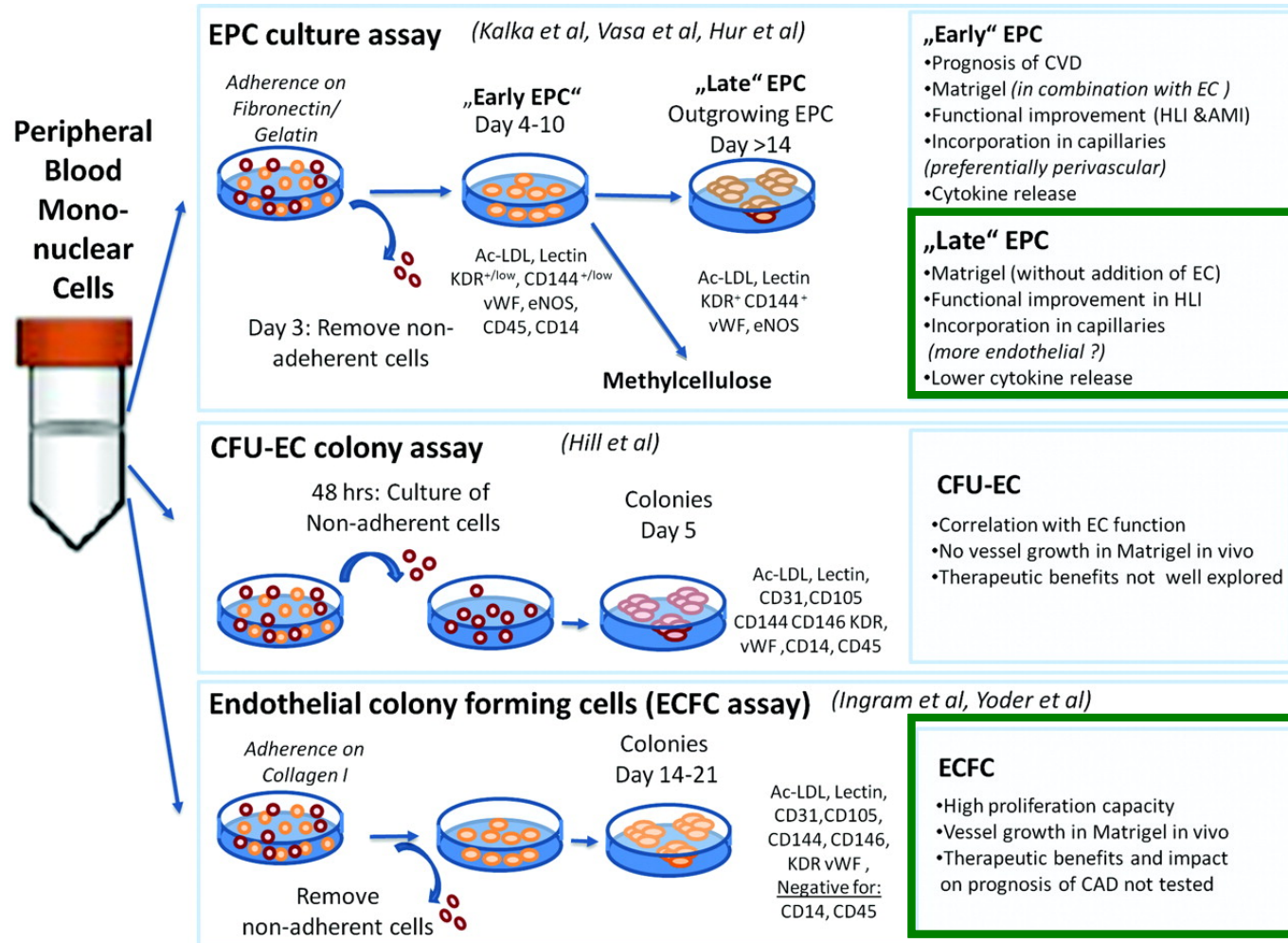
Main objectives:

- Determine Circulating Endothelial Cell (CEC) and circulating Endothelial Progenitor Cell (EPC) levels in stroke and Acute Myocardial Infarction (AMI) patients
- Determine the atherosclerotic burden in both AMI and stroke patients
- Isolate and culture EPC in order to determine EPC function in terms of adhesion, growth, proliferation and vasculogenesis



CULTURE PROTOCOLS

Overview of the most common protocols used to isolate EPCs.



Success

12-50%

EPC colonies

21 days

Culture time

45 days

CRITICAL STEPS ON EPC CULTURE PROCEDURES

Anticoagulant: EDTA, citrate and Heparin

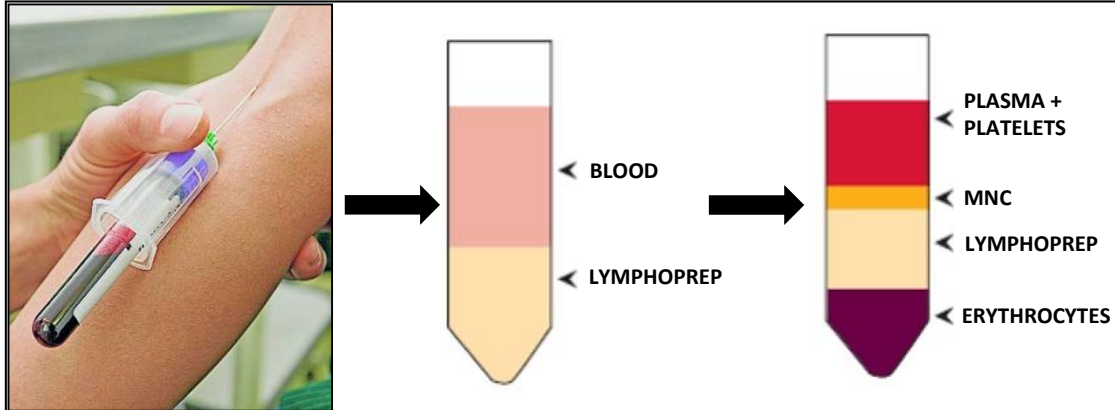
Blood volumes: 10, 20 and 30 mL

Blood processing time: 2 and 24 hours

Culture coating matrix: gelatin and fibronectin

Culture media supplementation: 2 and 20 % FBS

ISOLATION AND CULTURE PROCEDURE

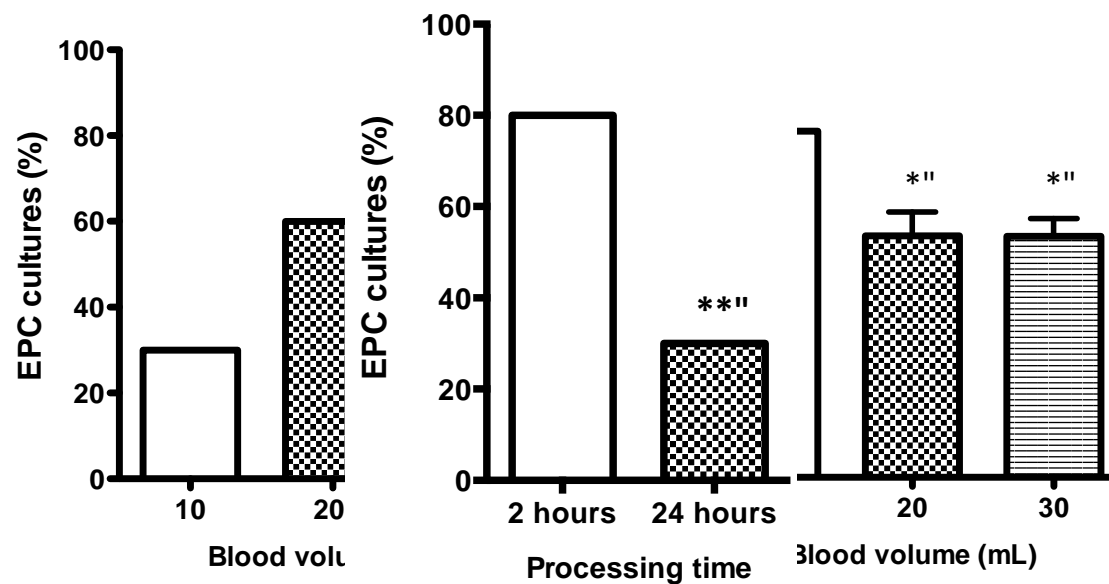


Blood recovery and processing

Anticoagulant: heparin

Blood volume = 30 mL

Processing time < 2 hours

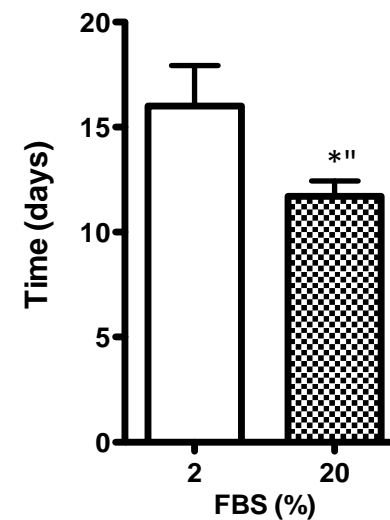
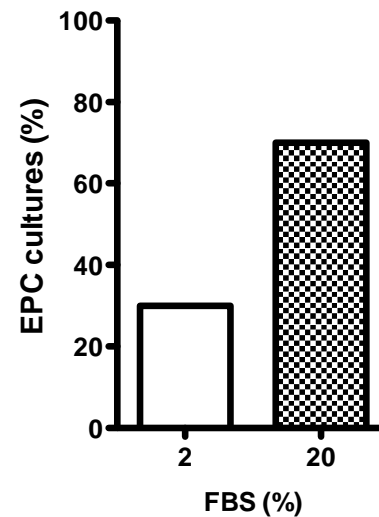
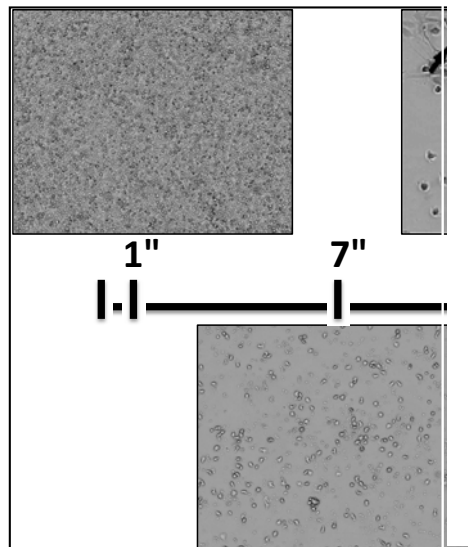


ISOLATION AND CULTURE PROCEDURE



EPC culture

Fibronectin coating matrix
20% Foetal Bovine Serum



Success

80%

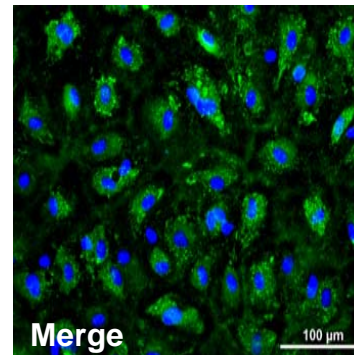
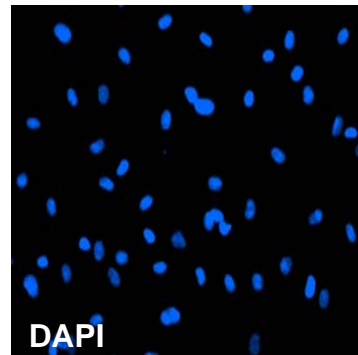
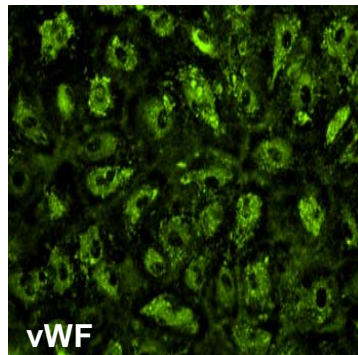
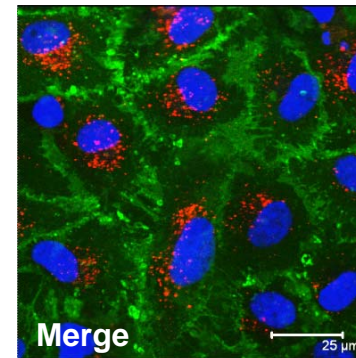
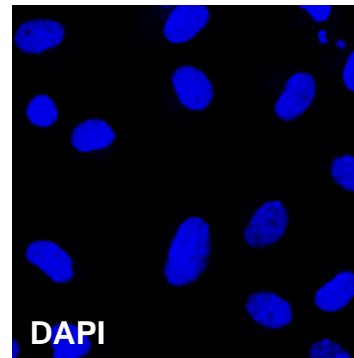
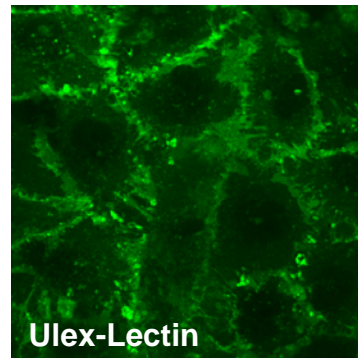
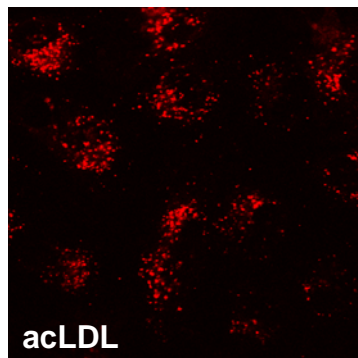
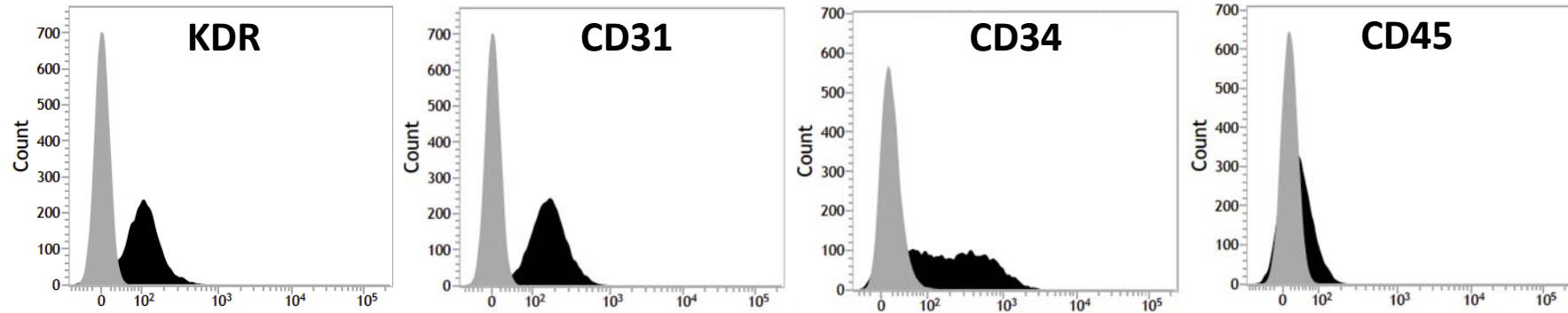
EPC colonies

13.5 days

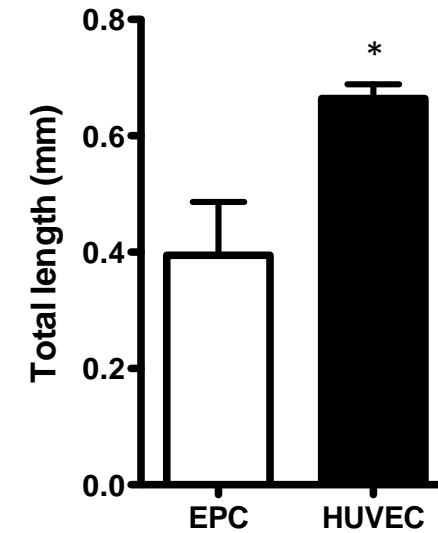
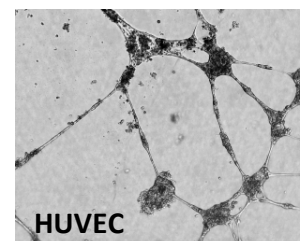
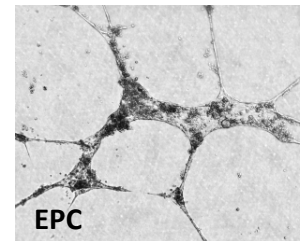
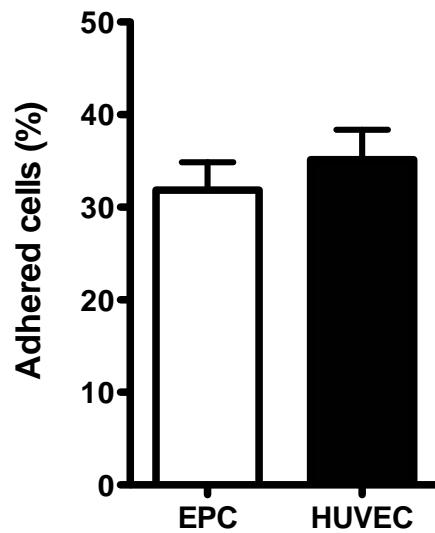
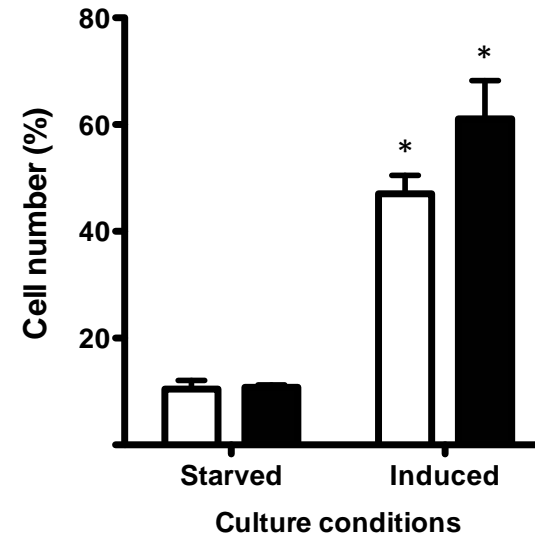
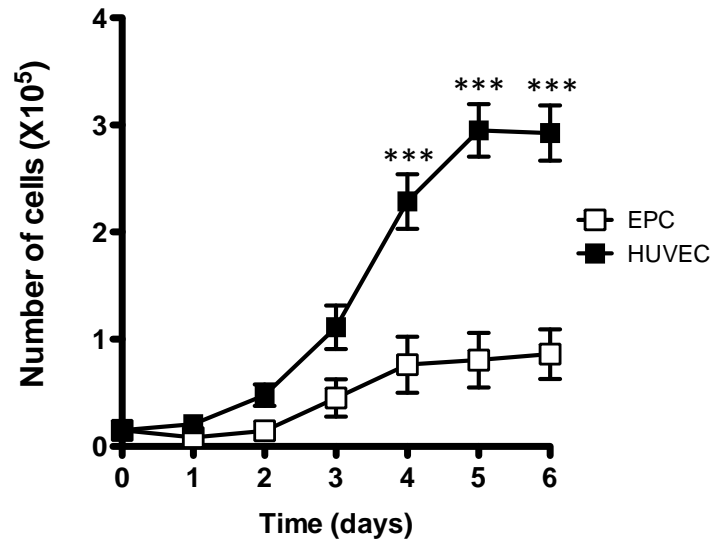
Culture time

25 days

EPC PHENOTYPIC CHARACTERIZATION



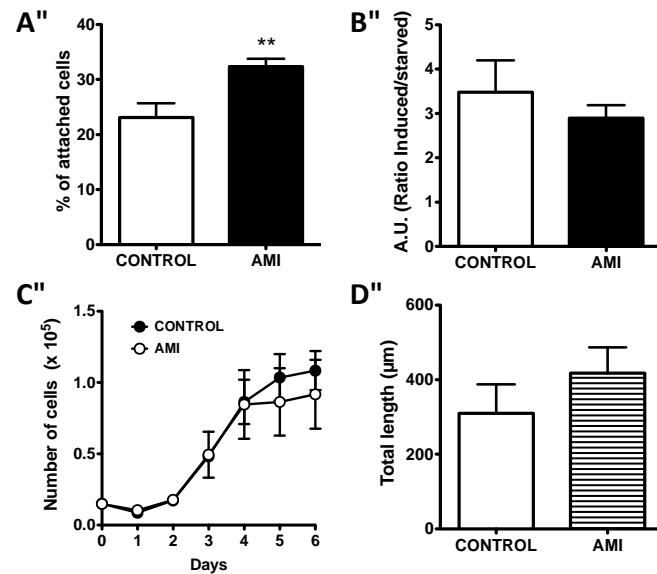
EPC FUNCTIONAL CHARACTERIZATION



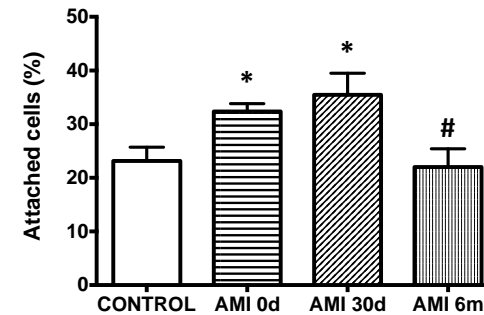
COMING SOON ...

Mobilization of endothelial progenitor cells in acute cardiovascular events in the Procell study: Time-course after acute myocardial infarction and stroke.

Authors: A Regueiro(1), E Cuadrado(2), Carlos Bueno-Betí(3), M Díaz-Ricart(4), A Oliveras(5), S Novella(3), G Gener(7), C Jung(7), I Subirana(8), JT Ortiz-Pérez(1), M Roque(1), X Freixa(1), J Núñez(6), G Escolar(4), J Marrugat (8), C Hermenegildo(3), MA Valverde(7), J Roquer(2), J Sanchís(6), M Heras(1).



“Endothelial progenitor cell function and acute myocardial infarction: 6-month follow-up”



CONTROL

EPC

CONCLUSIONS

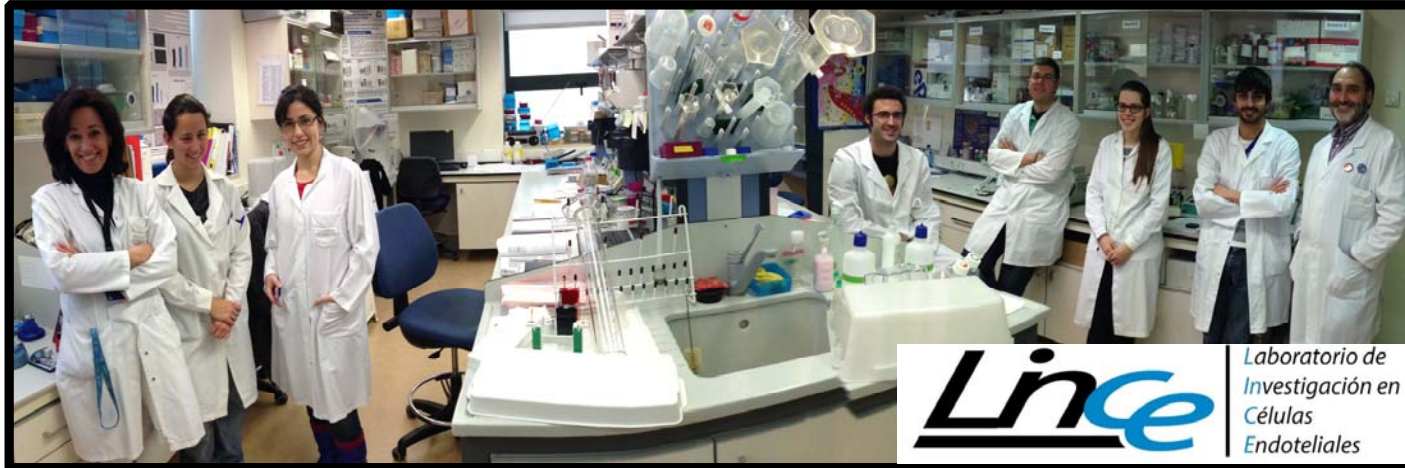
We propose the best conditions for EPC isolation and culture from peripheral blood samples.

Isolated EPC showed an endothelial cell-like phenotype

Isolated EPC behaved functionally like endothelial cells (HUVEC) do

In AMI patients, EPC maintain the functional behavior they were performing when they were isolated.

AKNOWLEDGEMENTS



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