Dr. Chandrasekhara Kartha

# My affiliation

Division of Cardiovascular Disease Biology,

Rajiv Gandhi Center for Biotechnology, Trivandrum

# Facilities available

Molecular biology facilities, DNA sequencer, FACS, electron microscopes, confocal microscope, MALDI-TOF

Animal house for experiments in small animals

A central cell line repository

A bioinformatics center

# My interests

- Delineate  pathogenic mechanisms of cardiovascular diseases, aimed at identifying therapeutic targets
- Develop cell-based regenerative therapies for cardiovascular diseases

## Cell-based Therapies for Ischemic Heart and Peripheral Vascular Diseases

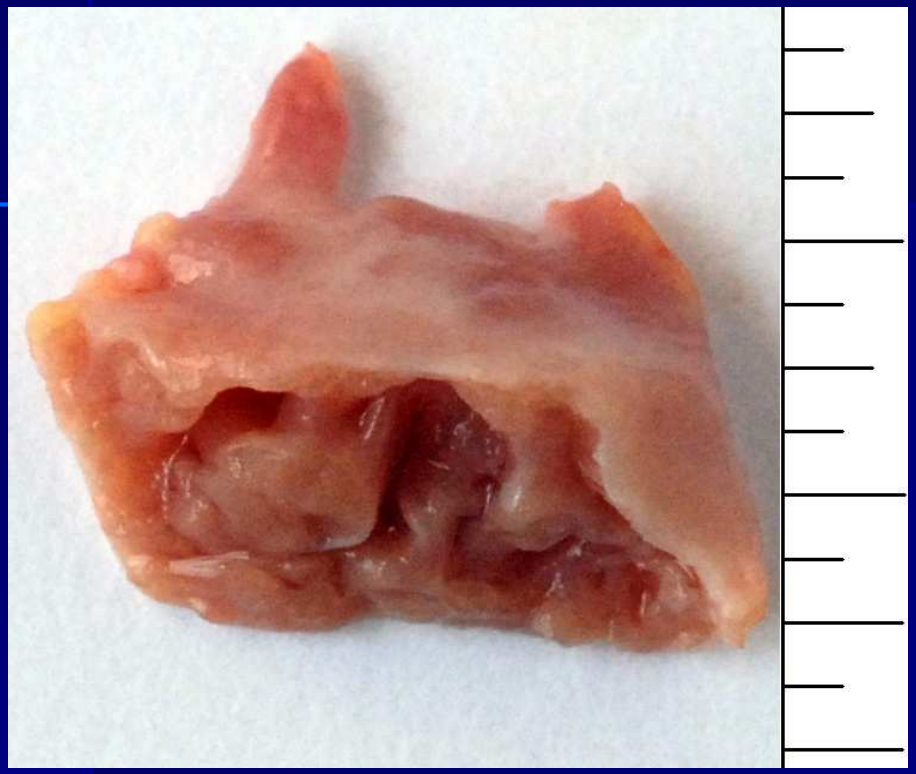
- Adult cardiac stem cells
- Engineered endothelial progenitor cells

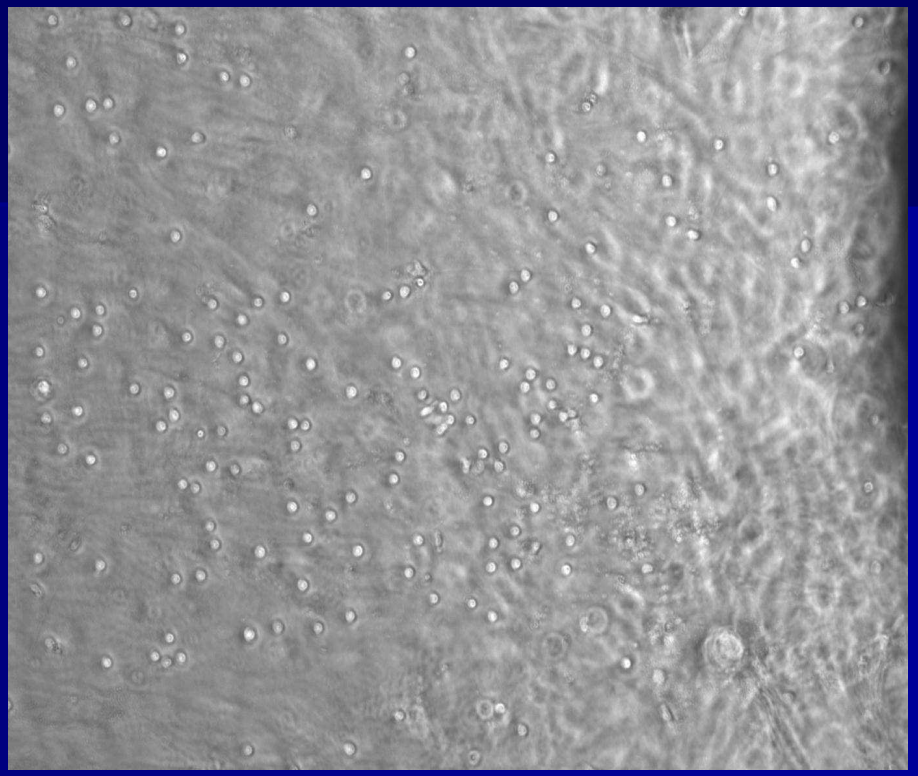
## Atherosclerosis Risk in Type 2 Diabetes Mellitus

- Mechanism: ? MCP-1
- A target to reduce risk
- Biomarker

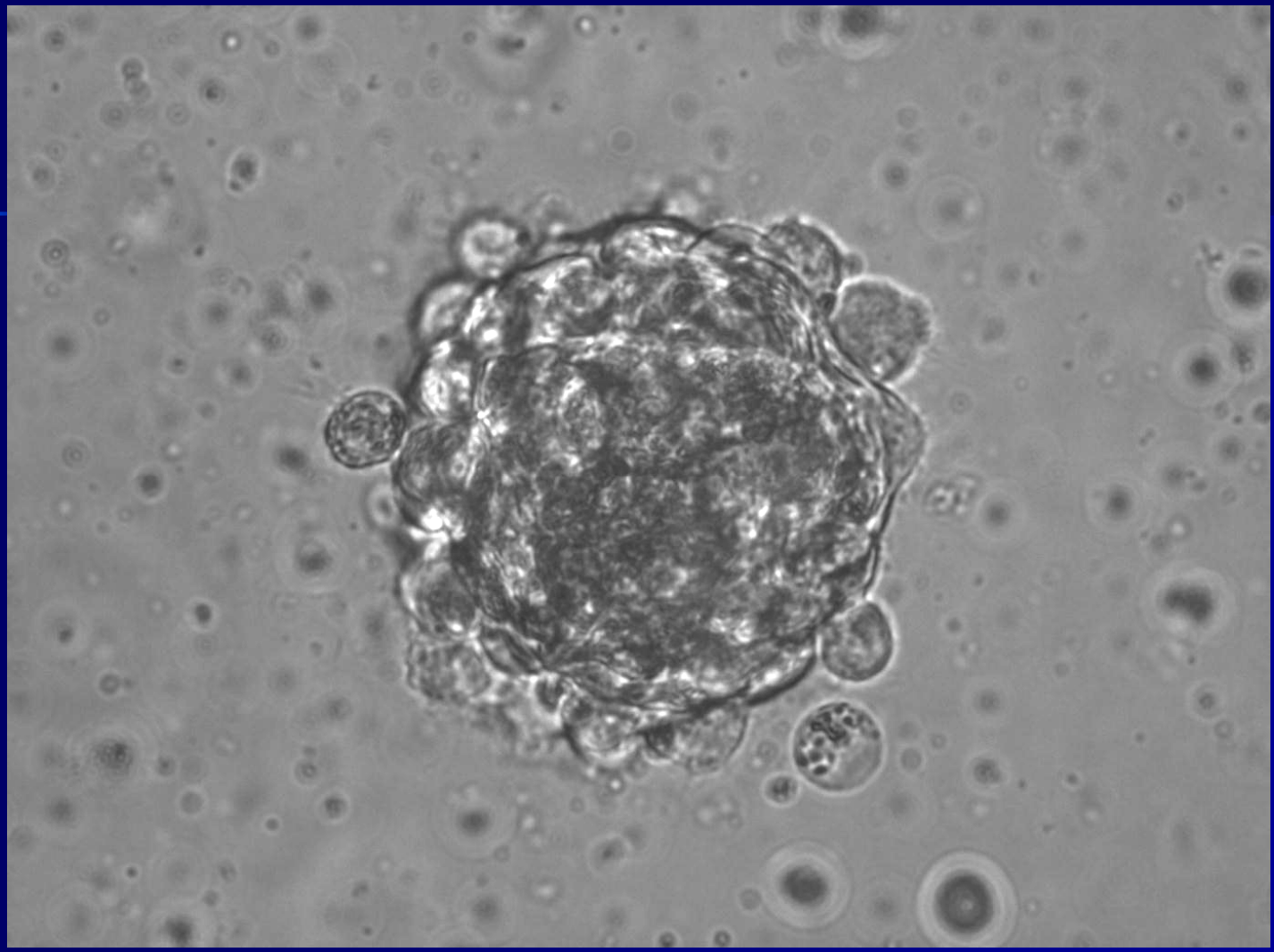
## Endothelial Remodeling in Cardiac Failure

- Characterization
- Influence on myocardial function

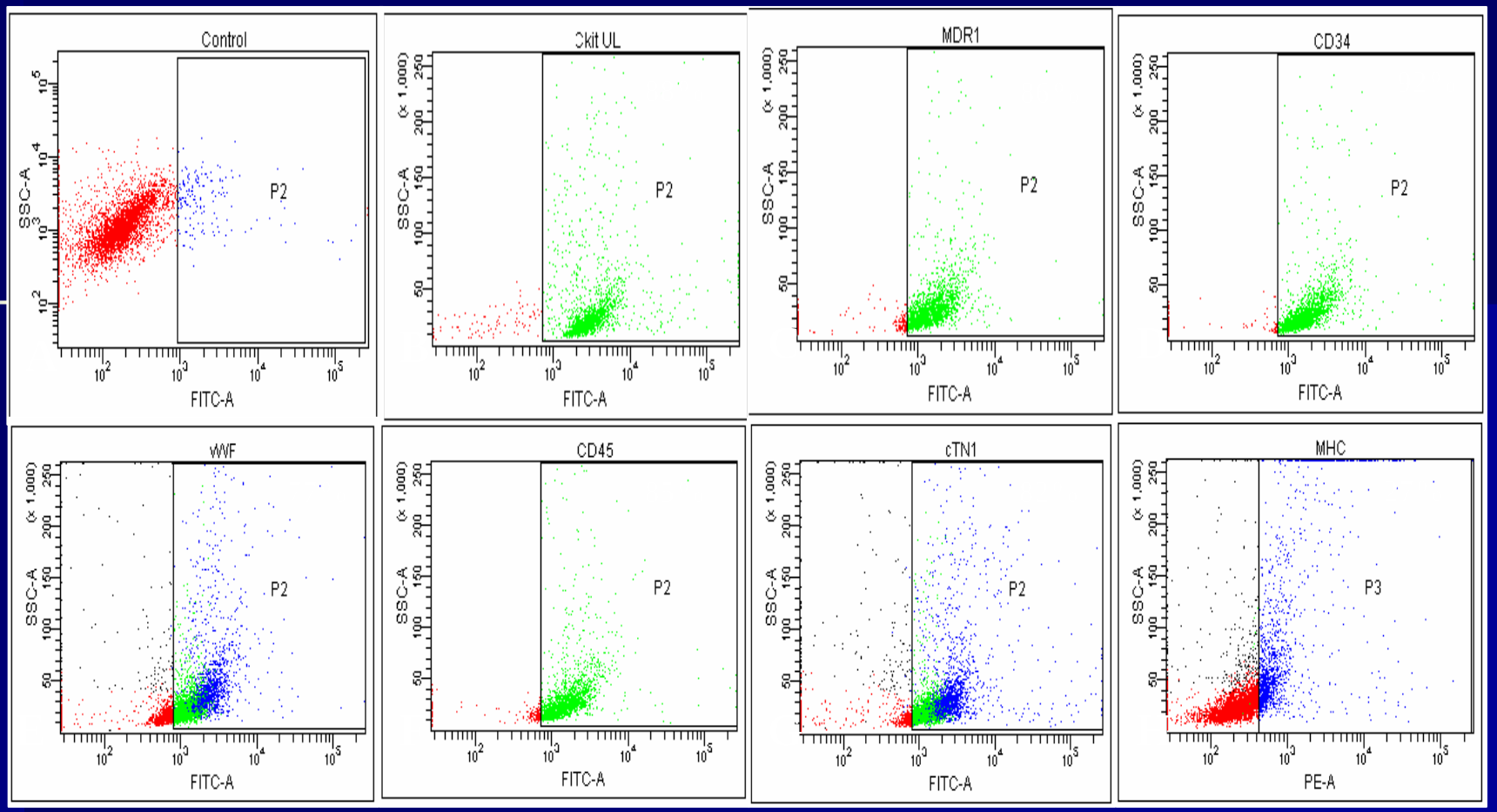




*Phase contrast micrograph of migrated phase-bright cells over adherent fibroblast cells, 3rd week Nonenzymatic method* (3.45 - 6.46 x$10^5$ cells/mL/g sample tissue)

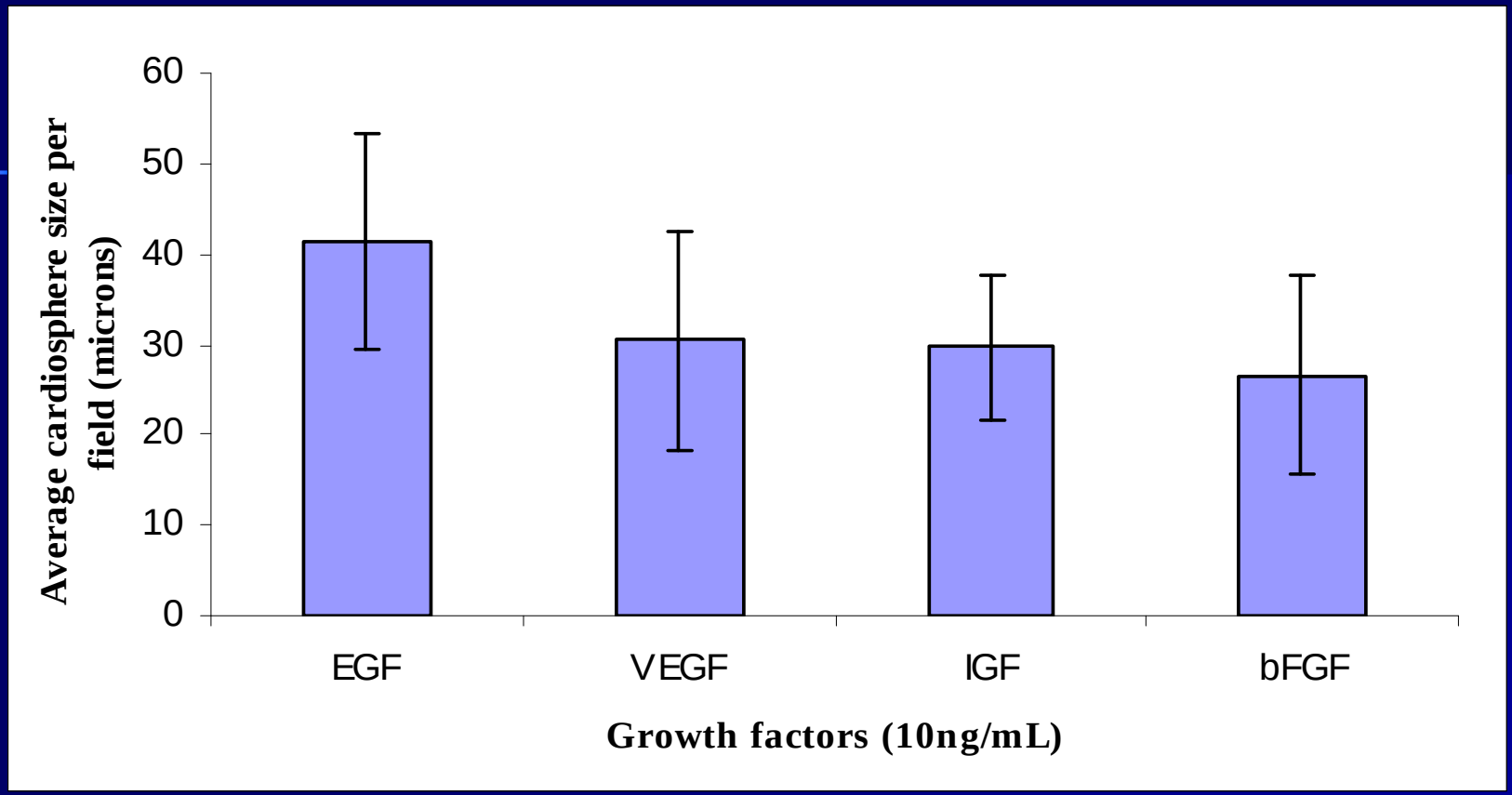


*Phase contrast micrograph of a floating Cardiosphere after 72 hrs in culture*



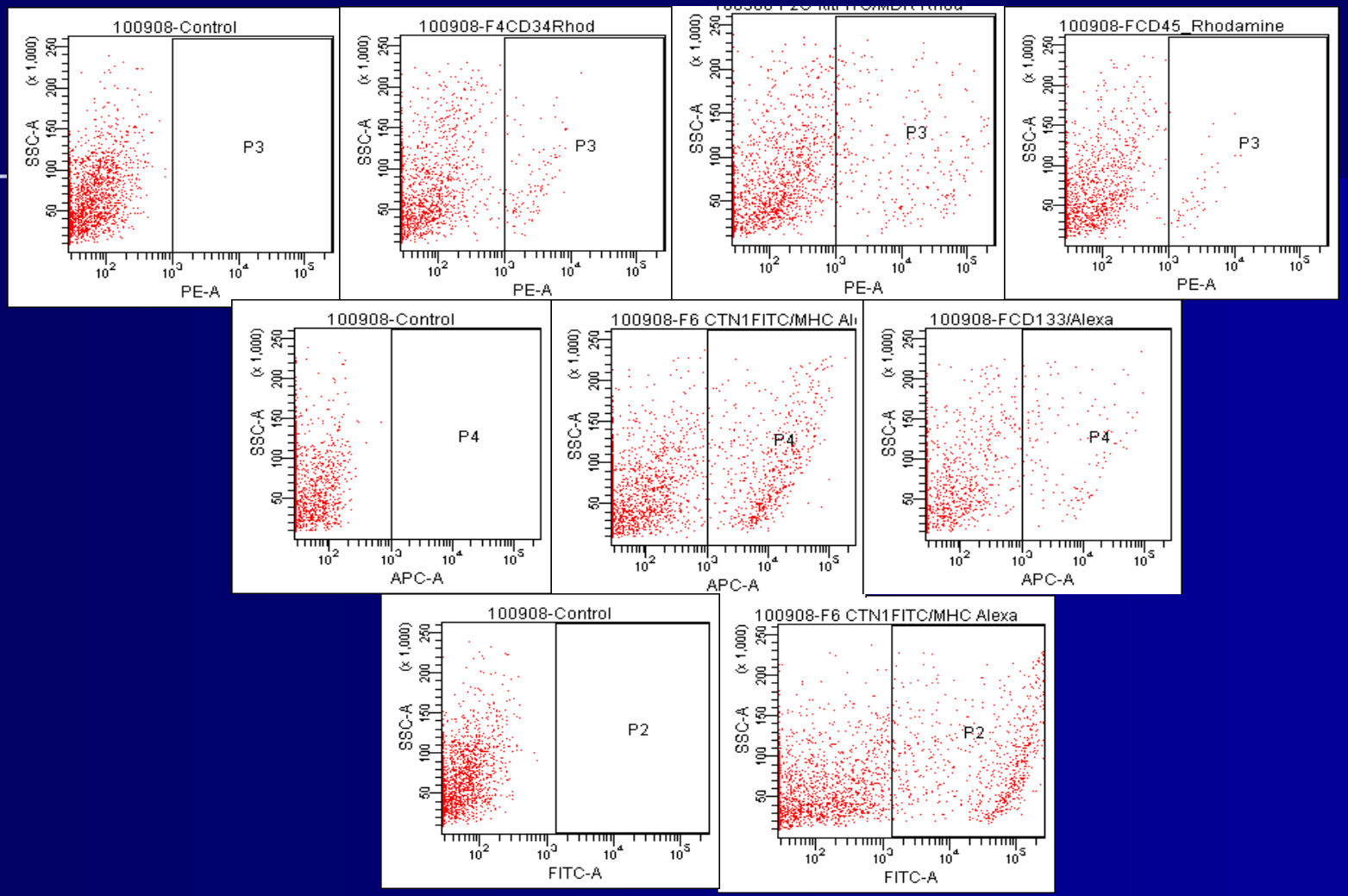

***Fluorescence activated cell-sorting analysis of cardiosphere cells***

***(A) FITC control. Phenotypic profile of CS cells stained positive for (B) ckit, (C) MDR1, (D) CD34, (E) vWF, (F) CD45, (G) cTN1 and (H) MHC expression***

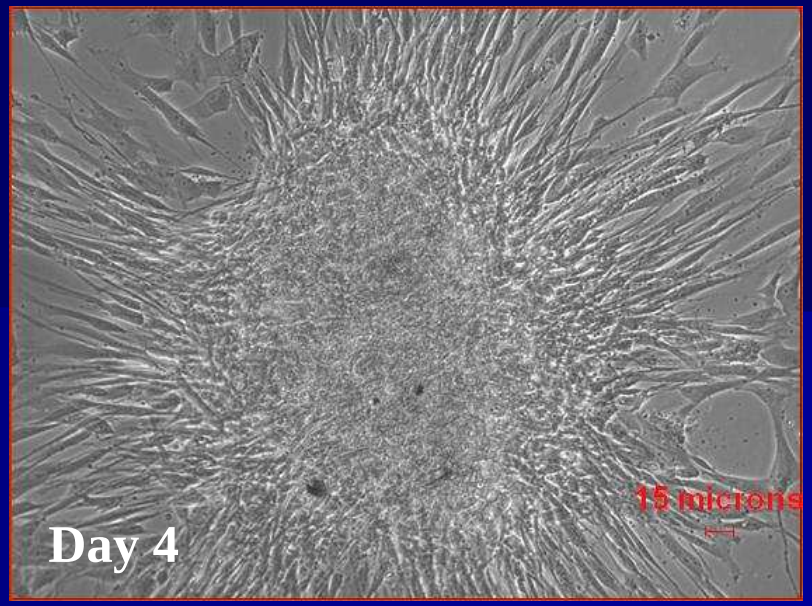


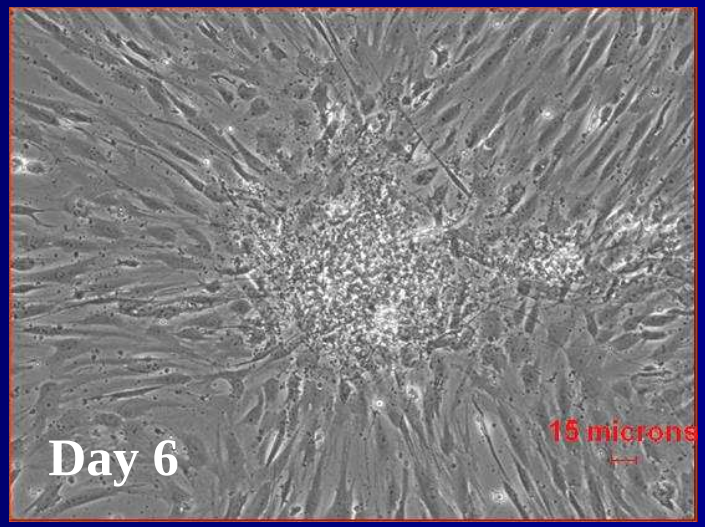

***Effect of growth factors on cardiosphere formation***

# Phenotypic profile of CDCs



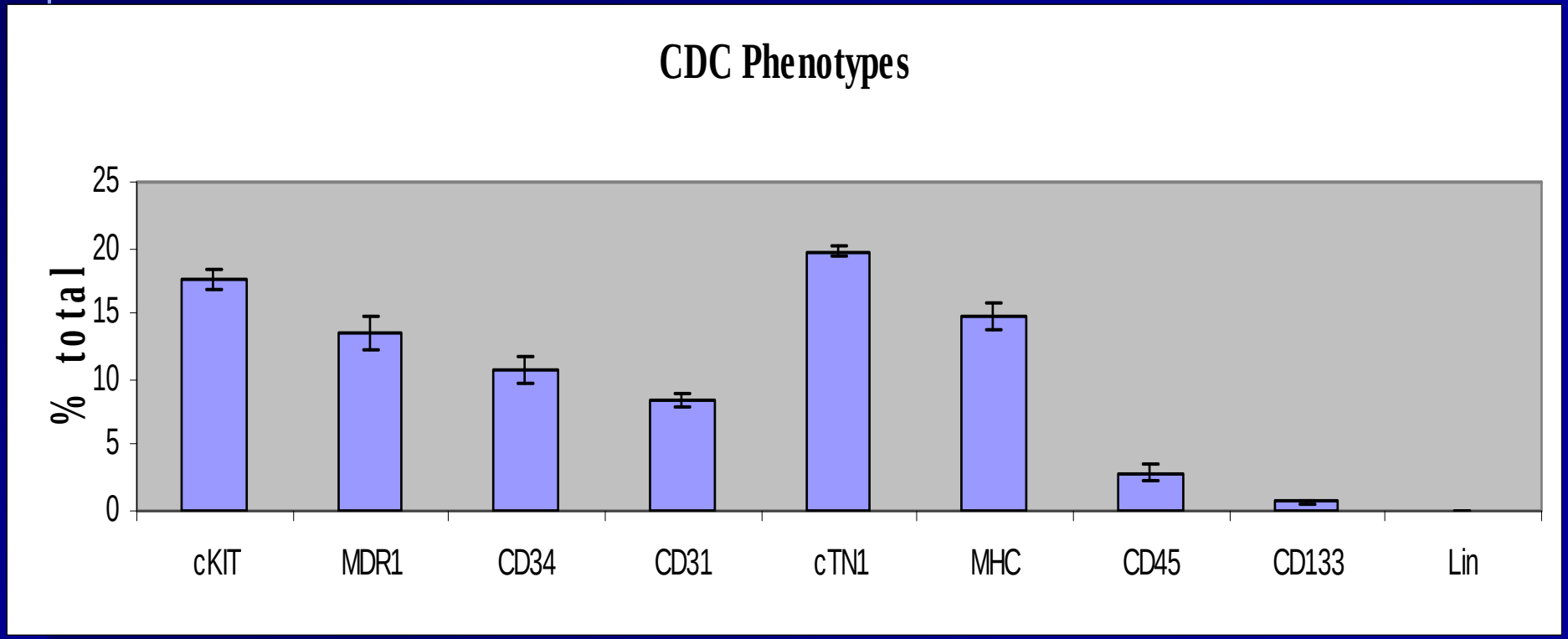

*A- Control PE, B- cells stained for CD34, C- ckit, D-CD45, E-control APC, F-cTN1, G-CD133, H-control-FITC and I- MHC*

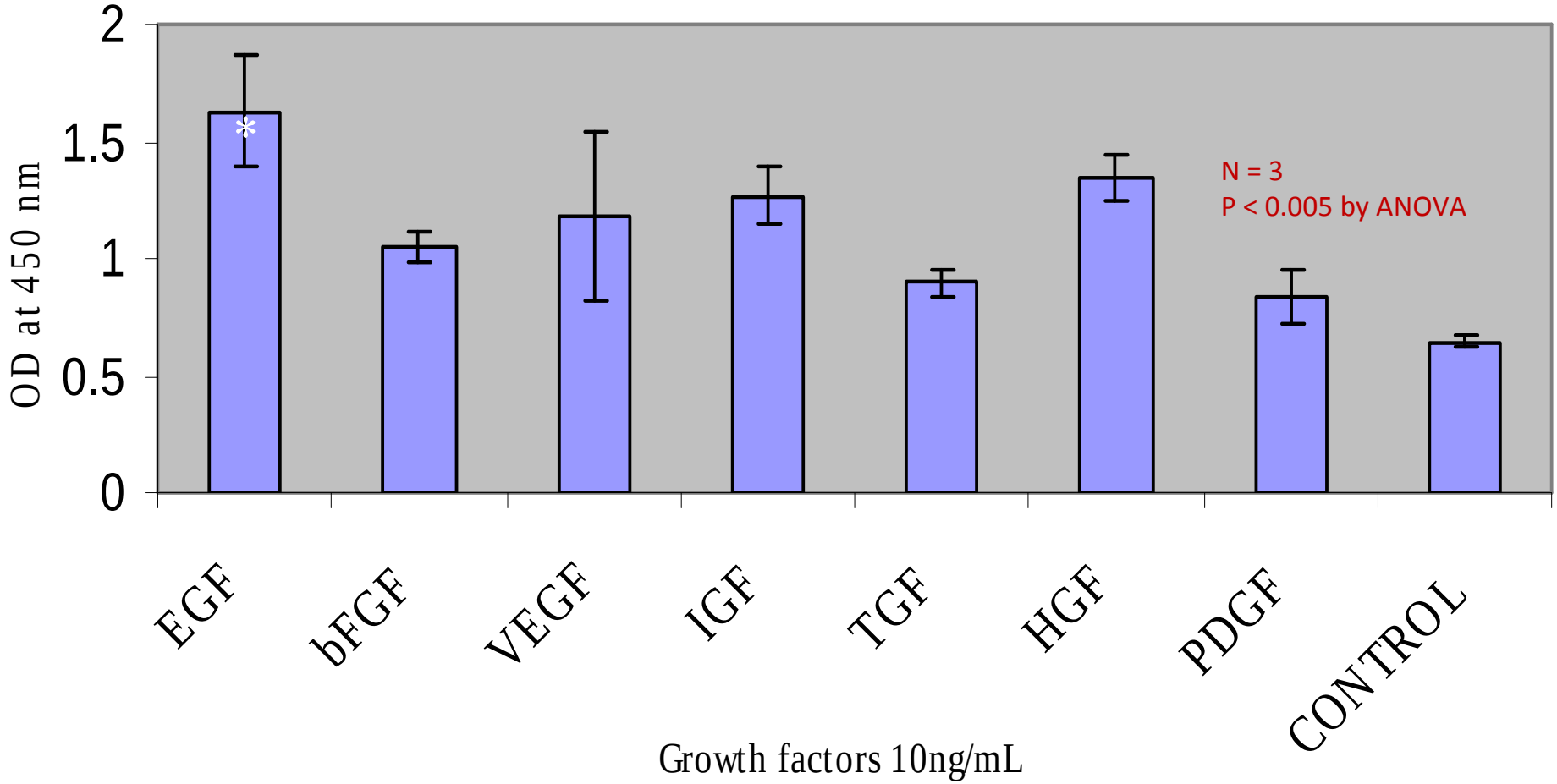

*Cardiospheres plated on fibronectin coated culture dish for expansion*

# Phenotypic profile of CDCs

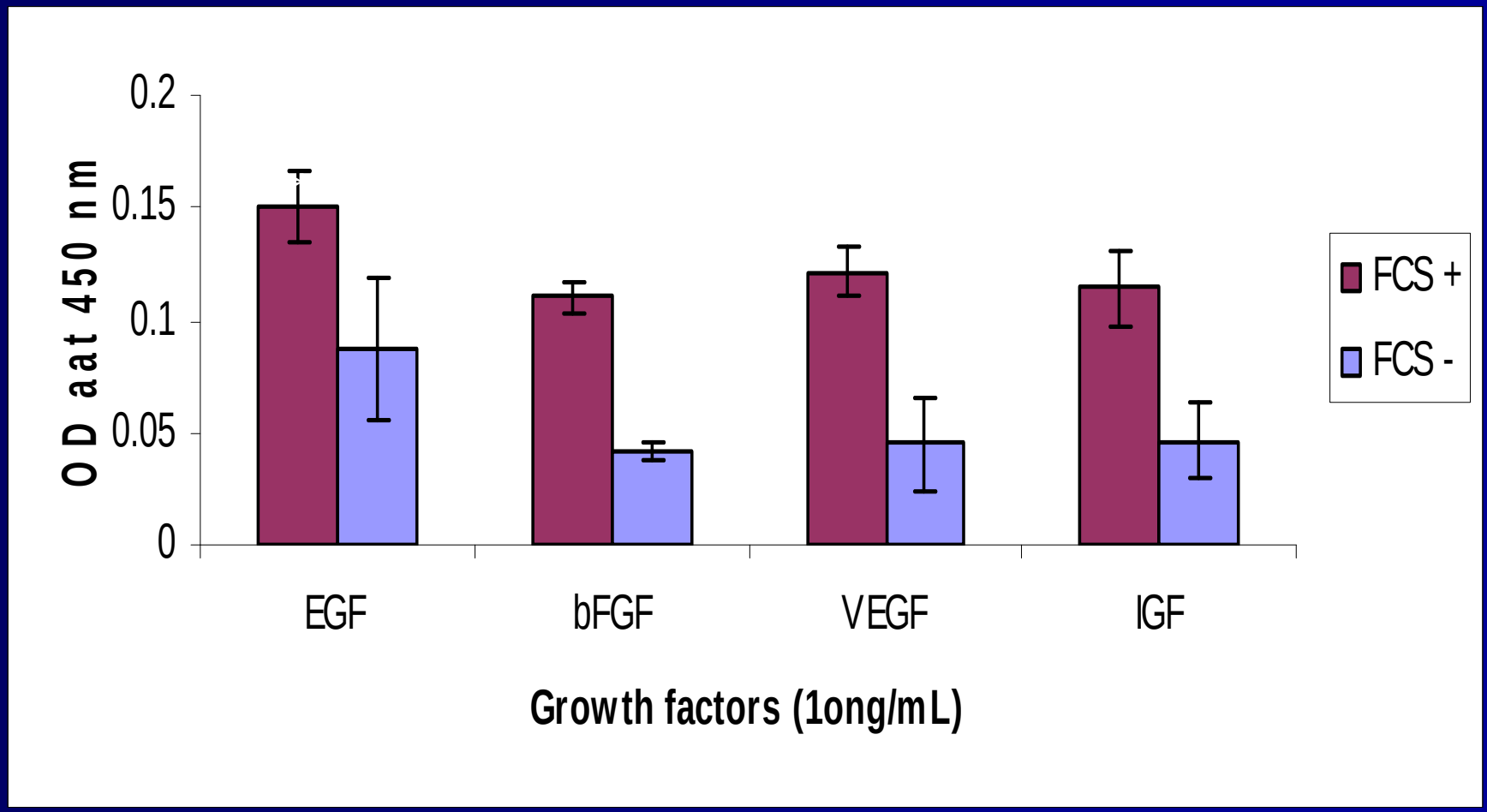


# Evaluation of the effect of growth factors on cardiosphere derived cell proliferation

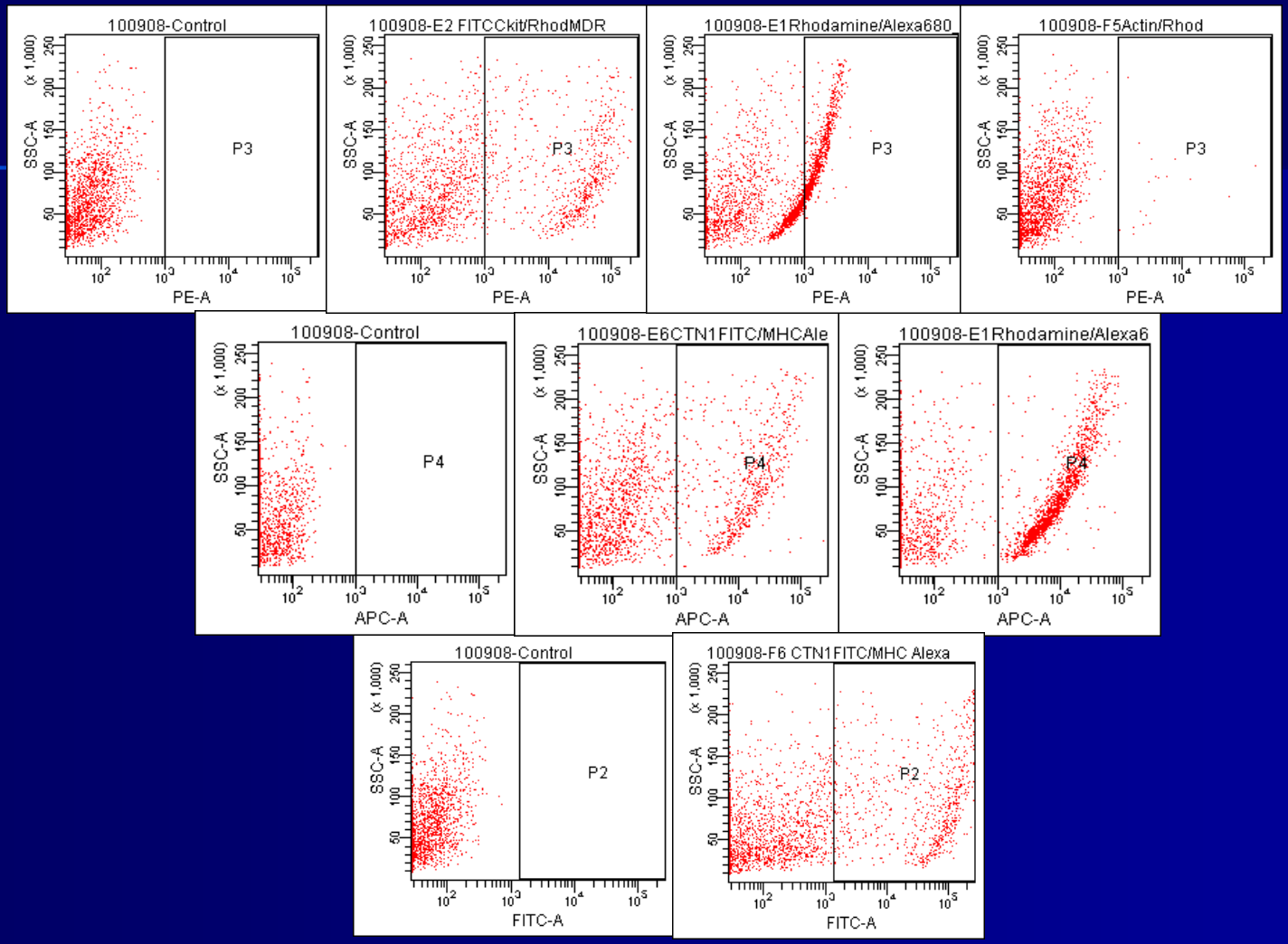
# Effect of growth factors on cardiosphere derived cell (CDC) proliferation



# Effect of growth factors on cardiosphere formation in serum reduced/deprived conditions by WST proliferation assay

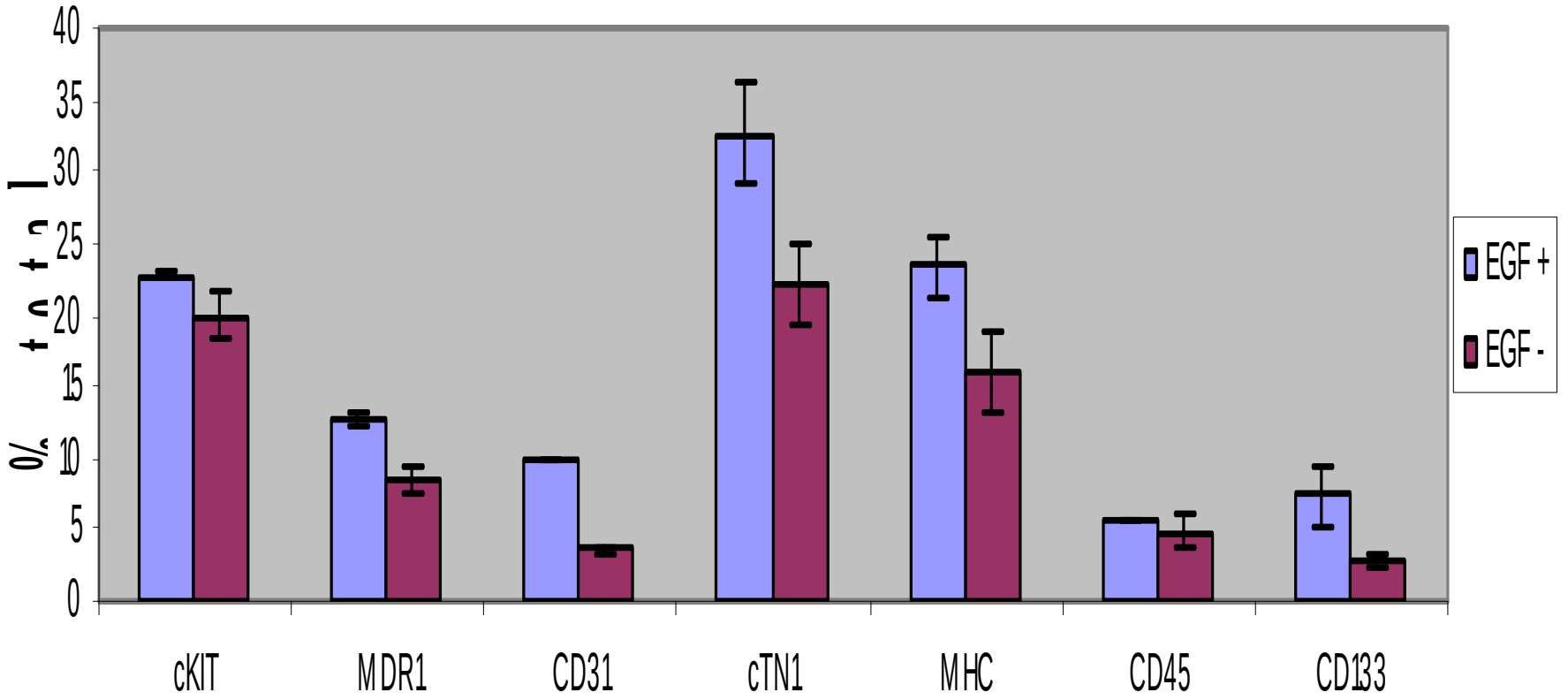


# Phenotypic profile of EGF treated CDCs

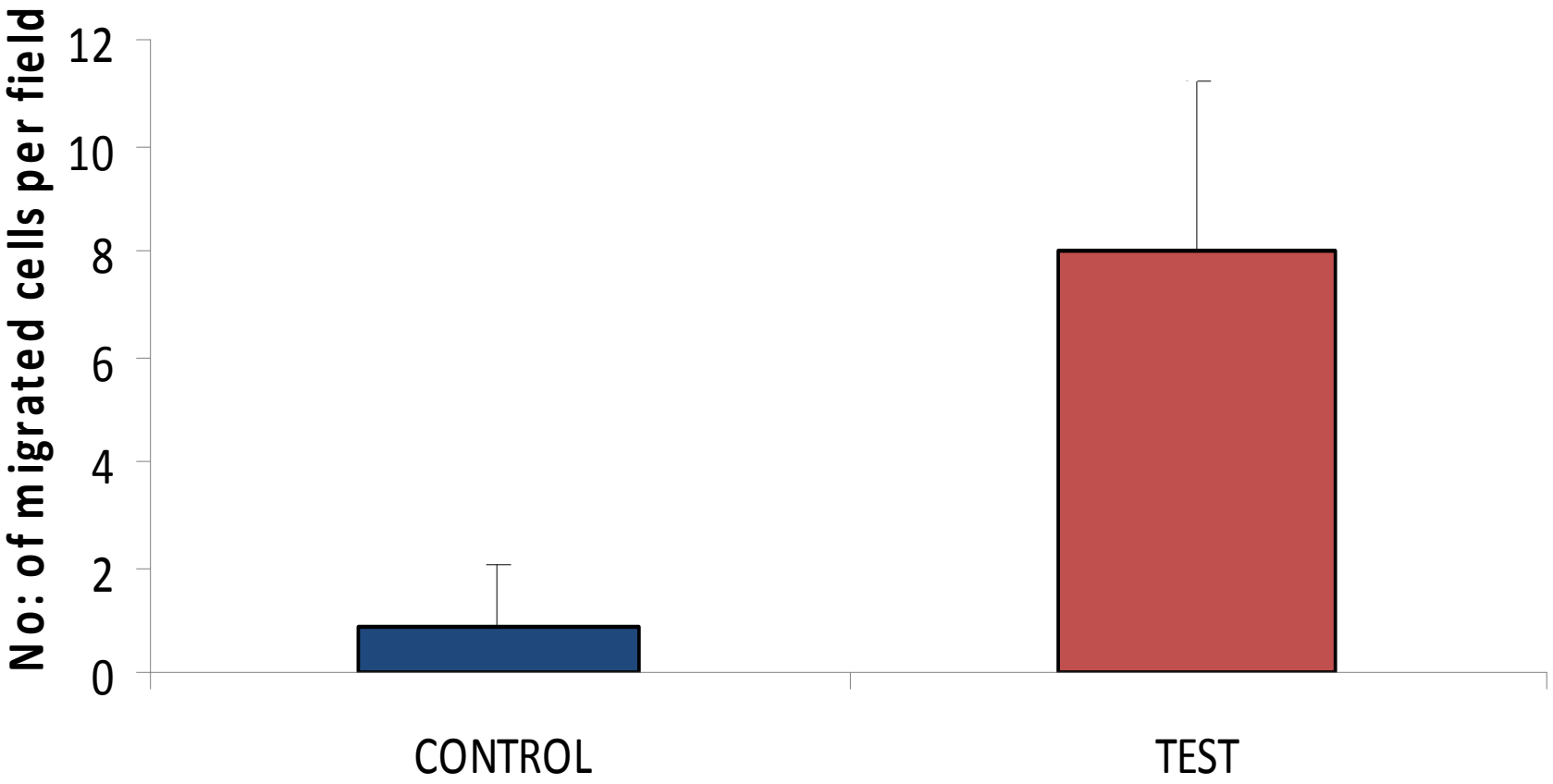



*A- Control PE, B- cells stained for CD34, C- CD31, D-CD45, E- control APC, F- ckit, G-cTN1, H-control-FITC and I- MHC*

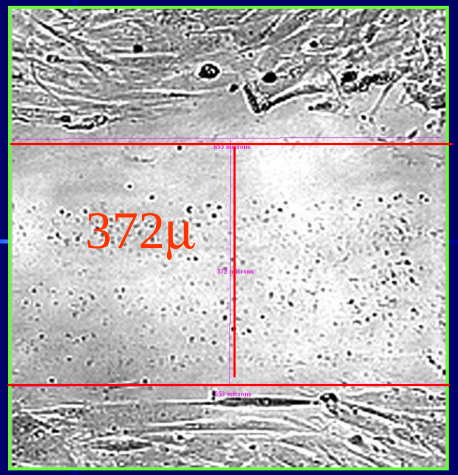
# Phenotypic profile of EGF treated and untreated CDCs



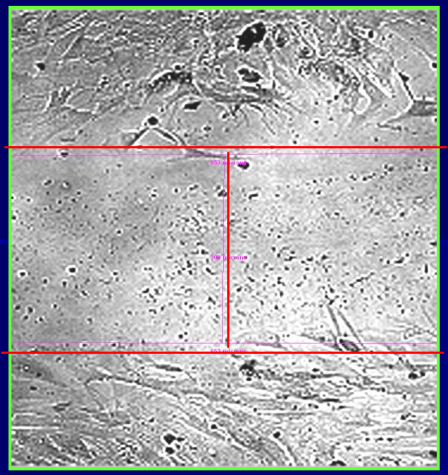
# Trans-well migration Assay



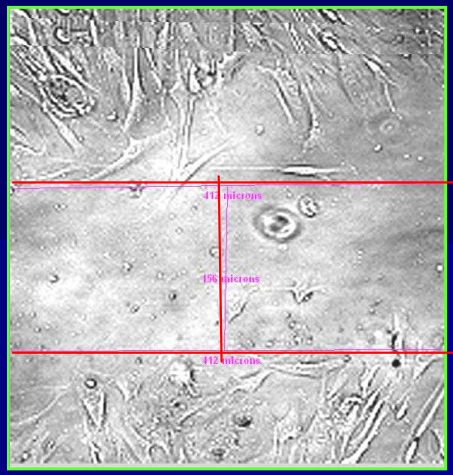
## Control - 2% FCS




0 hr



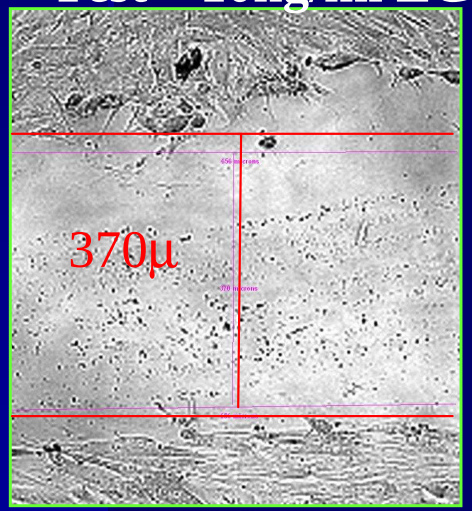
6 hr



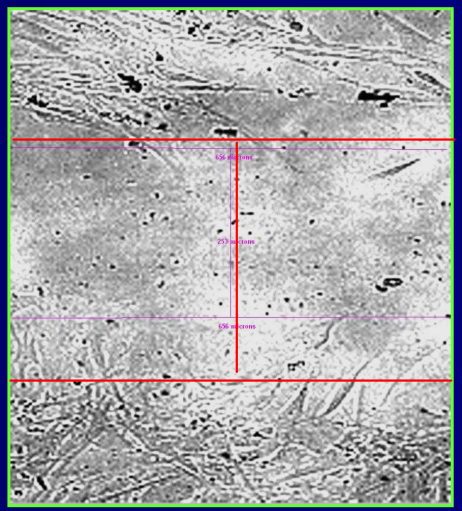
12hr


24hr

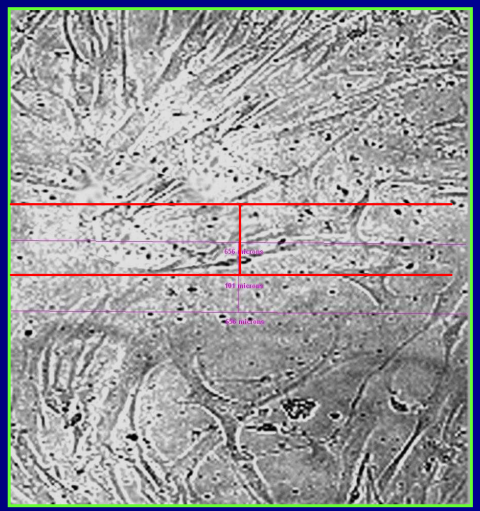
## Test – 10ng/ml EGF



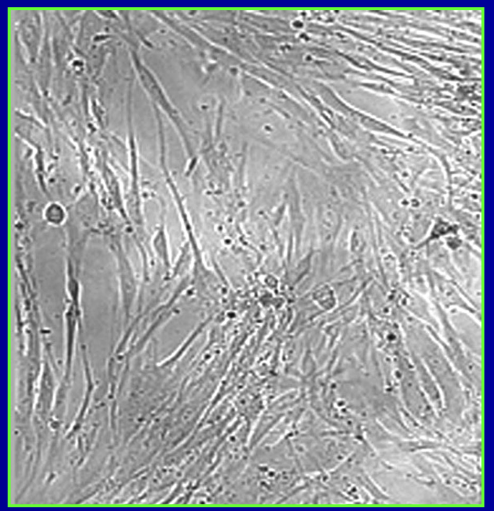

0hr



6 hr



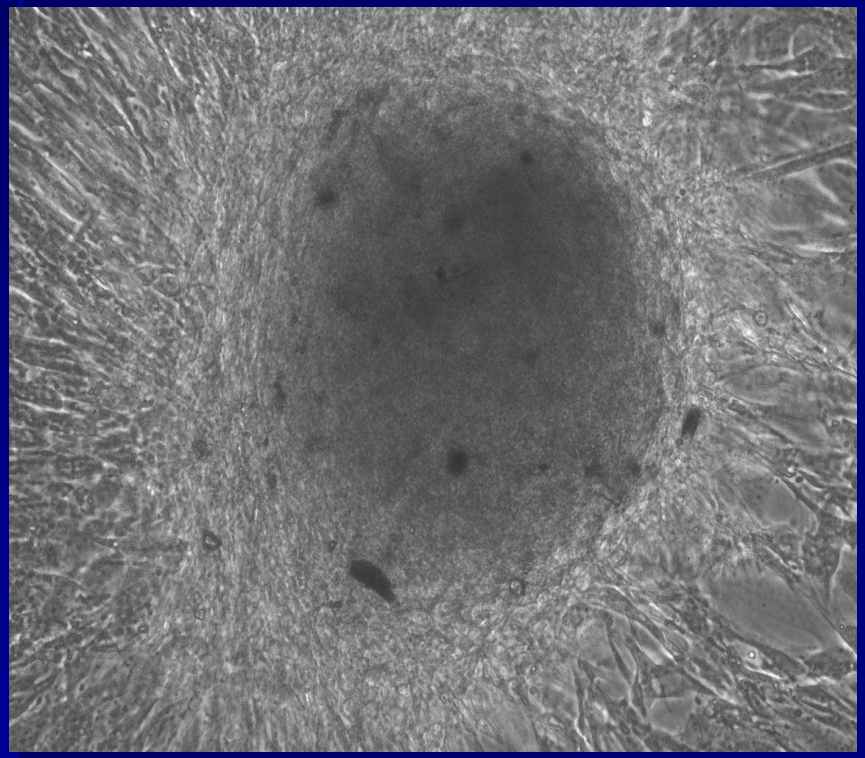
12 hr

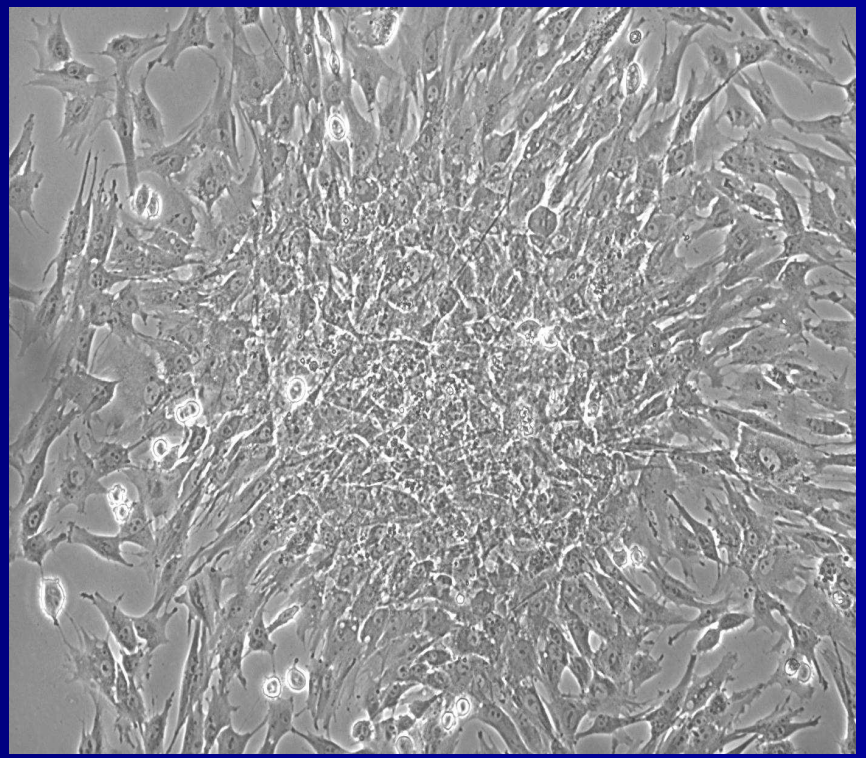


24hr

# Porcine ckit positive cells

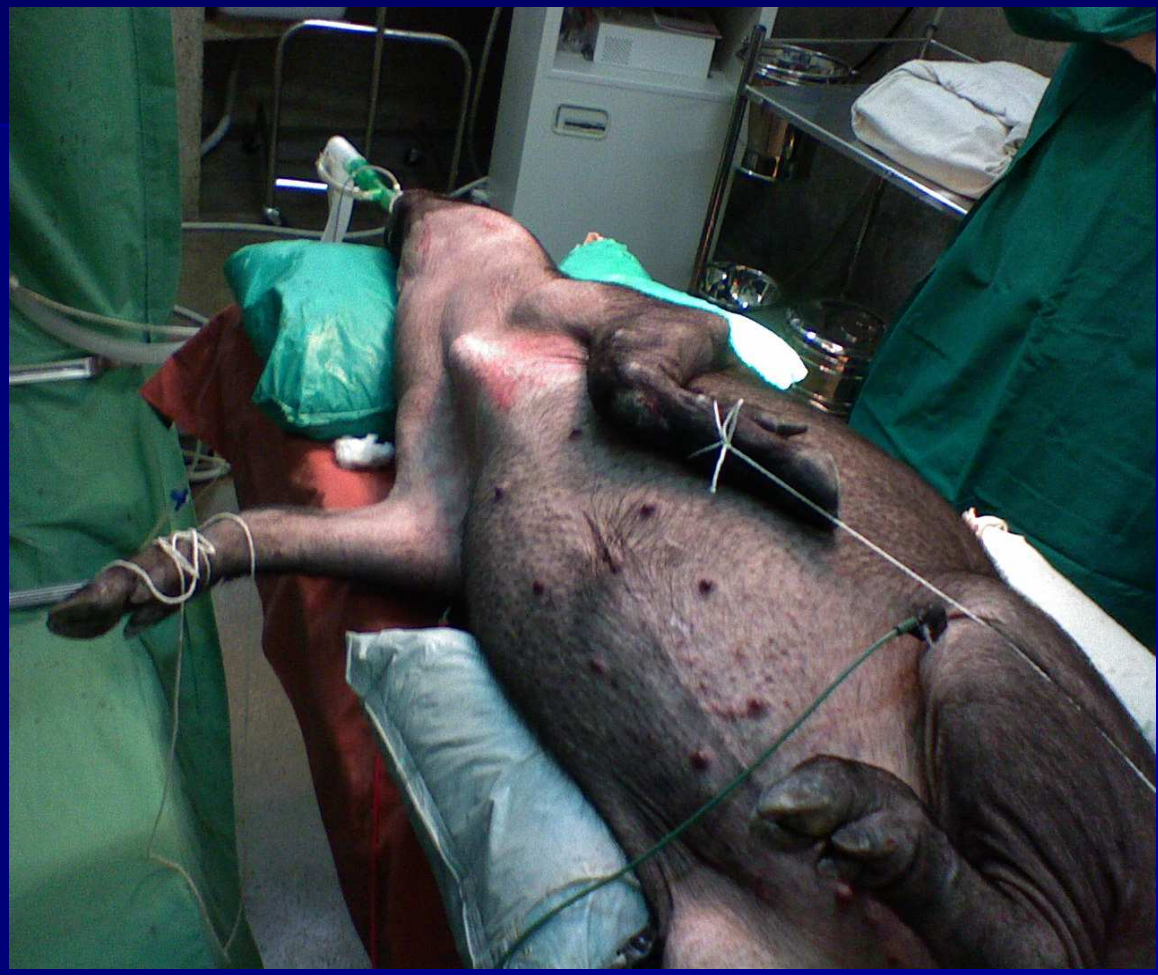
# Porcine cardiosphere-derived cells



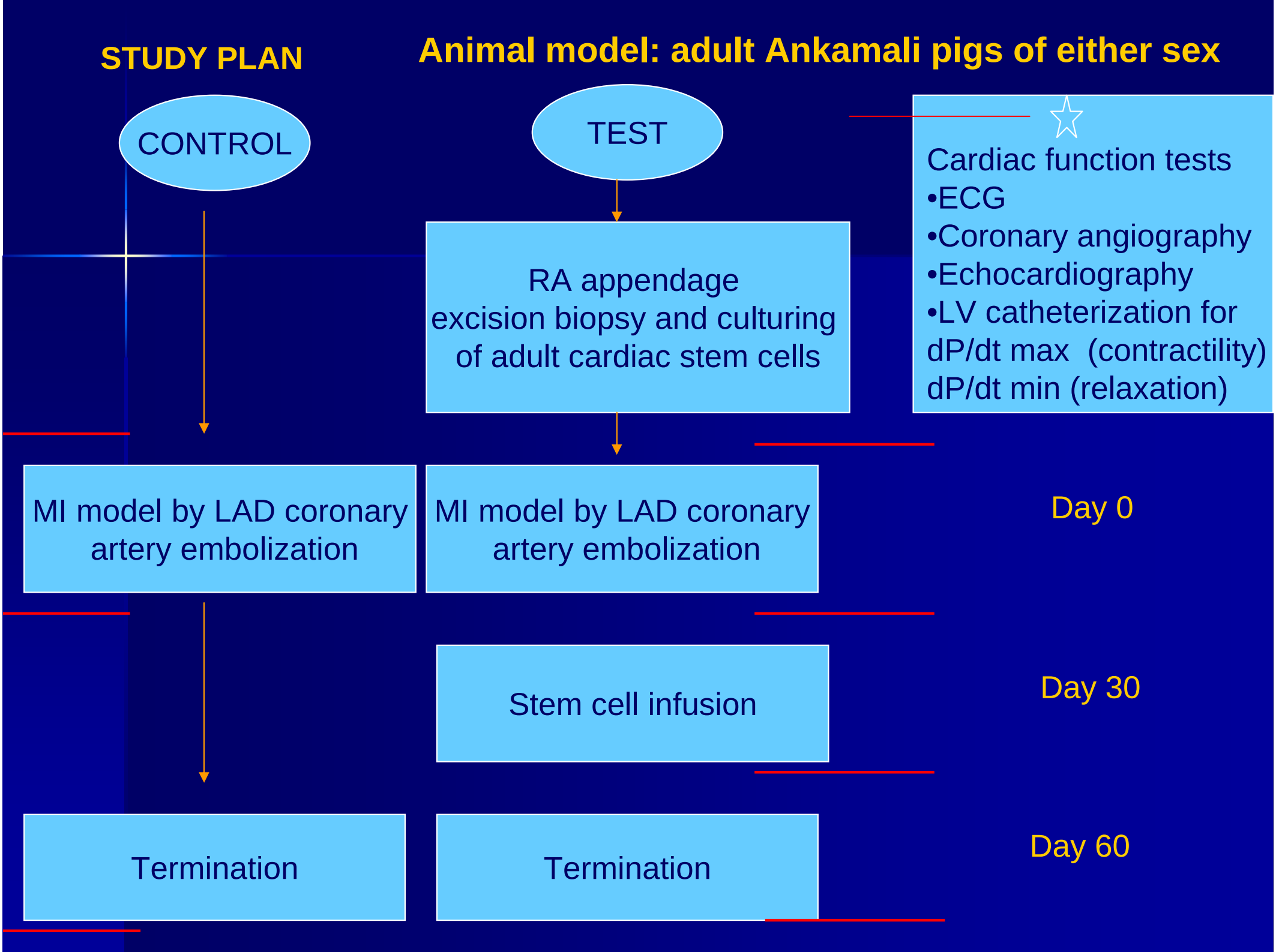


# Preparation of animals

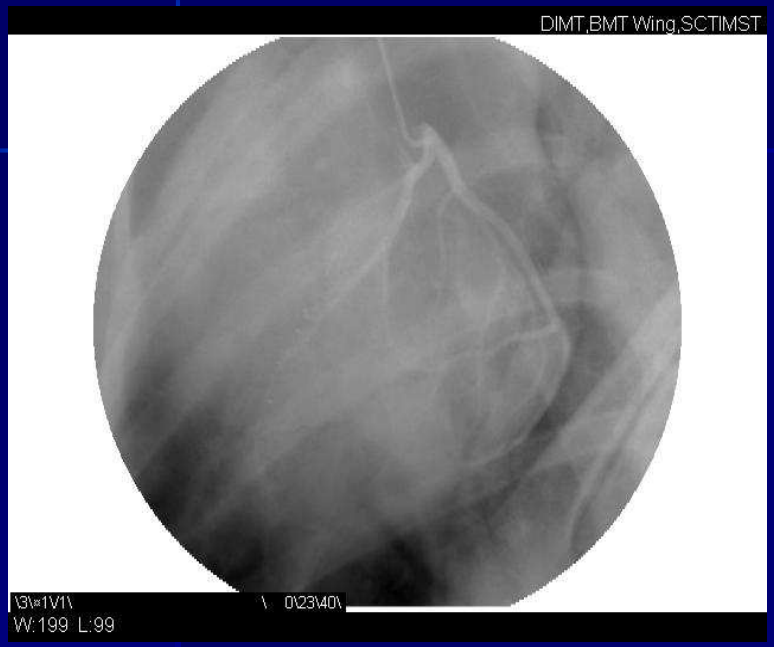
- Fasting for 12 hours
- Clinical examination
- Hematology and biochemistry
  - CBC
  - DLC
  - Hb
  - SGOT
  - SGPT
  - Creatinine
  - BUN
  - Random Blood glucose
  - Total cholesterol



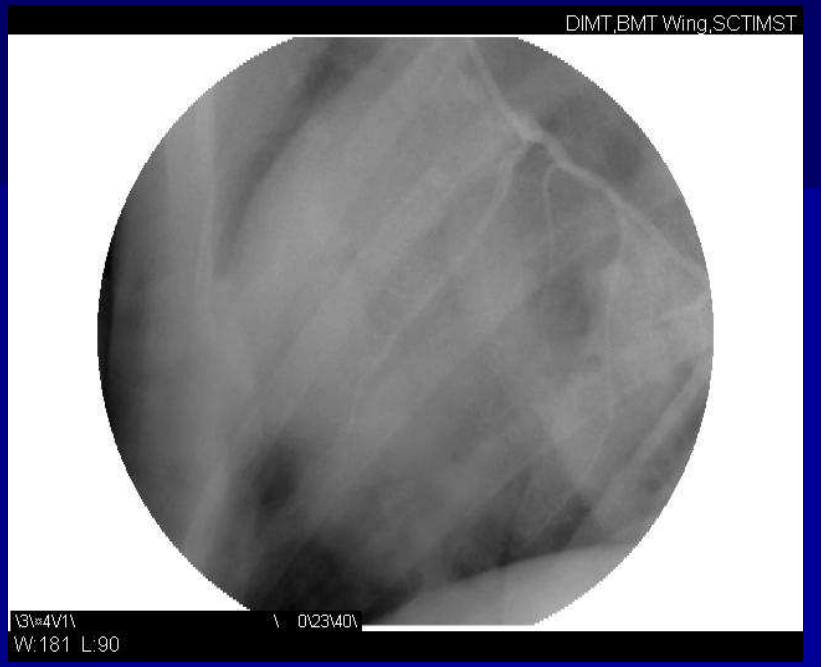
Exclusion criteria: Pregnant and animals not healthy as per above examinations are rejected from study


STUDY PLAN
Animal model: adult Ankamali pigs of either sex
CONTROL
TEST
Cardiac function tests
•ECG
•Coronary angiography
•Echocardiography
•LV catheterization for
dP/dt max (contractility)
dP/dt min (relaxation)
RA appendage
excision biopsy and culturing
of adult cardiac stem cells
MI model by LAD coronary
artery embolization
MI model by LAD coronary
artery embolization
Day 0
Stem cell infusion
Day 30
Termination
Termination
Day 60

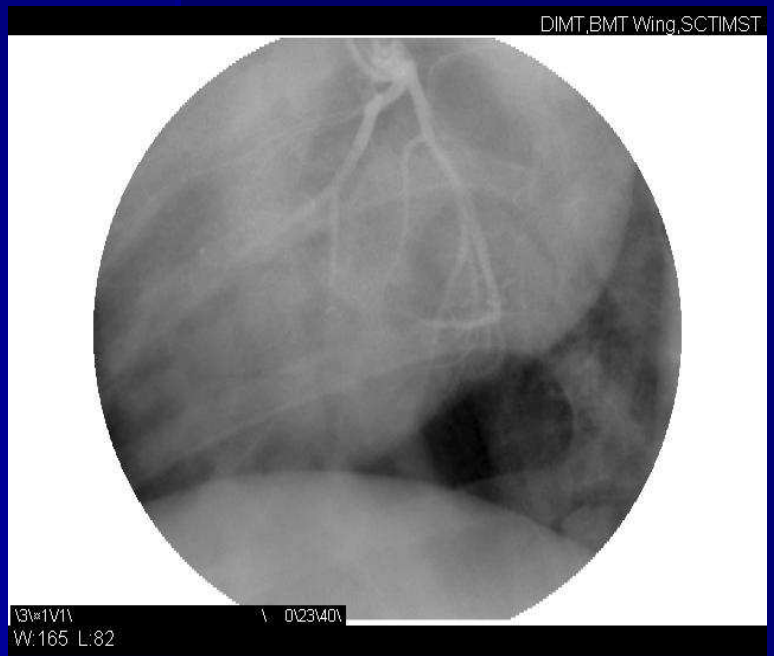
# Test Animal 11481-Coronary angiography (right oblique view)



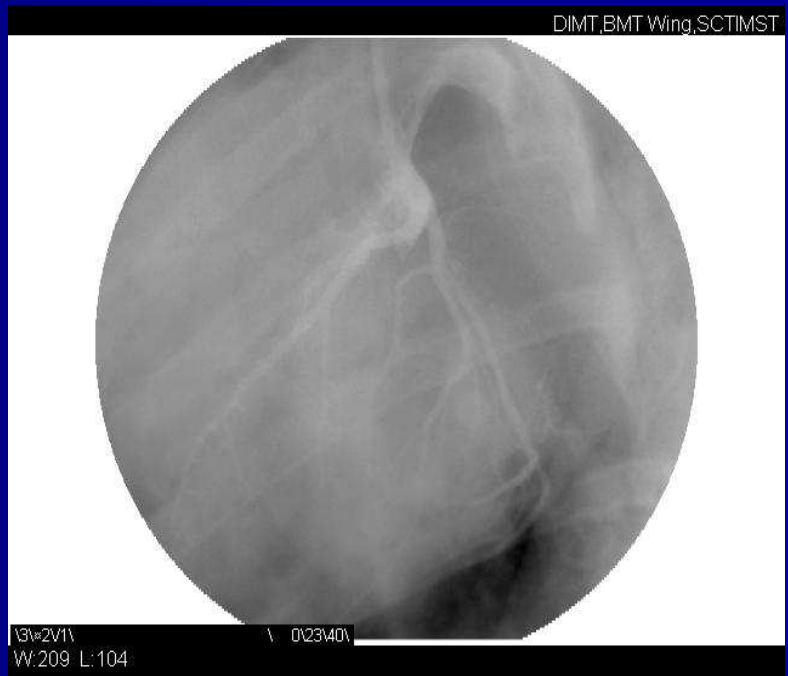

← Pre embolization




Post embolization →
Day 0

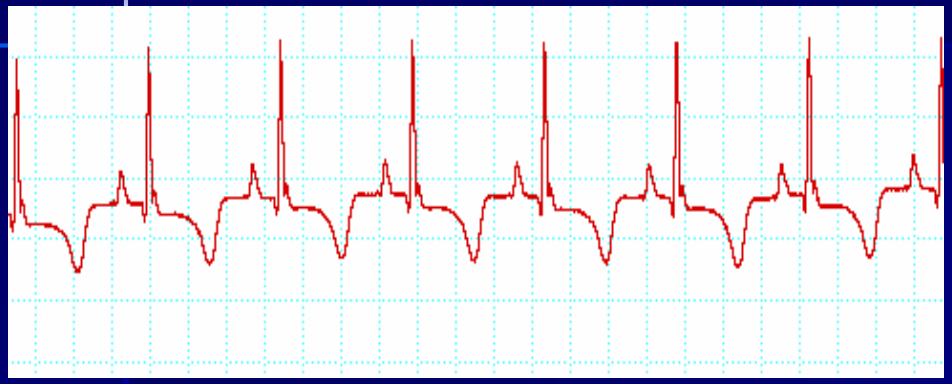



← Pre infusion
Day 30

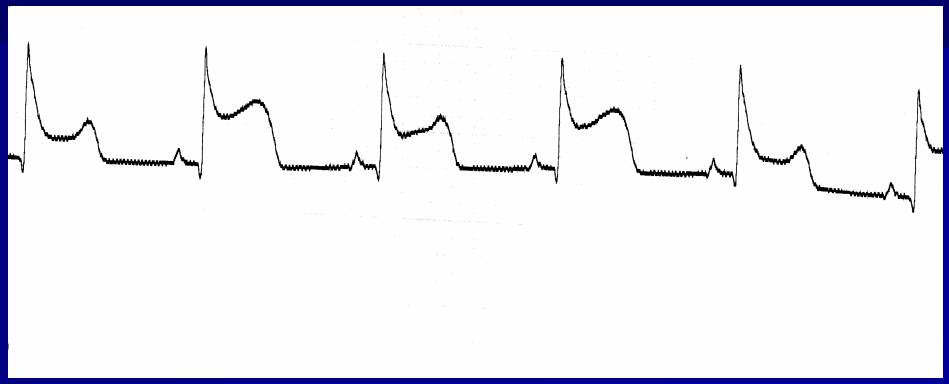



At termination →
Day 60

# Test Animal 11481- ECG lead II

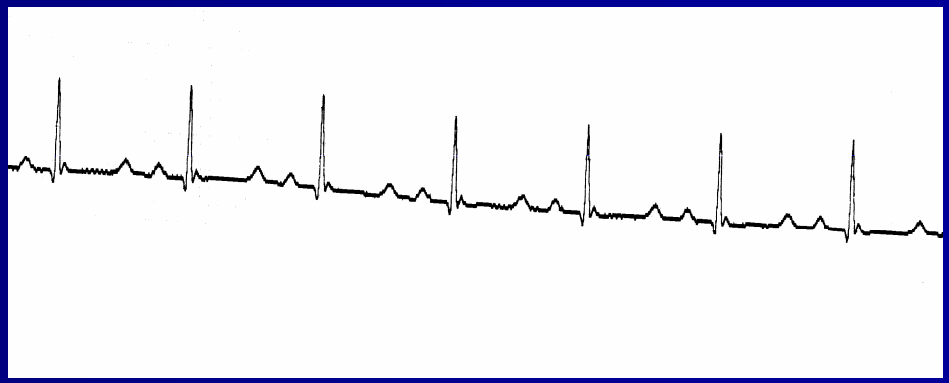


Pre embolization



Post embolization

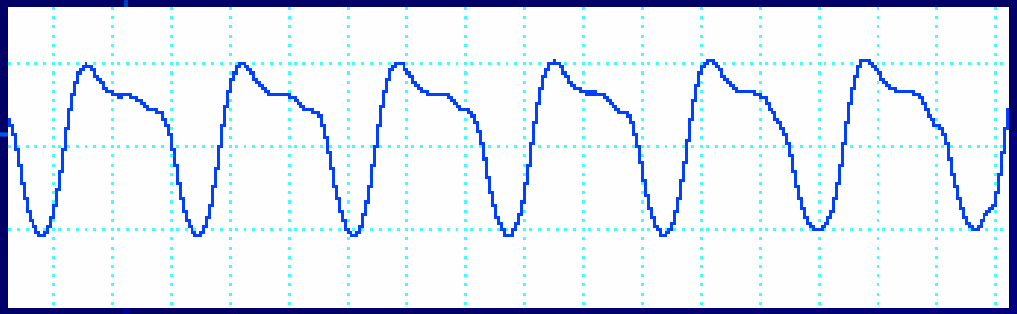
Preinfusion-Day 30



At termination 60 days

# Test Animal 11481- Ventricular function

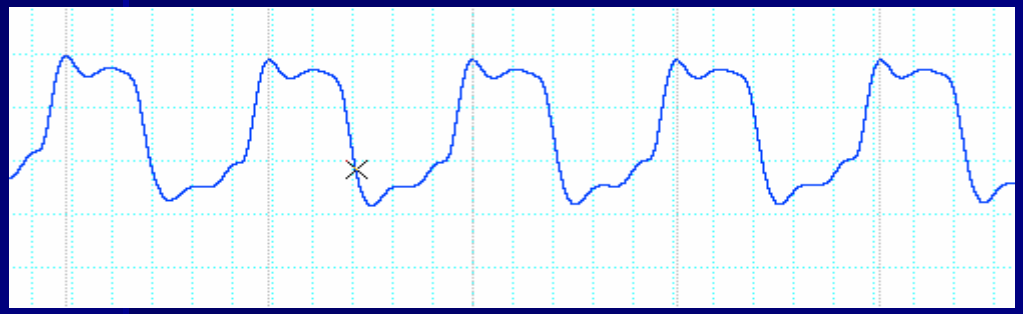
**(Power Lab, Chart 5 software)**



Normal animal

Max dP/dt 1298+/- 25 mmHg/Sec

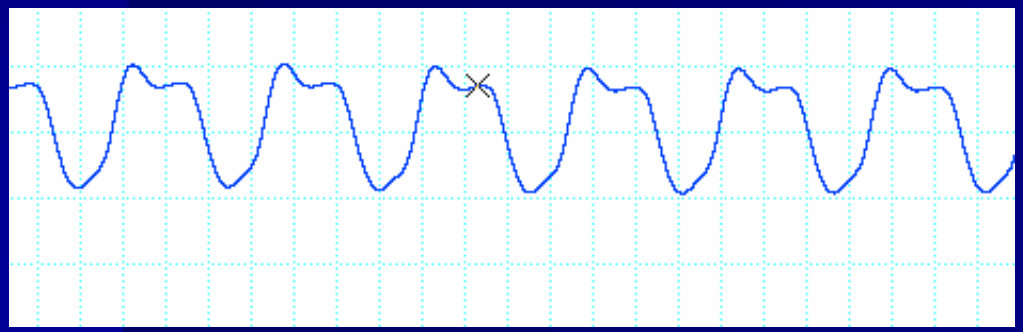
Min dP/dt -1069+/-38 mmHg/Sec



Post embolization, Preinfusion-Day30

Max dP/dt- 591+/-12mmHg/Sec

Min dP/dt -575+/- 5mmHg/Sec



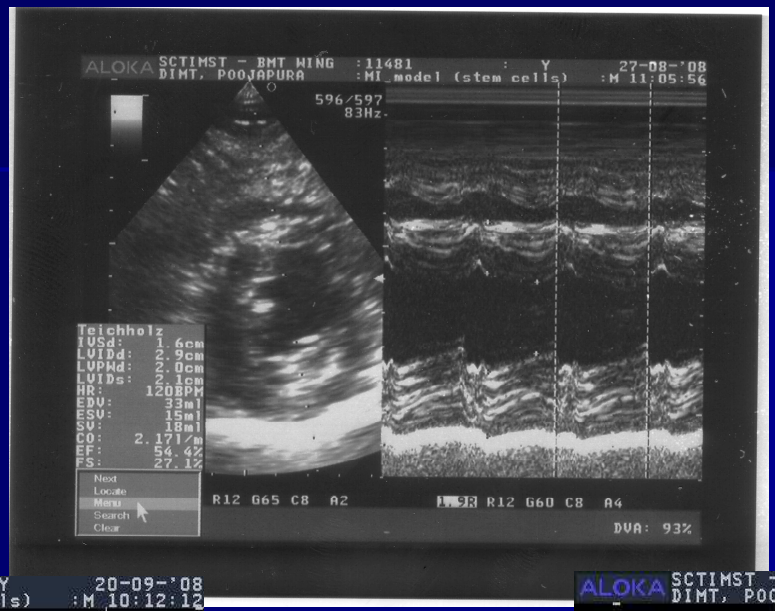
At termination 60 days

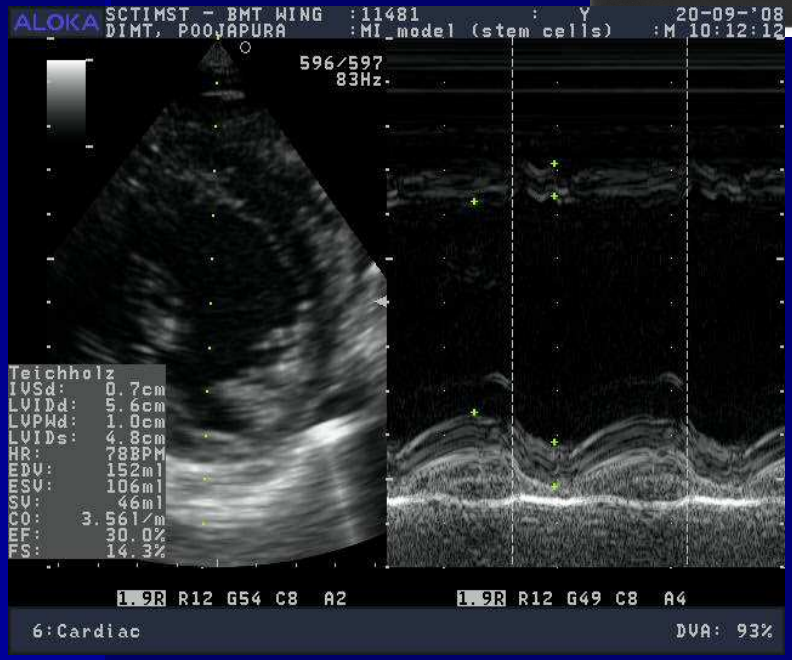
Max dP/dt- 849+/-10mmHg/Sec

Min dP/dt -693+/-7mmHg/Sec

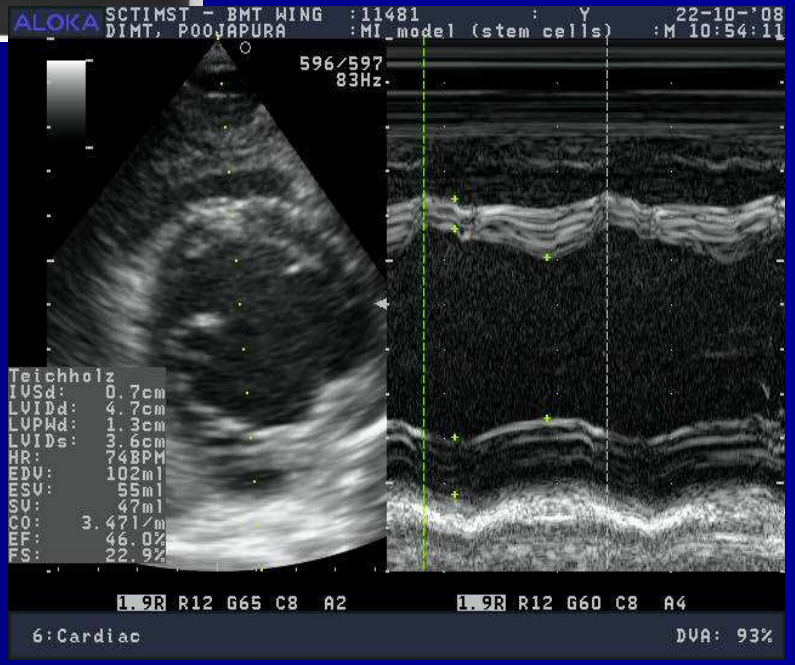
# Test Animal 11481- Echocardiography
## (right para-sternal short axis view)

Pre embolization

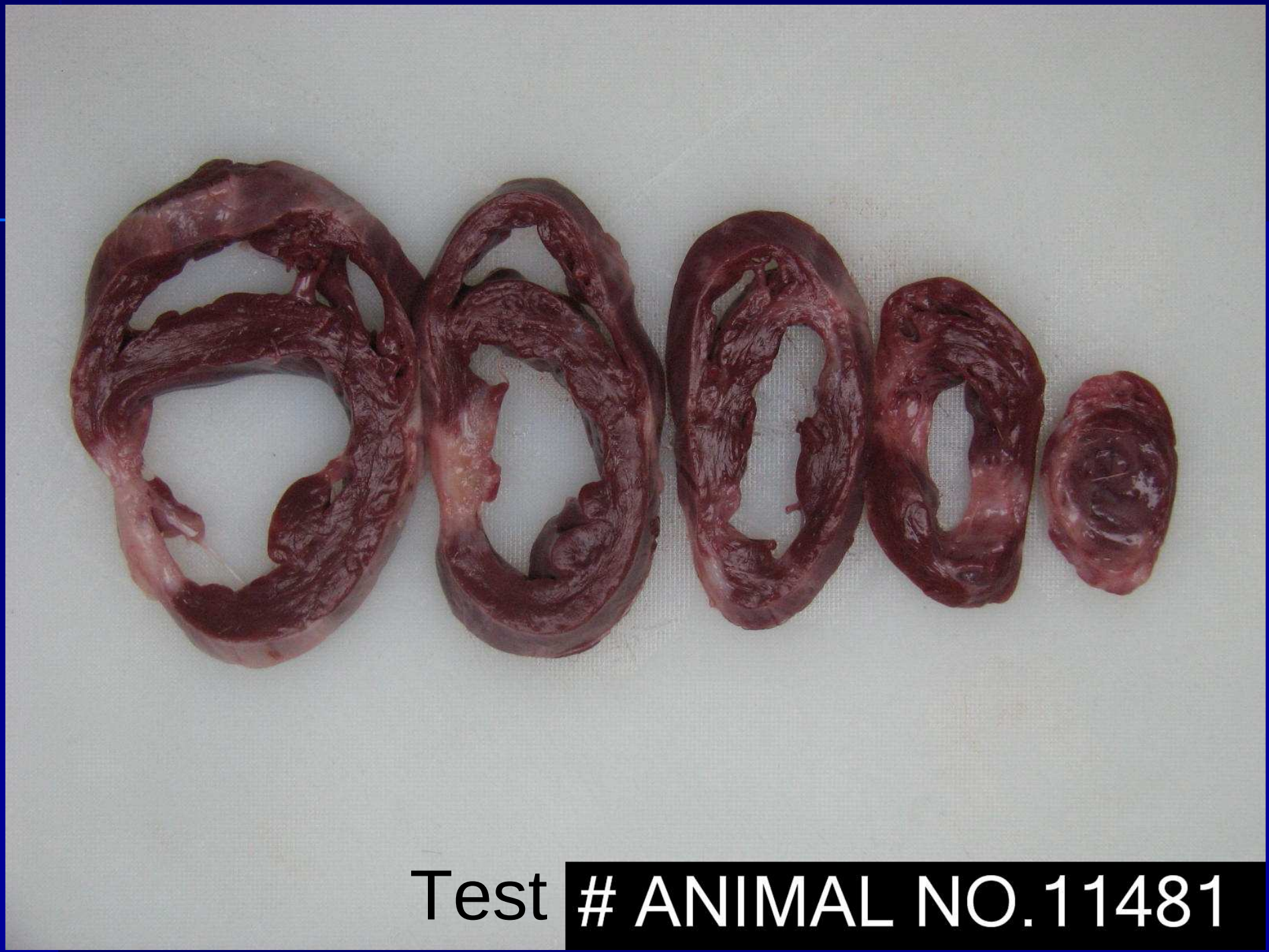


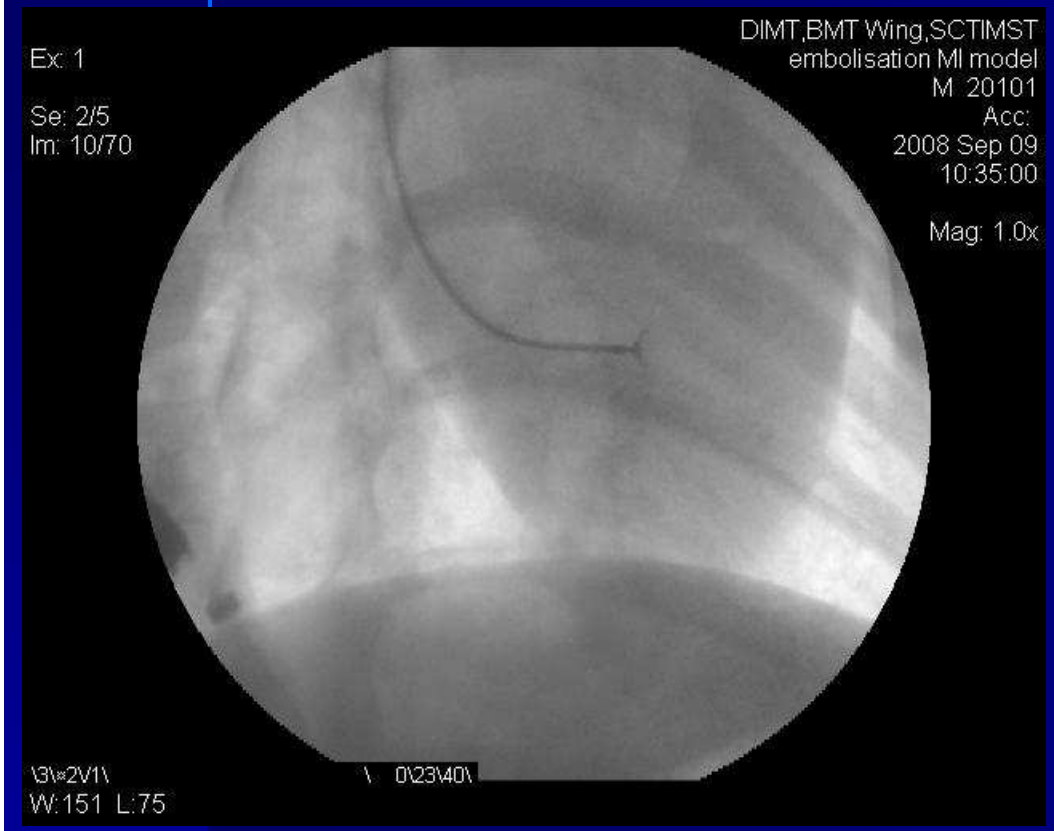

Post embolization-pre infusion 30 days

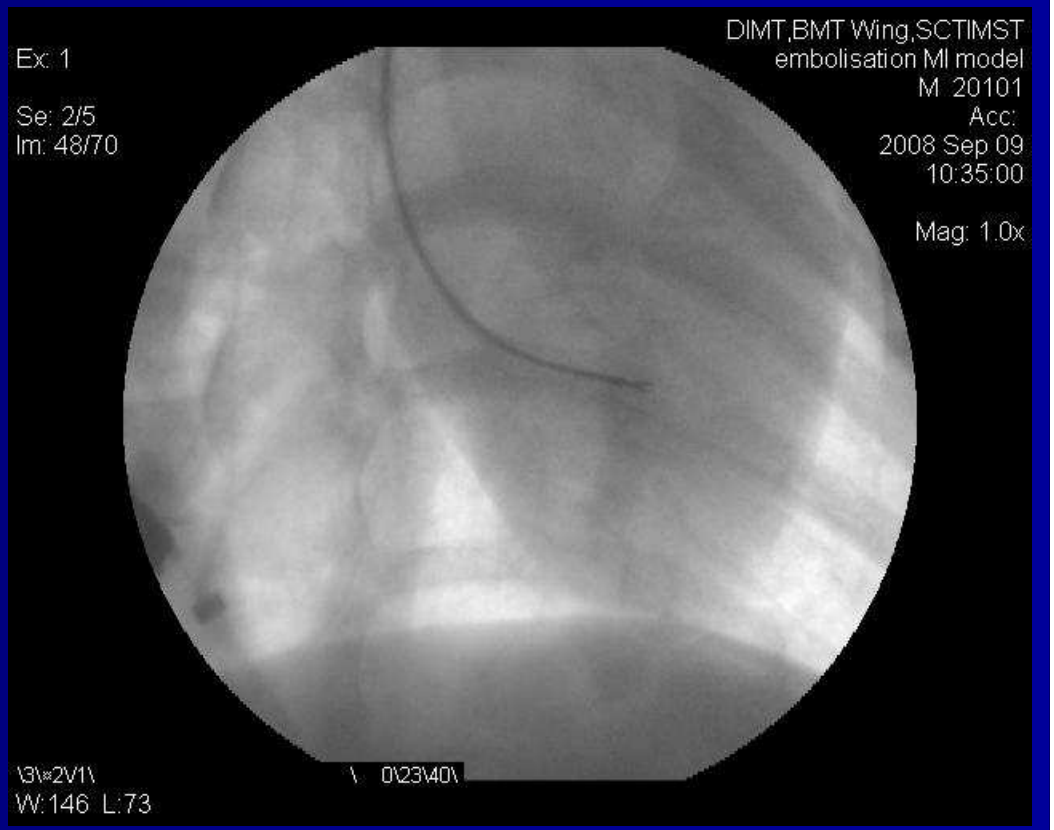



At termination 60 days

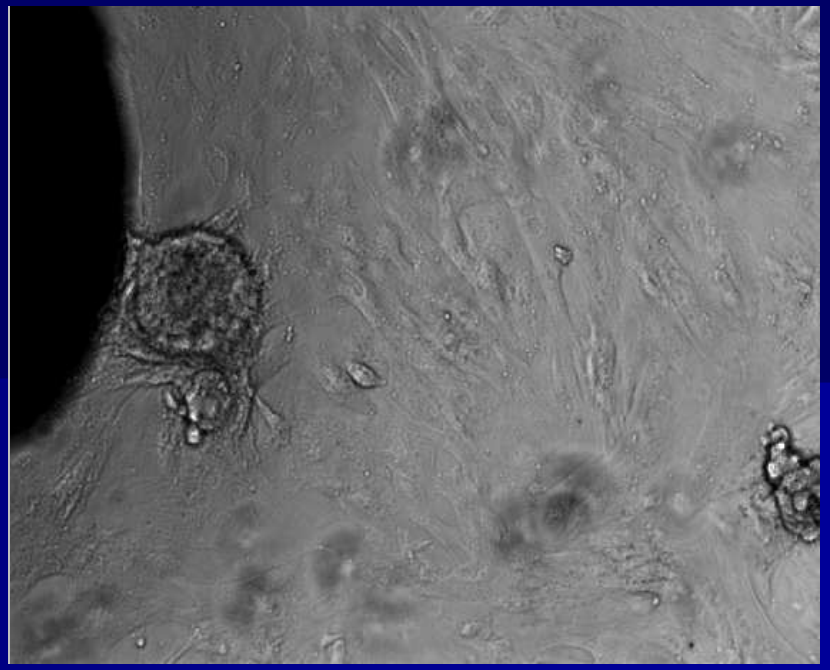

Test # ANIMAL NO.11481

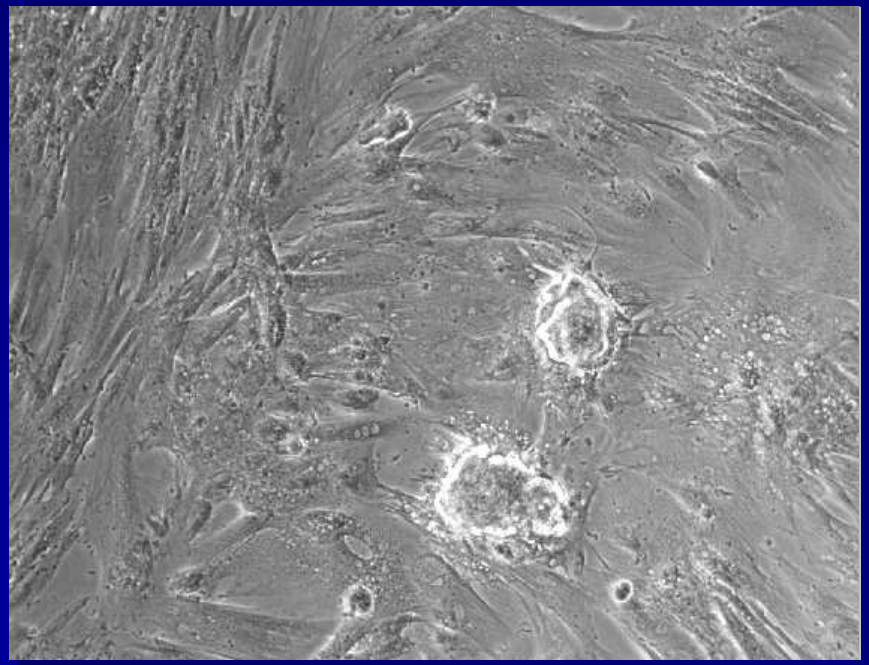
# Biopsy of right ventricular septum

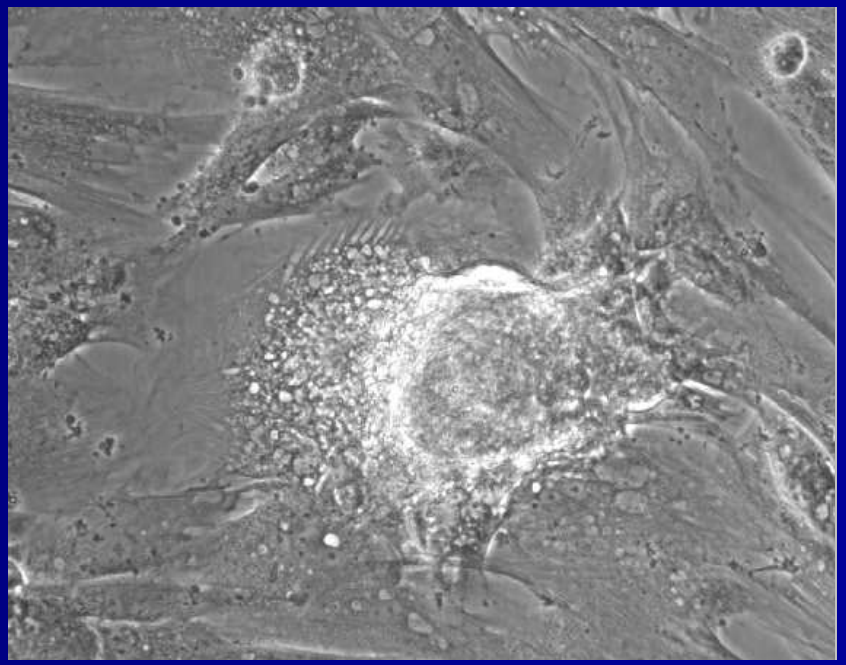


Cells from right ventricle biopsy (Pig) Day 21







# Transfection with endothelial nitric oxide synthase (eNOS) improves functions of circulating endothelial progenitor cells from coronary patients

Savneet Kaur, V.S. Harikrishnan,

Sachin J Shenoy, K. Jayakumar,

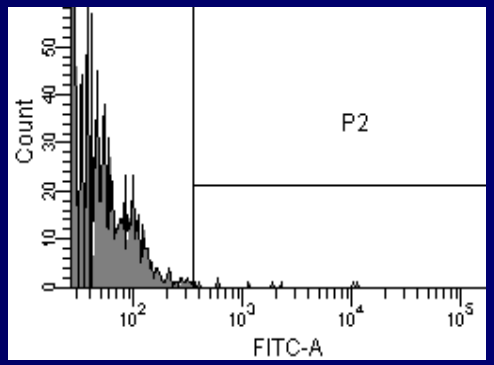
T. R. Santhosh Kumar and **CC. Kartha**

Sree Chitra Tirunal Institute for Medical Sciences & Technology and Rajiv Gandhi Center for Biotechnology, Trivandrum, INDIA

# EXPRESSION OF eNOS ON EPCs

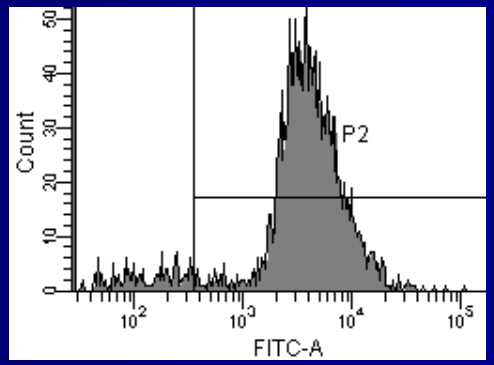
*Mean Fluorescence Intensity*

**823.5 ± 125.4**



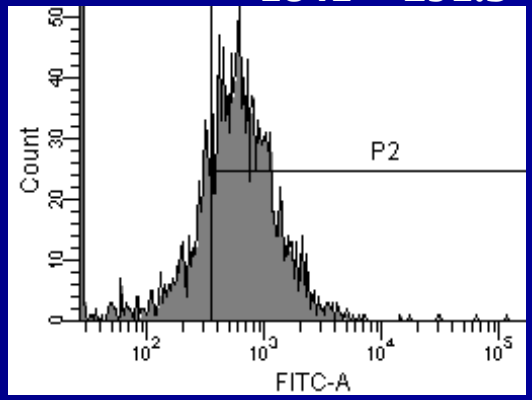

**Negative Control**

**5670.5 ± 574.8**




**Healthy Controls**

**1841 + 291.3**

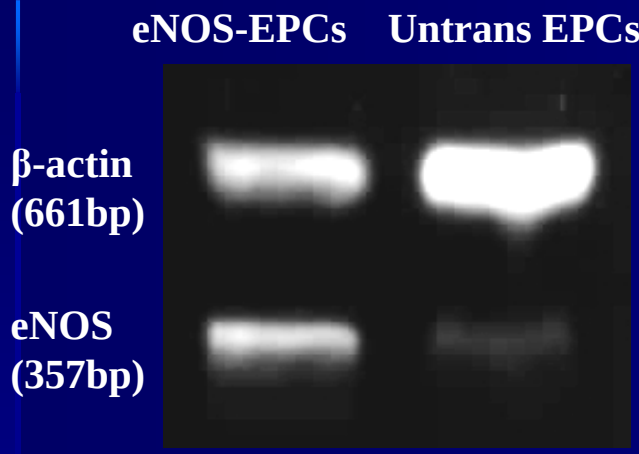



**CAD Patients**

- **The expression of eNOS on EPCs of CAD patients was 3-fold less than that on EPCs of healthy controls**

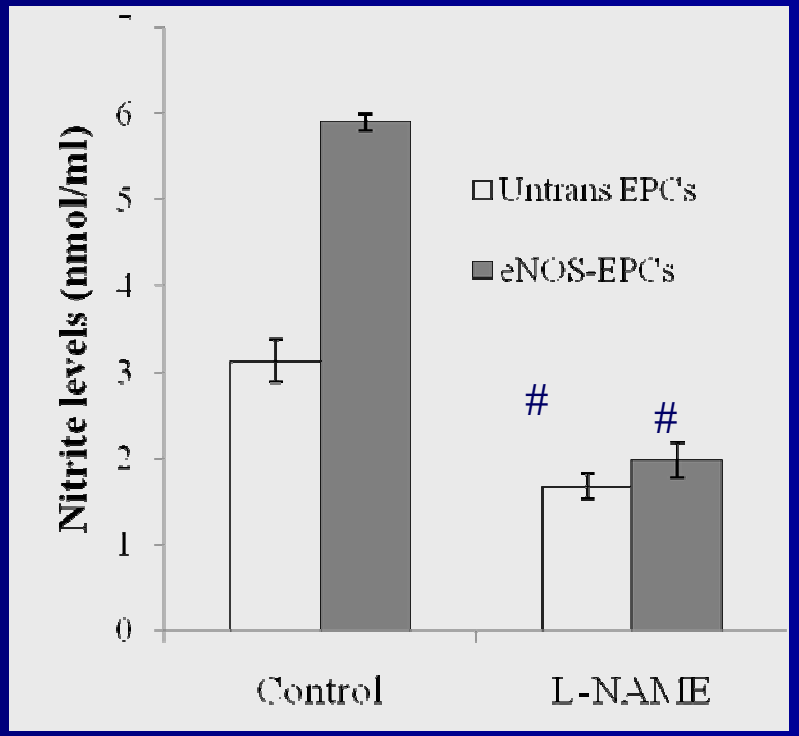
# TRANSFECTION OF EPCs WITH eNOS

## *RT-PCR*




- A 2-fold increase in the expression of eNOS in the transfected cells

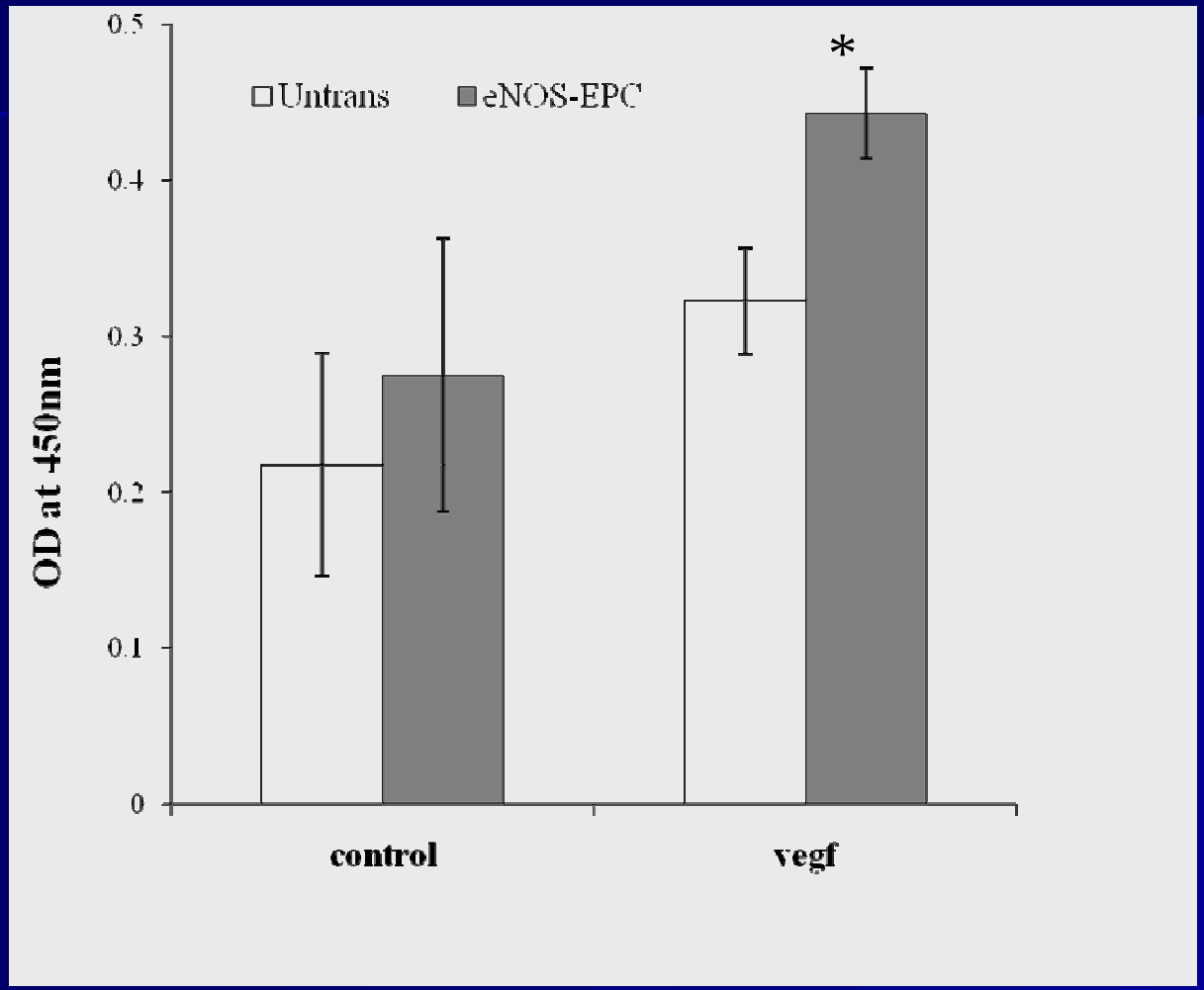
## *Nitrite levels- The Griess assay*




- A 3-fold increase in nitrite levels in the transfected cells

# CELL PROLIFERATION

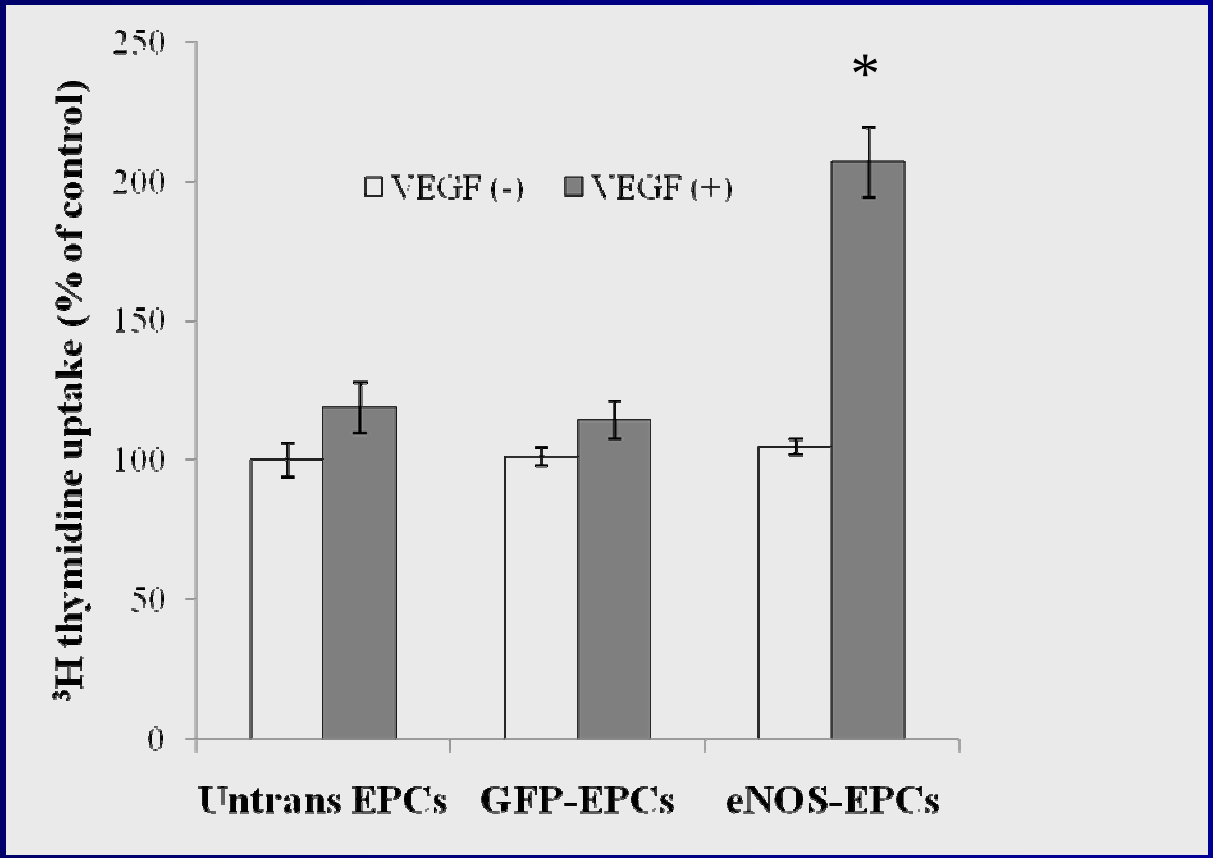
*WST-1 Colorimetric assay*




- **The transfection of EPCs with eNOS leads to a significant increase in their proliferation in presence of VEGF**

# CELL PROLIFERATION

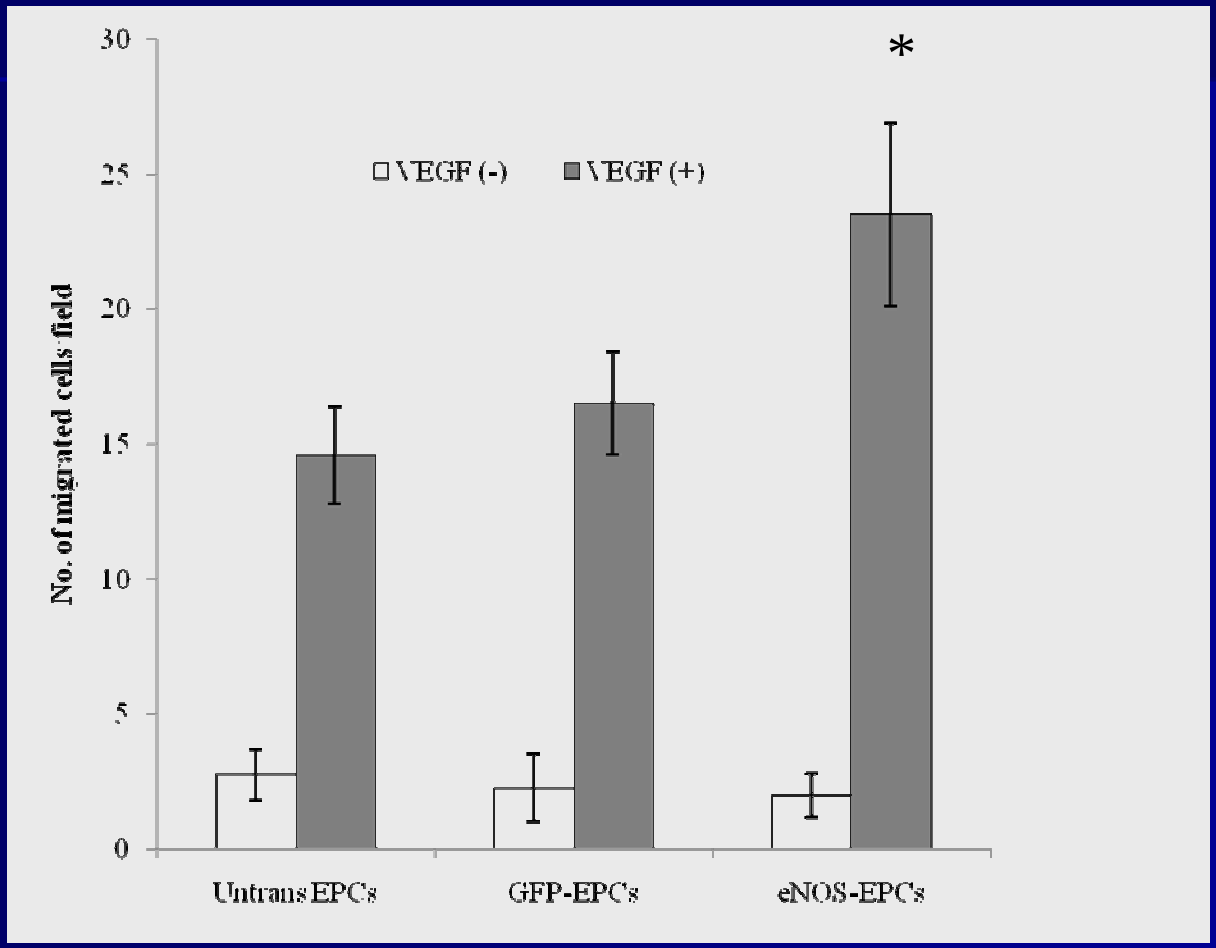
## *The $^{3}H$ Thymidine assay*




- **The transfection of EPCs with eNOS leads to a significant increase (1.8-fold) in their proliferation in presence of VEGF**

# MIGRATION IN RESPONSE TO VEGF

## *Modified Boyden Chamber Assay*

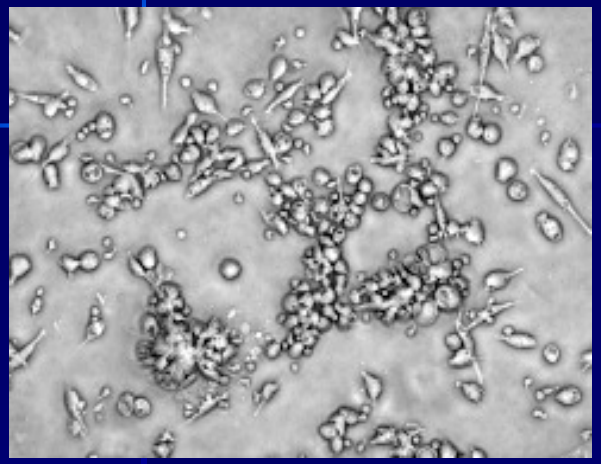



- **In response to VEGF, eNOS-EPCs show an enhanced migration (1.6-fold) as compared to that observed in the untransfected EPCs or GFP-EPCs**

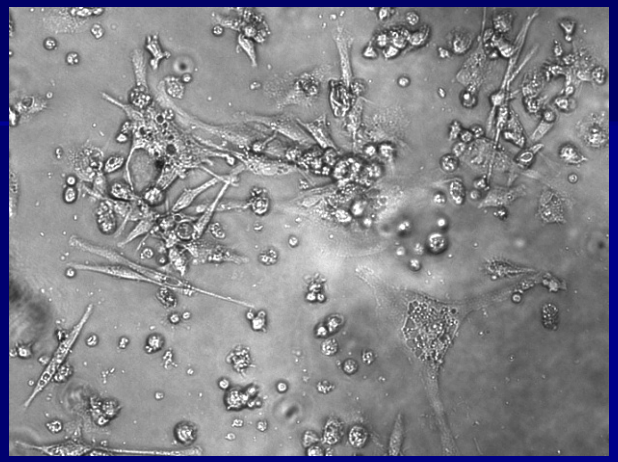
# DIFFERENTIATION

## *EPC-CFU Formation Assay*

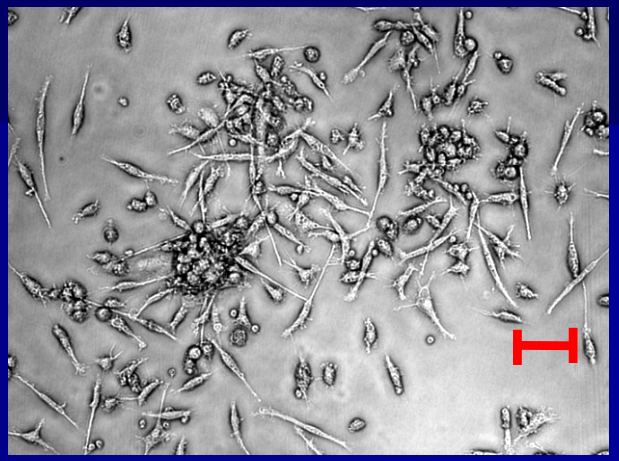
**EPCs**

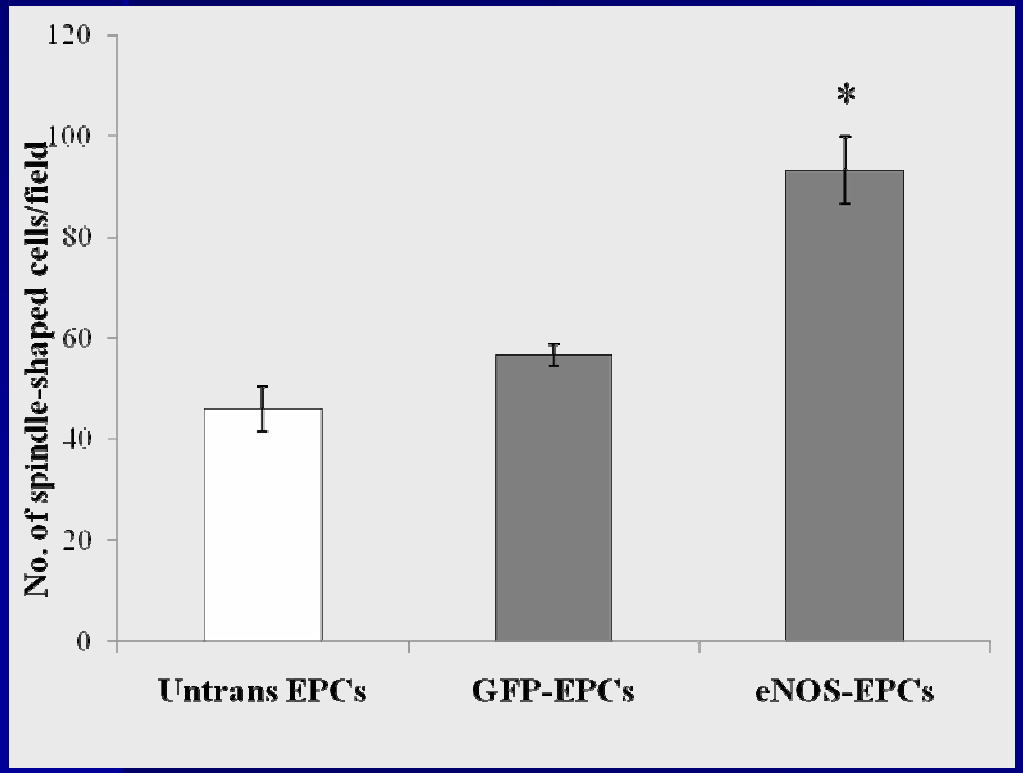


**GFP-EPCs**



**eNOS-EPCs**

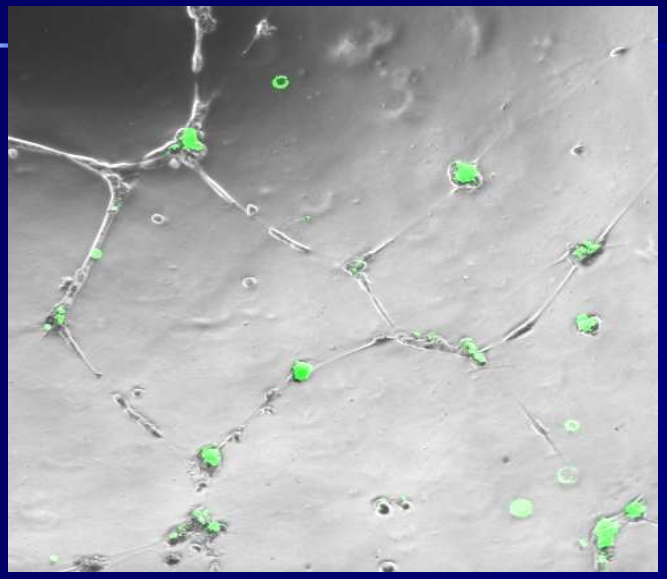




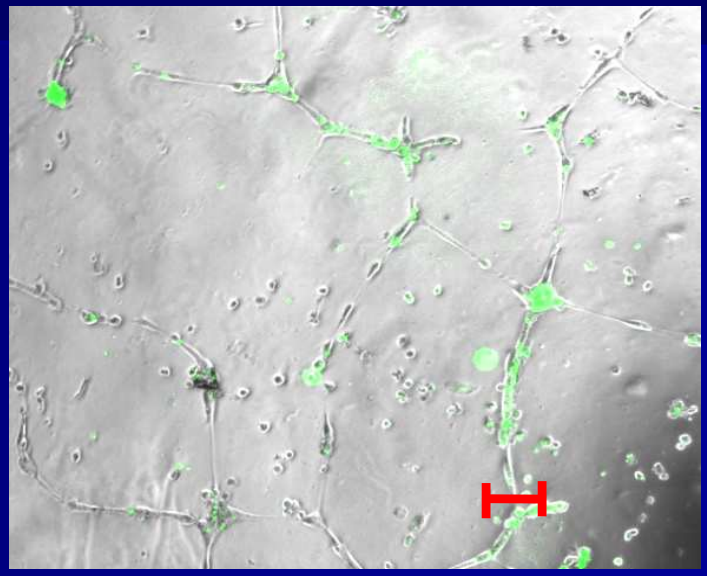

❖**The overexpression of eNOS in EPCs leads to an increased number (2-fold) of spindle-shaped cells on re-plating on fibronectin-coated plates**

# ANGIOGENESIS

**HUVECs + GFP-EPCs**



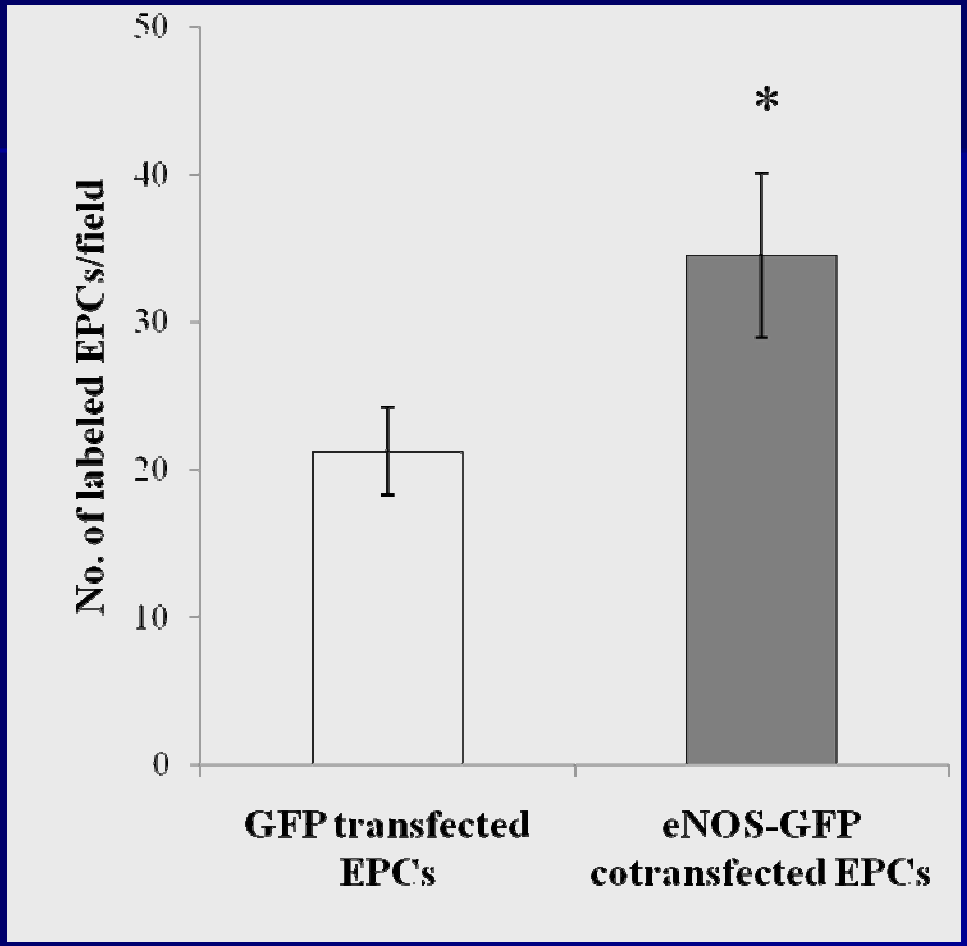
**HUVECs +GFP-eNOS-EPCs**



- **In presence of HUVECs, the incorporation of GFP-positive eNOS-EPCs in tube-like structures is more as compared to GFP-labeled-EPCs**

**(HUVECs: Human umbilical vein vascular endothelial cells)**

# ANGIOGENESIS

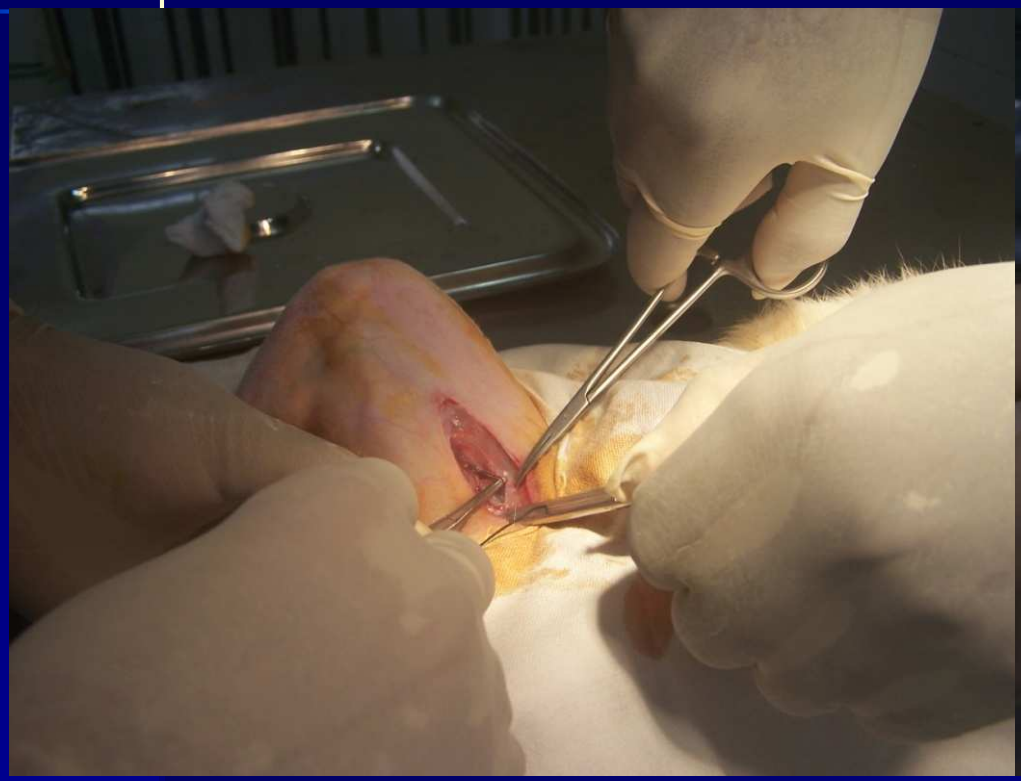


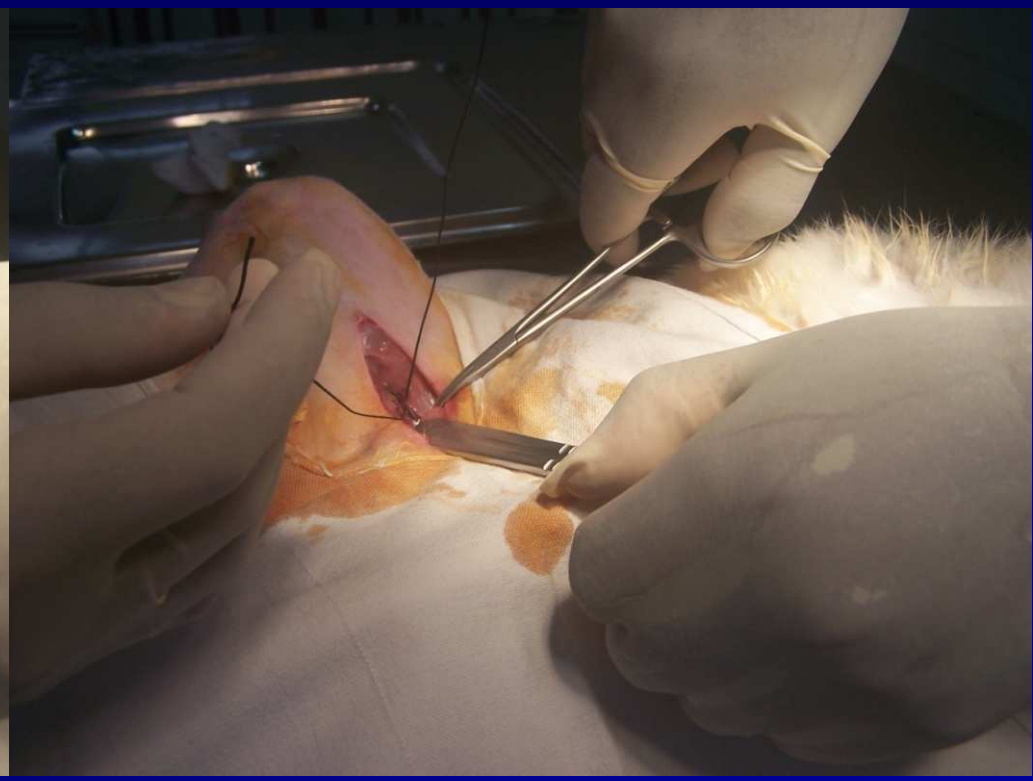

- eNOS-EPCs show augmented angiogenic property (1.6-fold) in comparison to that of the untransfected EPCs on the matrigel

# Evaluation of *in vivo* therapeutic angiogenesis efficacy of eNOS-EPCs

# PREPARATION OF HIND LIMB ISCHEMIA MODEL

*Pu et al, 1993*

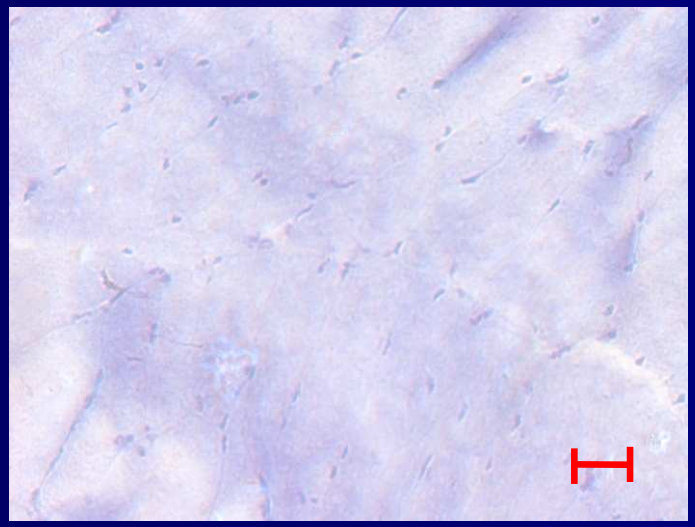




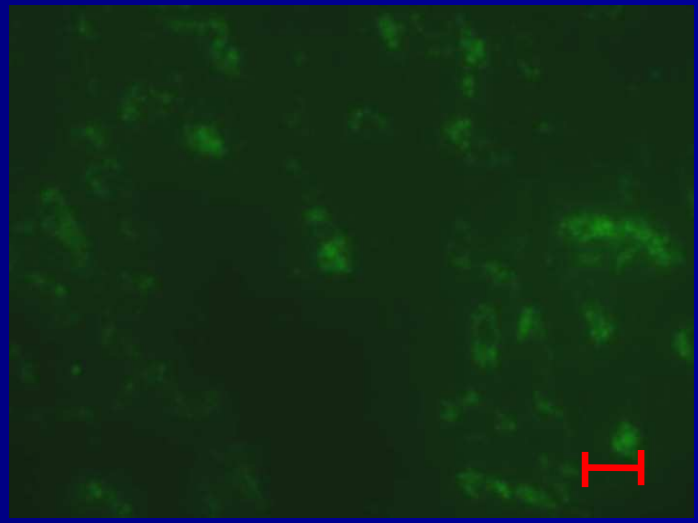
- Ligation of external iliac artery
- Complete excision of femoral artery and its branches

# IN VIVO EXPRESSION OF eNOS

*eNOS immunohistochemistry of tissue sections*



Hematoxylin-stained tissue section
(Phase contrast picture)

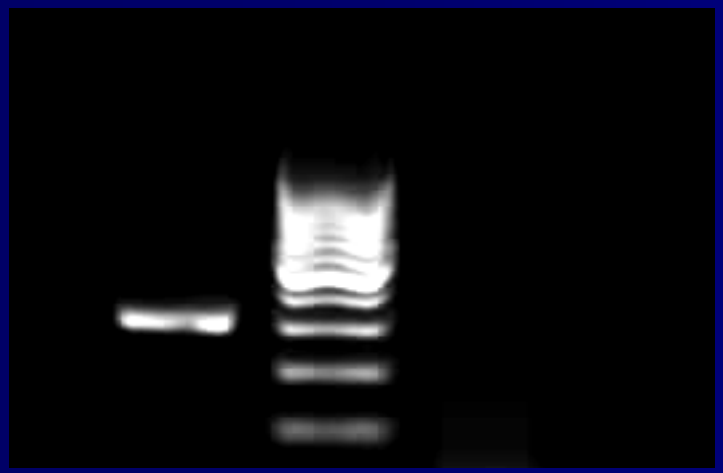


eNOS-stained tissue section
Fluorescence picture

- **FITC-fluorescence observed in eNOS-EPC treated animals**
- **No fluorescence in PBS or EPC treated animals**

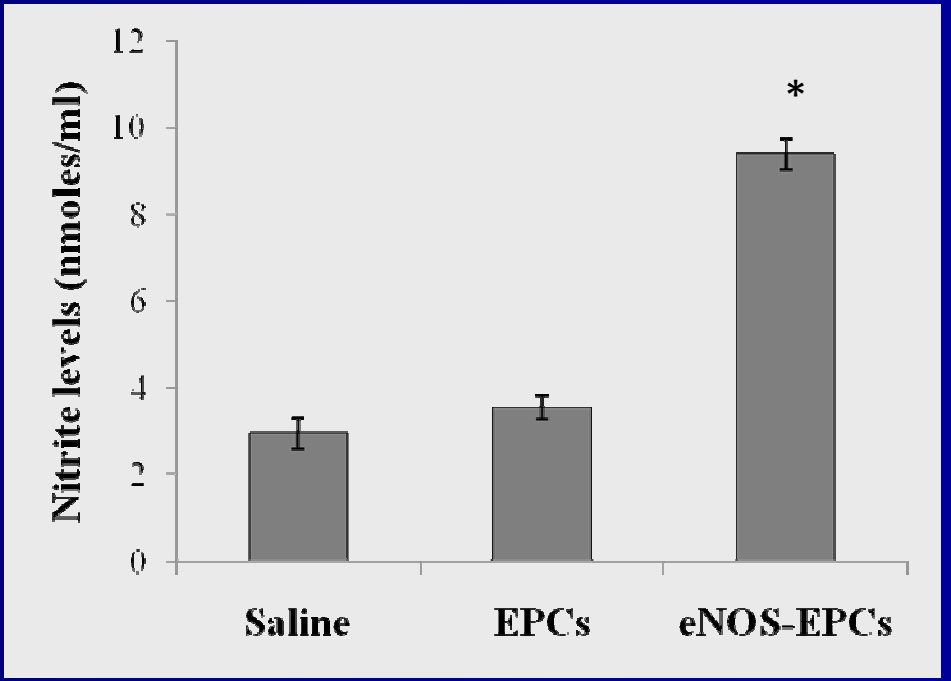
# IN VIVO EXPRESSION OF eNOS

## *RT-PCR*




❖ **eNOS-mRNA band appears in tissues from eNOS-EPC treated animals. No band observed in saline or EPC treated animals**

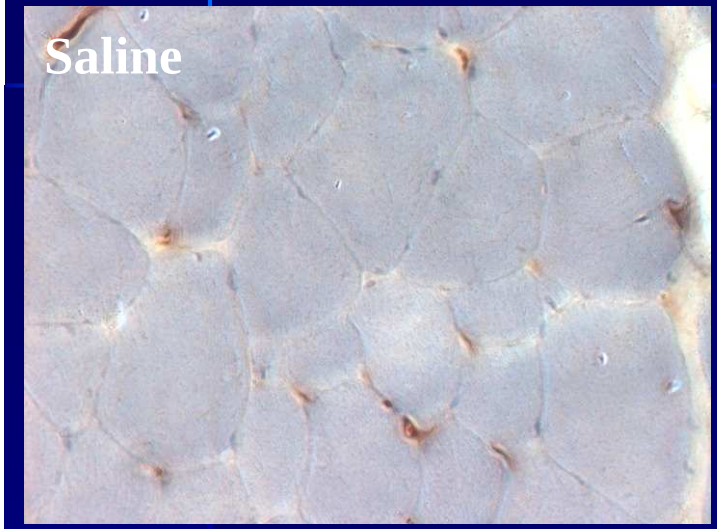
## *Tissue Nitrite levels- The Griess assay*

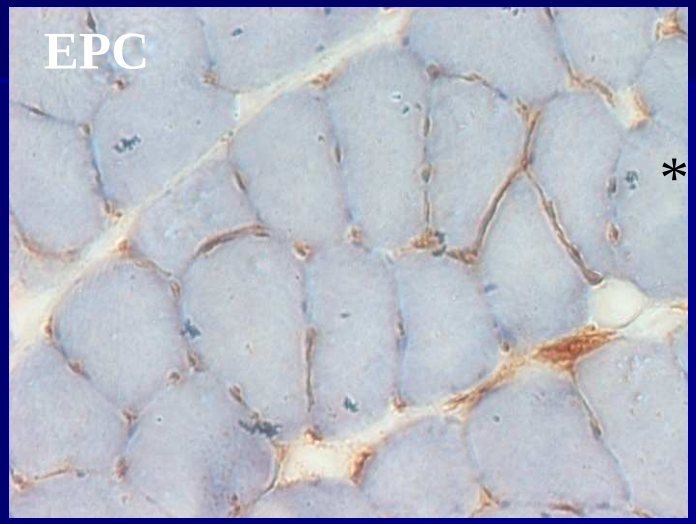


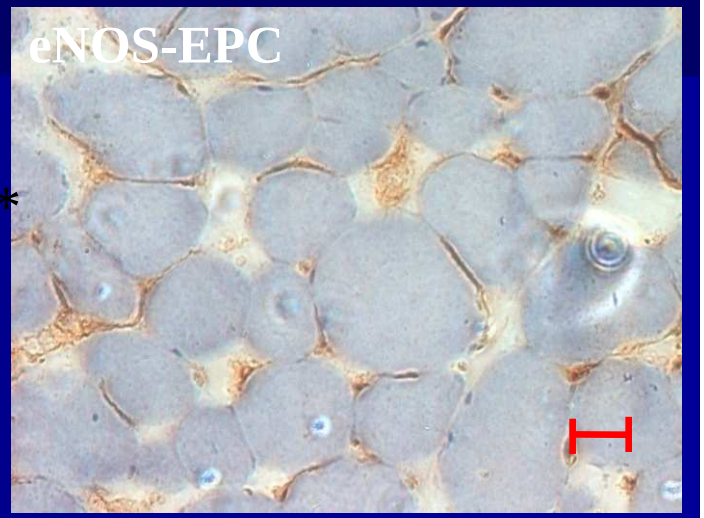

❖ **Tissue nitrite levels in eNOS-EPC treated rabbits are significantly higher than that observed in PBS or EPC treated animals**

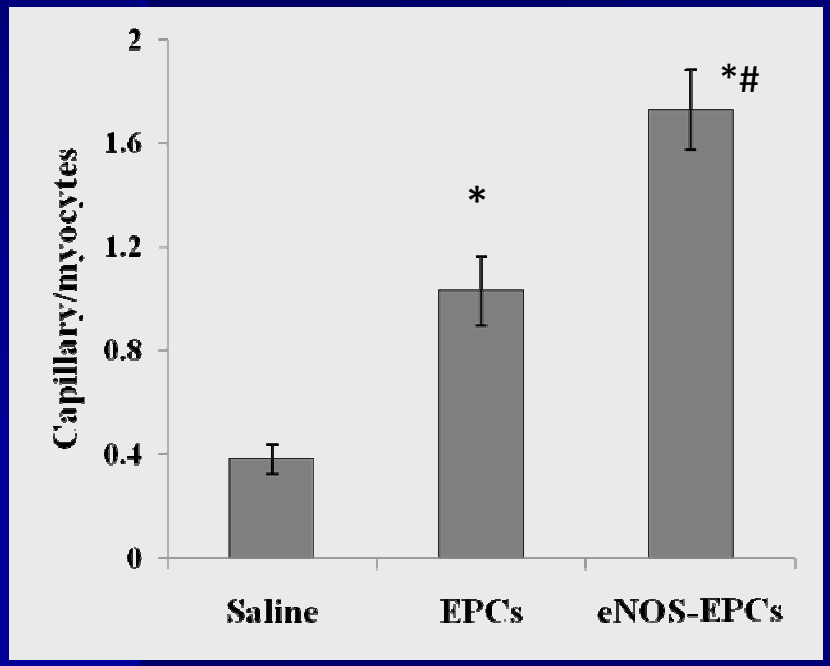
# CAPILLARY DENSITY OF LIMB TISSUES: CD31-STAINING ANGIOGENESIS

*CD31 immunohistochemistry of tissue sections*



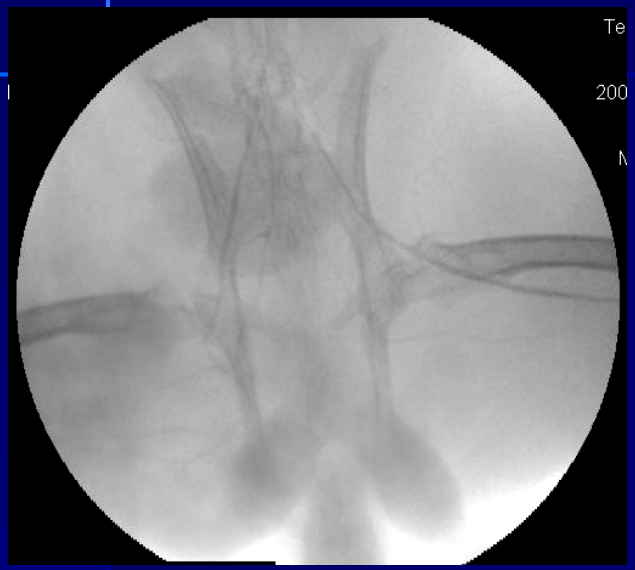



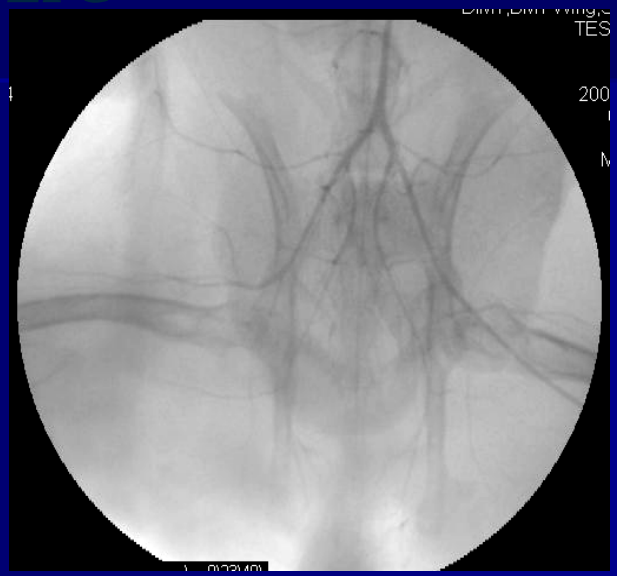

- **No. of CD31-postive cells or neo-capillary formation and angiogenesis in eNOS-EPC treated rabbits are significantly higher than that observed in PBS (4.5-fold) or EPC treated animals (2.7-fold)**

# CONTRAST ANGIOGRAPHY

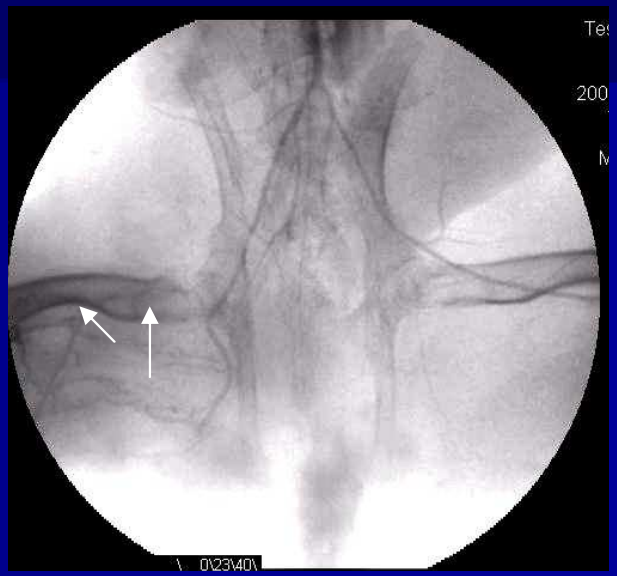
Saline



EPC

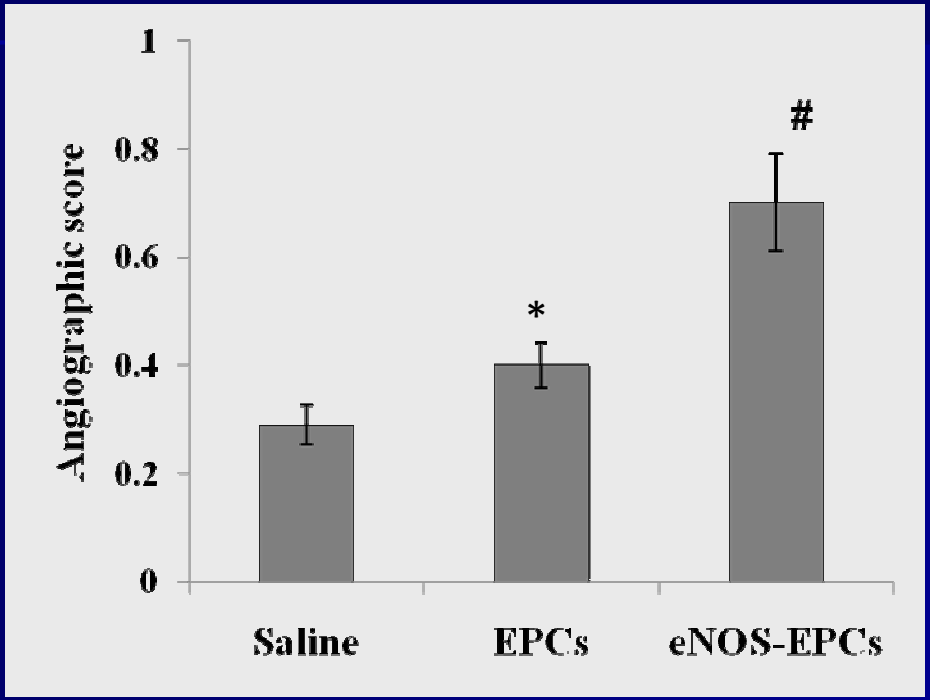


eNOS-EPC



- **A substantial increase in the distal arterial reconstitution of the ischemic hind limb in eNOS-EPC treated rabbits as compared to that observed in PBS or EPC treated animals**

# ANGIOGRAPHIC SCORE: ARTERIOGENESIS



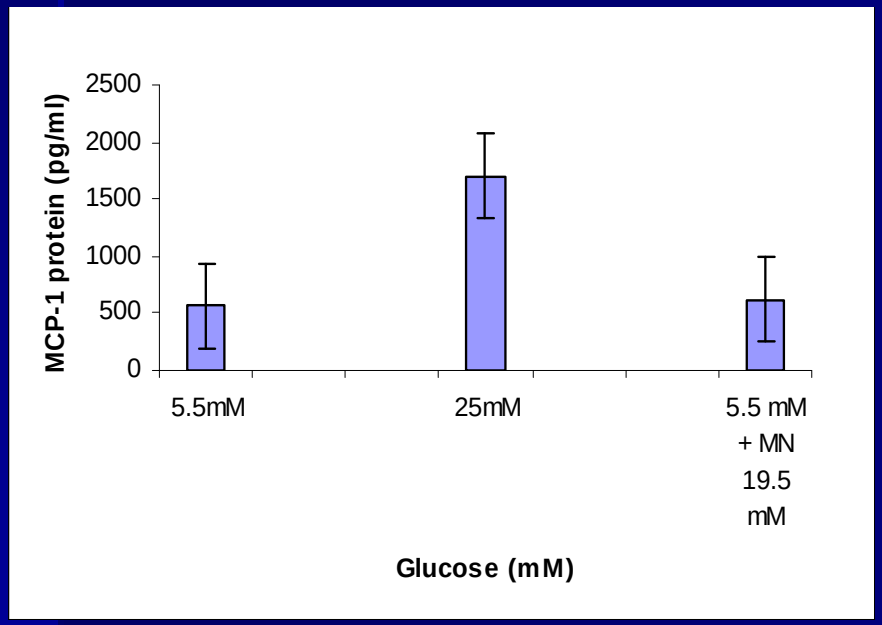

- No. of collaterals > 50 µm in diameter and hence arteriogenesis in eNOS-EPC treated rabbits are significantly higher than that observed in PBS (2.4-fold) or EPC treated animals (1.7-fold)

# Atherosclerosis Risk in Type 2 Diabetes Mellitus

Sumith R. Panicker and C.C.Kartha

- The role of MCP-1 in the increased risk of atherosclerosis in type 2 diabetes
- Whether MCP-1 could be a target for strategies to decrease the risk

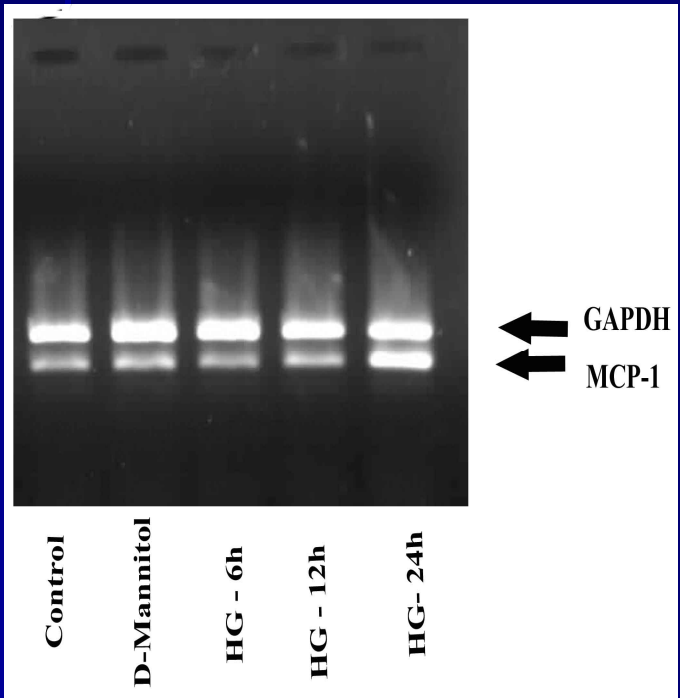
# Increased MCP-1 protein expression by RAECs upon HG induction




**HG (25mM) induces MCP-1 protein synthesis whereas Osmotic control , D-Mannitol (19.5mM) did not induce MCP-1 by RAECs**

*RAECs were cultured for up to 24 h in medium with control glucose (5.5mM), HG (25mM) and control glucose (5.5mM) + D-Mannitol (19.5mM) and the MCP-1 protein levels in the culture supernatant determined by ELISA. All results are expressed as mean ± SEM (n=3)*

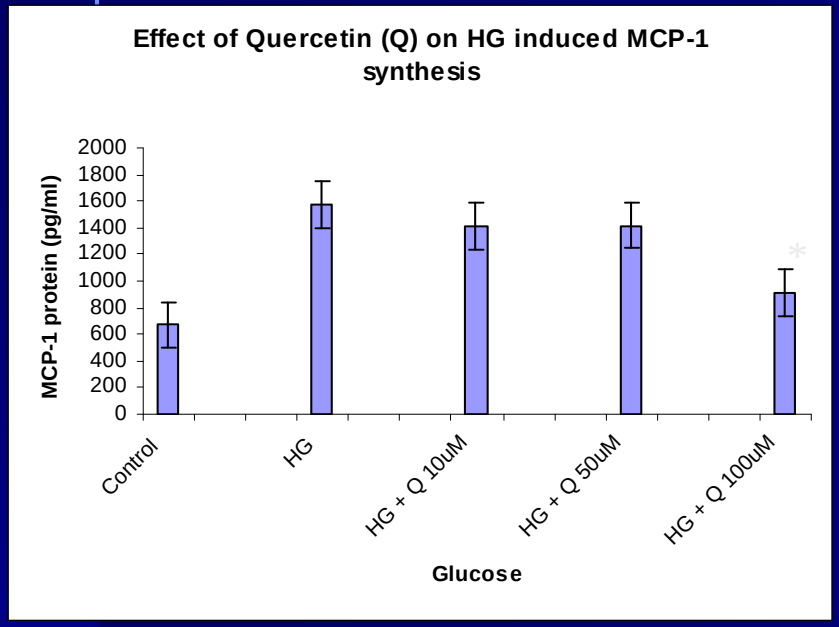
# Increased MCP-1 mRNA expression by RAECs upon HG induction




**Increased MCP-1 gene expression by RAECs exposed to HG**

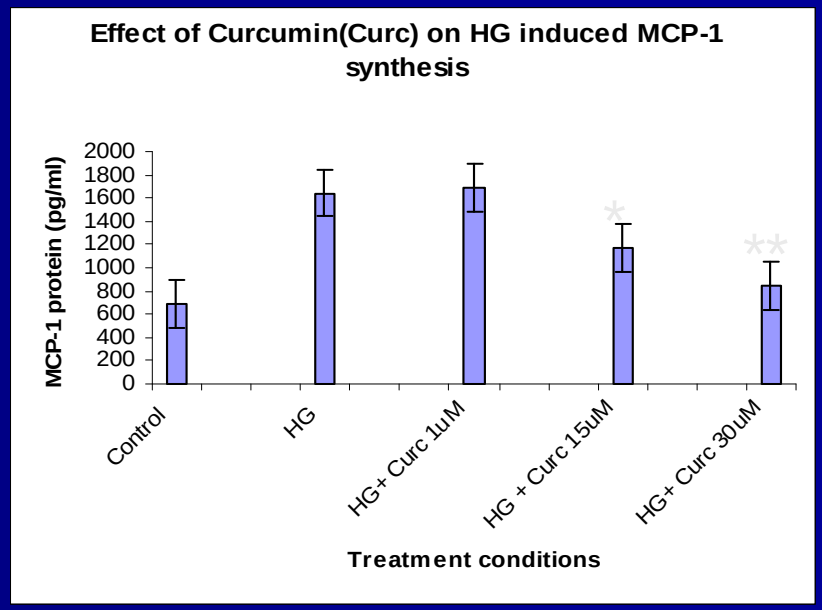
*RT-PCR products for MCP-1 and GAPDH were quantified using densitometry. HG (25mM) exposure upto 24h was found to induce MCP-1 mRNA by nearly 1.5 fold over control*

# Attenuation of HG induced MCP-1 expression by Quercetin & Curcumin



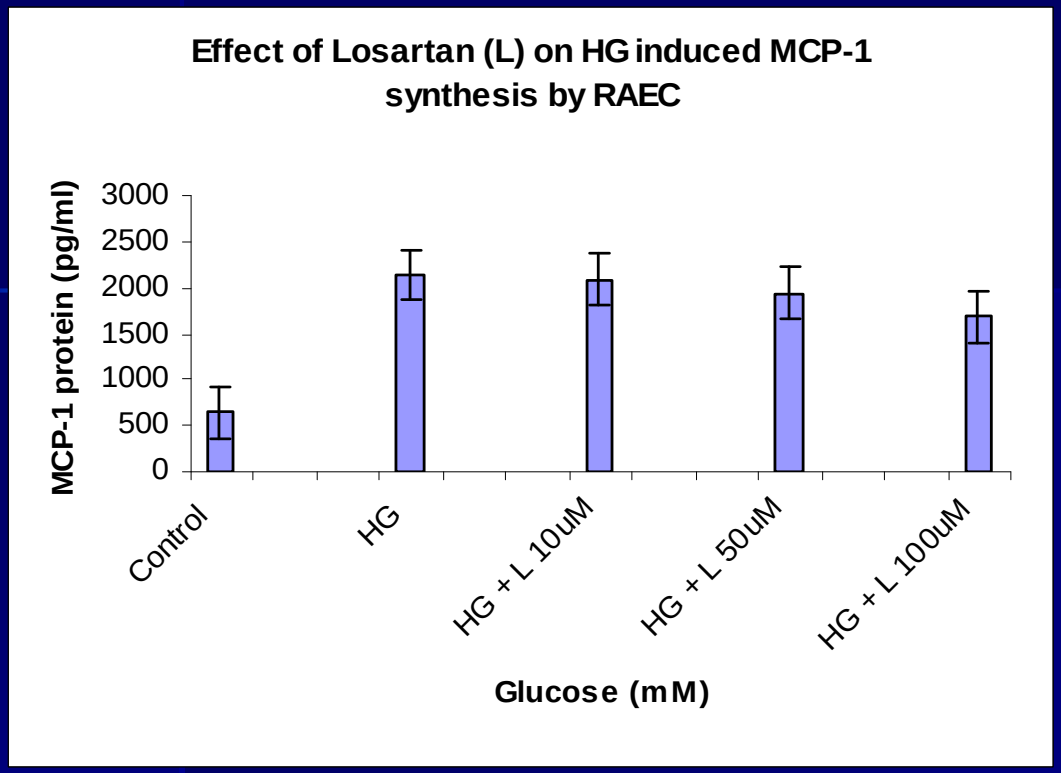

***Quercetin Inhibits HG induced MCP-1 protein synthesis by RAECs***

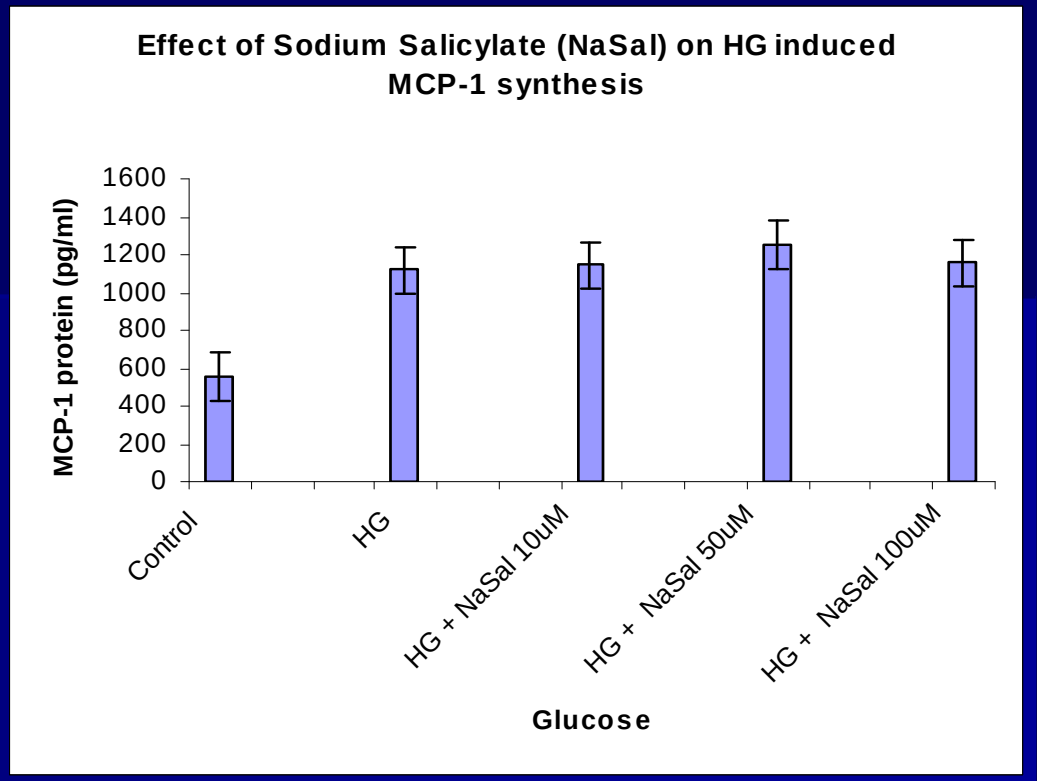
*RAECs were cultured for upto 24 h in medium with control glucose (5.5mM) and HG (25mM) concentrations in the absence or presence of indicated concentrations of Quercetin and the MCP-1 protein levels in the culture supernatant determined by ELISA. All results are expressed as mean ± SEM (n=3). *P<0.0001 when compared to HG treated group (25mM)*




***Curcumin Inhibits HG induced MCP-1 protein synthesis by RAECs***

*RAECs were cultured for upto 24 h in medium with control glucose (5.5mM) and HG (25mM) concentrations in the absence or presence of indicated concentrations of Curcumin and the MCP-1 protein levels in the culture supernatant determined by ELISA. All results are expressed as mean ± SEM (n=3). *P< 0.05 and **P<0.0001 when compared to HG treated group (25mM)*



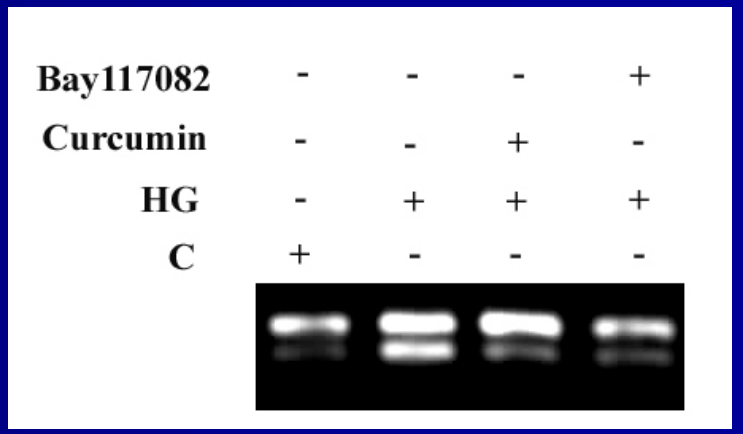



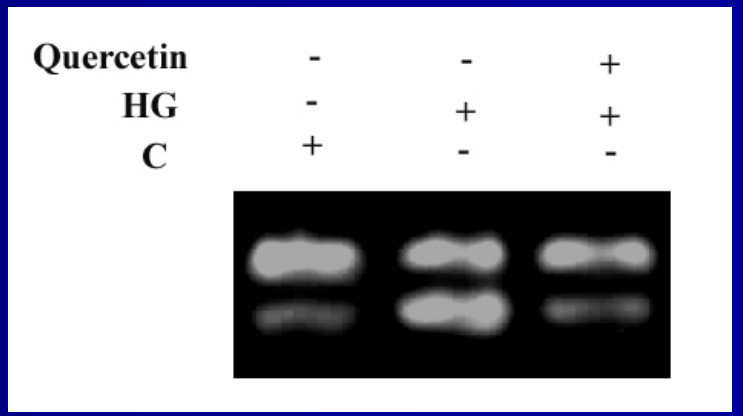

**Losartan and Sodium Salicylate does not inhibit HG induced MCP-1 expression by RAECs. RAECs were cultured for up to 24 h in medium with control glucose (5.5mM) and high glucose (HG) concentrations in the absence or presence of indicated concentrations of Losartan and the MCP-1 protein levels in the culture supernatant were determined by ELISA. All results were expressed as mean ± SEM (n=3).**

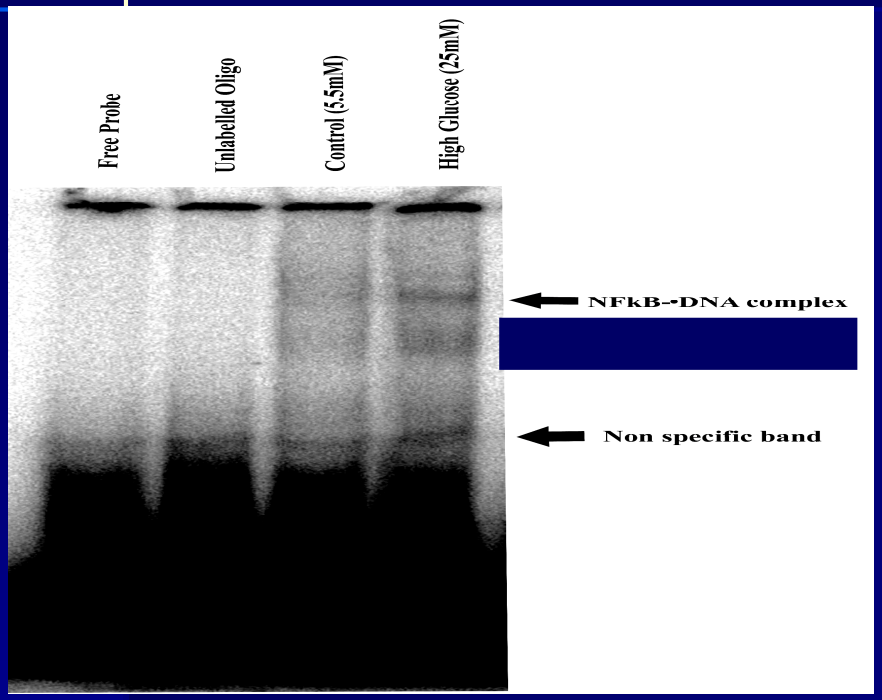
# Mechanism of MCP-1inhibition by Quercetin & Curcumin

***Quercetin and Curcumin inhibits HG induced MCP-1 mRNA synthesis by RAECs***

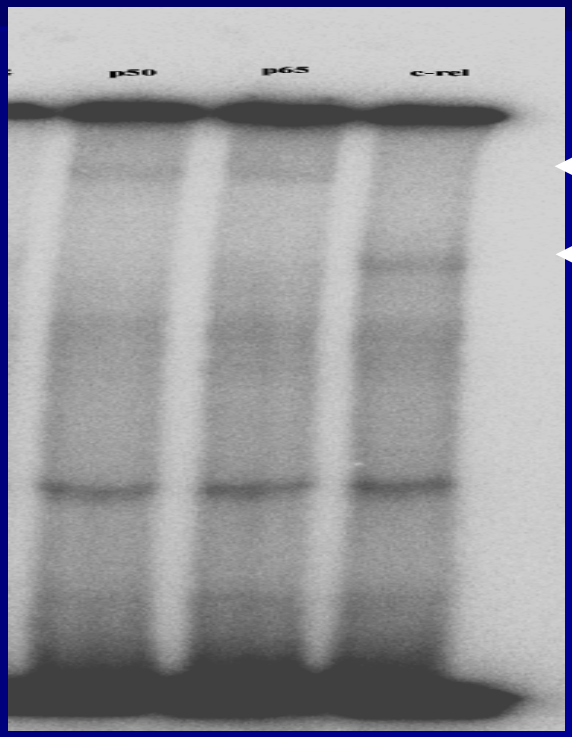
*RAECs were cultured for up to 24 h in medium with control glucose (5.5mM) and HG (25mM) concentrations in the absence or presence of Quercetin (100 uM), NF κ B inhibitor Bay11-7082(5 uM) and Curcumin (30 uM) and the MCP-1 mRNA levels determined by RT-PCR*



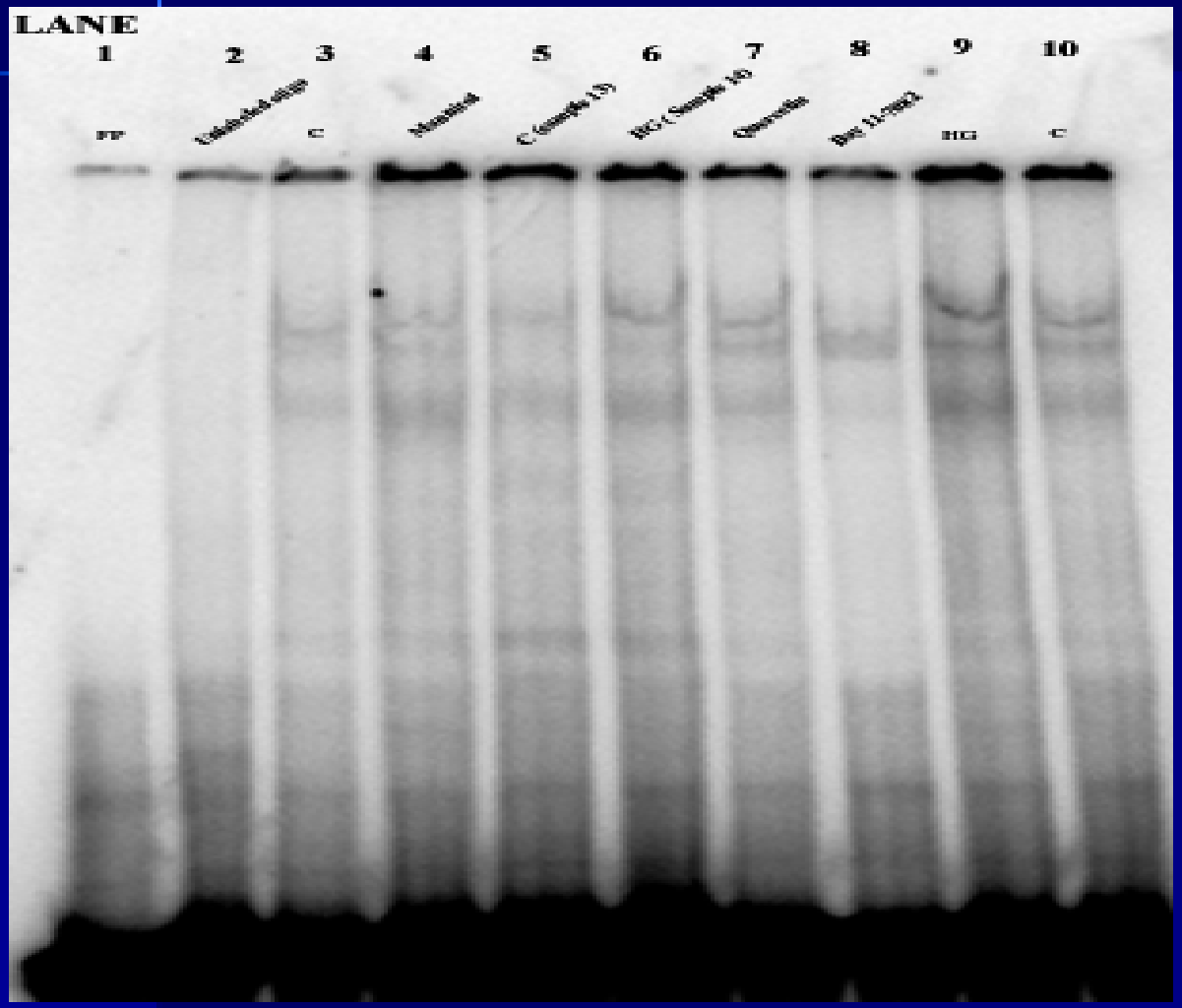






HG increases NFkB translocation into the nucleus as is shown by increased intensity of NFkB-DNA complex Upper arrow indicates the NFkB-DNA complex. This is representation of three different experiments.

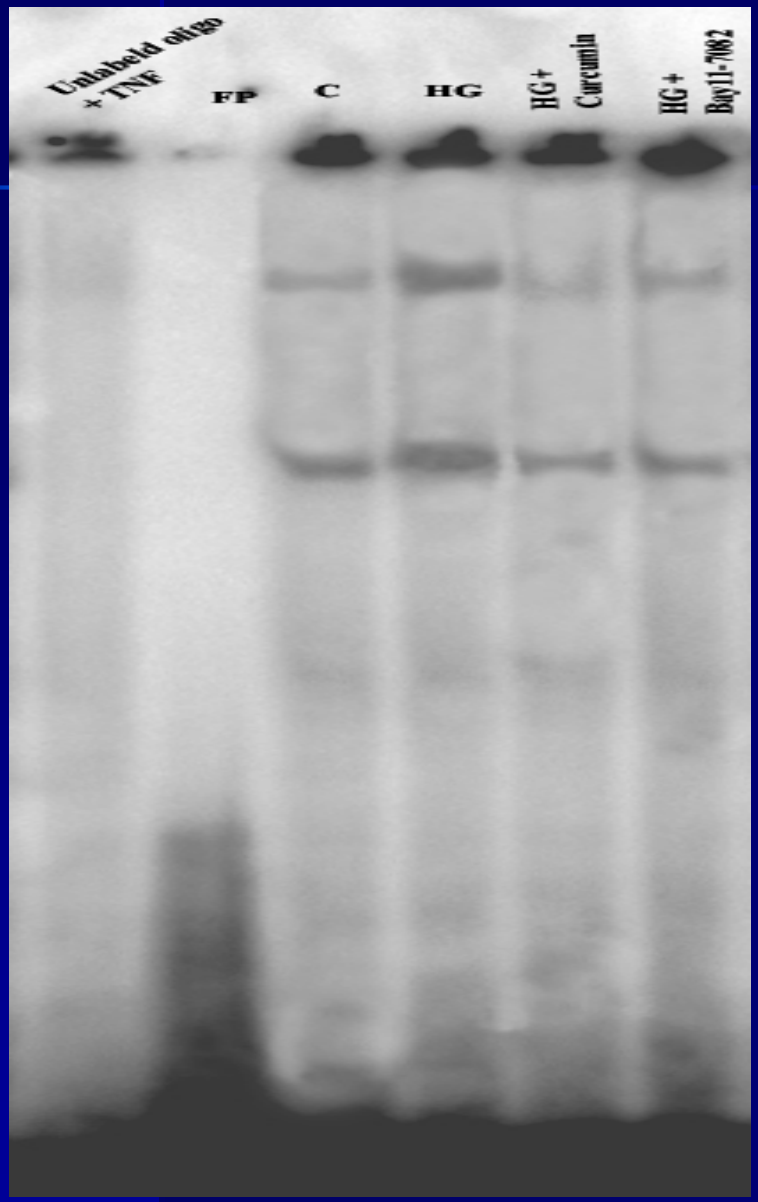



NFkB bands are composed of p50 and p65 subunits as revealed by supershift analysis. For determining the subunit composition of NFkB bands, supershift analysis using anti-p50, anti-p65 and anti-c-rel antibodies were done. Upper arrow indicates the supershifted bands and lower arrow indicates the NFkB bands. This is representation of three different experiments.




**Quercetin inhibits HG induced NFkB DNA binding activity in RAECs**

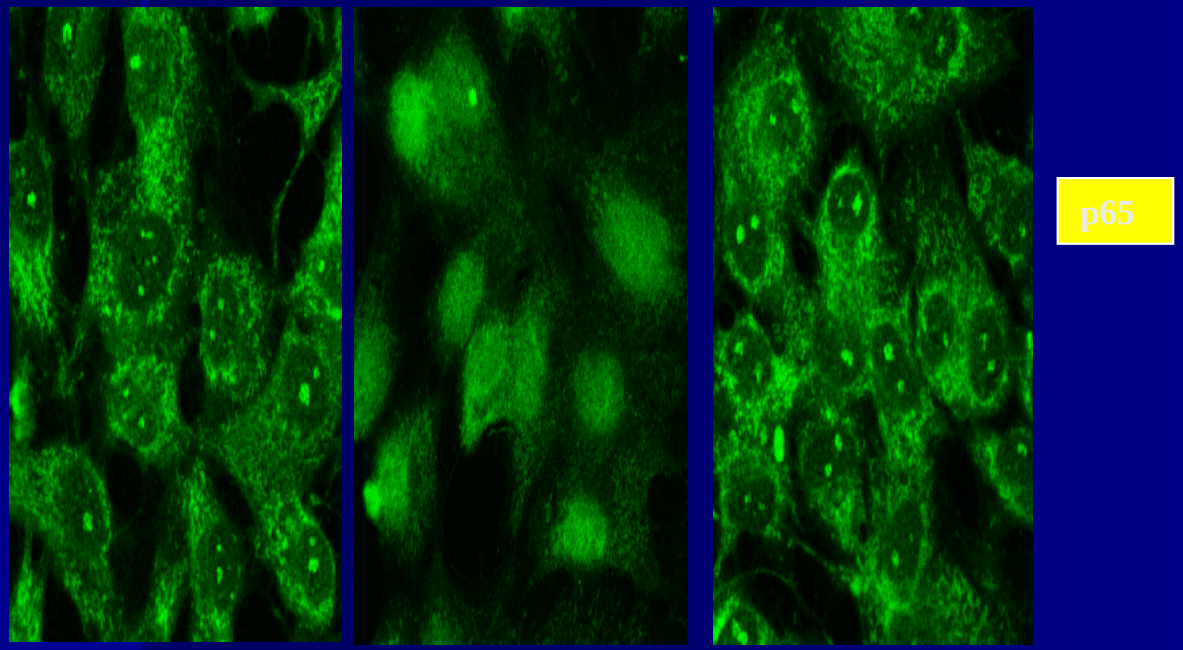
*RAECs were cultured for up to 24 h in medium with control glucose (5.5mM) and HG (25mM) concentrations in the absence or presence of Quercetin (100uM) and Bay11-7082(5uM) and NFkB DNA binding activity determined by EMSA. Bay11-7082 was used as positive control.*




***Curcumin inhibits HG induced NFkB DNA binding activity in RAECs***

*RAECs were cultured for up to 24 h in medium with control glucose (5.5mM) and HG (25mM) concentrations in the absence or presence of indicated concentrations of Curcumin (30uM) and Bay11-7082(5uM) and NFkB DNA binding activity determined by EMSA. Bay11-7082 was used as positive control.*

Control | HG | HG + Quercetin



p65

**Quercetin inhibits HG induced translocation of NFkB p65.**

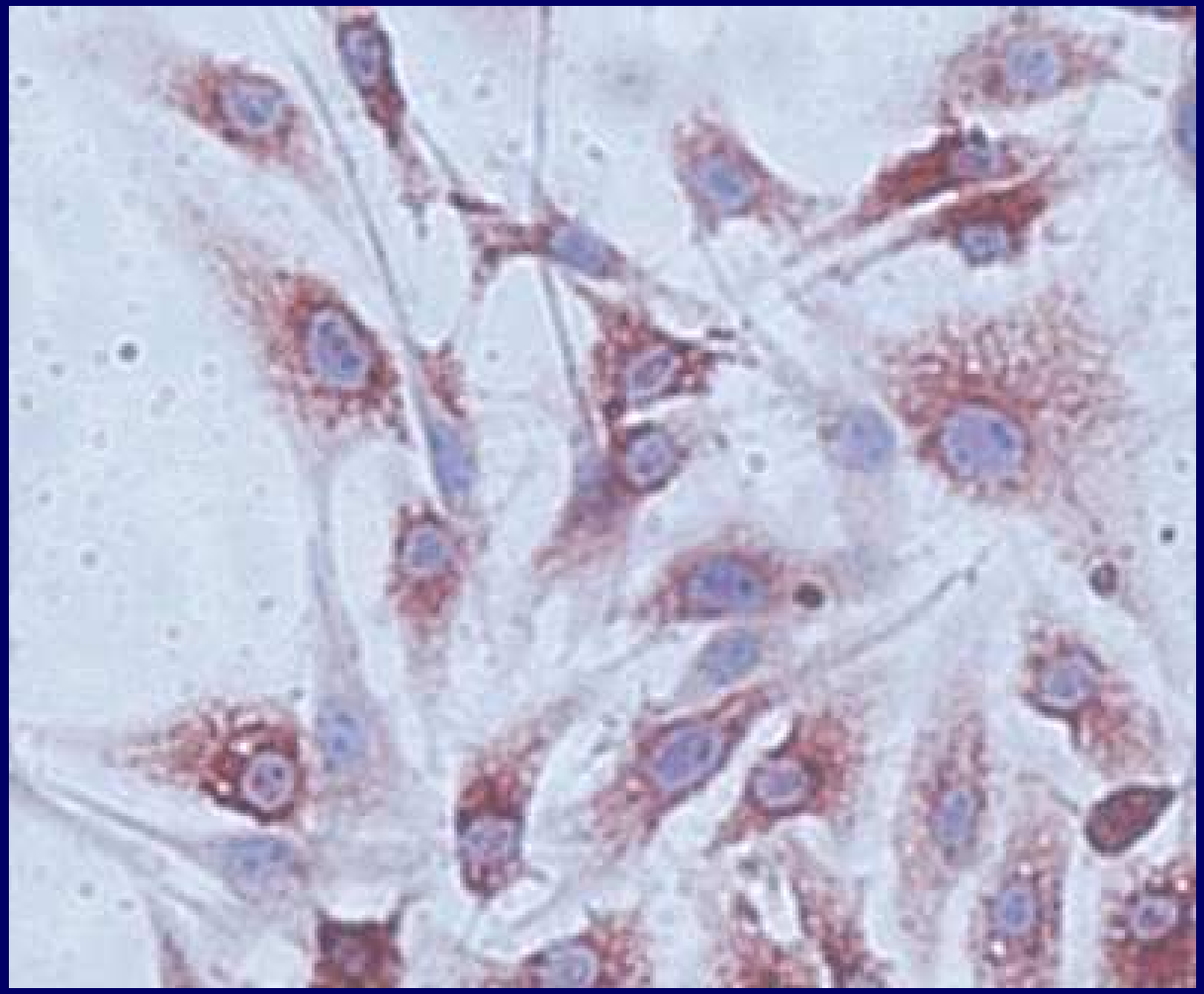
*RAECs were stimulated with HG for 24 h in the presence of Quercetin (100uM), fixed, stained with anti- p65 antibody, secondary labelled with FITC conjugated antibody and observed under confocal microscope. Arrows indicate nucleus. This is representative of 3 different experiments.*

# Does endocardial endothelium modulate ventricular remodeling in cardiac failure?

- Structure and functions of endothelial cells are region specific
- Endocardial endothelial cells are distinct from microvascular endothelial cells in the heart
- EECs are dissimilar with respect to their embryological origin, receptor mediated functions, electrophysiological properties and growth characteristics in culture

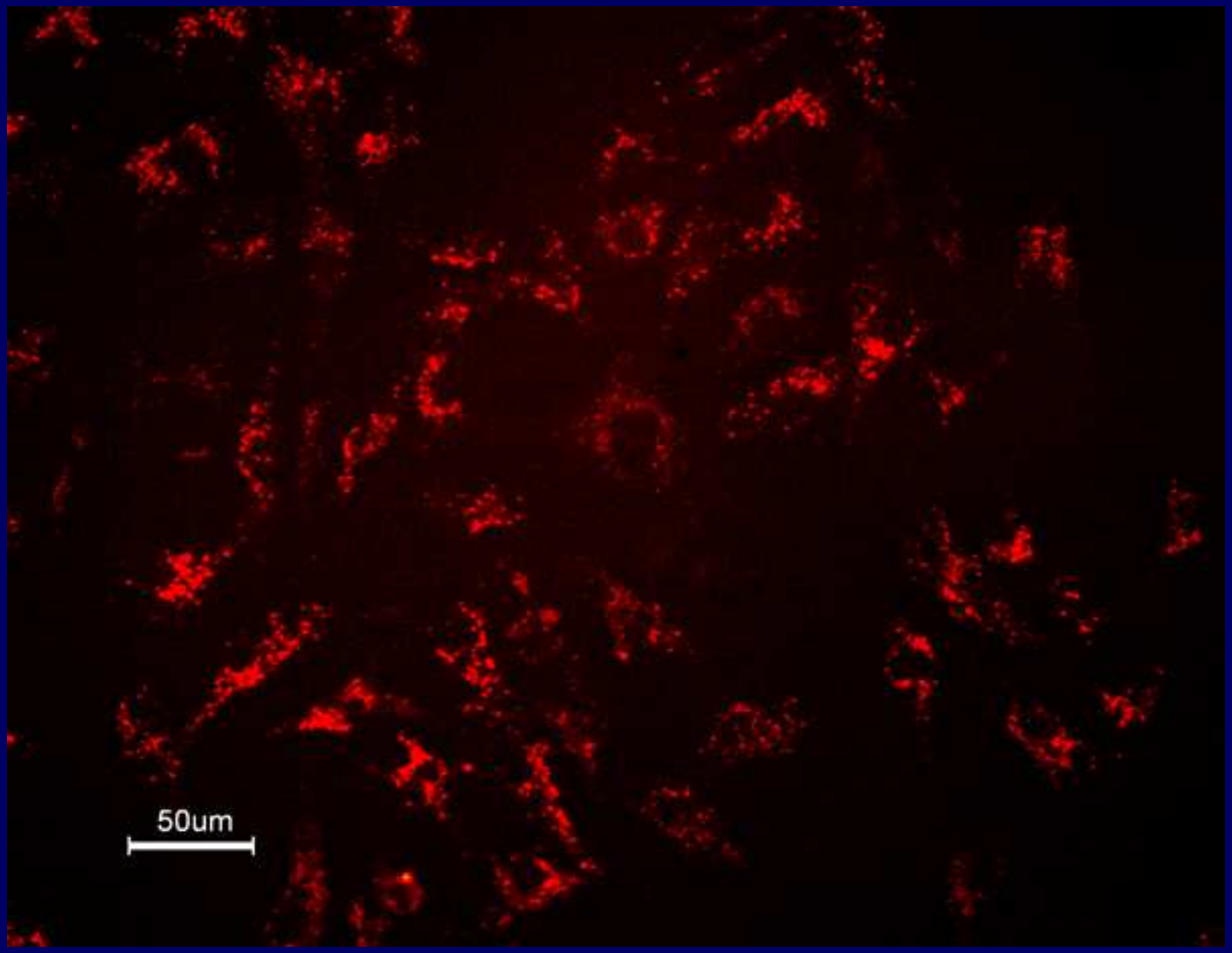
# Endothelial regulation of cardiac performance

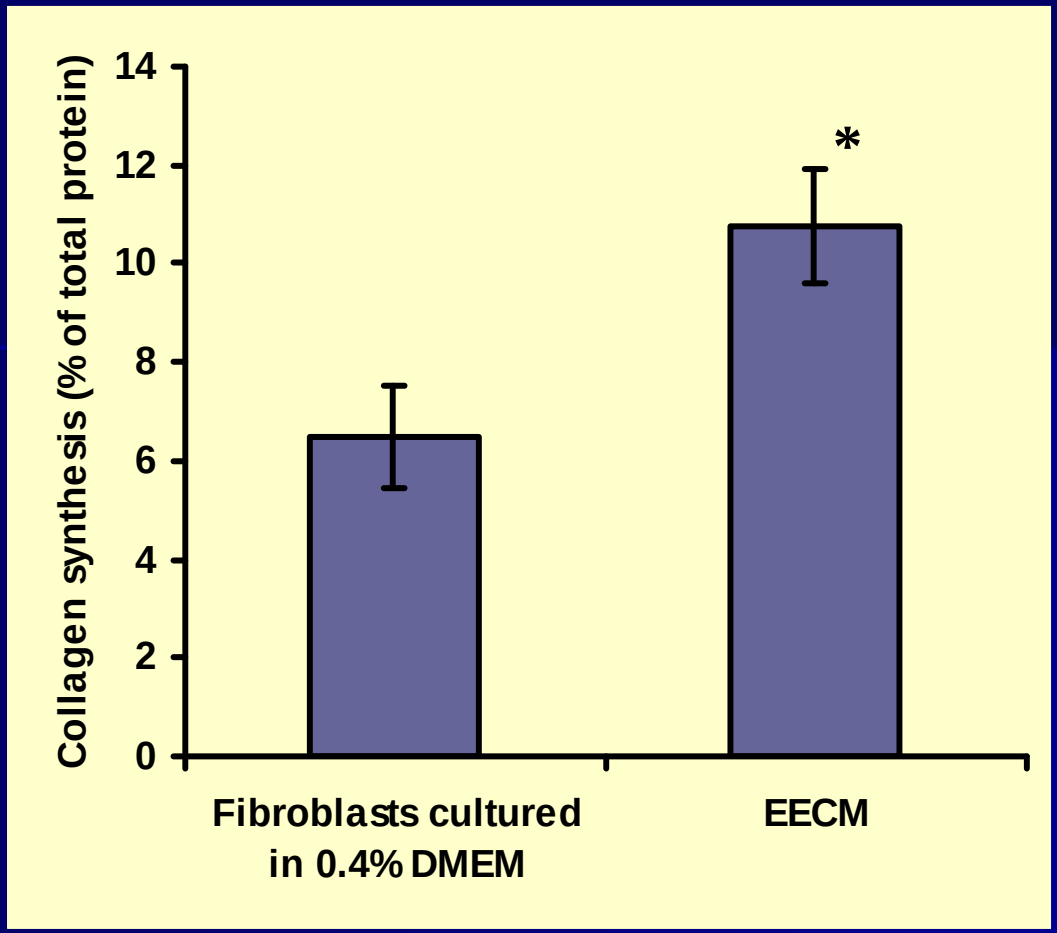
- Brutsaert *et al* (1988,1989) demonstrated that the endocardium is an important modulator of subjacent cardiac muscle performance
- Intact EE helps to modulate left ventricular function (LVF) by prolonging ejection duration, increasing end diastolic volume and slightly increasing systolic peak performance
- Several substances released or metabolized by cardiac endothelial cells have direct effects on cardiac myocyte function (Mc Clellan *et al.*1993;Ramaciotti *et al.1993)*
- While the modulator effects of endocardial endothelium on cardiomyocytes is firmly established, less well characterized is the the role of EE on cardiac interstitium and its component cells.



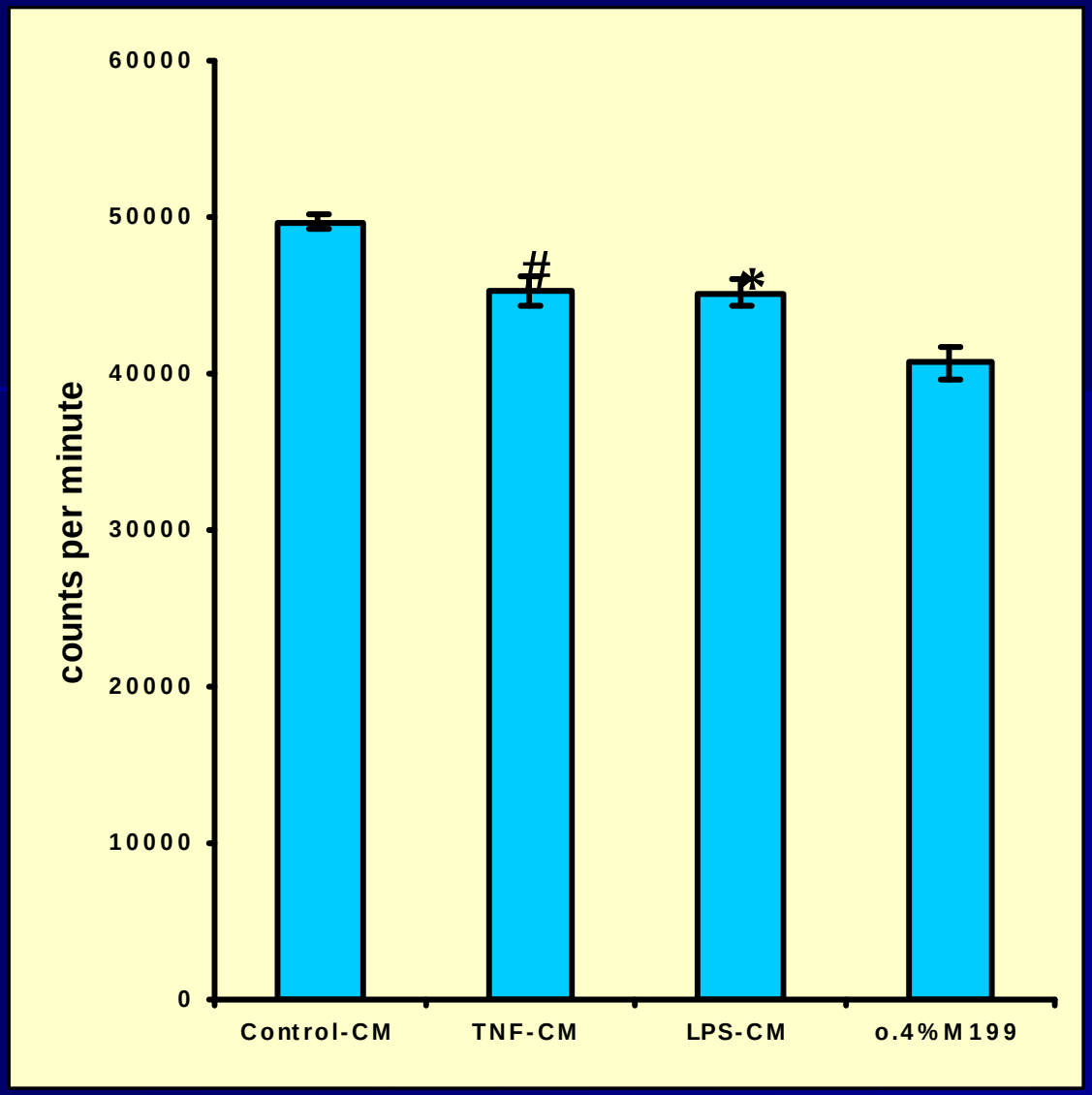
**Positive staining for von Willebrand factor by endocardial endothelial cells**

# DiI-Acetylated-LDL uptake by endocardial endothelial cells

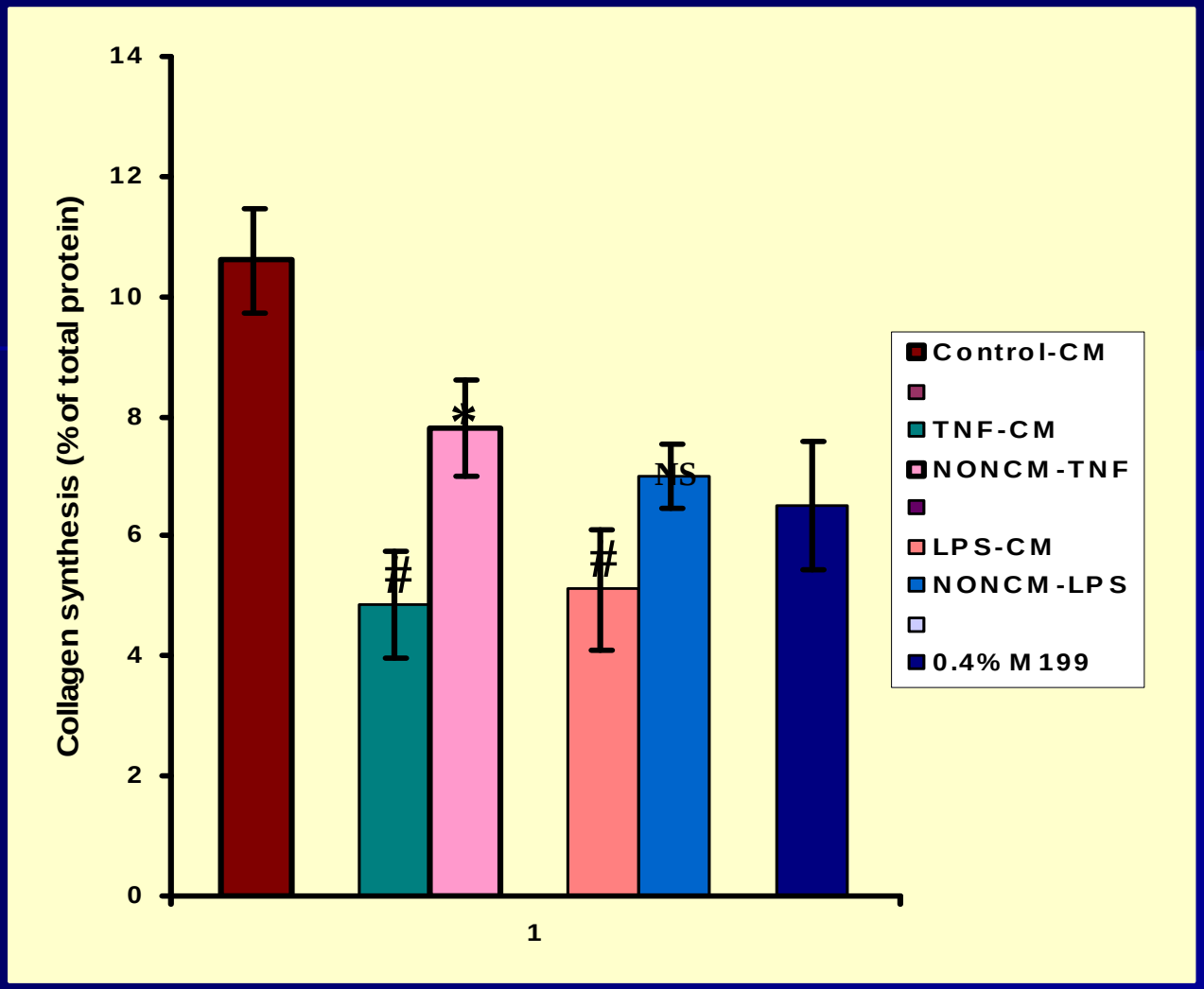





**Collagen synthesis in cardiac fibroblasts cultured in 0.4% DMEM and incubated with EEC conditioned medium (EECM). The percentage of collagen represented is the relative proportion of [$^3$H]-Proline incorporation into collagen vs. non-collagen protein. The values are mean ± SD for n=9. *p<0.001**

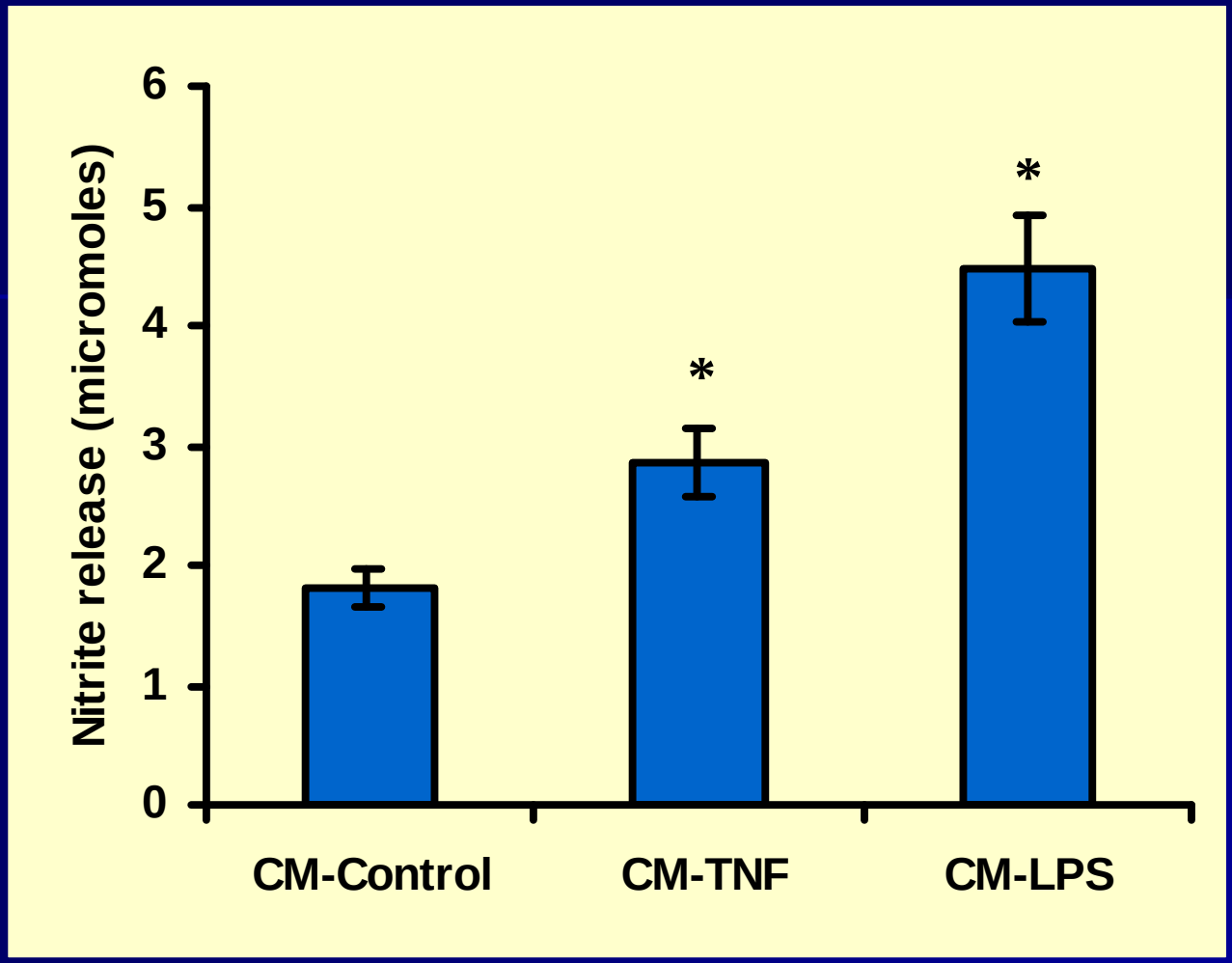



**Effect of CM from EECs treated with TNF-α and LPS on [$^3$H]- Thymidine uptake by cardiac fibroblasts. The values are mean ± SD.**
**For C-CM vs TNF-CM n=21, #p<0.05.**
**For C-CM vs LPS-CM, n=18 *p<0.001**

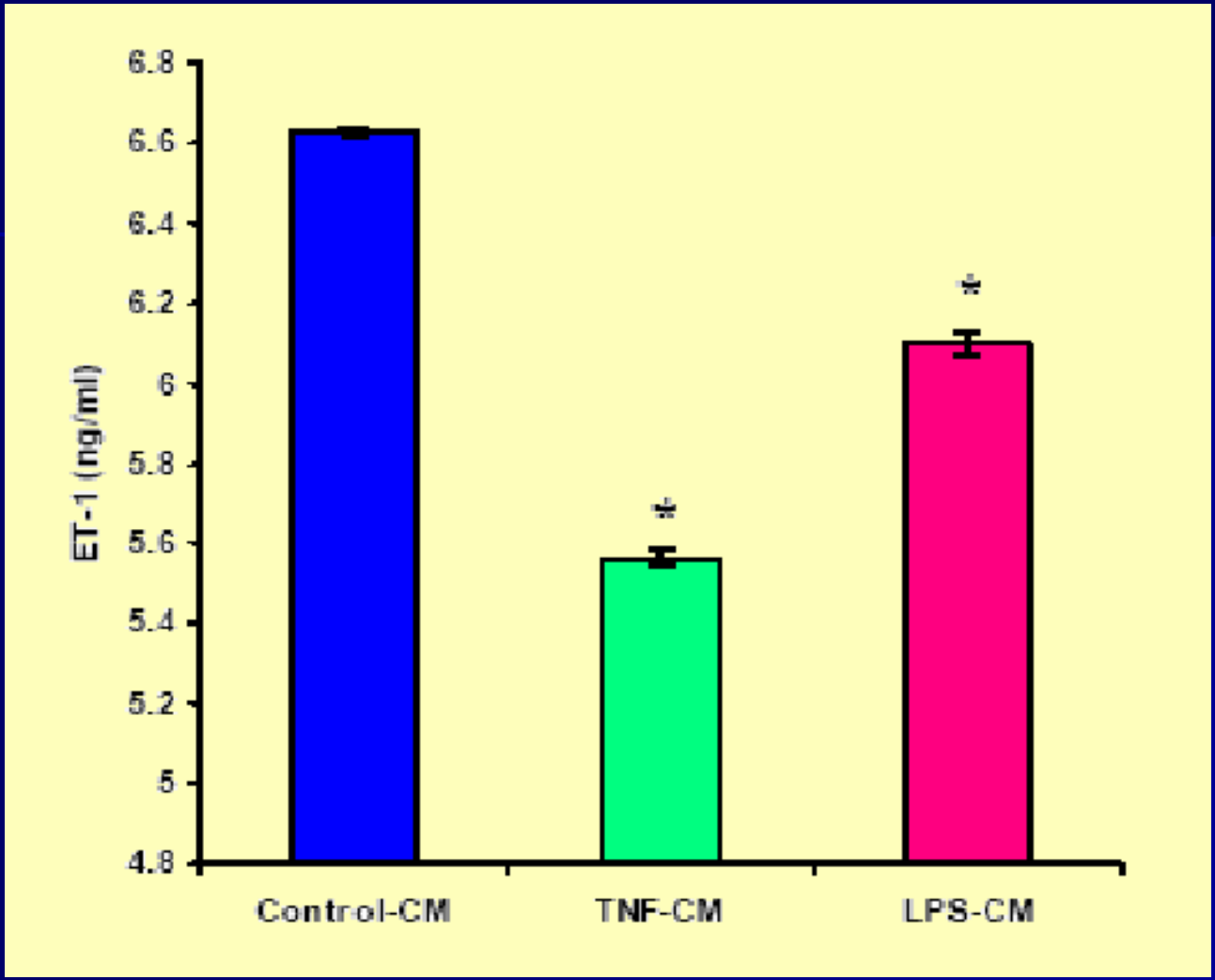



EECM TNF LPS 0.4% M199

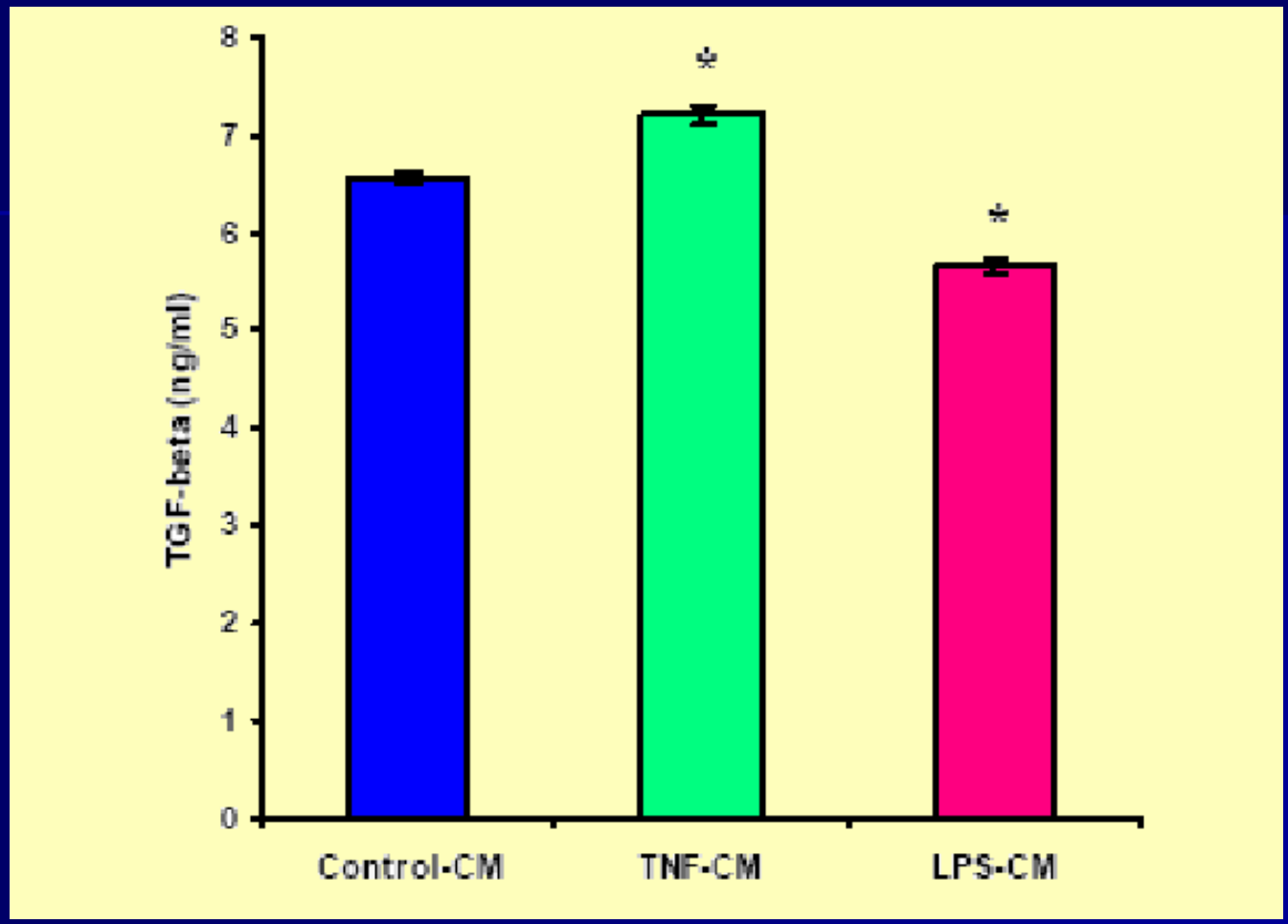
**Effect of CM from EECs treated with TNF-α and LPS on collagen synthesis by cardiac fibroblasts. The values are mean ± SD. n=6,**
**For C-CM vs TNF-CM & LPS-CM, #p<0.05**
**For TNF-CM vs NONCM-TNF, *p<0.001**




**Nitrite release by EE cells in response to proinflammatory agents TNF- α and LPS n=6;*p<0.05**




Effect of TNF-α and LPS on endothelin-1 release by endocardial endothelial cells. The values are mean ± SD (n=6;ANOVA;*p <0.01)




Effect of TNF-α and LPS on transforming growth factor-β release by endocardial endothelial cells. The values are mean ± SD (n=6;ANOVA;*p <0.01)

# Significance

- Several mechanisms that regulate myocyte and fibroblast growth, vasculogenesis, angiogenesis and matrix alterations during ventricular remodeling have been identified.
- However, transitions in cell-cell interactions during ventricular remodeling and consequent effects are less well known.
- Endocardial endothelial dysfunction associated with cardiac inflammation could alter the release of endothelial mediators such as NO and ET-1. The alterations could affect fibroblast response and perturb ventricular remodeling in pathological conditions of the heart.