

# **Heterogeneity of Endothelial Progenitor Cells (EPCs) & Synergism between Two Types of EPCs**

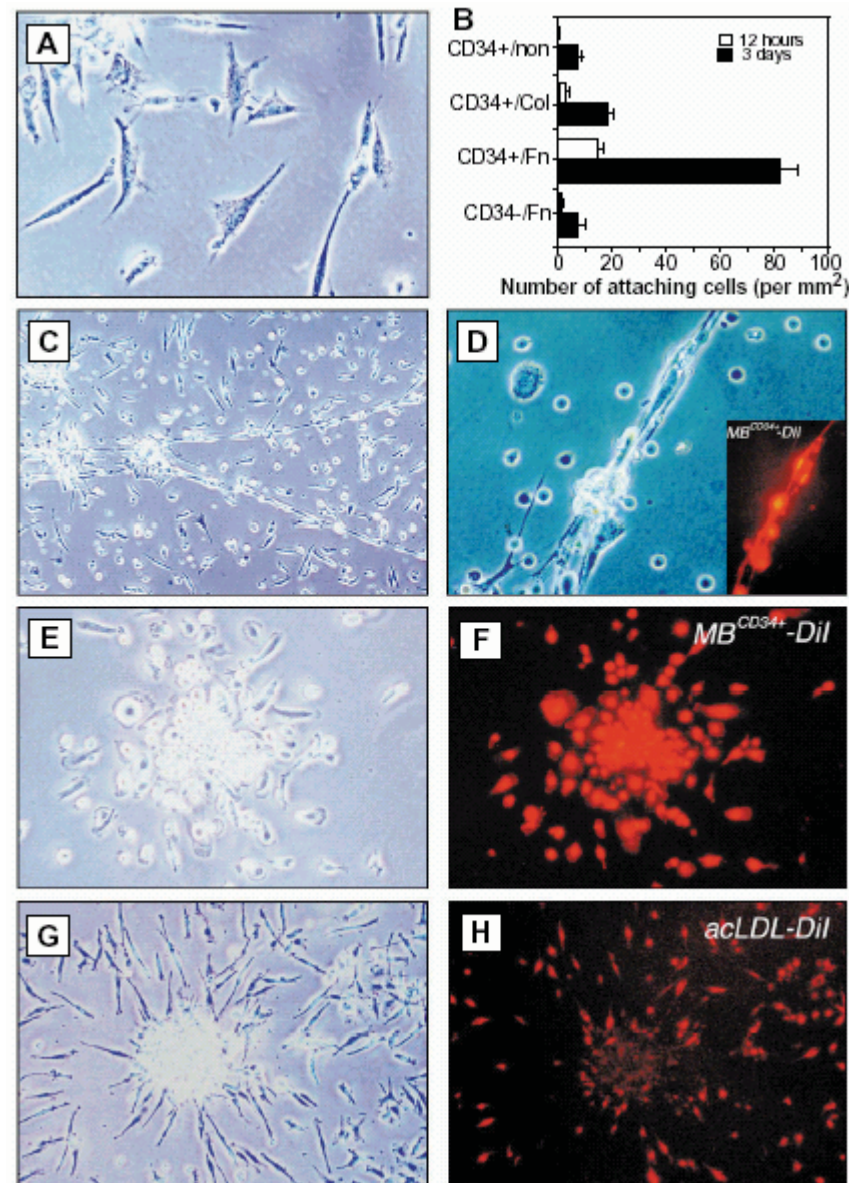
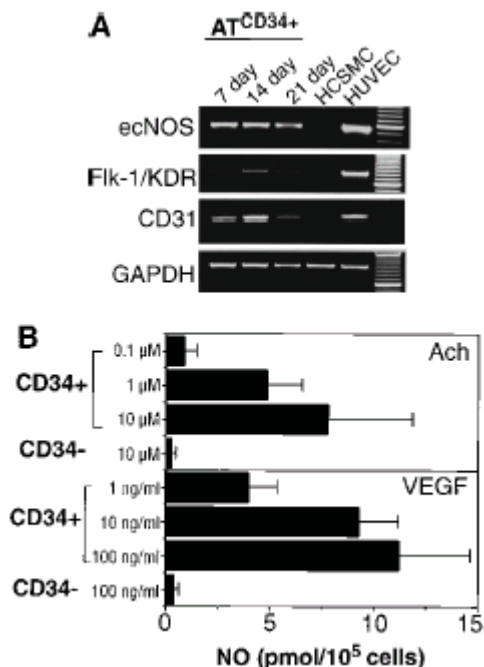
**Hyo-Soo Kim, MD/PhD**

**Cardiovascular Center / Cardiovascular Research Laboratory  
Seoul National University Hospital, Seoul, Korea**

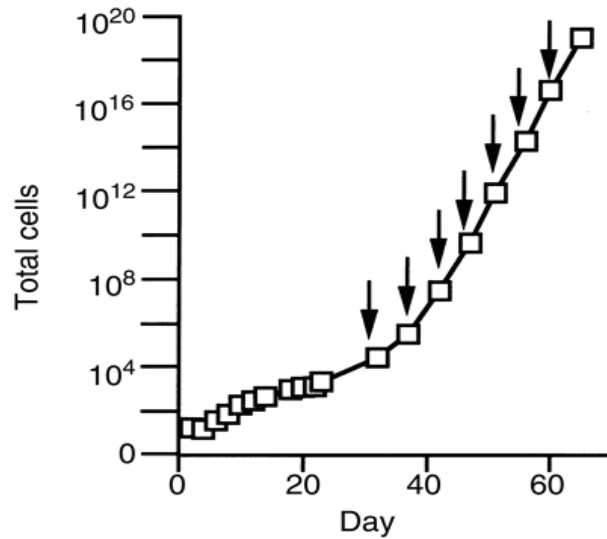
## Isolation of Putative Progenitor Endothelial Cells for Angiogenesis

Takayuki Asahara, Toyooki Murohara, Alison Sullivan,  
 Marcy Silver, Rien van der Zee, Tong Li,  
 Bernhard Witzenbichler, Gina Schatteman, Jeffrey M. Isner\*

Putative endothelial cell (EC) progenitors or angioblasts were isolated from human peripheral blood by magnetic bead selection on the basis of cell surface antigen expression. In vitro, these cells differentiated into ECs. In animal models of ischemia, heterologous, homologous, and autologous EC progenitors incorporated into sites of active angiogenesis. These findings suggest that EC progenitors may be useful for augmenting collateral vessel growth to ischemic tissues (therapeutic angiogenesis) and for delivering anti- or pro-angiogenic agents, respectively, to sites of pathologic or utilitarian angiogenesis.



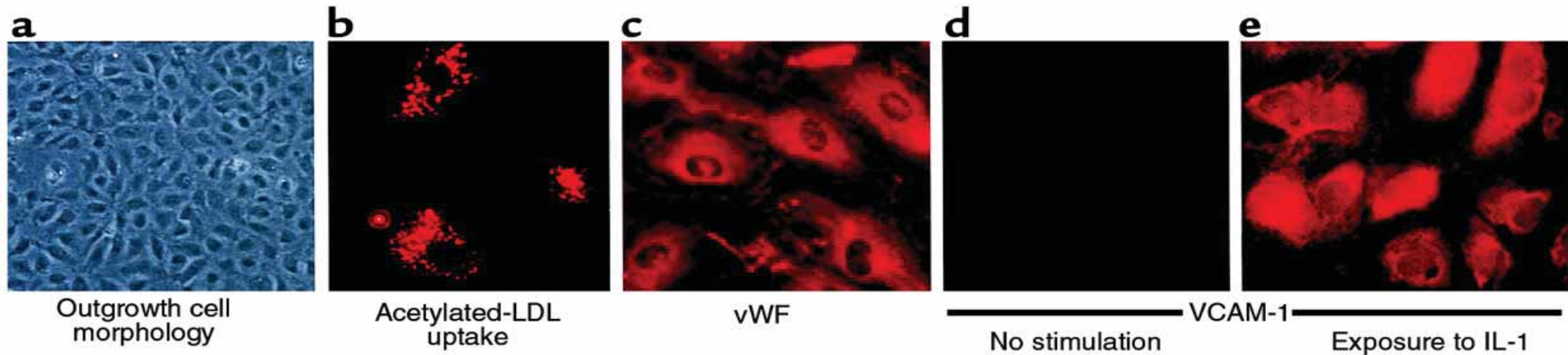
# Another Type of Circulating Endothelial Progenitor Cells in Blood



Yi Lin. et. al. J Clin Invest. 2000

endothelial outgrowth after ~1 month from total mononuclear cells

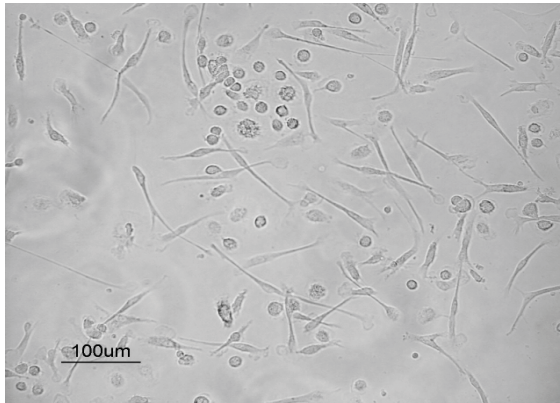
1000-fold expansion, cobblestone appearance



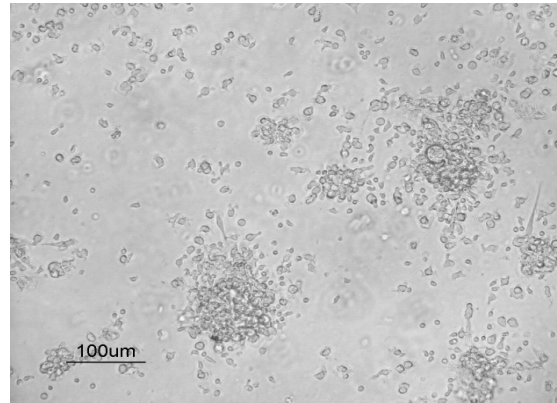
# Identification of two distinct types of EPC

## initial growing cells (early EPC)

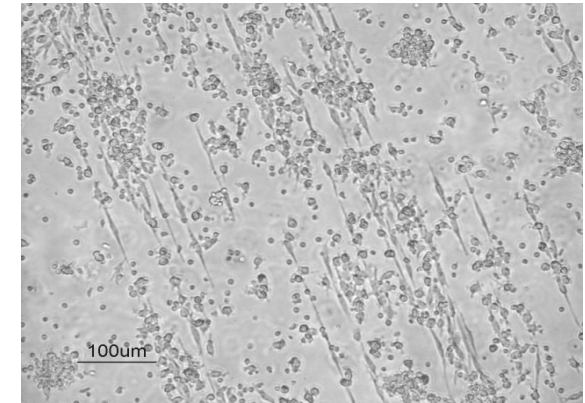
*Hur J, Yoon CH, Kim HS, et al. ATVB 2004;24;288-293.*



**spindle-like cells**

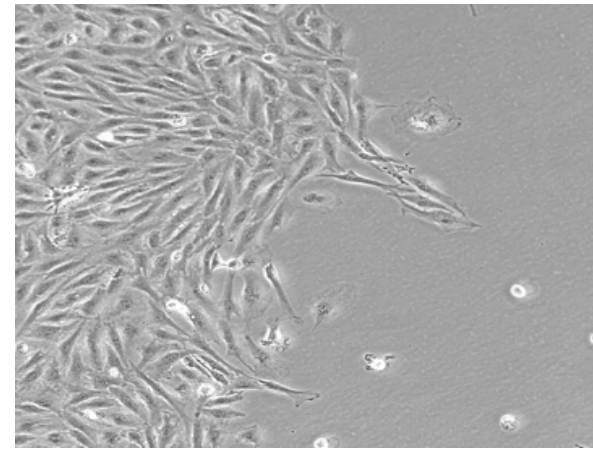
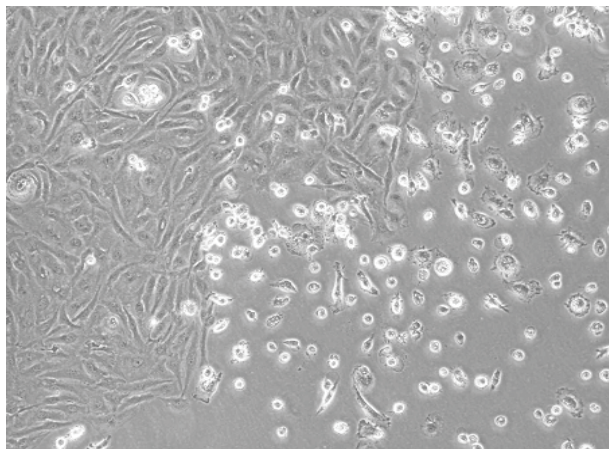


**cluster formation**



**linear arrangement**

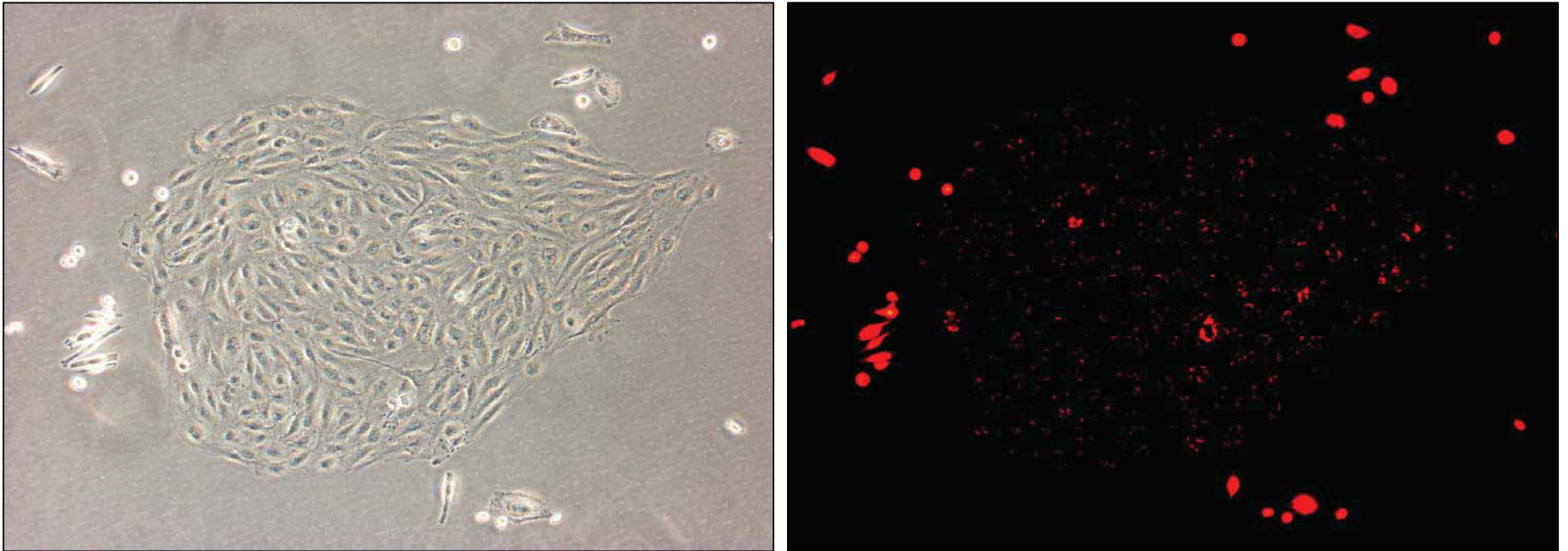
## late outgrowing cell (late EPC)



**outgrowing cobble-stone like cells and disappearing early spindle-like cells**

# Do Late EPC derive from Early EPC ?

*Hur J, Yoon CH, Kim HS, et al. ATVB 2004;24;288-293.*



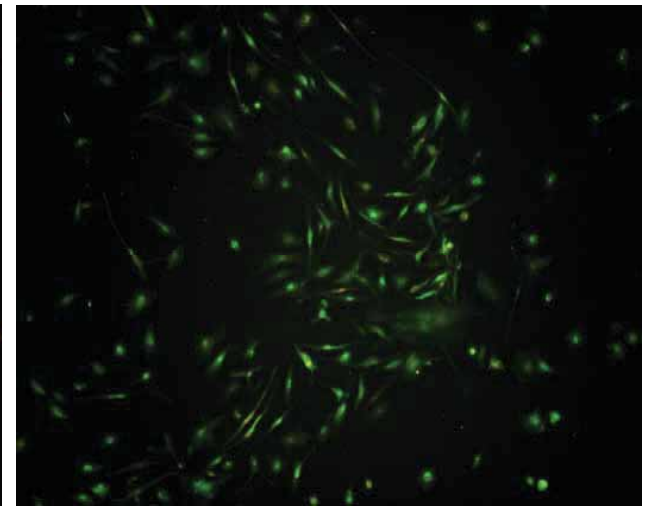
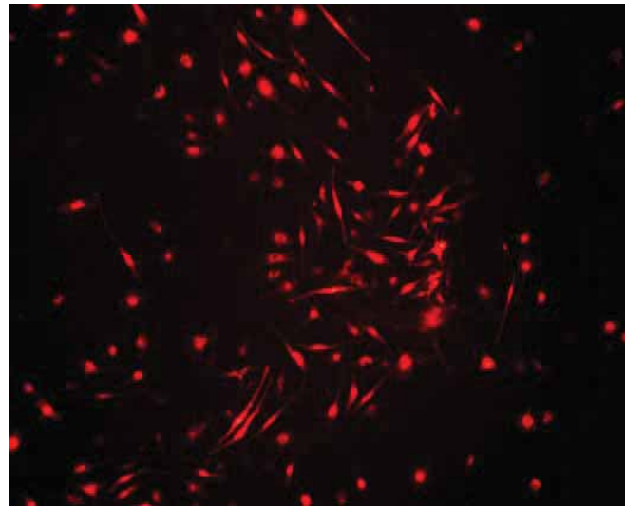
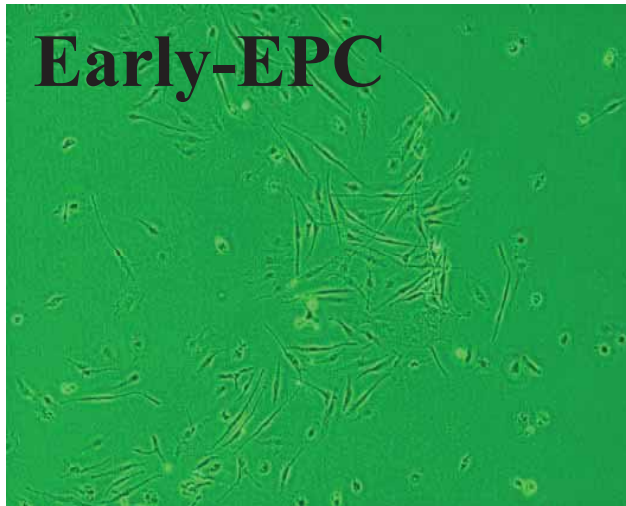
Di I-labeled early EPC were subcultured in a very low cell density.

Late EPC appeared later as a cluster of cells probably from a clone of early EPC.

This fact suggests that early EPC might be a population of heterogeneous cells  
which contain clones that can differentiate to late EPCs.

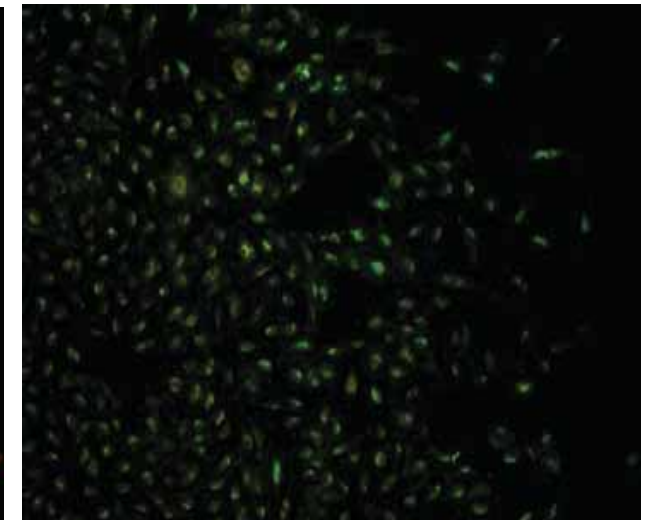
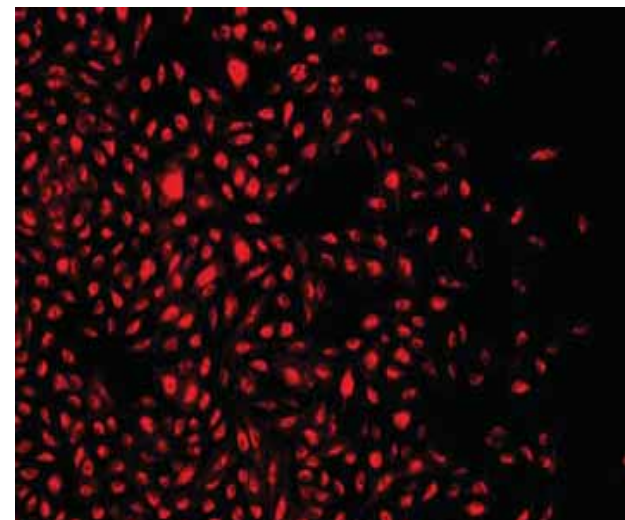
# Both have EC phenotype

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**DiI-acLDL**

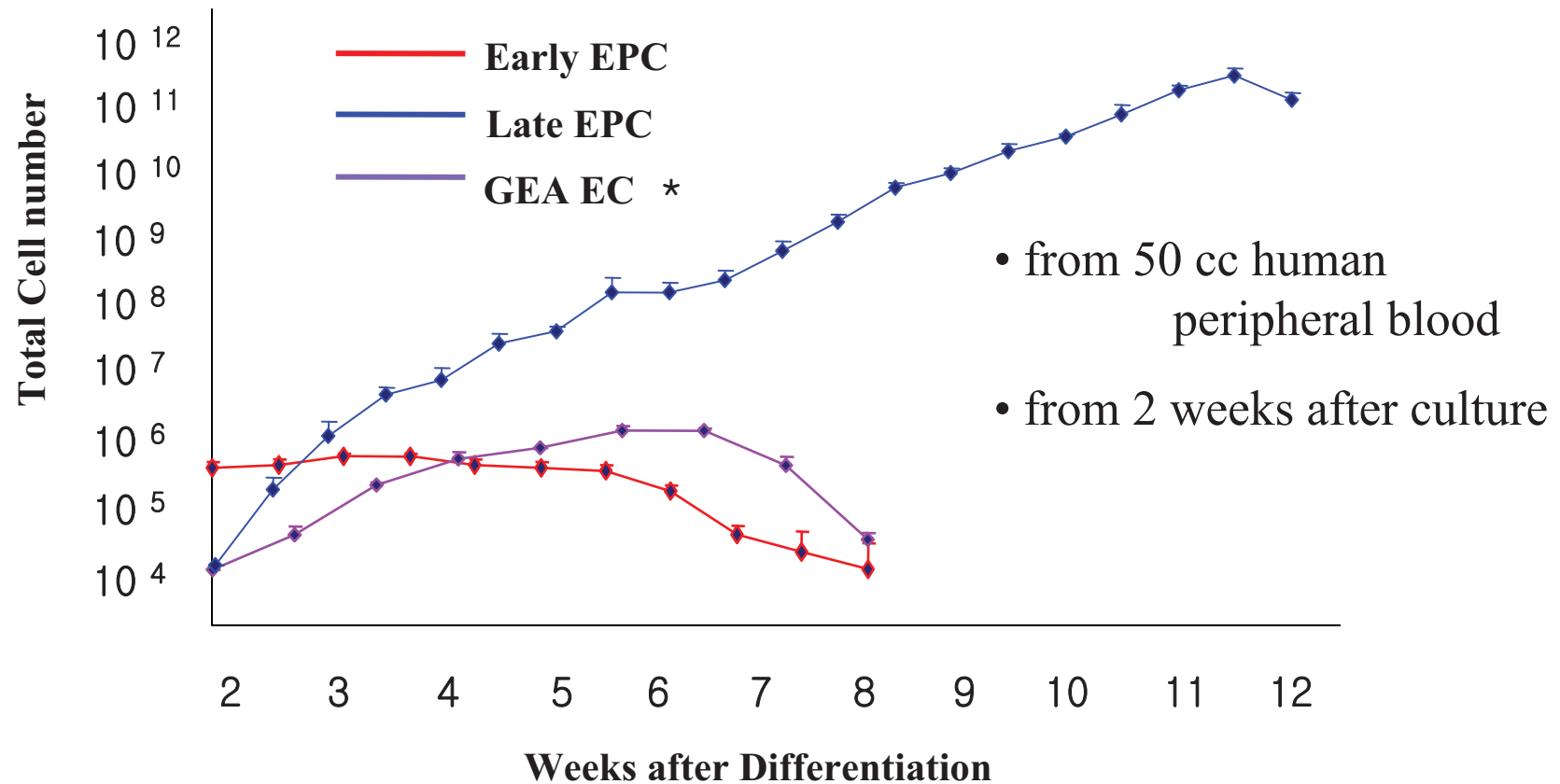
**UEA-1 Lectin/FITC**



# Growth curve of the two types of cells

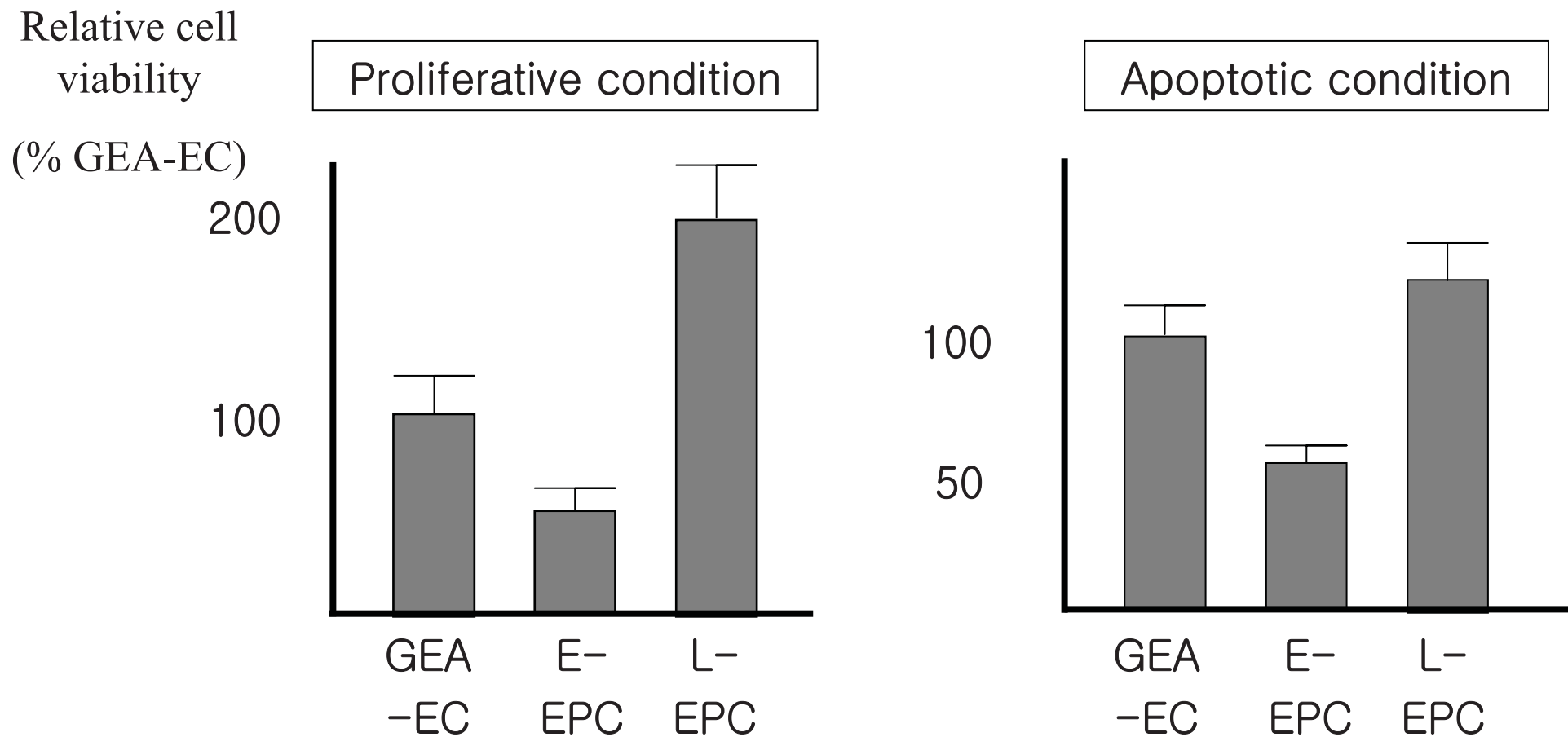
*Hur J, Yoon CH, Kim HS, et al. ATVB 2004;24;288-293.*

**Growth curves**



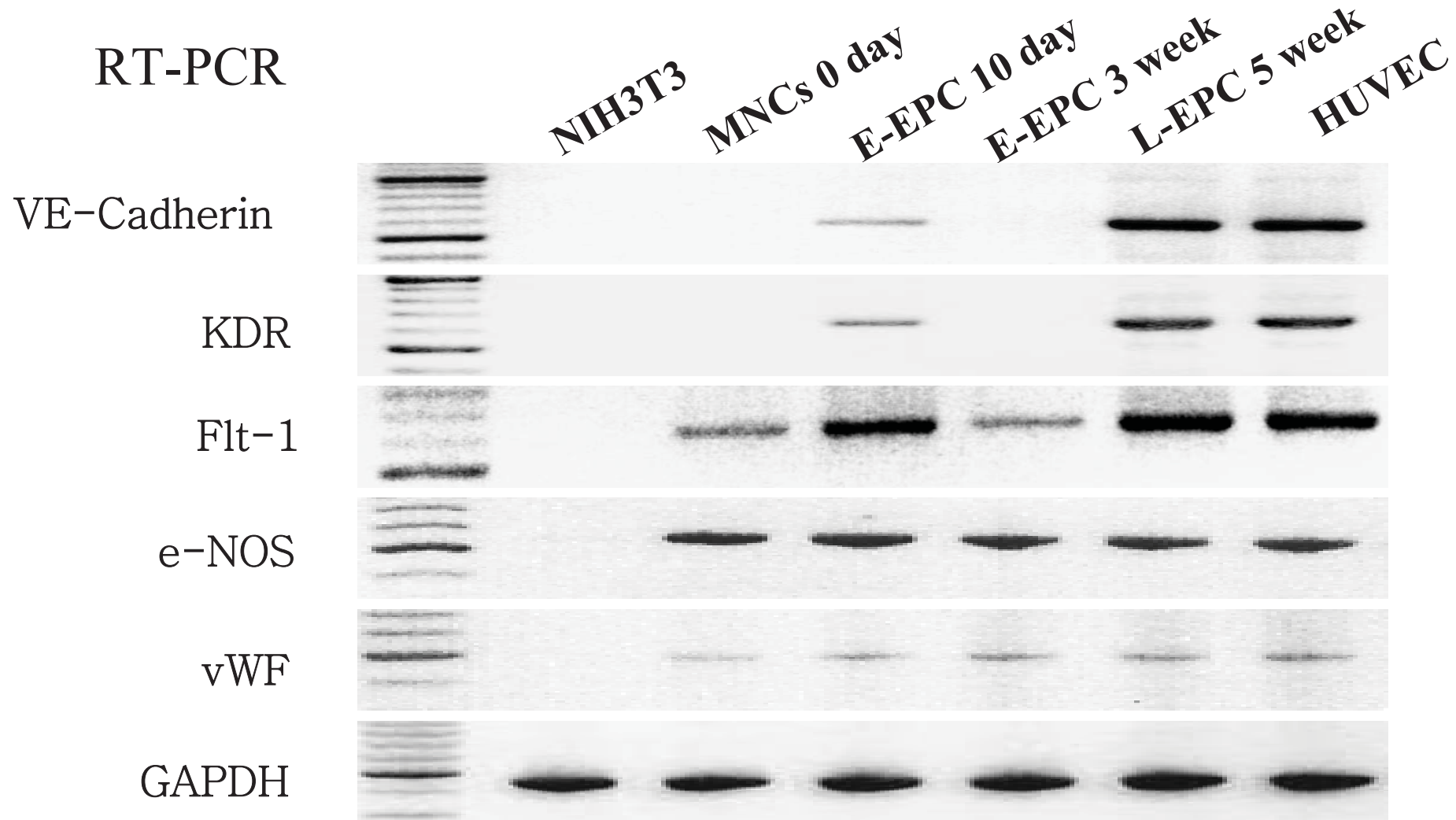
\* GEA EC : gastroepiploic artery endothelial cell from totally resected stomach

# Difference of proliferation/survival potency between two types of EPCs

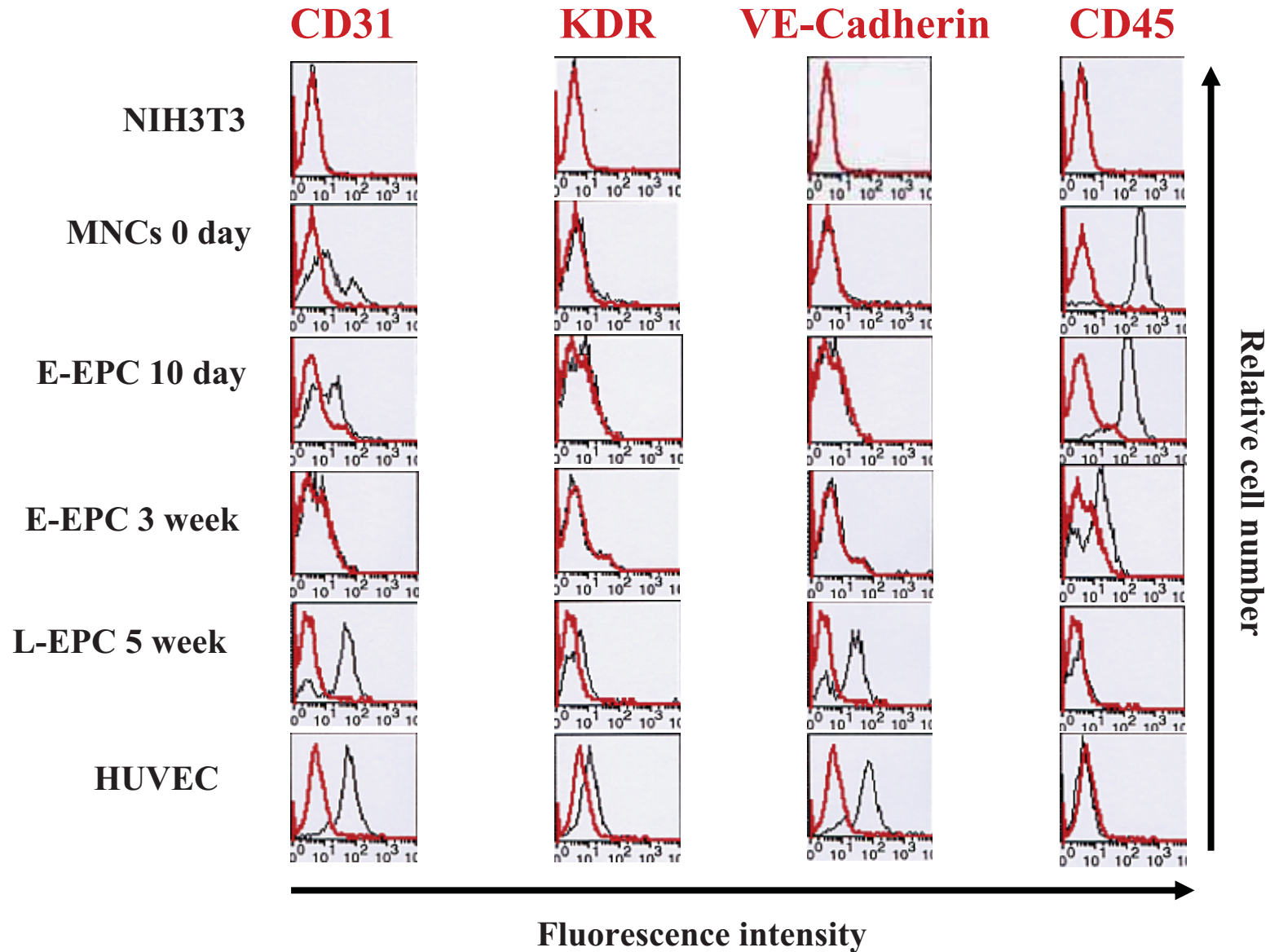




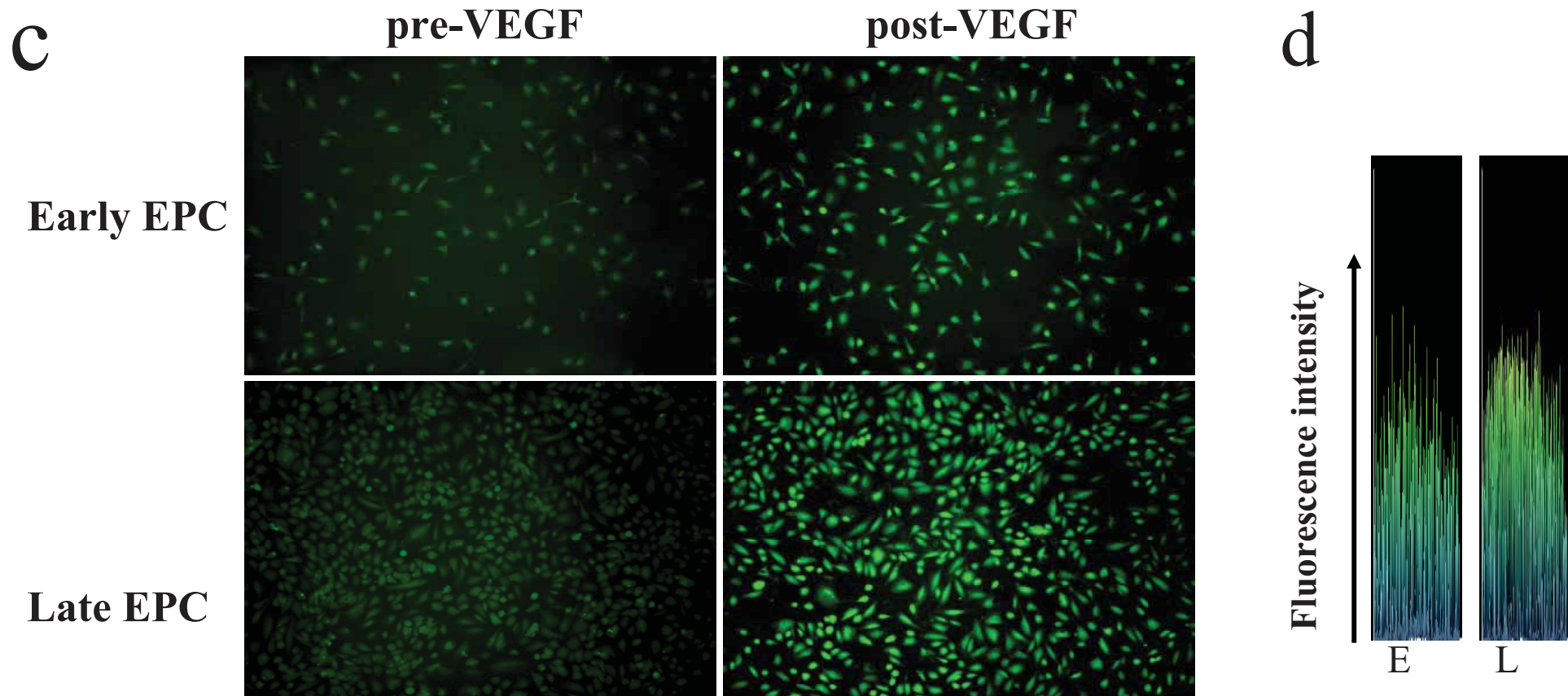
# Difference of gene expression pattern btw early-EPC & late-EPC



# Difference of protein expression pattern btw early-EPC & late-EPC

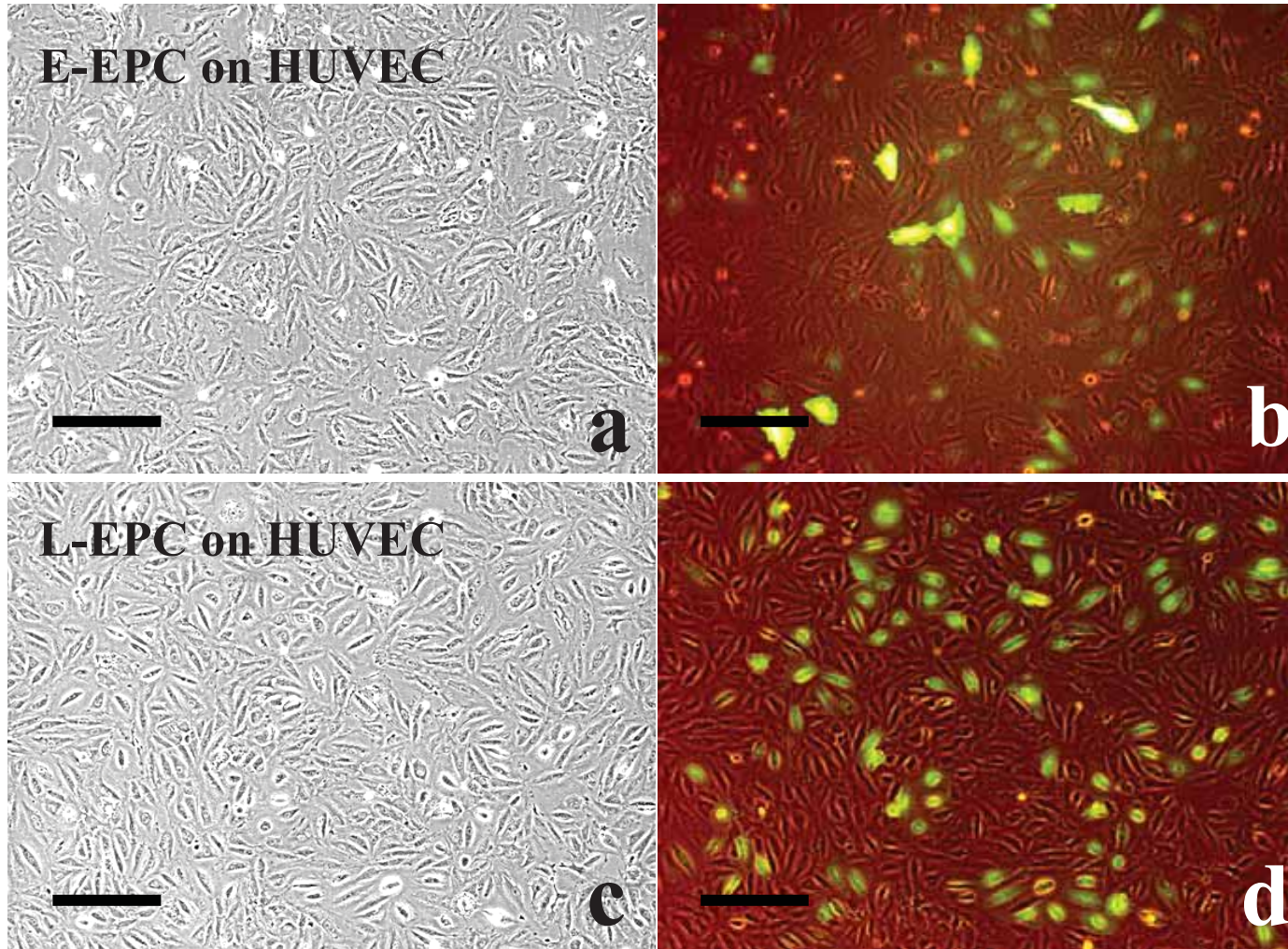


Difference in capacity of **NO** production in response to VEGF  
btw early-EPC & late-EPC



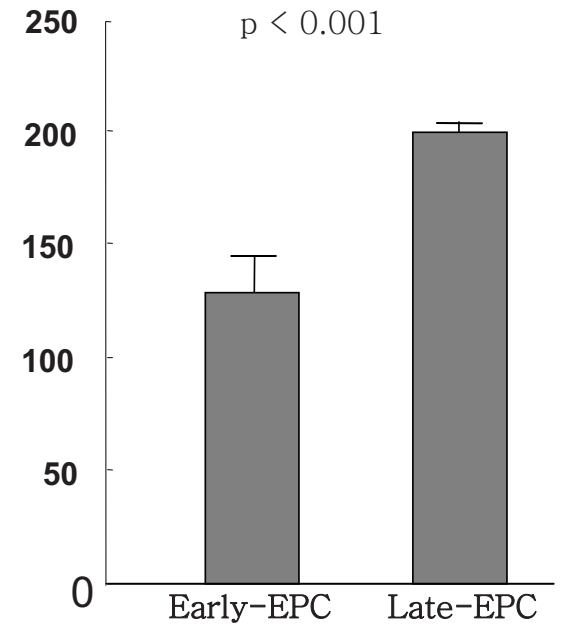
Using NO-specific fluorescence indicator, DAF2-DA (DiAminoFluorescein2 DiAcetate)

# Difference in **capacity of adhesion** to HUVEC btw early-EPC & late-EPC



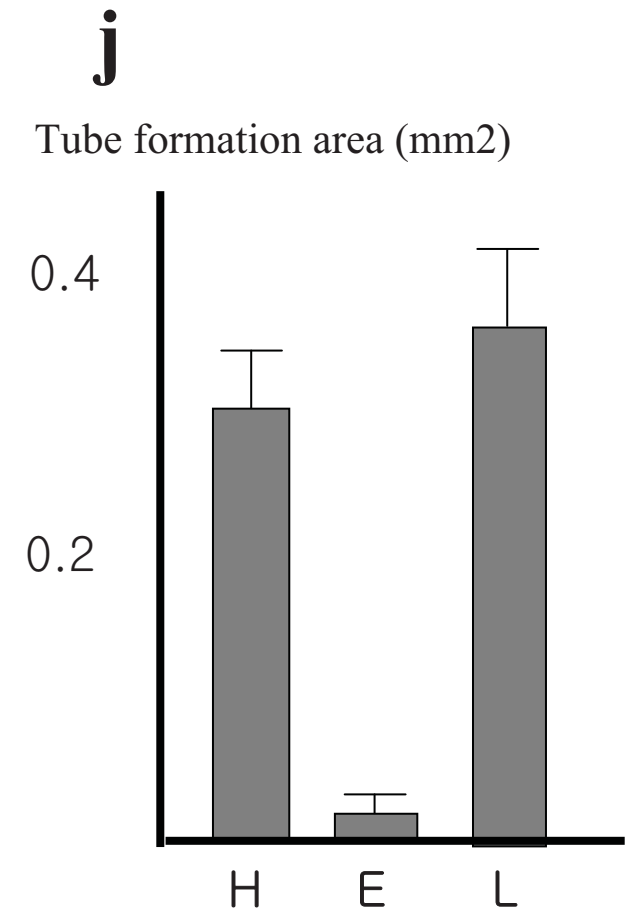
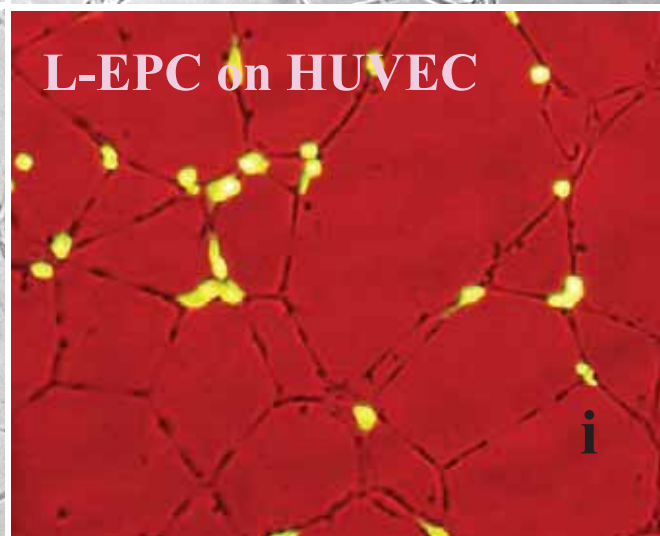
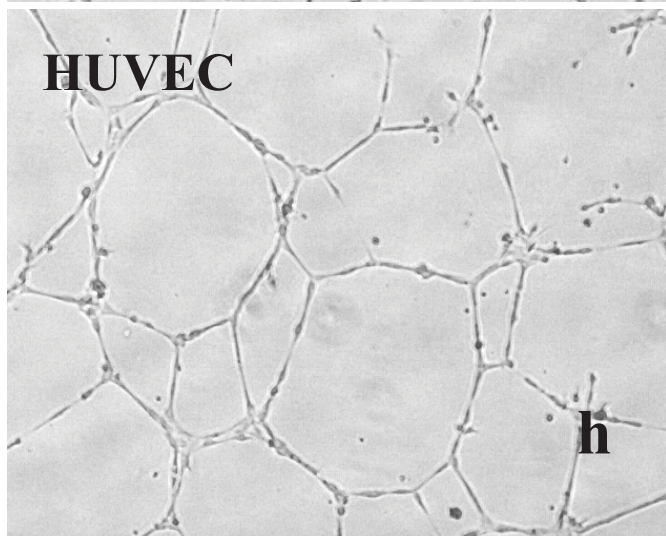
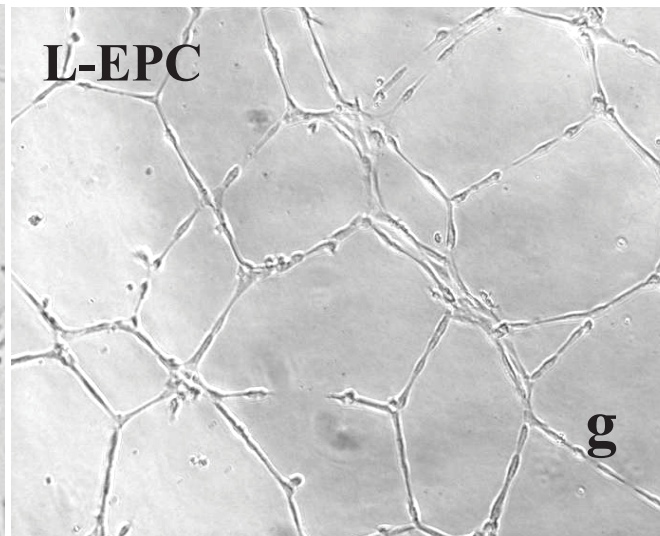
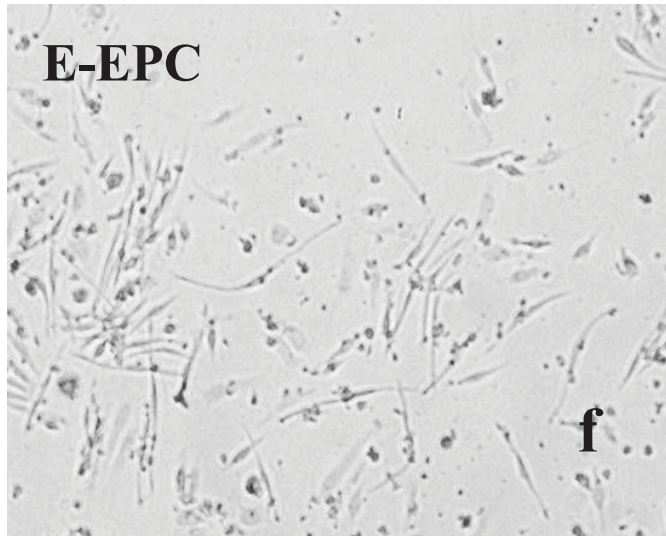
**e**

Incorporated cell  
number / 0.4 mm<sup>2</sup>



E- or L-EPC were transduced with Adv-GFP.

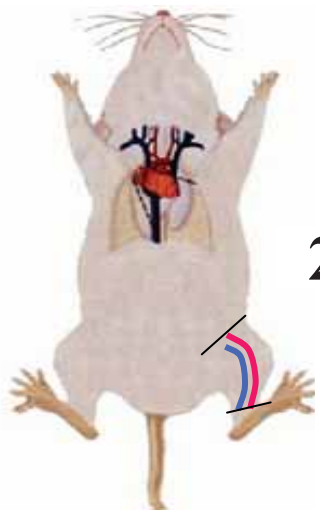
# Difference in **capacity to form a capillary tube** btw early-EPC & late-EPC



L-EPC were transduced with Adv-GFP.

# Hindlimb Ischemia Model of Nude Mouse

## Analysis of salvage of ischemic limb



21 days



limb salvage



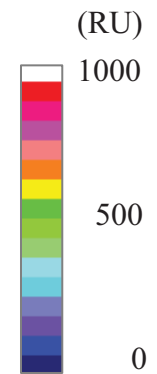
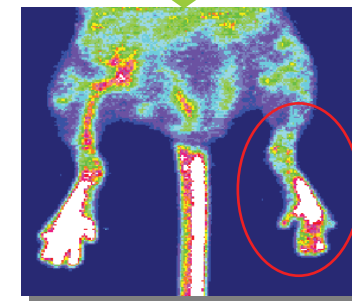
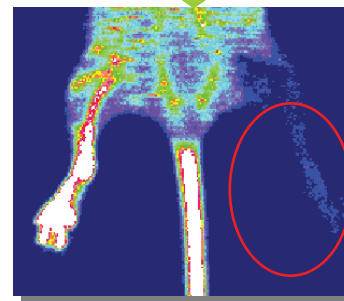
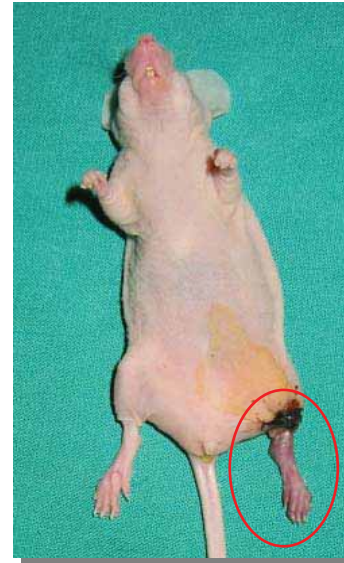
tip necrosis



limb necrosis

hindlimb ischemia

# Laser Doppler perfusion imager : non-invasive evaluation of blood flow



**Index of neovascularization :**

$$= \frac{\text{perfusion of ischemic limb}}{\text{perfusion of non-ischemic limb}}$$

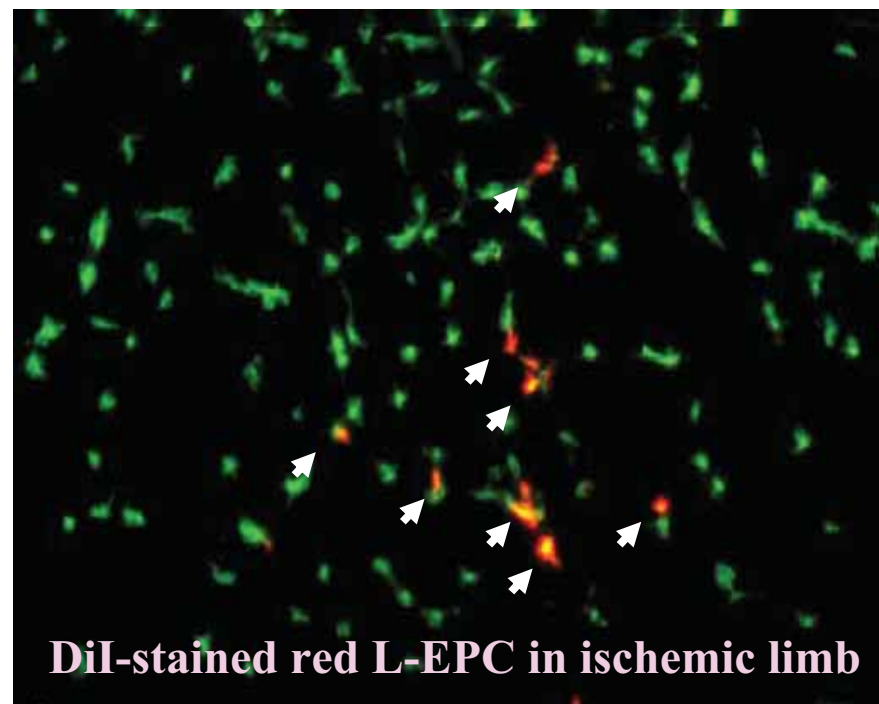
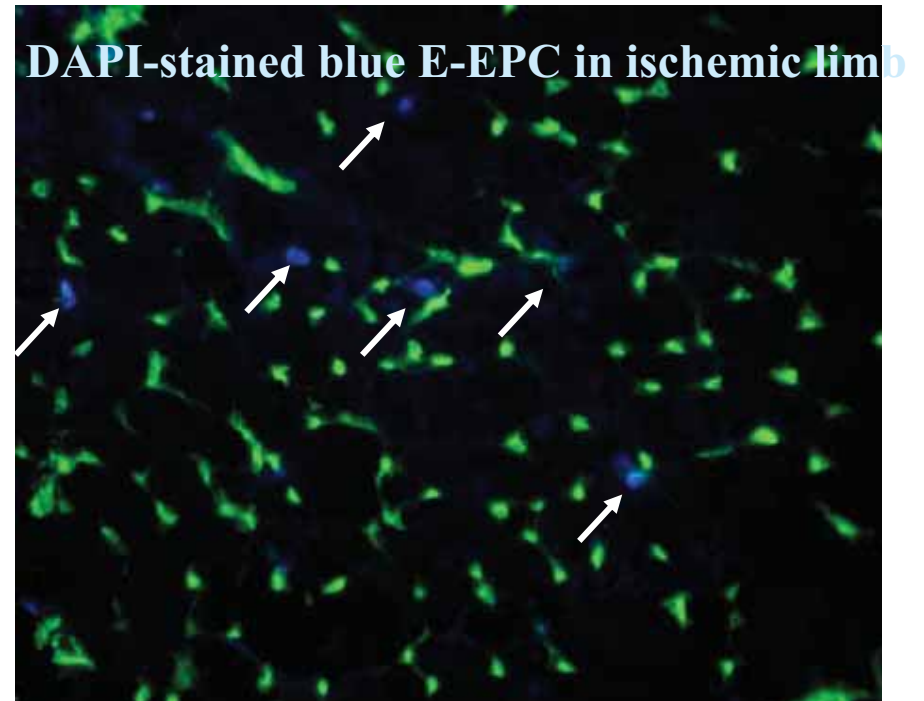
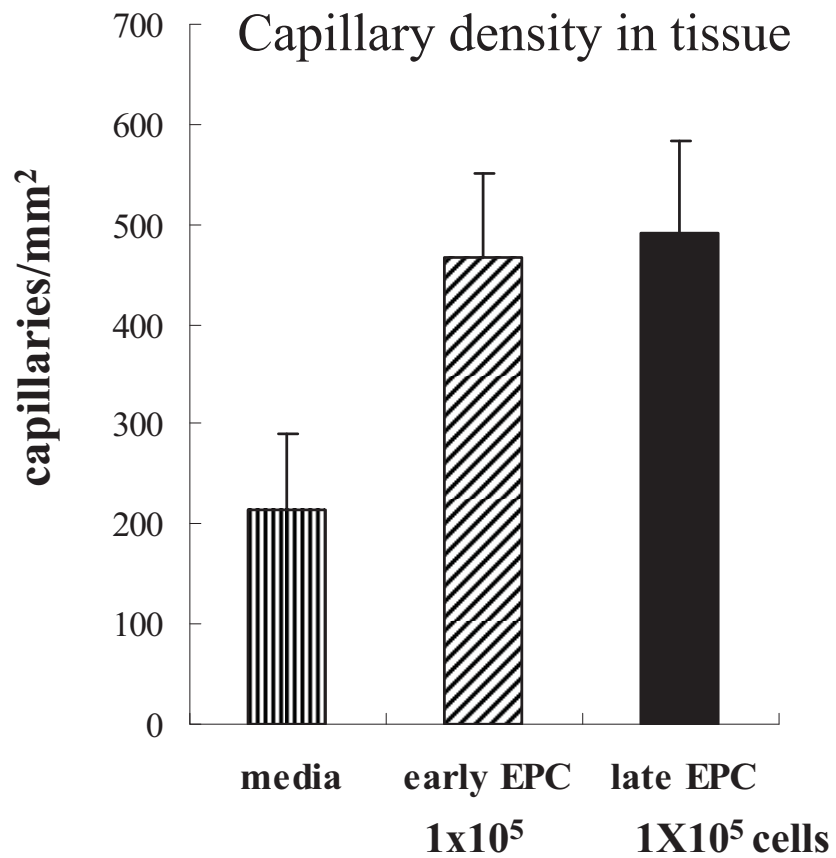
0 % : no flow

100 % : normal

decreased blood flow in ischemic limb  
(4 %)

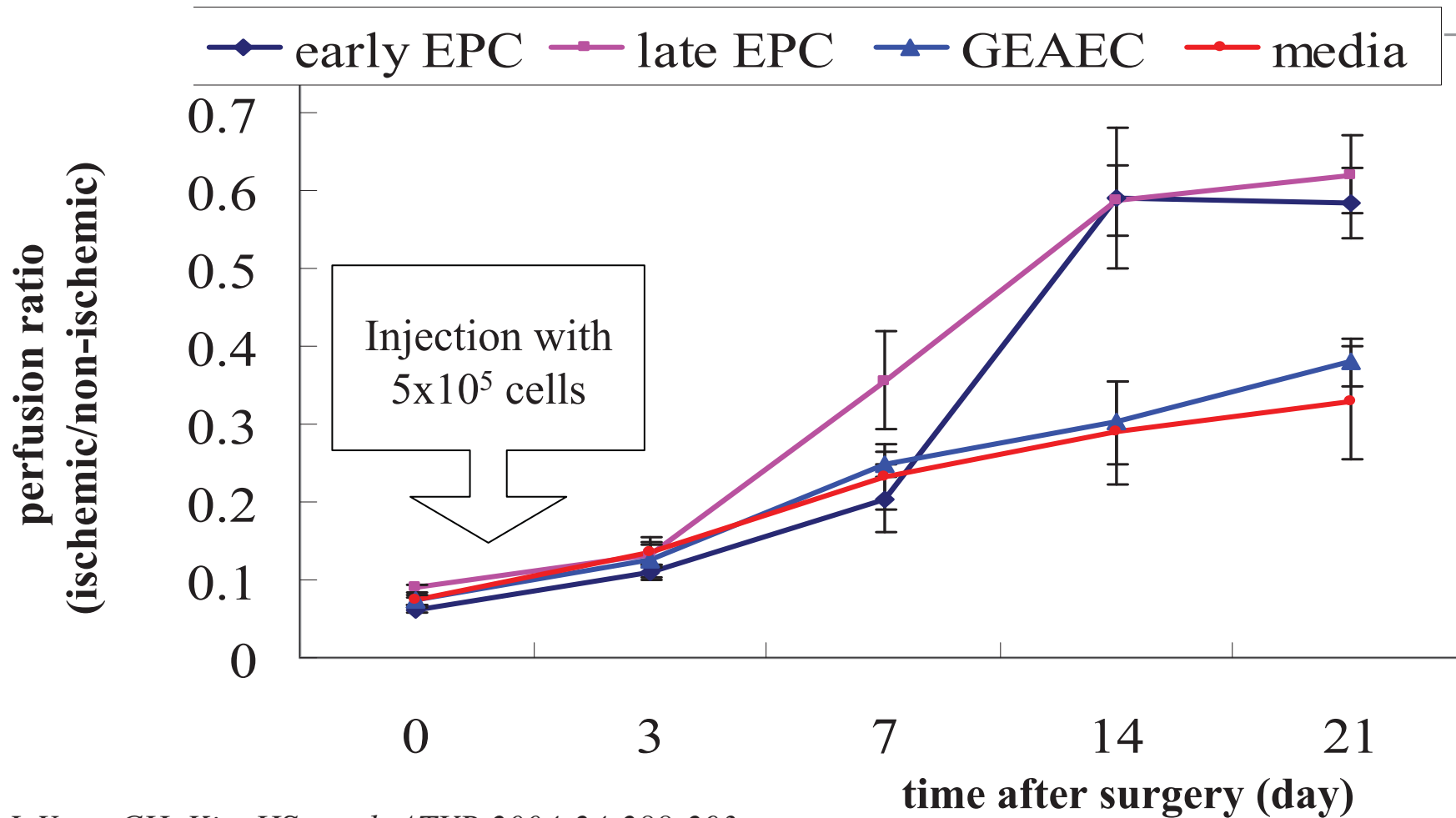
improved blood flow (59 %)

**in vivo vasculogenic potency & differentiation to endothelial cell**  
of early-EPC & late-EPC  
in hind limb ischemic model



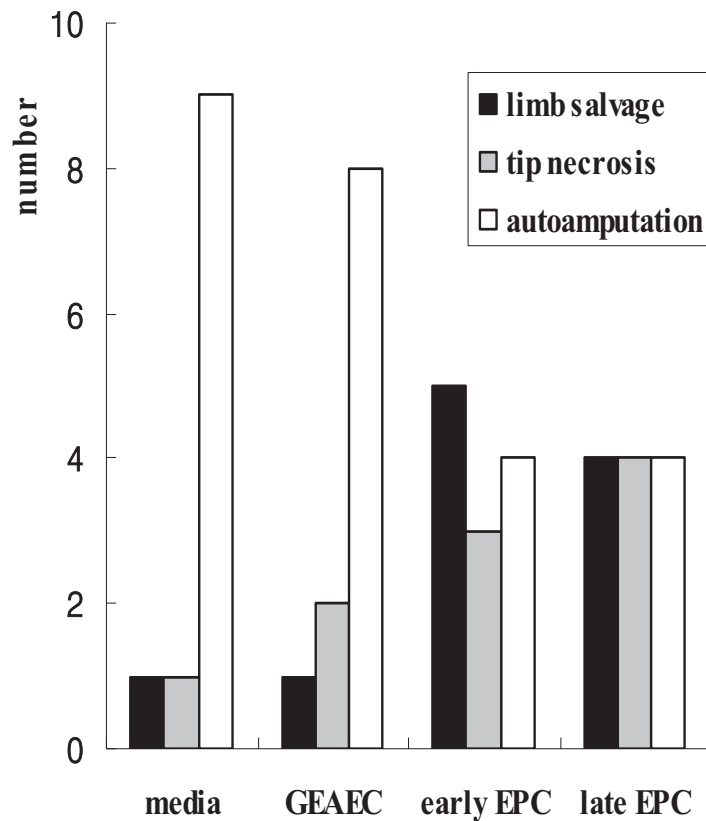


# Increased perfusion by transplantation of EPC

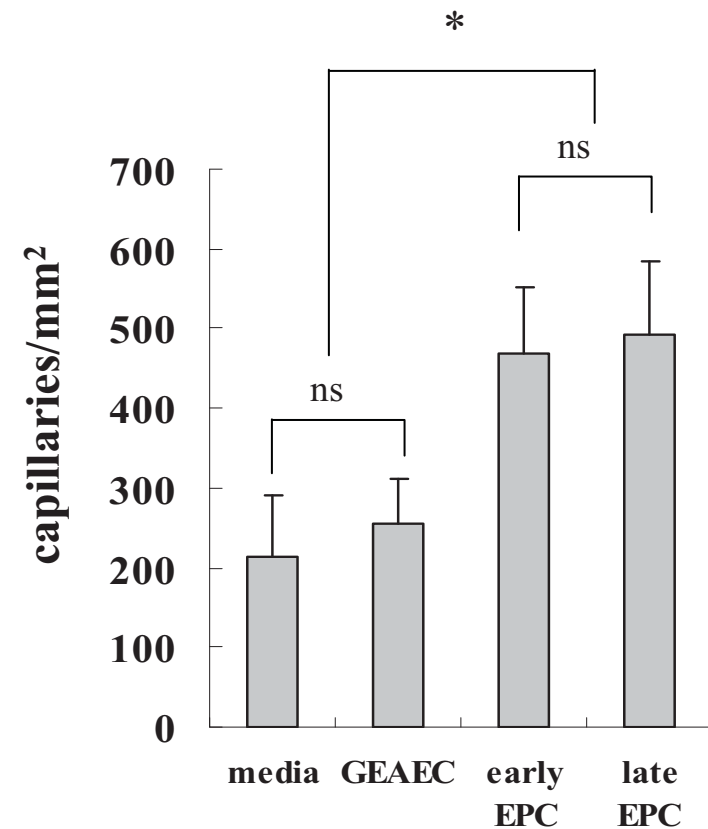


# EPC transplantation saves the ischemic limbs

## limb salvage



## capillary density



# Two different roles of Early & Late EPC in vasculogenesis

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## Early EPC

### In vitro function

**incomplete** gene expression

**poor** proliferation/survival

**poor** incorporation to EC

**poor** differentiation to EC

### In vivo function

**good** angiogenic potency

## Late EPC

### In vitro function

**complete** gene expression

**good** proliferation/survival

**good** incorporation to EC

**good** differentiation to EC

### In vivo function

**good** angiogenic potency