Ischemic Protection in Cardiovascular Disease

- An Overview for Therapeutic Application -

충북의대 황 경 국

- **Is Cardioprotection from severe ischemia possible ?**

- **What is the adaptive behavior of the Cell in protecting itself from ischemia ?**

Cardioprotection: Genesis of Concept

- Major paradigm shift by Braunwald et al (1971)

  Extent & severity of tissue damage after coronary occlusion

  :**modified therapeutic manipulation during ischemia**

- Experimental interventions for cardioprotection

  Exception of **“ early (timely) reperfusion”,**

  none has been translated into clinical practice

- Considerations for effective clinical treatment

  Survive in any arrhythmia

  Minimize damaged functional myocardium

Ischemic Protection in Cardiovascular System

- **Myocardial Response to Ischemic Injury**

  Ischemic Preconditioning (PC)
  Hibernation
  Stunning

  Regulation of Ischemia–induced Oxidative Stress

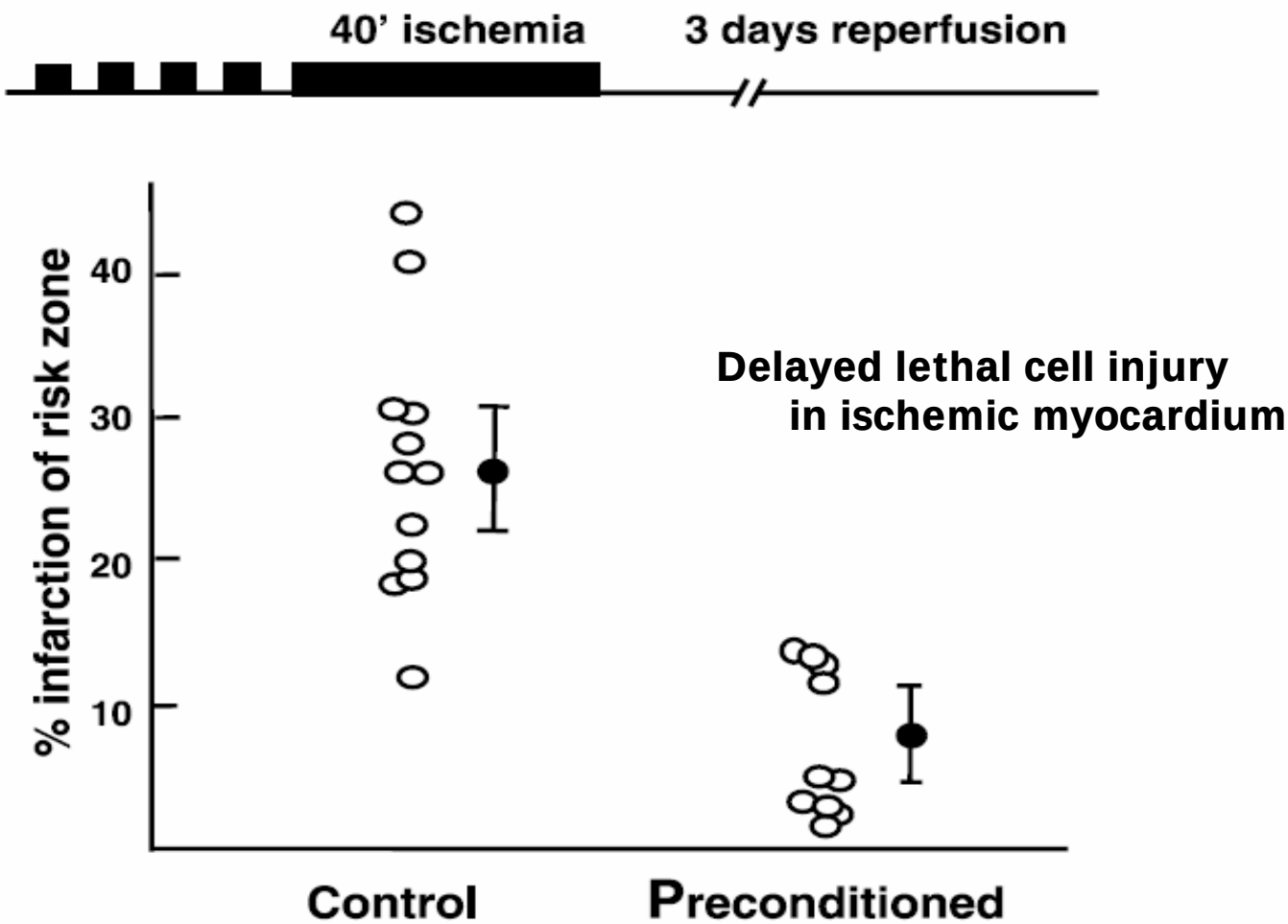
- **Therapeutic Strategy for Ischemic Protection**

  Gene therapy
  Cell therapy : Angiogenesis vs Myocardial regeneration

- **Future perspectives**

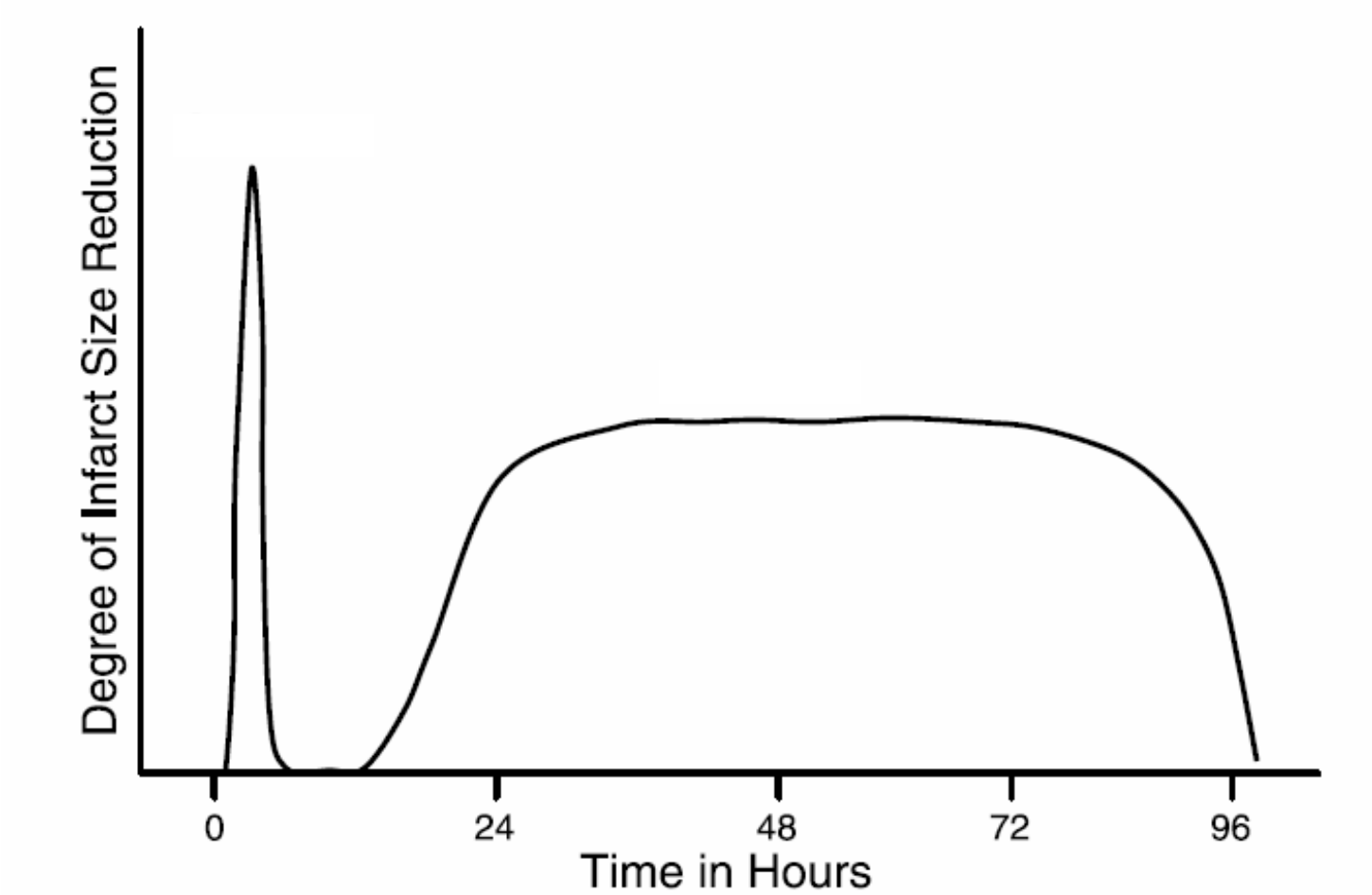
  Considerations for Effective treatment

Ischemic Preconditioning



(Murry et al *Circulation. 1986*:74:1124-1136)

Temporal Nature of 2 windows of Preconditioning



Biphasic Pattern of Ischemic Preconditioning

- **Classic or Early phase of IPC**

  1-2hrs after PC stimuli

- **Late Phase of IPC** (Second Window)

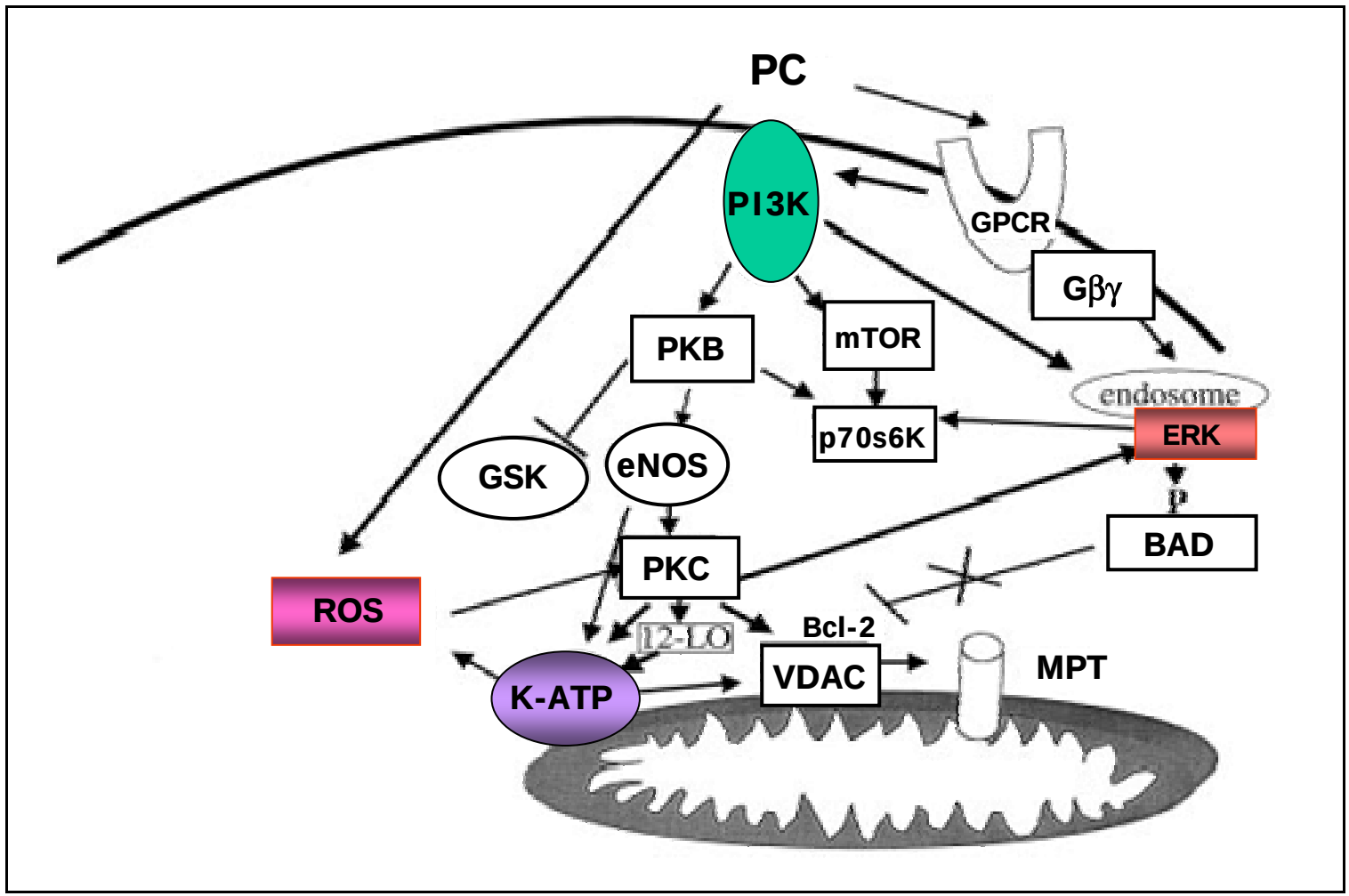
  "Universal response of the heart to stress in general"

  12-72 hrs after PC stimuli

  PKC-$\varepsilon \rightarrow$ NF-κB $\rightarrow$ iNOS / COX-2

  Increased Bcl-2 expression $\rightarrow \downarrow$ MPT opening

Primary Signaling Pathway in Preconditioning



End Effect of Cardioprotection: reduced cell death both necrosis & apoptosis

Secondary Signaling Pathway in Preconditioning

Converge to a few related mitochondrial proteins.

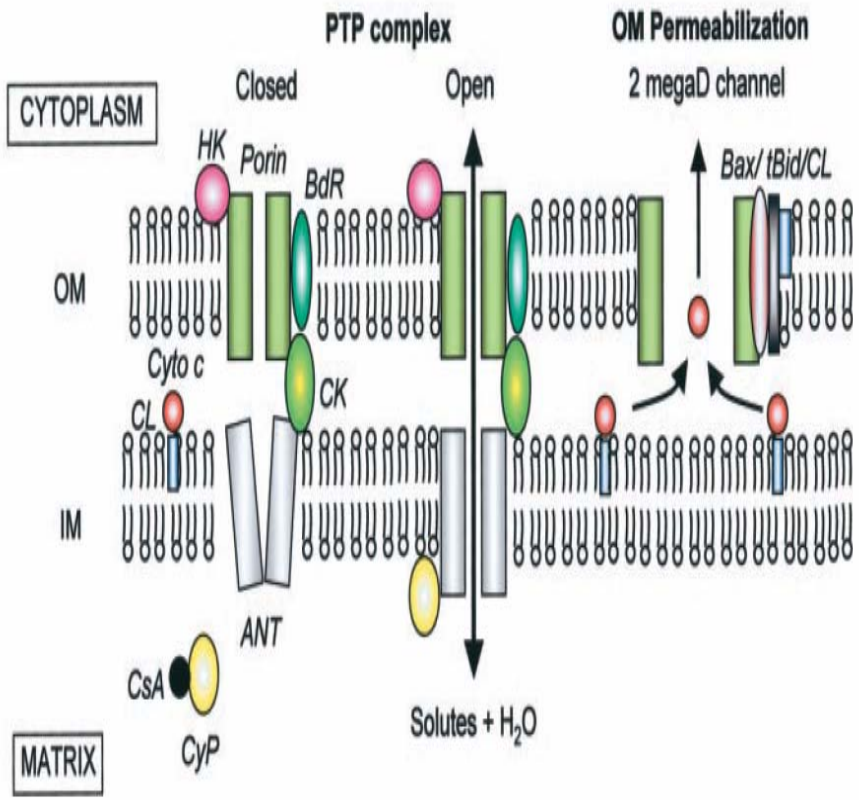
- ERK: phosphorylate mitochondrial-associated BAD
  which would cause it to dissociate from bcl-2,
  leaving bcl-2 free to bind and inhibit VDAC and the MPT.

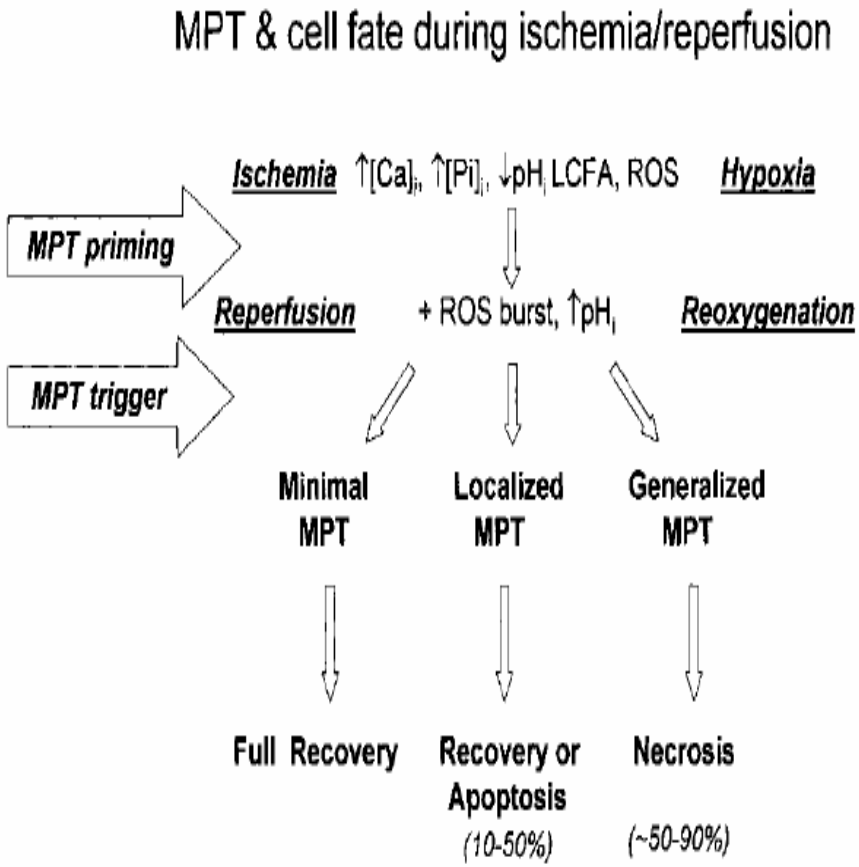
- PKC, NO, 12-LO metabolites, ROS: activate the mito K_{ATP} channel,
  reduce apoptosis and cell death.

- Mito K_{ATP}: maintain mitochondrial structure
  VDAC is in a low conductance state, synergize with the bcl-2
  VDAC in a closed state: reduce apoptosis
  (either via VDAC association with BAX or as part of the MPT).

- PC signaling : mito K_{ATP}, VDAC, and MPT
- Delayed PC: additional targets, such as NF-kB, iNOS, COX-2, p70s6K,

Mitochondrial Permeability Transition (MPT)





Weiss JN et al. Cir Res**. 2003;93:292-301**

Mitochondrial Permeability Transition (MPT)

Opening of PMTP in the inner mitochondrial membrane
- $\rightarrow$ matrix swelling, outer membrane rupture
- $\rightarrow$ Release of apoptotic signaling molecules (cytochrome *c*)
- $\rightarrow$ Irreversible injury to the mitochondria.

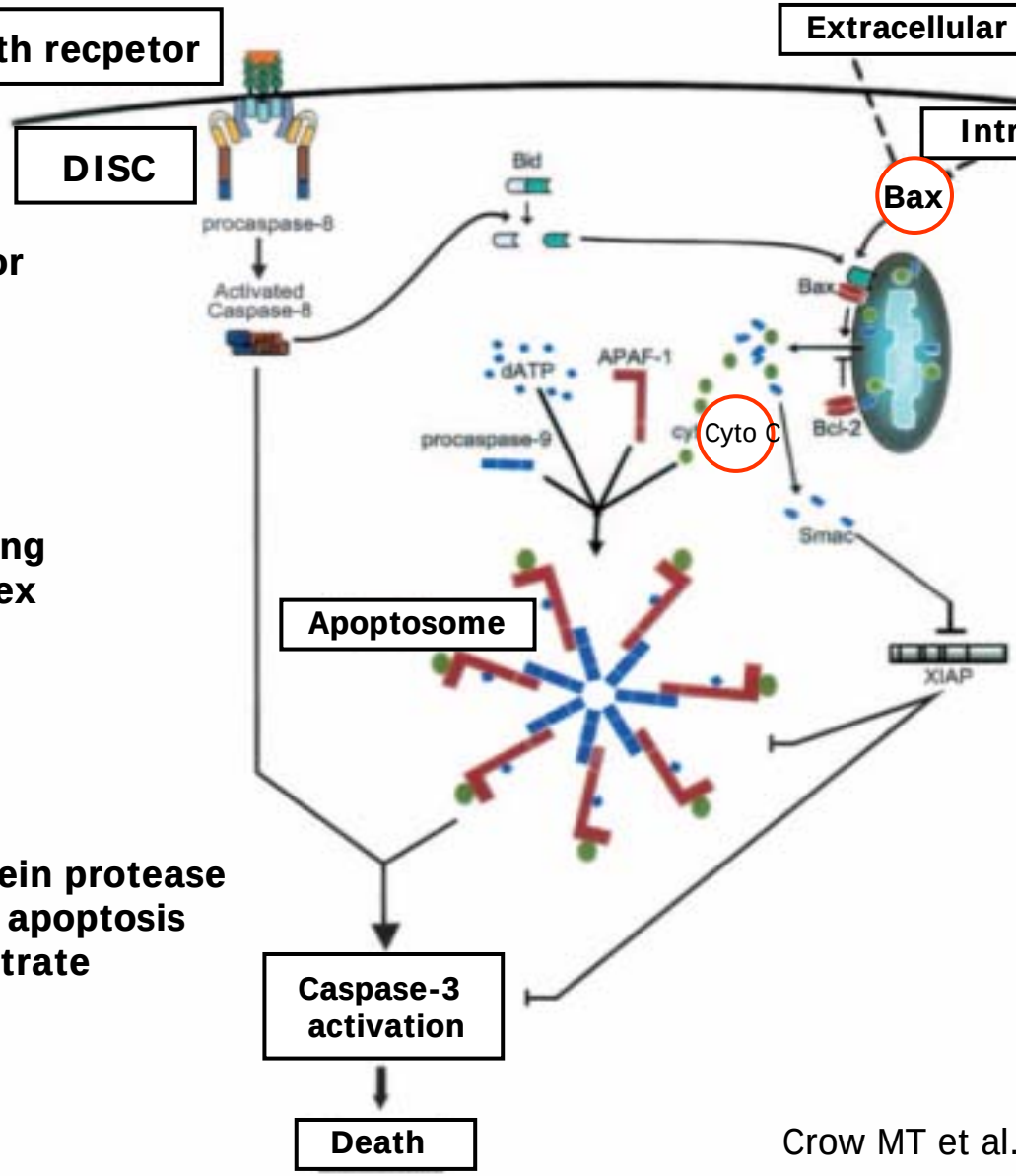
During ischemia

Intracellular Ca^{2+}, long-chain FA accumulation, ROS
- $\rightarrow \uparrow$ mitochondrial susceptibility to MPT,
- $\rightarrow \uparrow$ the likelihood that MPT will occur on reperfusion

Functional cardiac recovery : depends on mitochondrial recovery

Cardioprotection by ischemic preconditioning must ultimately involve the prevention of MPT.

Mitochondrial Death Pathway



Death recpetor
Fas

DISC
Death-inducing signal complex

Caspase: cytein protease
executor of apoptosis
cleave substrate

Intrinsic pathway
mitochondrial

Bcl-2 family

Anti-apoptotic
Bcl-2, Bcl-XL

Proapototic
Bax, Bak
Bid, Bad

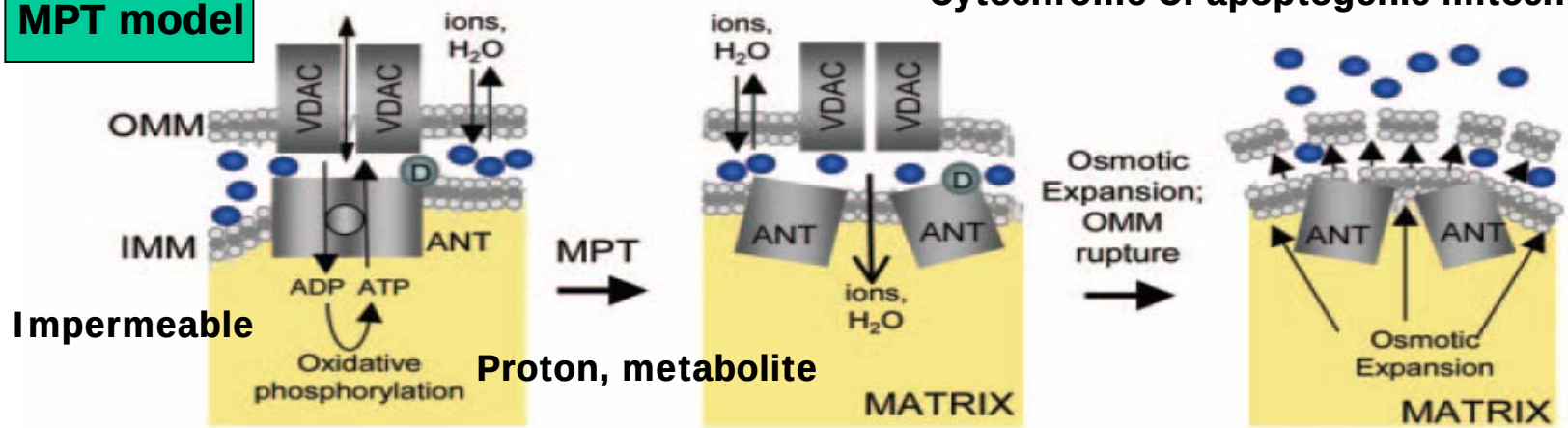
APSF-1
apoptotic protease
activating factor-1

Crow MT et al. Cir Res. **2004;95:957-970**

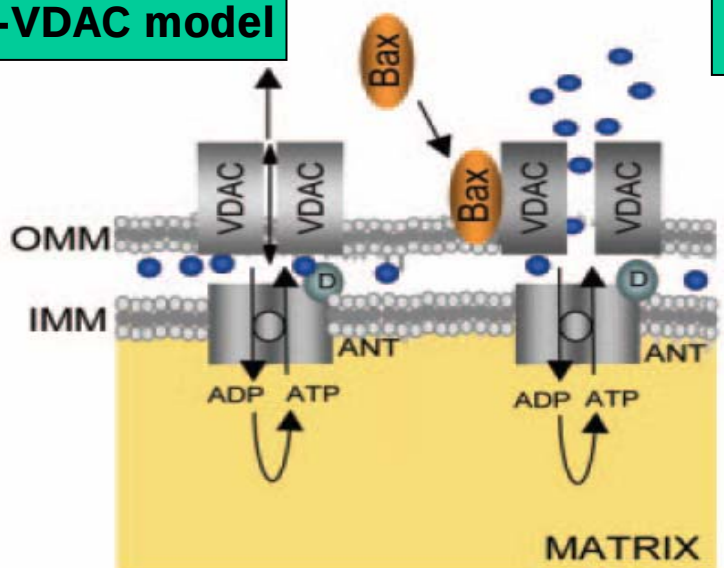
Mitochondrial Death Pathway: Apoptosis

MPT model

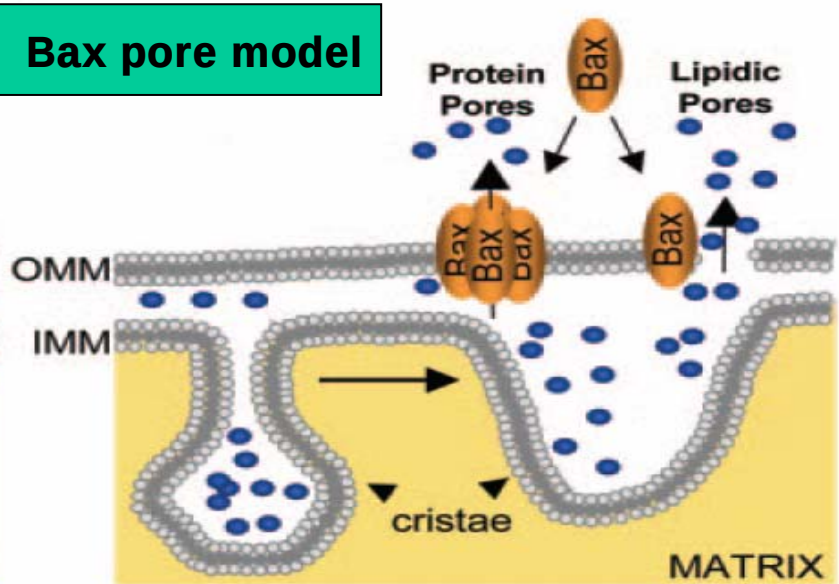
Cytochrome C: apoptogenic mitochrial protein



Bax-VDAC model

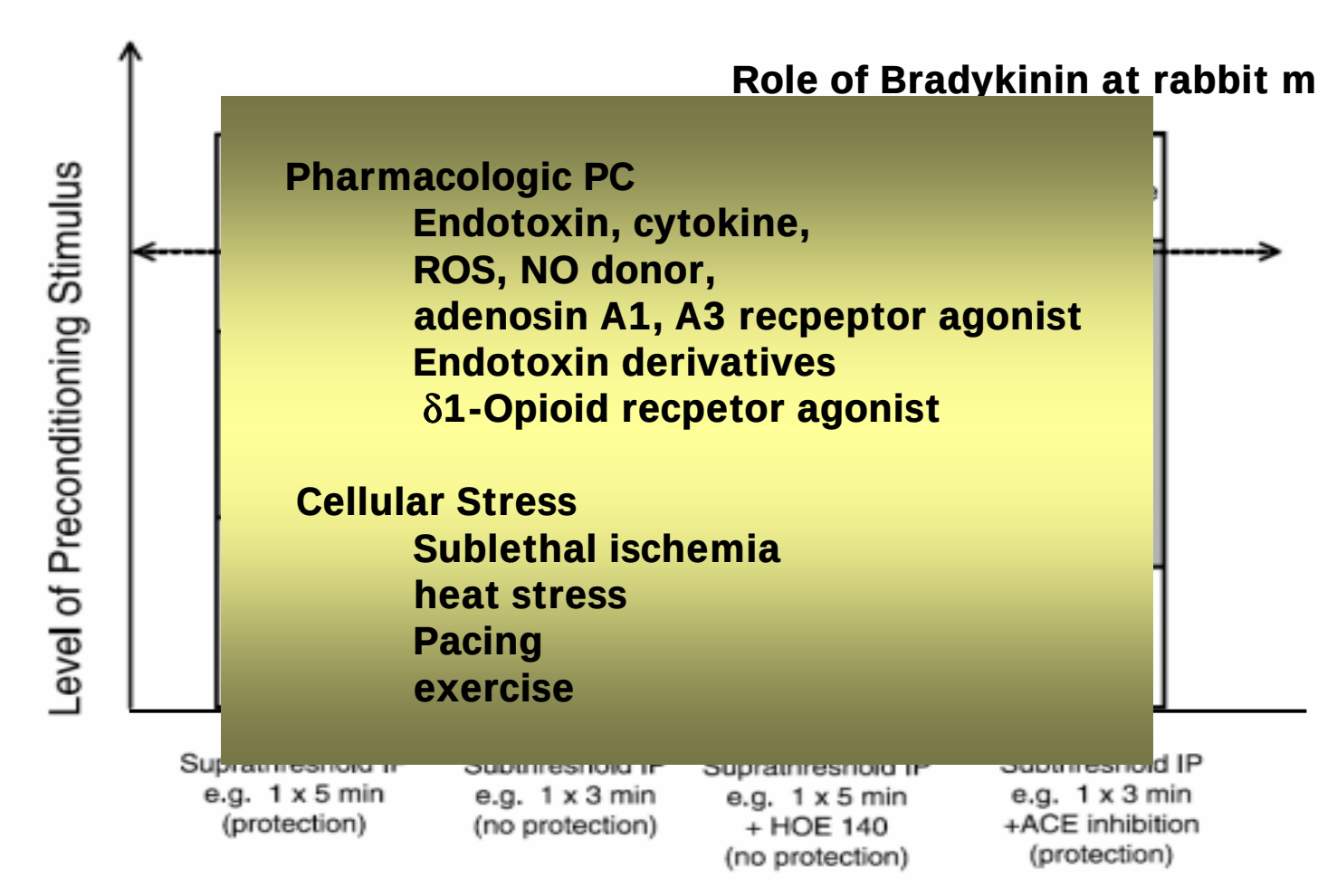


Bax pore model



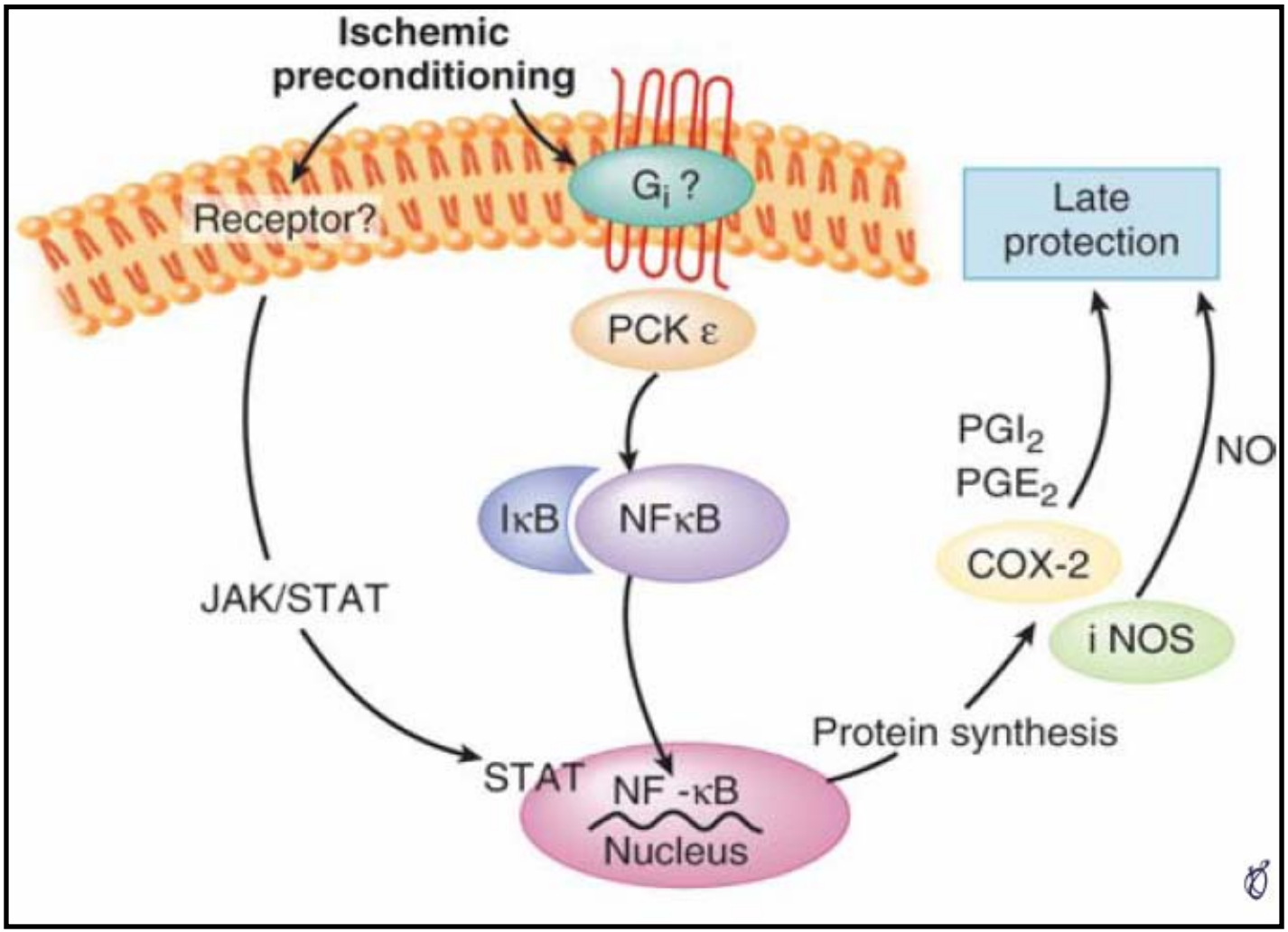
Crow MT et al. Cir Res**. 2004;95:957-970**

Trigger Mechanism of Early IPC

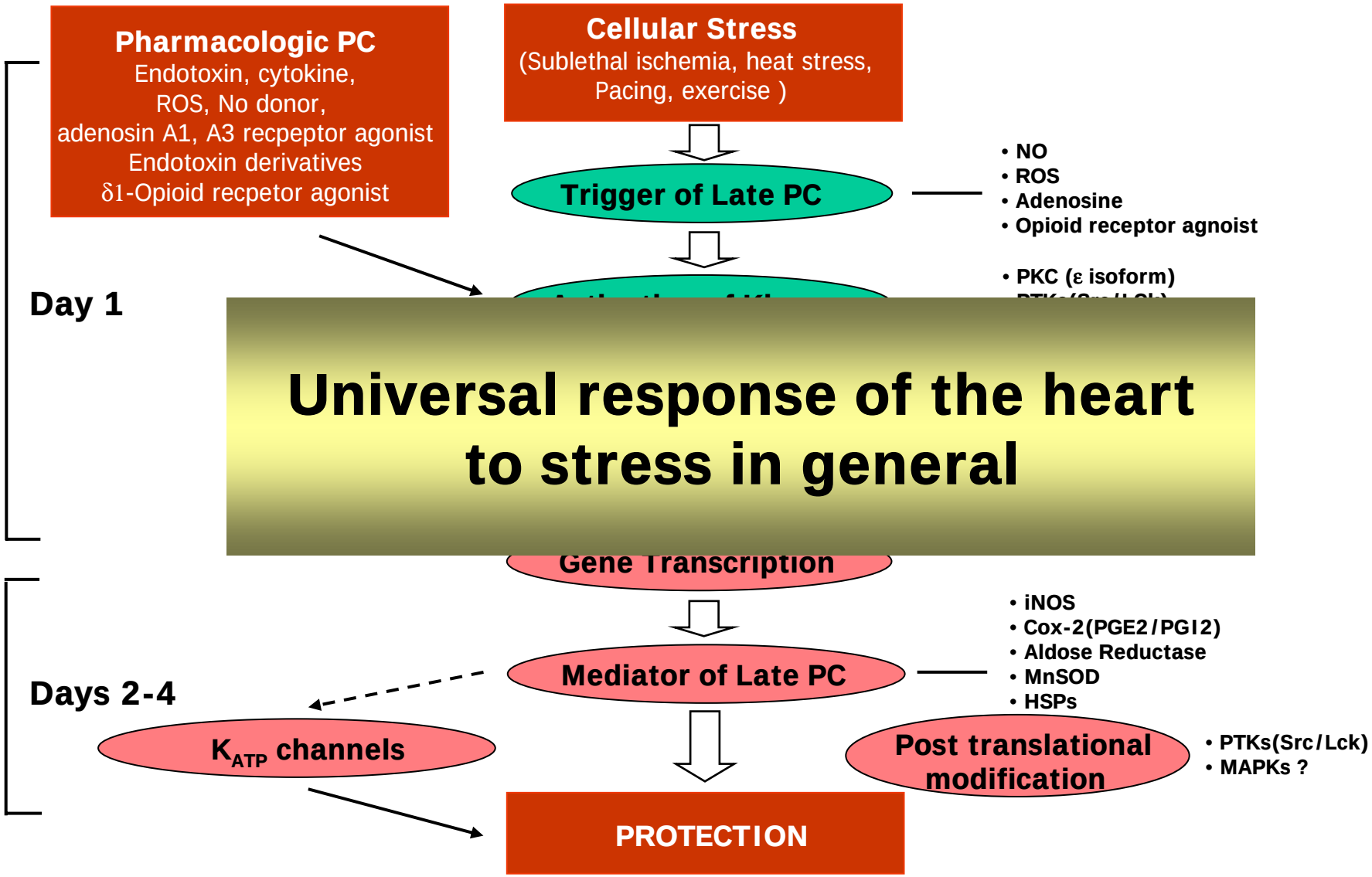


(Goto et al *Circ Res. 1995*:77:611-621)

Mechanism of Late IPC



Cellular Mechanism of Late Ischemic Preconditioning



Hibernation and Stunning

- **Hibernation :** metabolic adaptation

  first described by Rahimtoola

  Loss of contractile fx to signaling by inflammatory-like process

- **Stunning** (prolonged post-ischemic ventricular dysfunction)

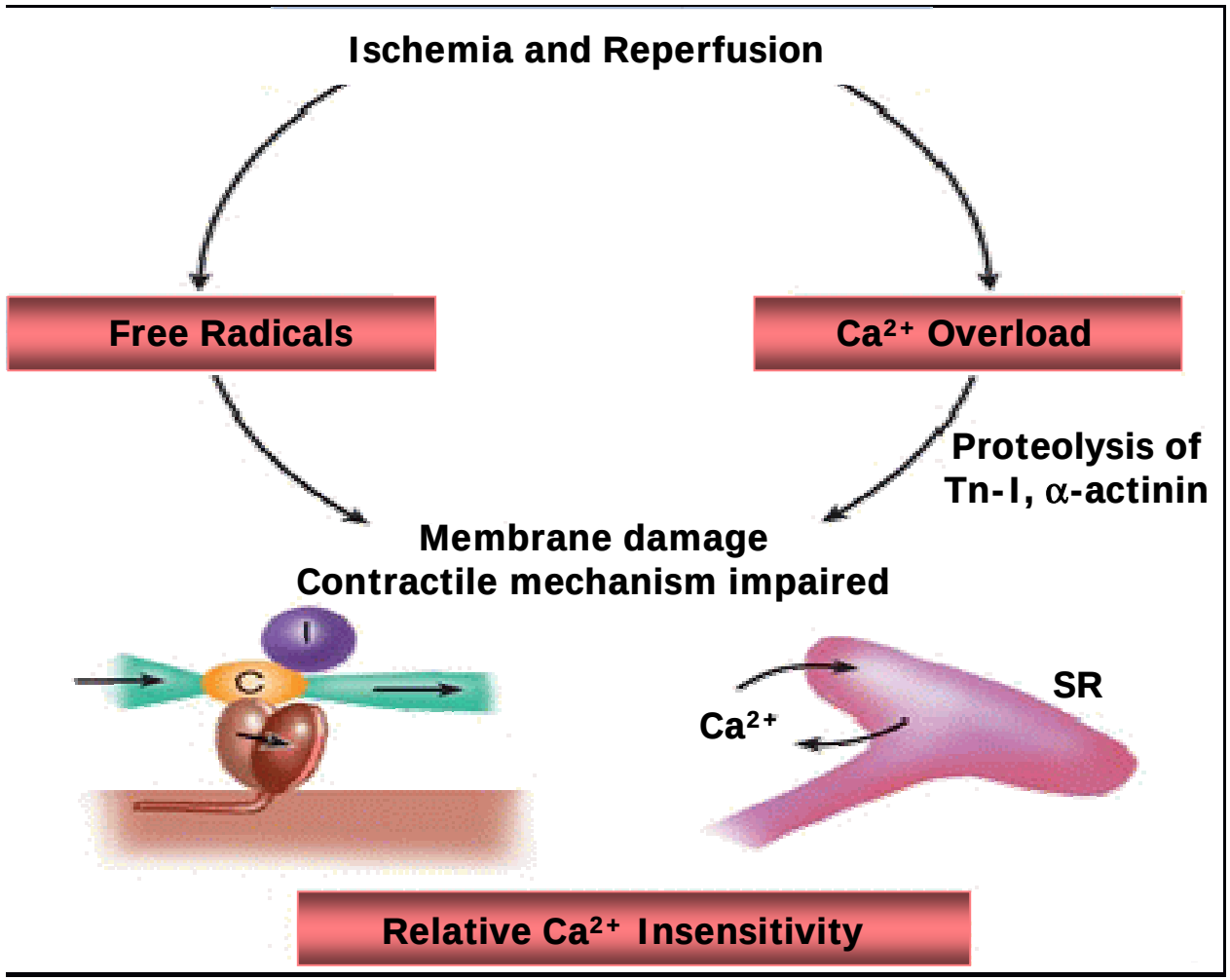
  defined by Braunwald and Kloner

  Oxygen free radicals (oxyradical hypothesis)

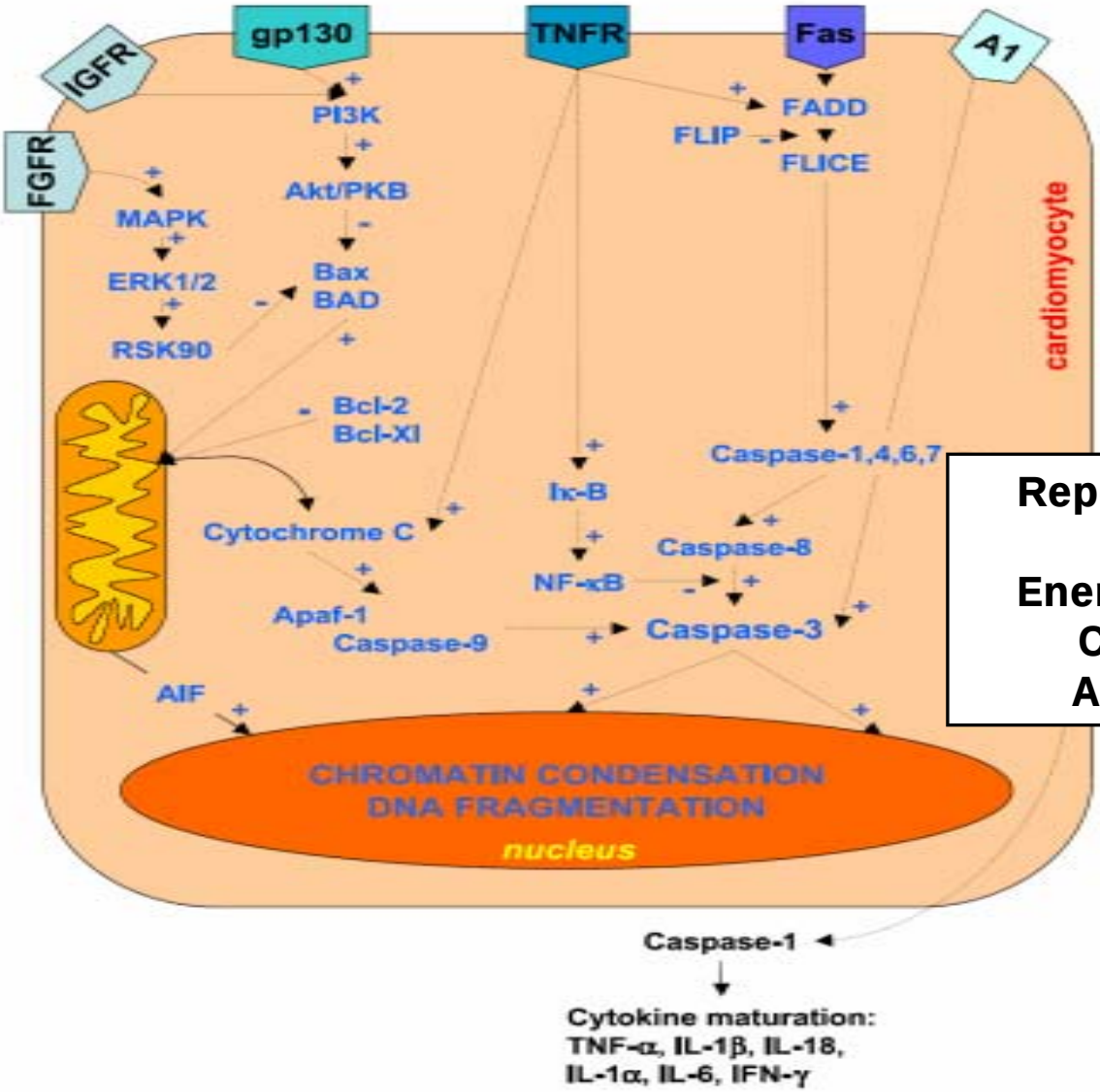
  Cytosolic calcium overload (calcium hypothesis)

- **Ischemia-reperfusion Injury**

Mechanism of Ischemic Reperfusion Injury



Apoptosis: Ischemic Reperfusion Injury



Reperfusion: oxygen, glucose

Energy required
- **Completion of apoptosis**
- **Acceleration of apoptosis**

- **Cardioprotection**
  from severe ischemia is possible

- **Adaptive behavior of the Cell**
  in protecting itself from ischemia

  - not only cardiomyocytes -

Gaps in Knowledge That Hinder Translation

- ? Reproducibly effective in clinical relervant setting
  DM, HT, Hypercholesterolemia, LVH, old age
- Uncertainty regarding the magnitude of reperfusion injury
- Reliablity of methods to measure infarct-size
- Relevant model of sudden cardiac death
- Lack of appropraite biosensors in the setting of ischemia and cardiac arrest

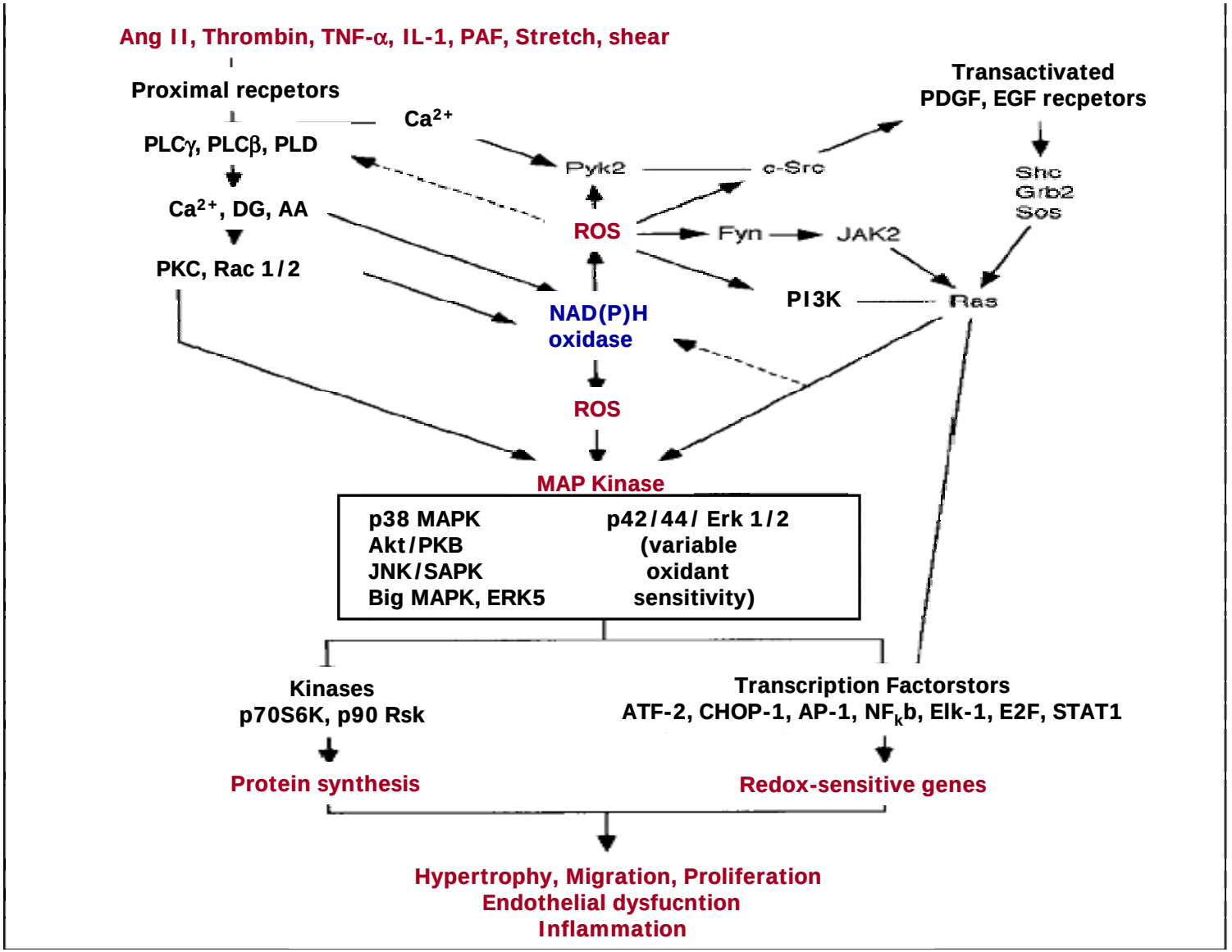
Opportunities

- Infract size reduction is feasible
- Preconditioning
- Reliability of methods to measure infarct-size
- Progress in unraveling the mechanism
  of ischemia/reperfusion injury and protection
- Encouraging clinical data
  GIK, GUARDIAN, EXPEDITION

Regulation of Oxidative Stress: Role of ROS

ROS molecule	Main Sources	Enzymatic defense system	Products
Superoxide ($O_2^{\cdot -}$)	'Leakage' of electron from the electron transport chain	Superoxide dismutase (SOD)	$H_2O_2 + O_2$
		Superoxide reductase	H_2O_2
	Activated phagocytes Xanthin oxidase Flavoenzyme		
Hydrogen peroxide (H_2O_2)	From $O_2^{\cdot -}$ via SOD	Glutathion peroxidase	$H_2O + GSSG$
	NADPH-oxidase Glucose oxidase Xanthin oxidase	Catalsae	$H_2O + O_2$
		Peroxiredoxin (Prx)	H_2O
Hydroxyl radical ($^{\cdot}OH$)	From $O_2^{\cdot -}$ and H_2O_2 via transition metals (Fe,Cu)		
Nitirc Oxide(NO)	Nitirc oxide synthases	Glutathion/TrxR	GSNO

Redox Sensitive Signaling Pathway in Vascular Cell



Redox Sensitivity of Gene Expression in Cardiovascular Cells

Gene	Cell Type	Stimulus
VCAM-1	Endothelial cells	TNF-α, IL-1α, IL-1β, IL-4
ICAM-1	Endothelial cells	TNF-α, NO, lactosylceramide
E-selectin	Endothelial cells	IL-1α, LPS, PMA, TNF-α
MCP-1	Mesangial cells	TNF-α
	VSMCs	PDGF
	VSMCs	Ang II
	VSMCs	TNF-α
MCSF	Endothelial cells	TNF-α, ox-LDL
	Endothelial cells	H_2O_2, TNF-α
	Mesangial cells	TNF-α
eNOS	Endothelial cells	Xanthine/xanthine oxidase
iNOS	Mesangial cells	IL-1β
Cu/Zn-SOD	Endothelial cells	H_2O_2
Catalase	Endothelial cells	H_2O_2
Glutathione peroxidase	Endothelial cells	H_2O_2
Mn-SOD	Endothelial cells	Thioredoxin
HO-1	Endothelial cells	H_2O_2, shear stress
	Macrophages	Ox-LDL
	VSMCs	PDTC
COX-2	Mesangial cells	IL-1β
	VSMCs	Catalase overexpression
HSP-70	Endothelial cells	H_2O_2
		Xanthine/xanthine oxidase
Scavenger receptor	VSMCs	PMA, H_2O_2/vanadate
	Macrophages	
IL-8	Microvascular endothelial cells	H_2O_2
HB-EGF	Endothelial cells	H_2O_2
	VSMCs	Methylglyoxal
Atrial natriuretic factor	Cardiac myocytes	Ouabain
VEGF	Endothelial cells	H_2O_2
	VSMCs	H_2O_2, 4-hydroxynonenal

(Griendling KK et al ATVB. 2000;20:2175-2183)

Targets for gene-based therapy for myocardial protection and rescue from ischemia induced Injury

Strategy/Therapeutic target		Genetic Manipulation	Vector	Application
Protection/Prevention				
Anti-oxidant genes	HO-1, SOD, Catalase, GPX	Overexpression	ADV, AAV, LV, α-virus	CAD, ACS, I/R injury
Heat shock proteins	HSP70, HSP90,HSP27	Overexpression	ADV, AAV, LV, α-virus	CAD, ACS, I/R injury
Survival genes	Bcl-2, Akt, HGF	Overexpression	ADV, AAV, LV, α-virus	CAD, ACS, I/R, HF
Inflammatory cytokines Adhesion molecules Tissue Factors	ICAM,VCAM,TNF-α,NF-$_K$B	Inhibition	AS-ODN, Decoy ODN, ADV-AS-ODN, RV-AS-ODN	MI, I/R injury, Graft atherosclerosis, Transplantation
Proapoptotic genes	Bad, P53, Fas ligand	Inhibition	AS-ODN, Decoy ODN, ADV-AS-ODN	CAD, ACS, I/R, HF
Coronary vessel tone	e-NOS, adenosin receptors	Overexpression	RV, ADV, AAV(?)	CAD, I/R injury, HF
Rescue				
Proangiogenic gene	$VEGF_{121}$, $VGEF_{165}$, FGF-1, FGF-2, FGF-4, FGF-5, HGF, eNOS Ang-1, MCP-1, G-CSF, PDGF-BB, IGF-1, IGF-2, HIF-1α	Overexpression	Plasmid, ADV, AAV, LV (?)	CAD, MI, HF

(Melo LG et al Circulation. 2004;109:2386 -2393)

Therapeutic Strategy For Ischemic Protection

- **Ischemic Protection by Gene Therapy**

  Gene therapy: VEGF를 이용한 ischemic limb에서만 임상 효과가 보고.

  Heart Failure : target gene (3 biologic different pathway)

  ① Ca^{2+} homeostasis: Sarcoplasimic reticulum Ca^{2+}- ATPase (SERCA 2A)

  ② β-adrenergic receptor: β-adrenergic receptor kinase 1 (β-ARK1)

  ③ Reducing apoptosis : Bax, Bcl-2, Akt, p53 etc

  허혈성 손상 및 이에 따른 2차 신호전달 기전을 통해 발현되는 심근보호 유전자를 고려하면, 상기 3가지는 그대로 적용할 수 뿐 아니라 혈관내피세포, 혈관 평활근 및 심근세포에서 발현되는 Redox sensitive gene도 적용할 수 있겠다.

Target gene of Heart Failure

- Ca^{2+}homeostasis: Sarcoplasimic reticulum Ca^{2+}-ATPase (SERCA 2a)

  SERCA (115 kDa) : ↑ Ca^{2+} uptake to SR, ↓ Cytosolic Ca^{2+}

  Reduced expression or activity of SERCA 2a at failing heart

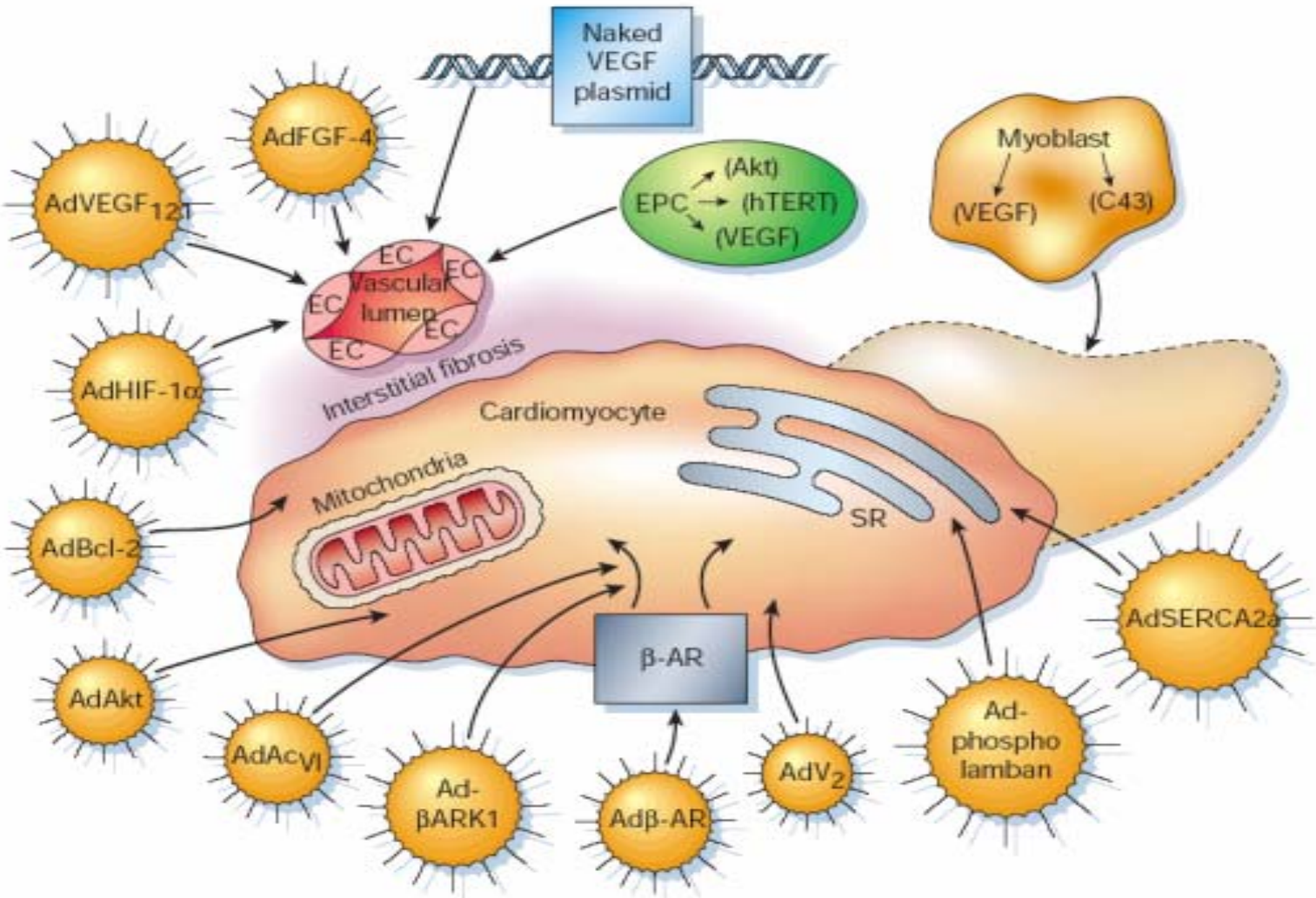
  Phospholamban: endogenous inhibitor of SERCA 2a

- β-adrenergic receptor: β-adrenergic receptor kinase 1 (β-ARK1)

  Down regulation of β-AR, Up regulation of β-AR Kinase at CHF

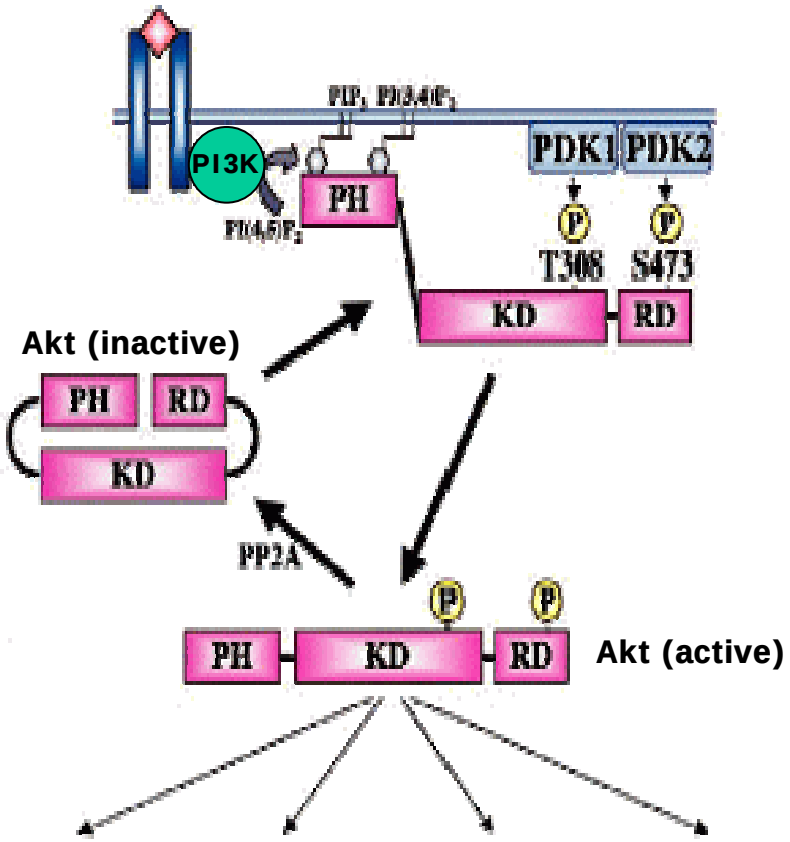
- Reducing apoptosis : Bax, Bcl-2, Akt, p53

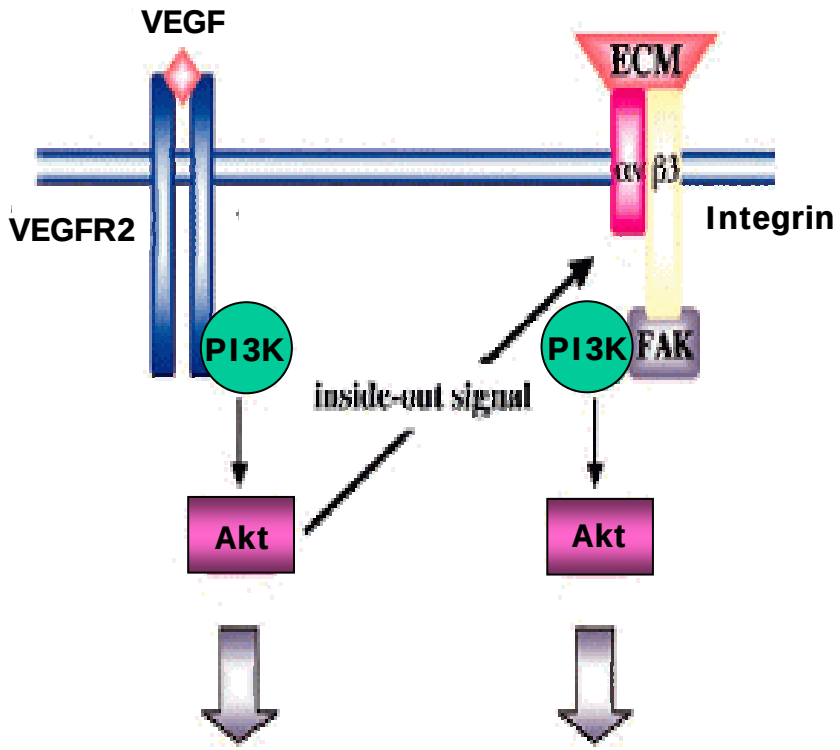
Myocardial Gene Therapy



Isner JM. ***Nature*. 2002;415:234-239**

Role of Akt Signaling in Vascular Homeogenesis





Cell survival

Bad
Forkhead
IKKα
FLIP

Cell cycle

E2F
P21
MDM2
hTERT

Glucose metabolism

GSK3
GLUT4

Protein synthesis

mTOR
S6K1
4E-BP1

Growth factor-dependent survival pathway

Attachment-dependent survival pathway

(Shiroma et al *Cir res.* 2002:90:1234-1250)

Regenerative Medicine

Functional loss = disease

Organ damage

⇩

Medical approach
- drug

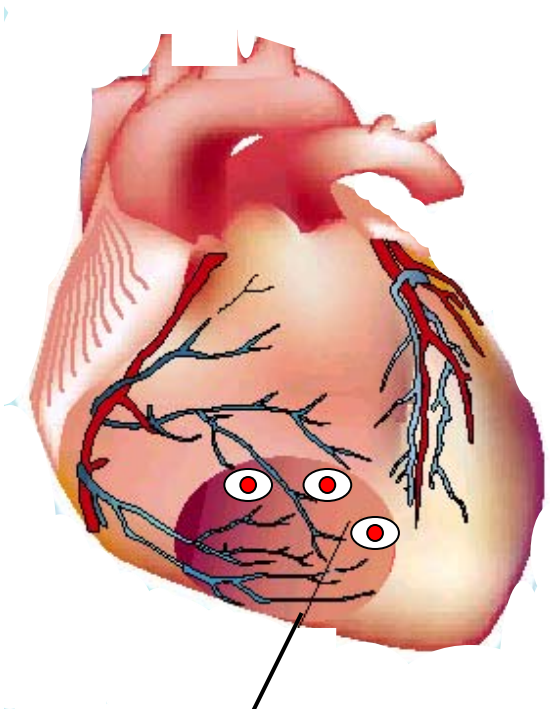
Intervention, Surgery

Organ Transplantation

⇩

Regeneration of Organ

Cell Therapy: stem cell



Ischemic heart disease

Major Advances in Real World of Cardiology

[1] **Intervention :** Drug-eluting stent (DES)

[2] **Cell (Stem cell) therapy in human trial**

TACT study (2001), BM-derived MNCs local injection

TOPCARE- AMI, BM-derived MNCs intracoronary injection (2002)

TOPCARE- CHF

MAGIC- cell (2003)

BOOST trial (2004)

AC 133(+) cell intracoronary injection (2003)

Skletal myoblast transendocardial injection

transvenous myocardial injection

EPC Ab coated stent: AC 133(+) (2003)

More Question than Answer

Which patient should be considered for cell therapy ?

Which type of stem cell should be used ?

Which quantity and concentration should be used ?

By what mechanism do stem cell engraft, survive, differentiate ?

? Improvement : active (by increasing contractility)

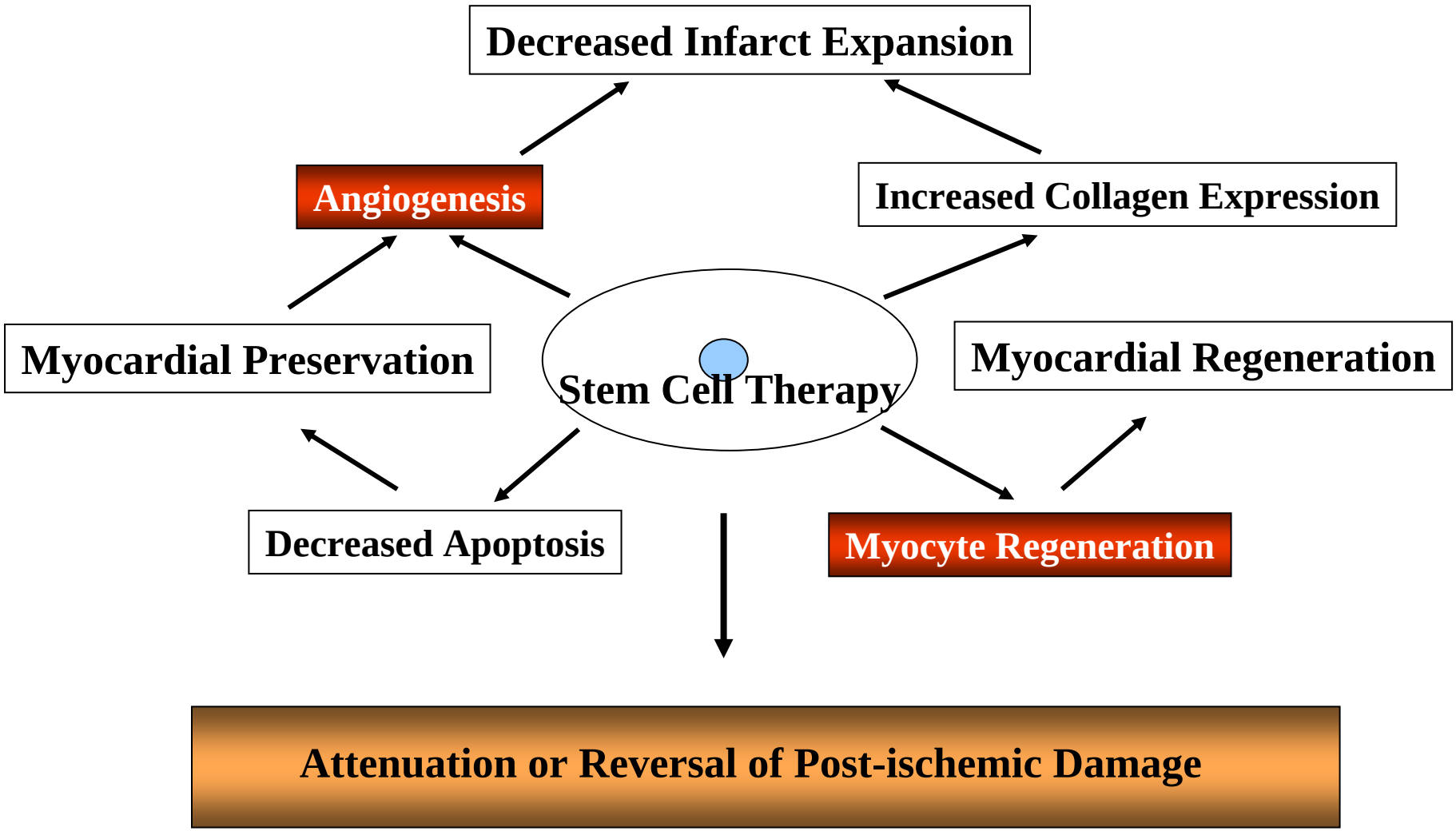
passive (by limiting infarction and remodeling)

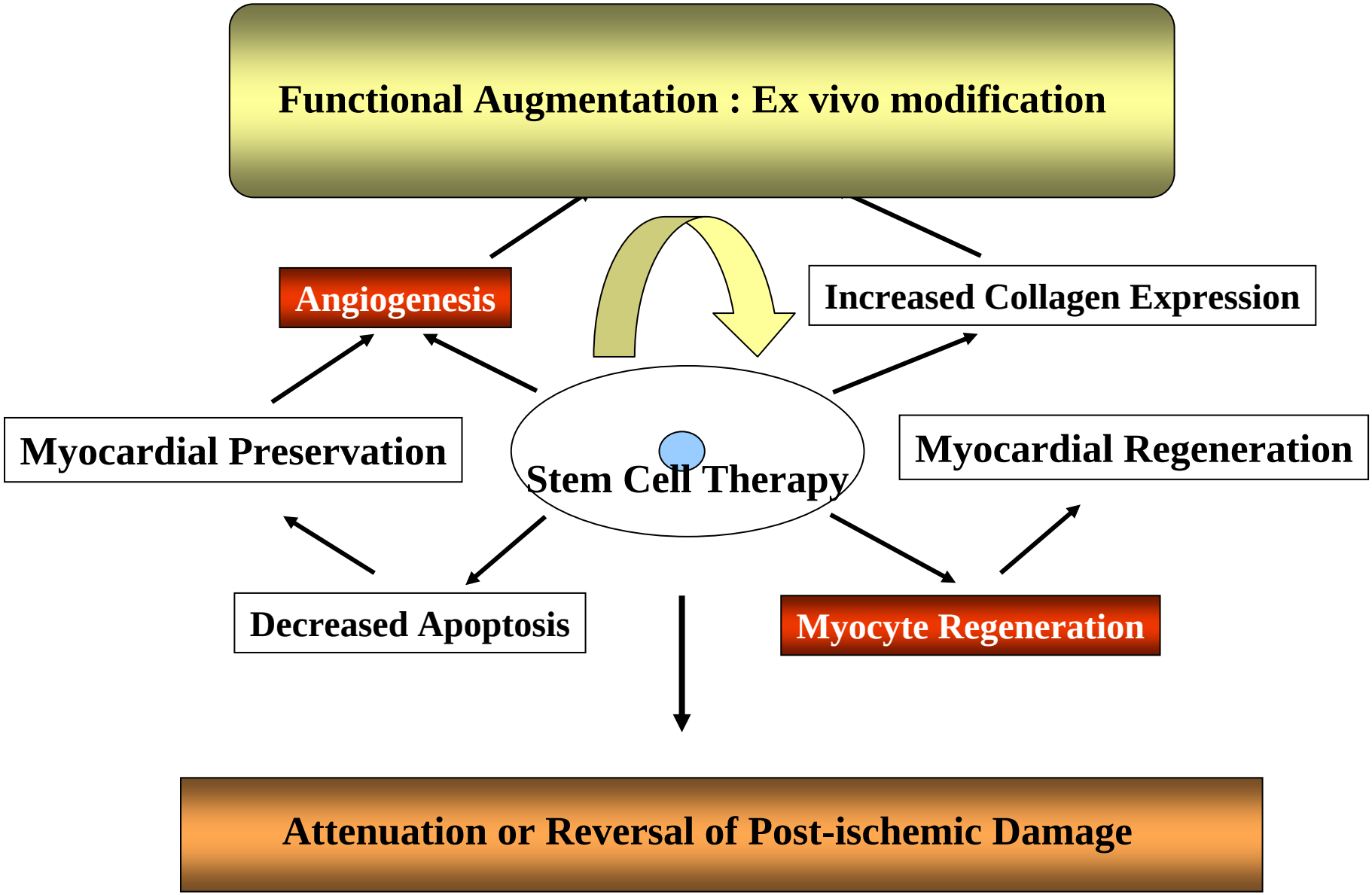
? Life span of transplanted stem cell

? Safe (long-term safety)

? Potential tumorigenesis

? Potential benefit in non-ischemic heart failure

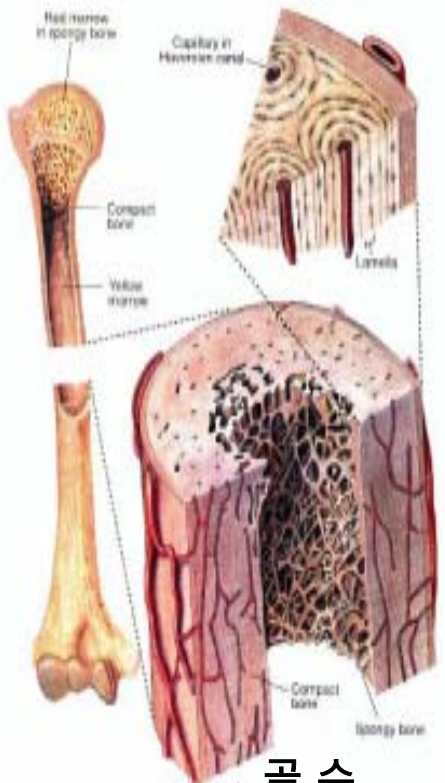

Decreased Infarct Expansion
Angiogenesis
Increased Collagen Expression
Myocardial Preservation
Stem Cell Therapy
Myocardial Regeneration
Decreased Apoptosis
Myocyte Regeneration
Attenuation or Reversal of Post-ischemic Damage


Functional Augmentation : Ex vivo modification
Angiogenesis
Increased Collagen Expression
Myocardial Preservation
Stem Cell Therapy
Myocardial Regeneration
Decreased Apoptosis
Myocyte Regeneration
Attenuation or Reversal of Post-ischemic Damage

Future Perspectives

Genetic Modification

Adv-GSK , Ex Vivo modification



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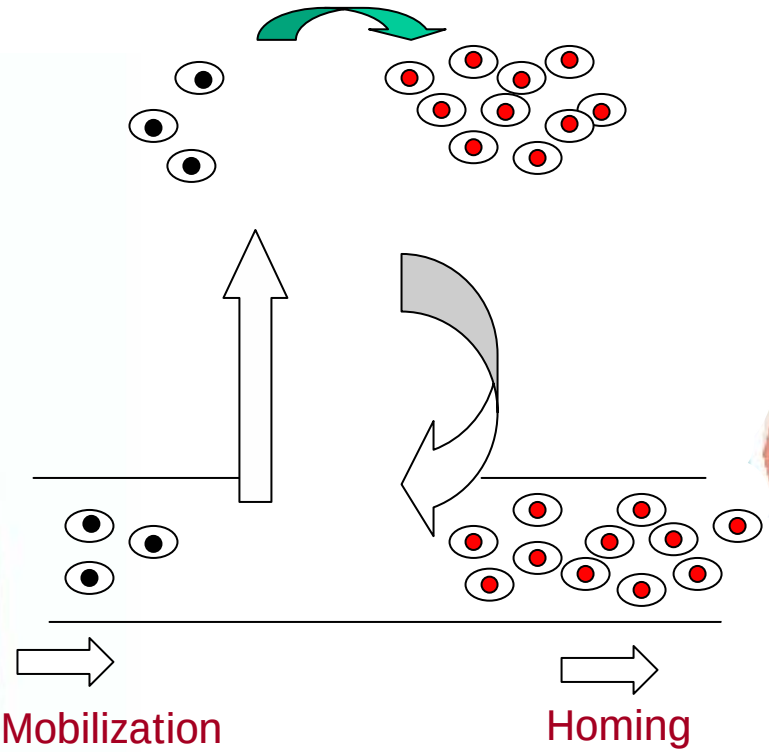
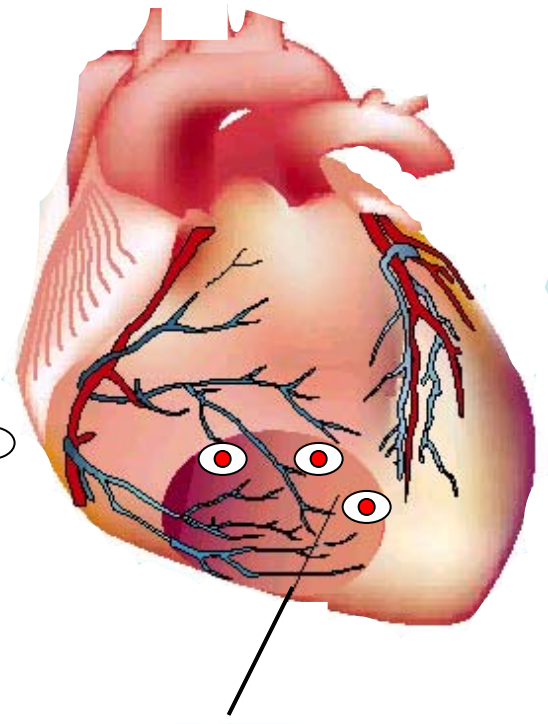
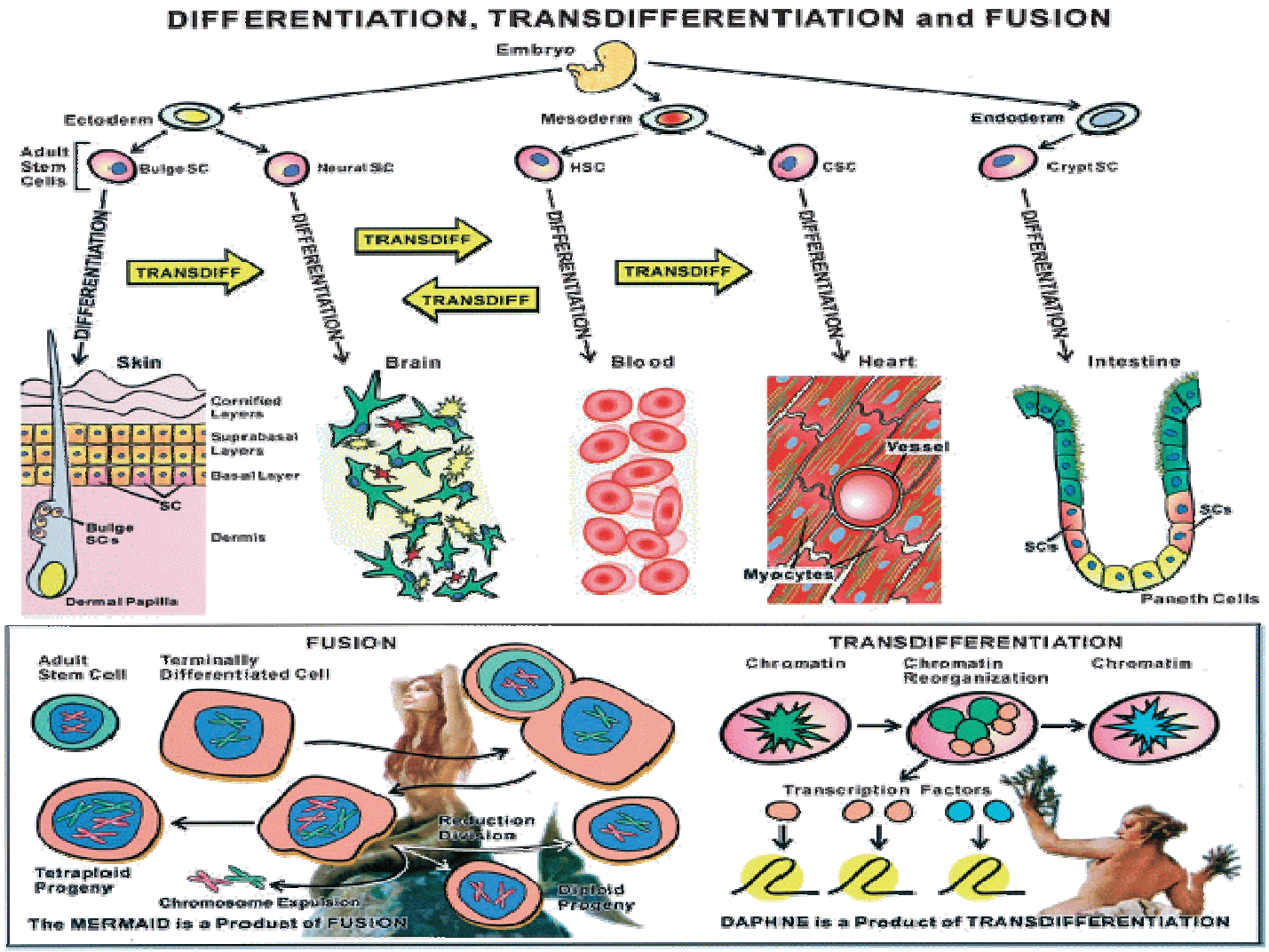




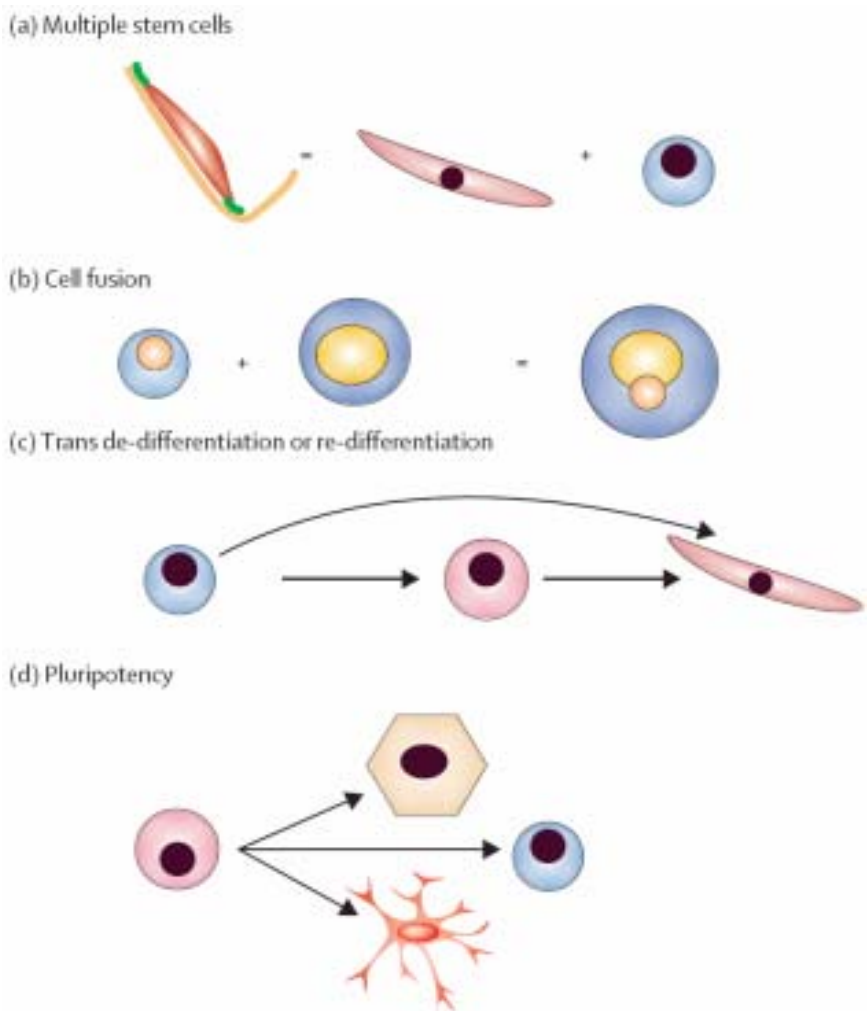
허혈 심근

Plasticity of Adult Stem Cell



(Anversa et al *Circulation* 2004:109:2832-2838)

? Possible Mechanism of the Plasticity



① 여러 종류의 조직-특이 줄기세포가
서로 다른 장기에도 존재한다.

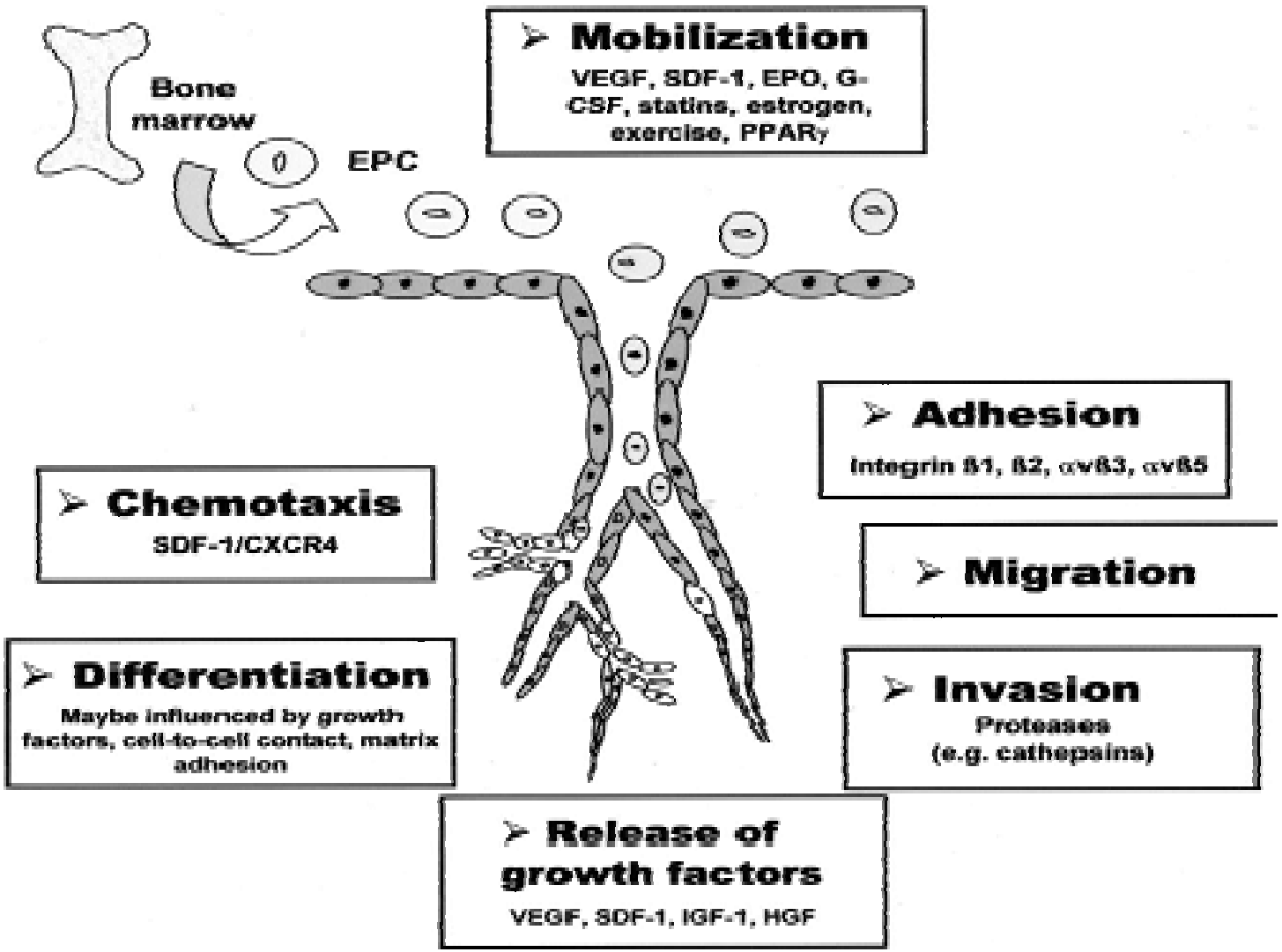
② 융합 가능성 **(fusion):(4n: 2n+2n)**
기존 세포와 주입된 줄기세포의 융합

③ 세포의 탈분화 및 재분화 과정

④ **True pleuripotent or multipotent** 줄기세포가 출생 후에도 지속적으로 존재한다.

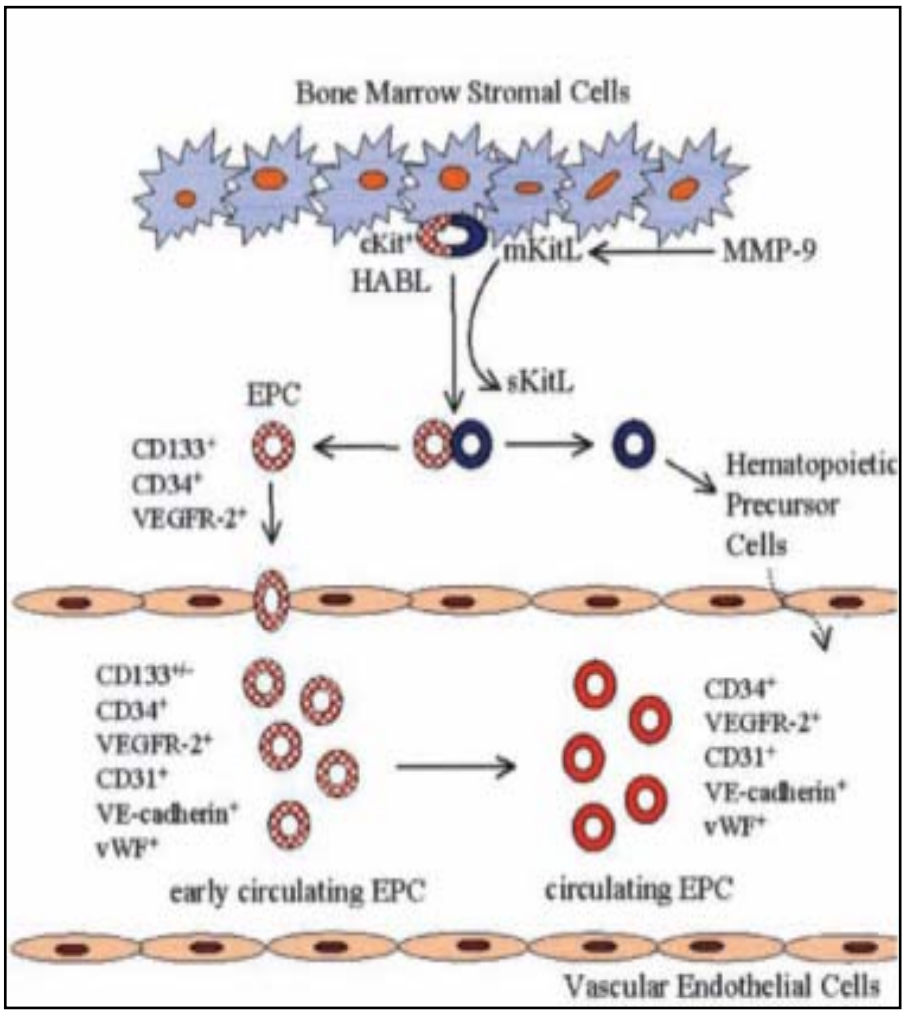
(Mathur et al *Lancet* 2004:364:183-192)

Homing and Differentiation of EPC



(Urbich and Dimmeler et al, *Circ Res*. 2004;95:343-353.)

Mobilization of EPC from Bone Marrow



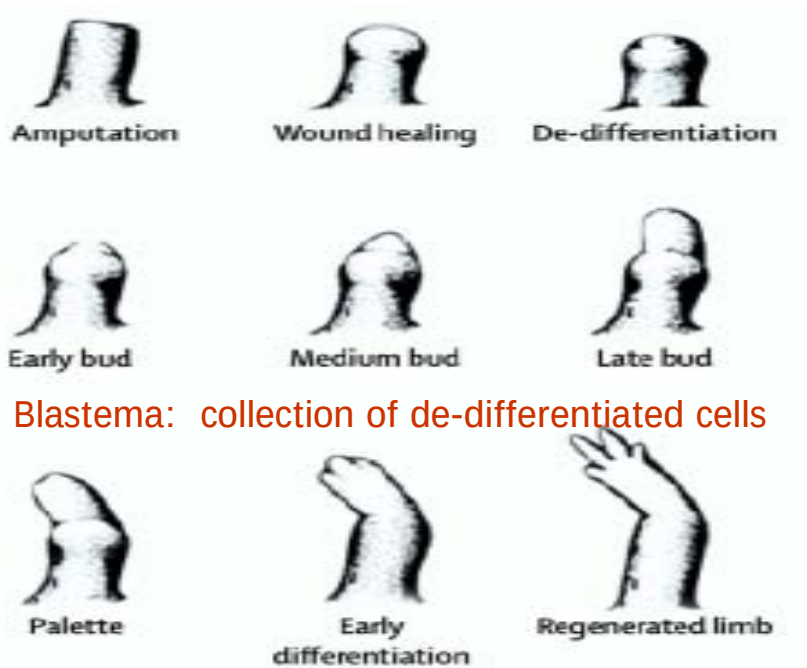
- Activation of (MMP-9)
- mKitL to a soluble Kit ligand (sKitL)
- cKit-(+) stem and progenitor cells (hemangioblast, HABL), move to the vascular zone of BM
- from a quiescent to a proliferative state
- Early EPCs: CD133/CD34/VEGFR-2.
- Circulating EPCs: CD34/VEGFR-2/CD31/VE-cadherin/vWF

(Hristov et al, *Aterioscleroo Thrombo Vasc Biol*. 2003;23

Candidates for Mobilization

- SCF, c-kit and MMP-9
  - Role for stem cell mobilization after MI
- SDF-1 and CXCR-4
  - Migration of CD 34+ cells
- G-CSF
  - Via SDF-1 and CXCR-4
- VEGF and Flk-1
  - Angiogenesis

Myocardial Regeneration



Regeneration in the brain, spinal cord, intestine, heart, limb, lens & retina.

Blastema: collectection of dedifferentaisted cells at injury site
phosphorylation of the proteins of retinoblastoma tumor suppress gene

Limited supply of diagnostic antibody

(Mathur et al *Lancet* 2004:364:183-192)

Natural Repair of the Heart

- **Traditional concept of the cardiomyocytes (CMCs)**

  terminally differentiated cells

  number of CMCs at birth only decrease with age

  no house-keeping mechanism to repair any damage

  only Hypertrophy rather than hyperplasia

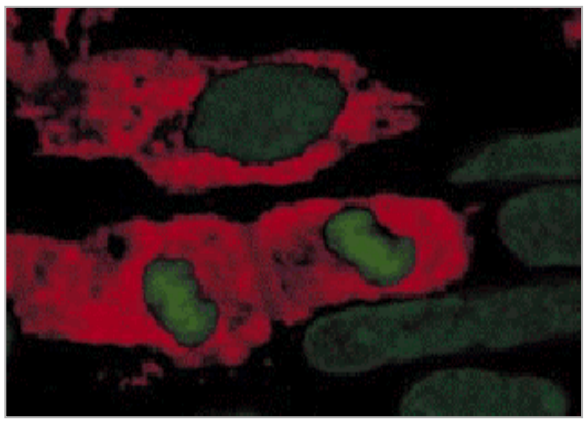
- **Improved LV function after MI**

  Process of remodeling that

  combination of hypertrophy and fibrosis

Myocardial Regeneration

- Human Cardiomyocytes divide after MI. Beltrami et al. NEJM 2001

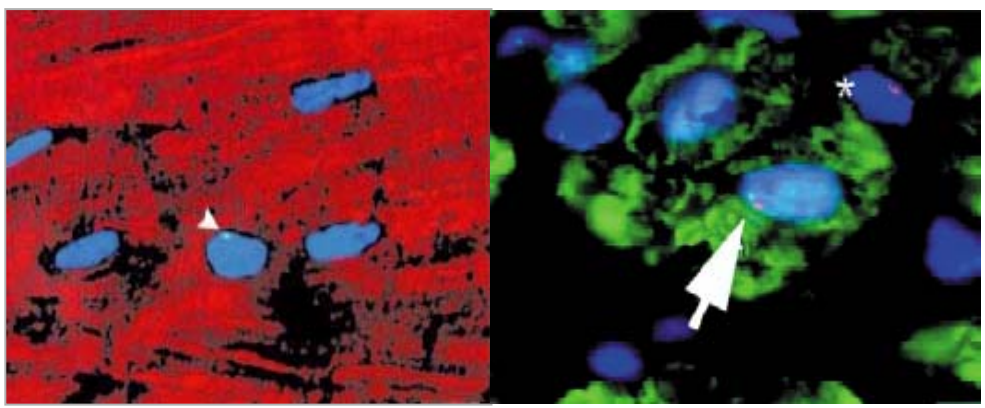


10–60 fold increase in mitotic figures was recorded in patients dying from heart failure,

The mitotic proportion was low, 0·015-0·08%

? Act as an effective repair mechanism.
? The source of dividing Cell: unclear

- Chimerism of Transplanted Heart Quaini et al. NEJM 2002



Derived form Extra-cardiac Origin

2 potential sources
Bone-marrow
Residual cardaic stem cell

Male recipient + female donor Heart

Candidate Cells of Myocardial Regeneration

- Autologous cells
  - Differentiated cells
    - – Skeletal muscle cells
    - – Cardiomyocytes
    - – Fibroblasts...
  - **Endothelial progenitor cells**
  - **Skeletal myoblasts**
  - **Stem cells**
    - **– BM hematopoietic or mesenchymal stem cells**
    - **– Peripheral blood stem cells**
- Allogenic cells
  - Stem cells from umbilical cord blood
  - Embryonic stem cells

Questions ?

- **? Possible origin of proliferating cells**
- **? Clinical Significance**
- **? Safety**

  Functional and electrical integration

  : hypocontractile and proarrhythmic consequences

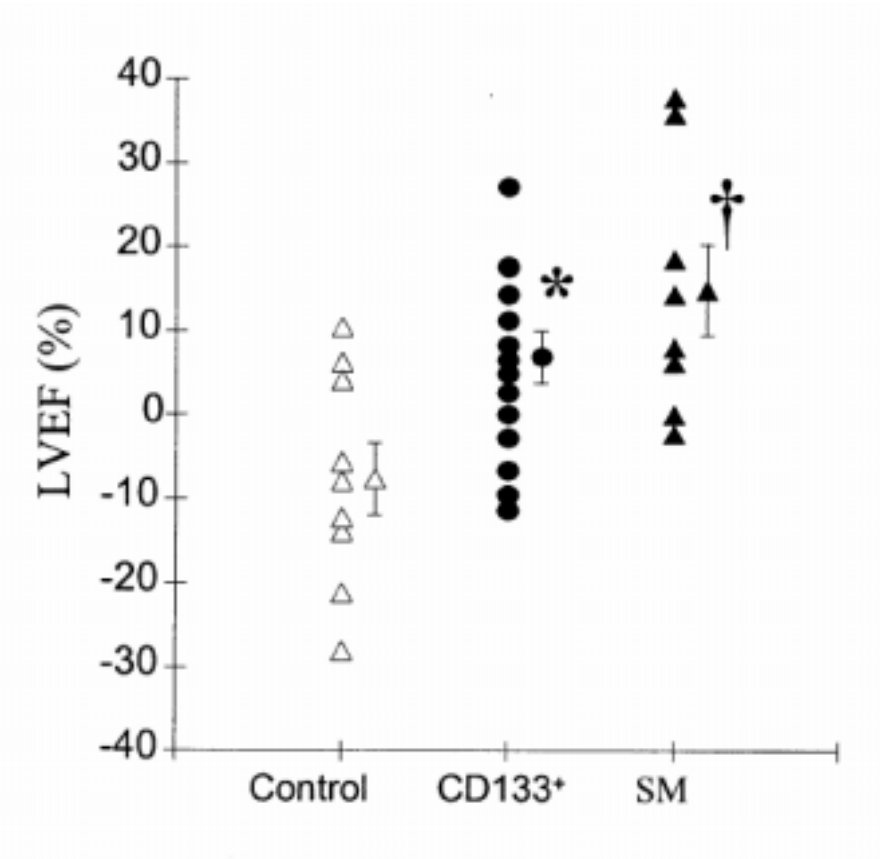
  implanted stem cell

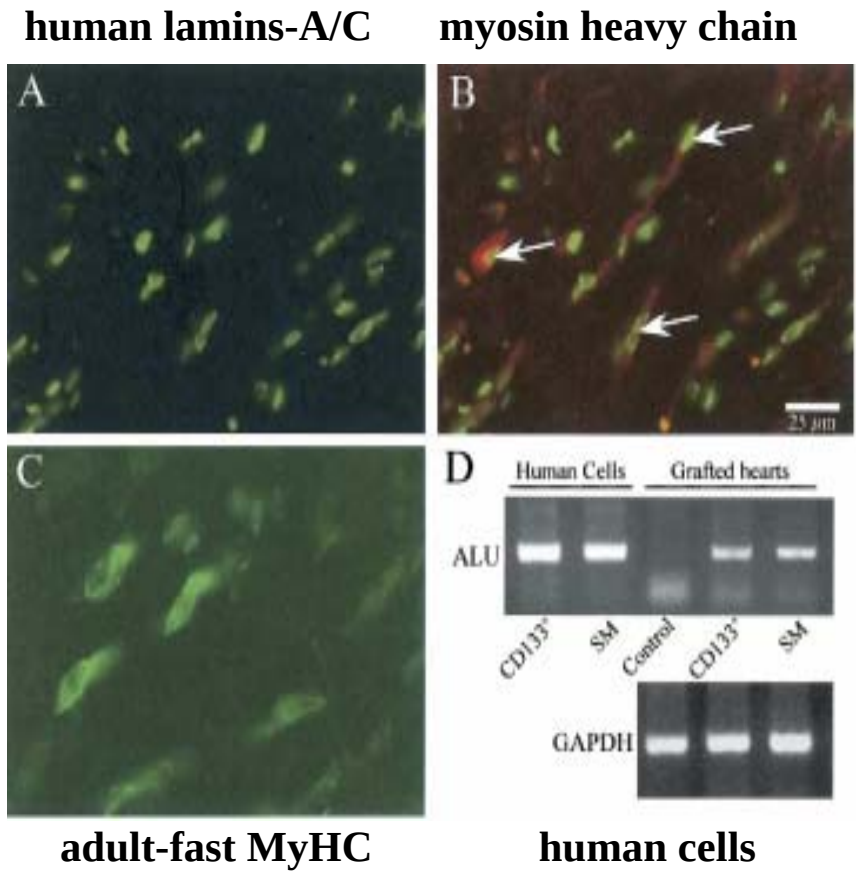
  ? differentiated to fibroblast: increased scar

  ? differentiated to myocyte

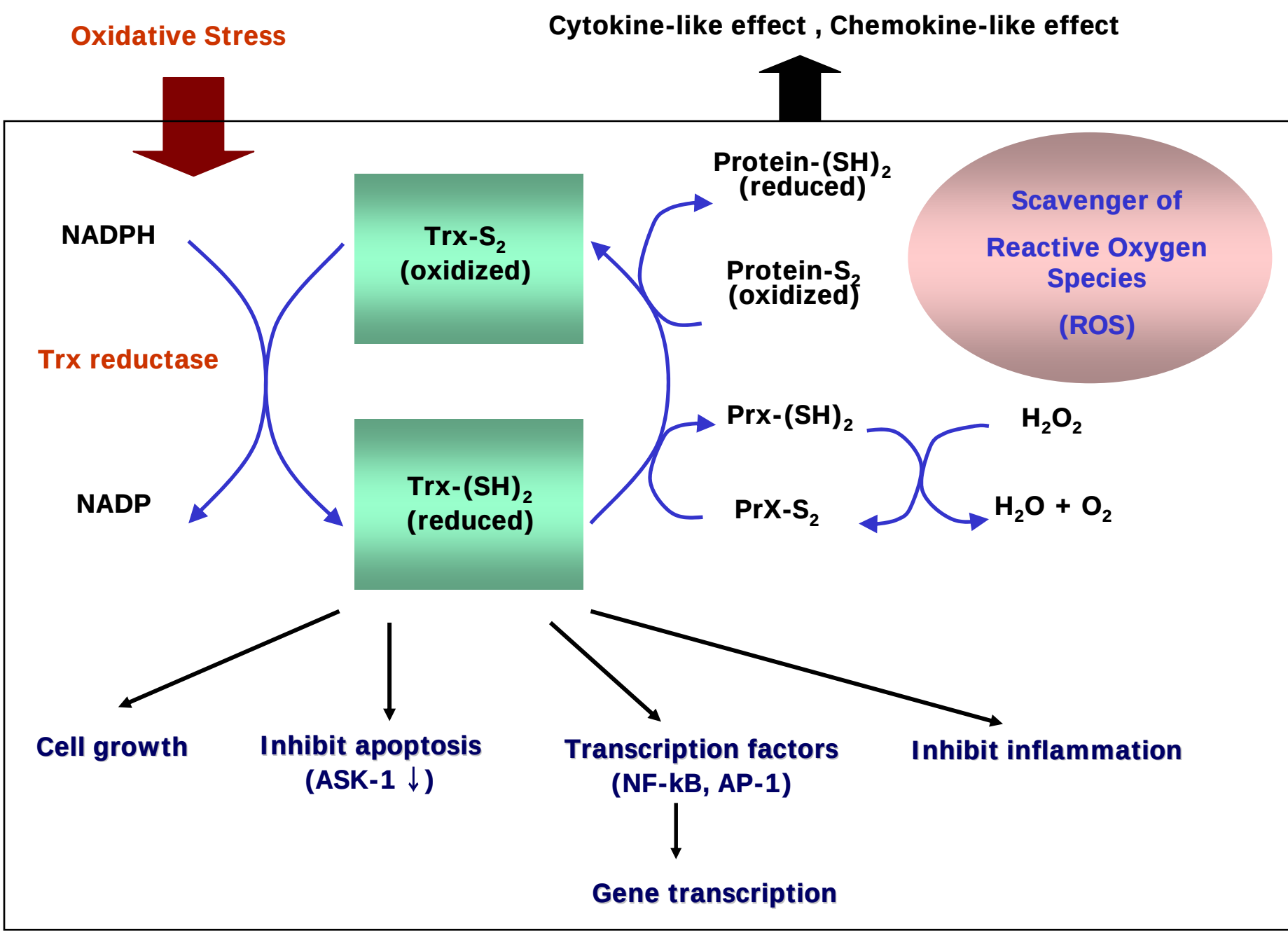
  discordance b/w structural contractile property

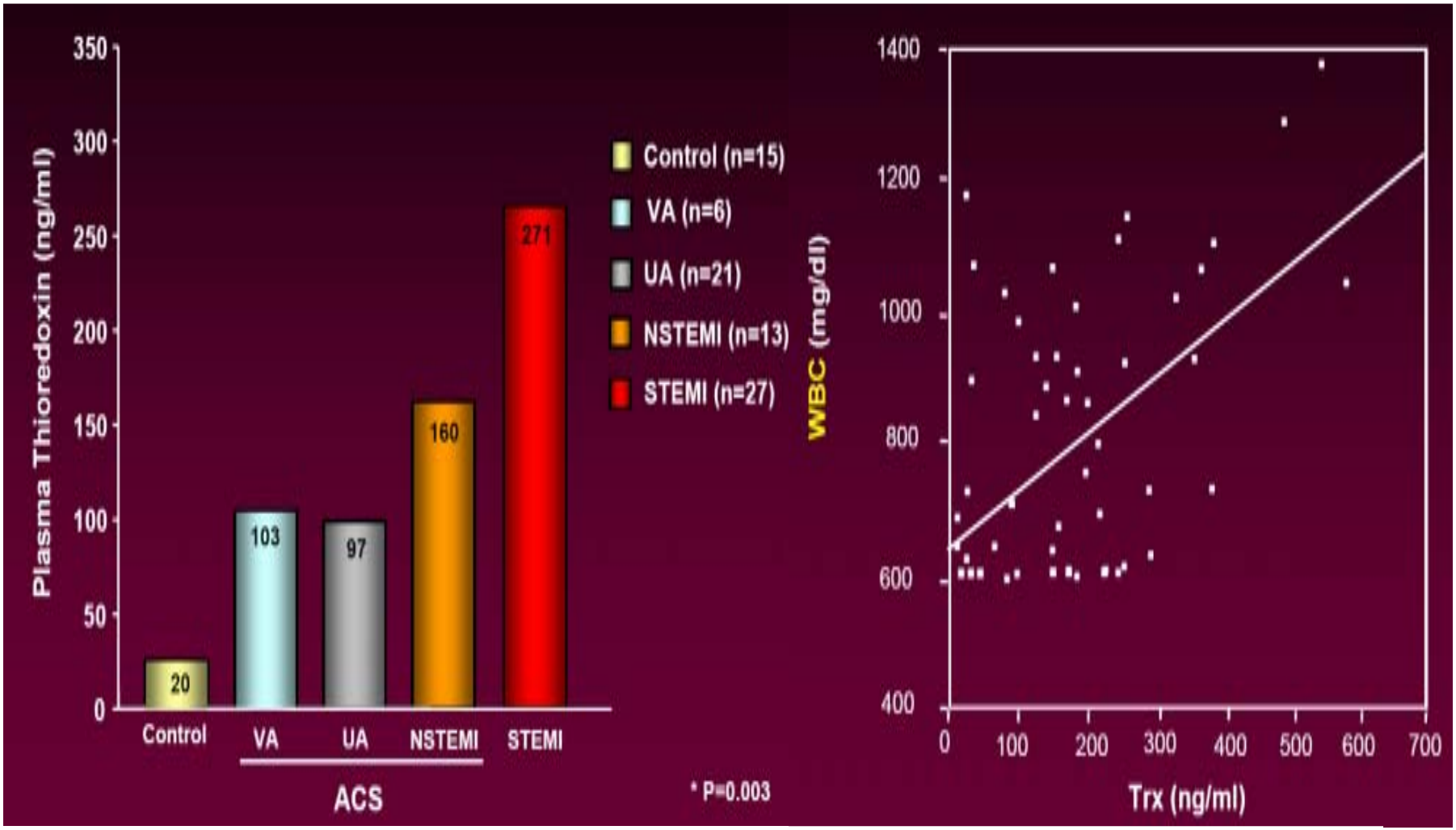
Human Skeletal Myoblasts and BM-derived CD133 Progenitors for the Repair of Infarcted Myocardium





Agbulut O et al *J Am Coll Cardiol* 2004;44:458–63


Oxidative Stress
Cytokine-like effect , Chemokine-like effect
NADPH
Trx reductase
NADP
Trx-S2 (oxidized)
Trx-(SH)2 (reduced)
Protein-(SH)2 (reduced)
Protein-S2 (oxidized)
Prx-(SH)2
PrX-S2
H2O2
H2O + O2
Scavenger of Reactive Oxygen Species (ROS)
Cell growth
Inhibit apoptosis (ASK-1 ↓)
Transcription factors (NF-kB, AP-1)
Gene transcription
Inhibit inflammation



급성 관동맥 증후군 환자 (N=67)에서의 혈장 Trx의 농도는 질환의 중증도와 관련이 있고,

심근경색의 크기 및 초기 백혈구 수치와도 유의한 상관 관계가 있다

§ 3 types of MI model

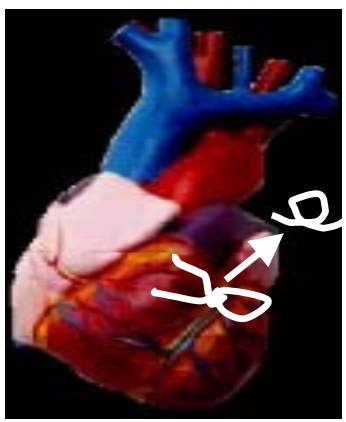
Coronary artery (LAD) ligation in Sprague-Dawley rats (N=71, n=3 at each time point)

- Sequential analysis: Control (normal), 0 hr, 30 min, 1hr, 2hr, 4hr, 12hr, 24hr, 48hr

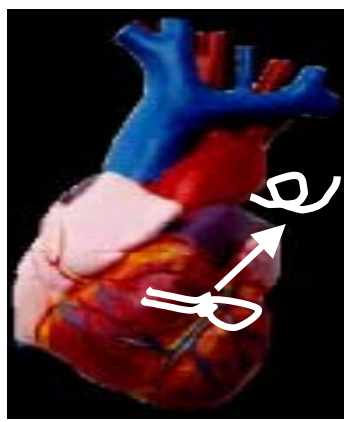
Non-reperfused transmural MI
- MI -

Early (45 min)-reperfused nontransmural MI
- EMI -

Late (5 hrs)-reperfused transmural MI
- LMI -



after 45 min



After 5 hrs

Myocardial infarction

ROS overproduction

↓

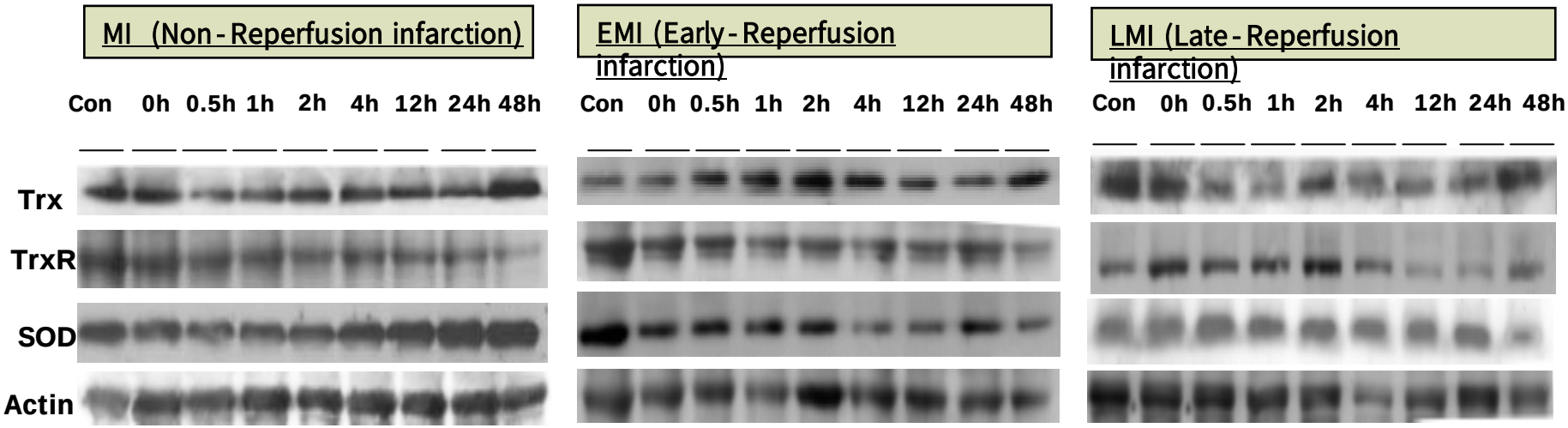
Myocardial injury

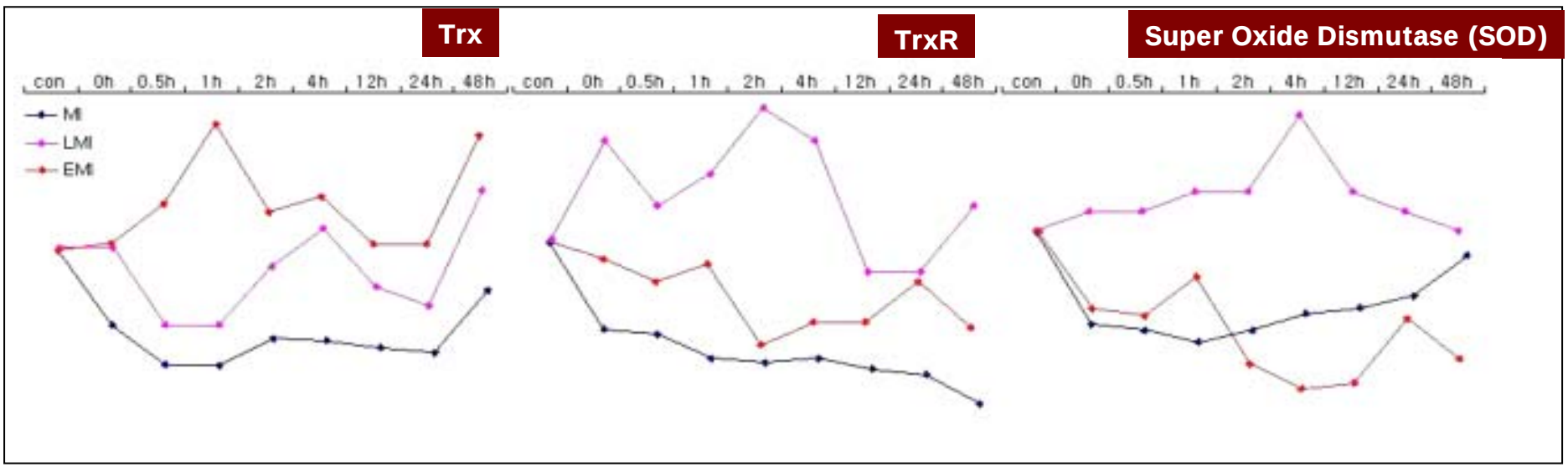
LV remodeling

↓

Protein extraction for Western blot analysis

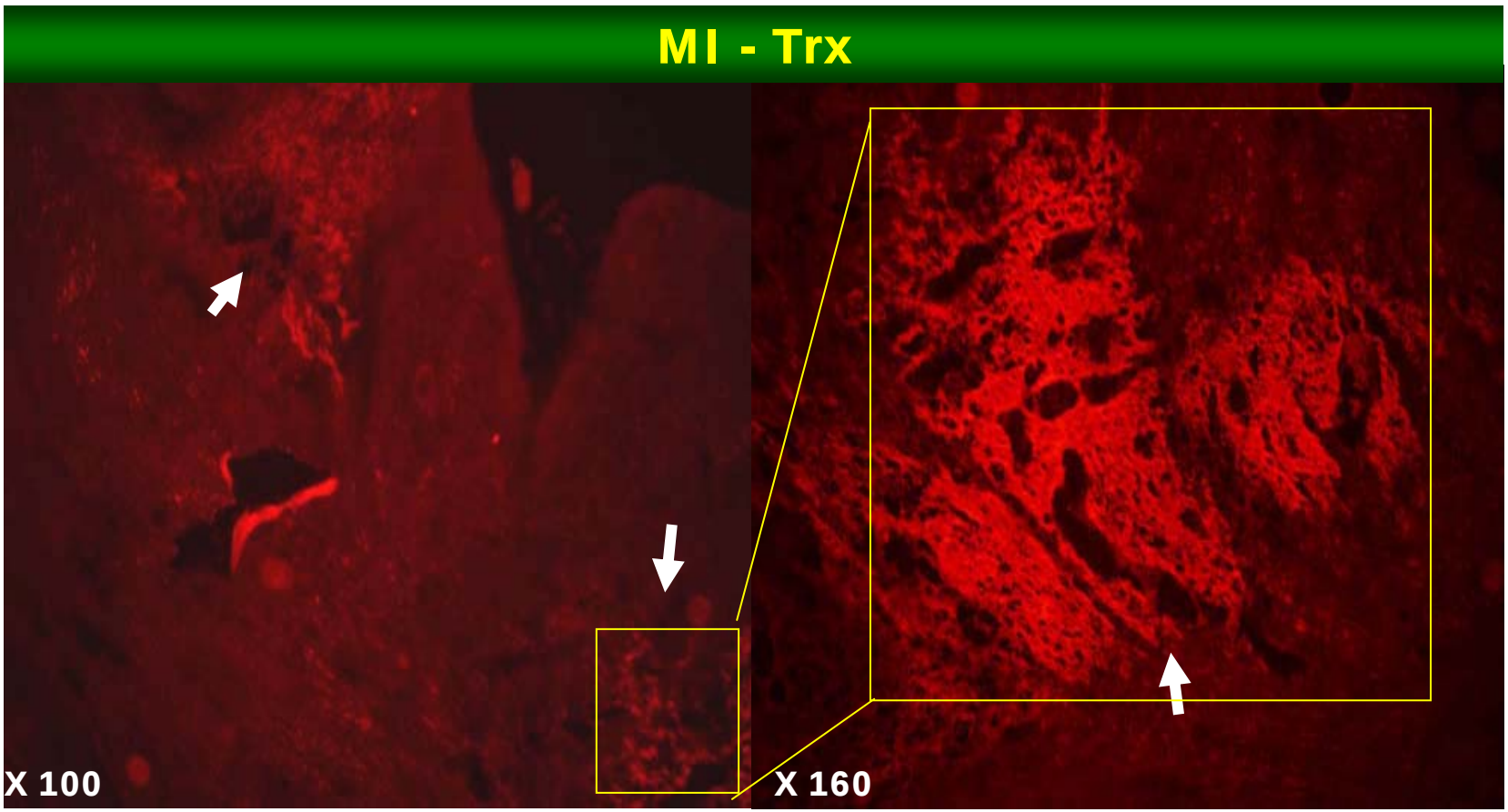
Expression of Myocardial Trx and TrxR in 3 MI models





Immunohistochemistry for Trx localization

- Trx expression in infarct border-zone



Trx = Red Fluorescence

§ Hypoxic injury by anaerobic chamber

HUVECs (Human Umbilical Vein Endothelial Cells)

EPCs (Endotherial Progenitor Cells)

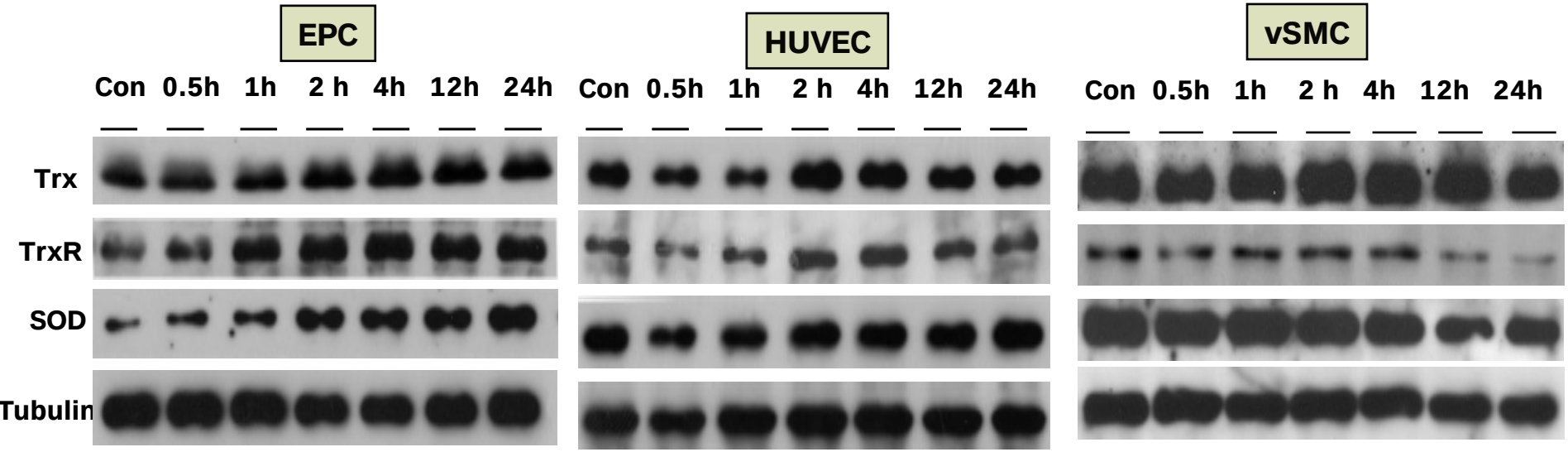
VSMCs (Vascular Smooth Muscle Cells)

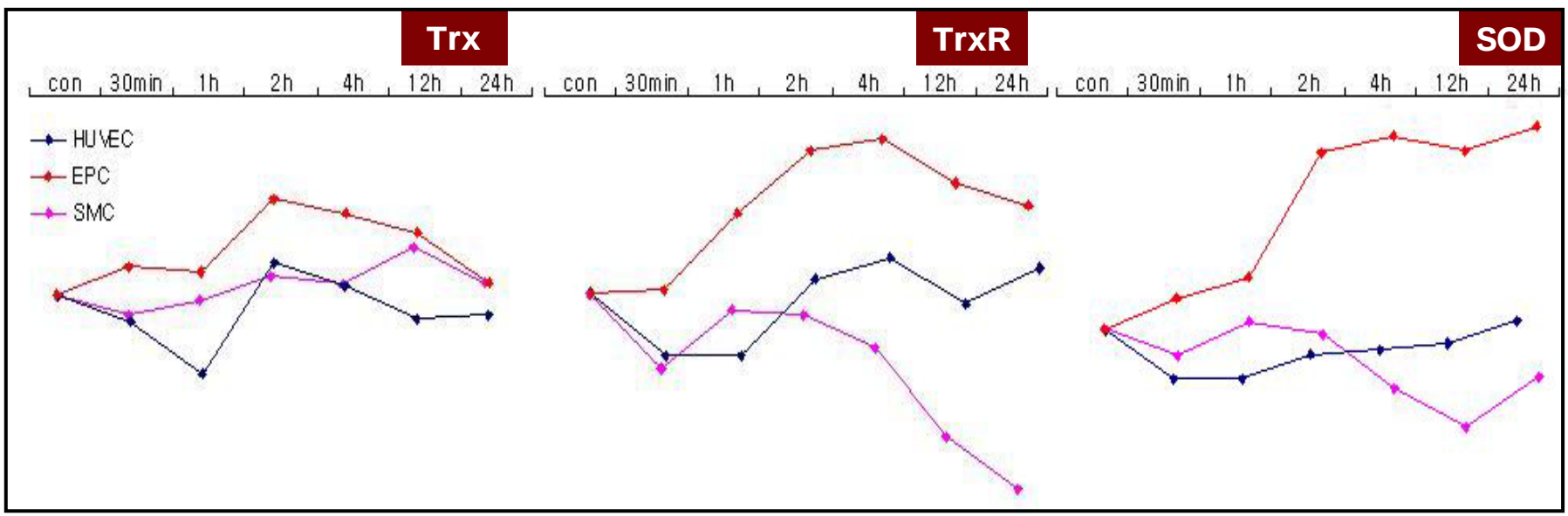
Hypoxic injury (1% O_2)
0h, 0.5 hr, 1hr, 2hr, 4hr, 12hr, 24hr

Cell harvest

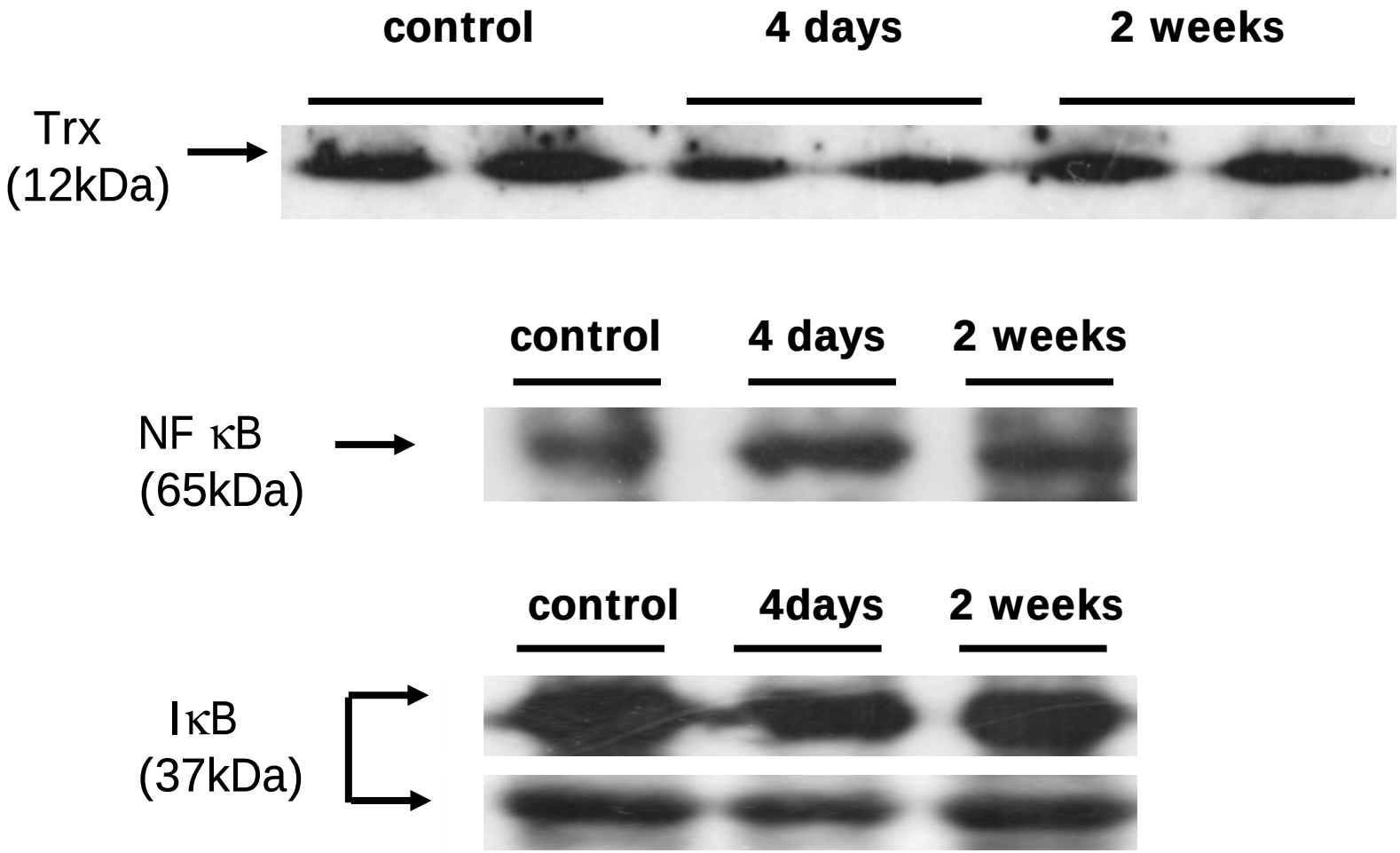
Protein Extraction for Western blot analysis

Hypoxic injury of 3 cell types (vascular cell)

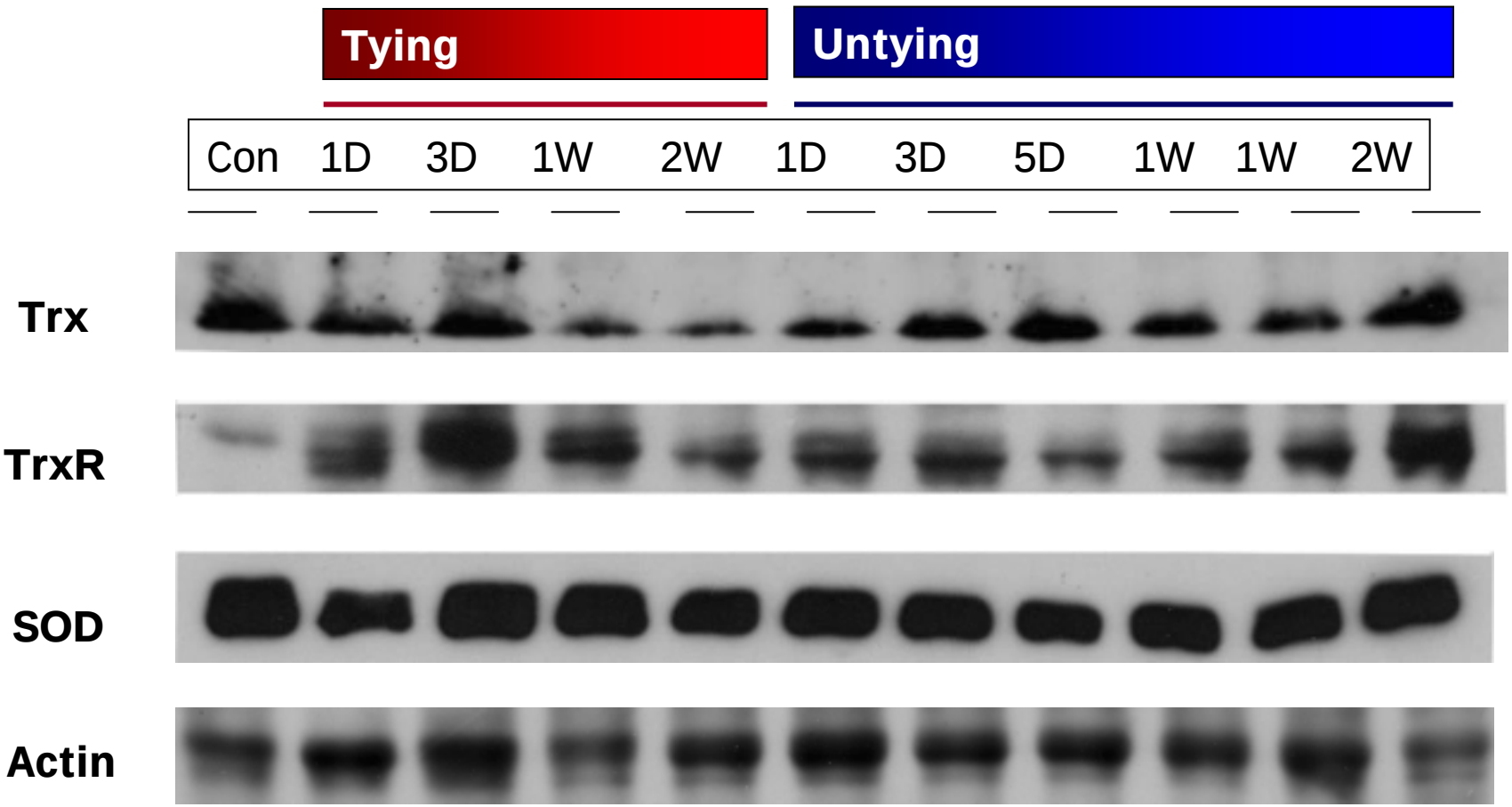




Expression of Trx and Its downstream pathway in Balloon injury models



Expression of Trx and TrxR in TAC model



eNOS production at 293 T cells and NO secretion VSMCs

- SHR - 6wks old male, 2 group. Lenti-eNOS virus vs Control (1 x TBS injection)
  Receiving L-arginine hydrochloride (35.6mmol/L) in drinking water

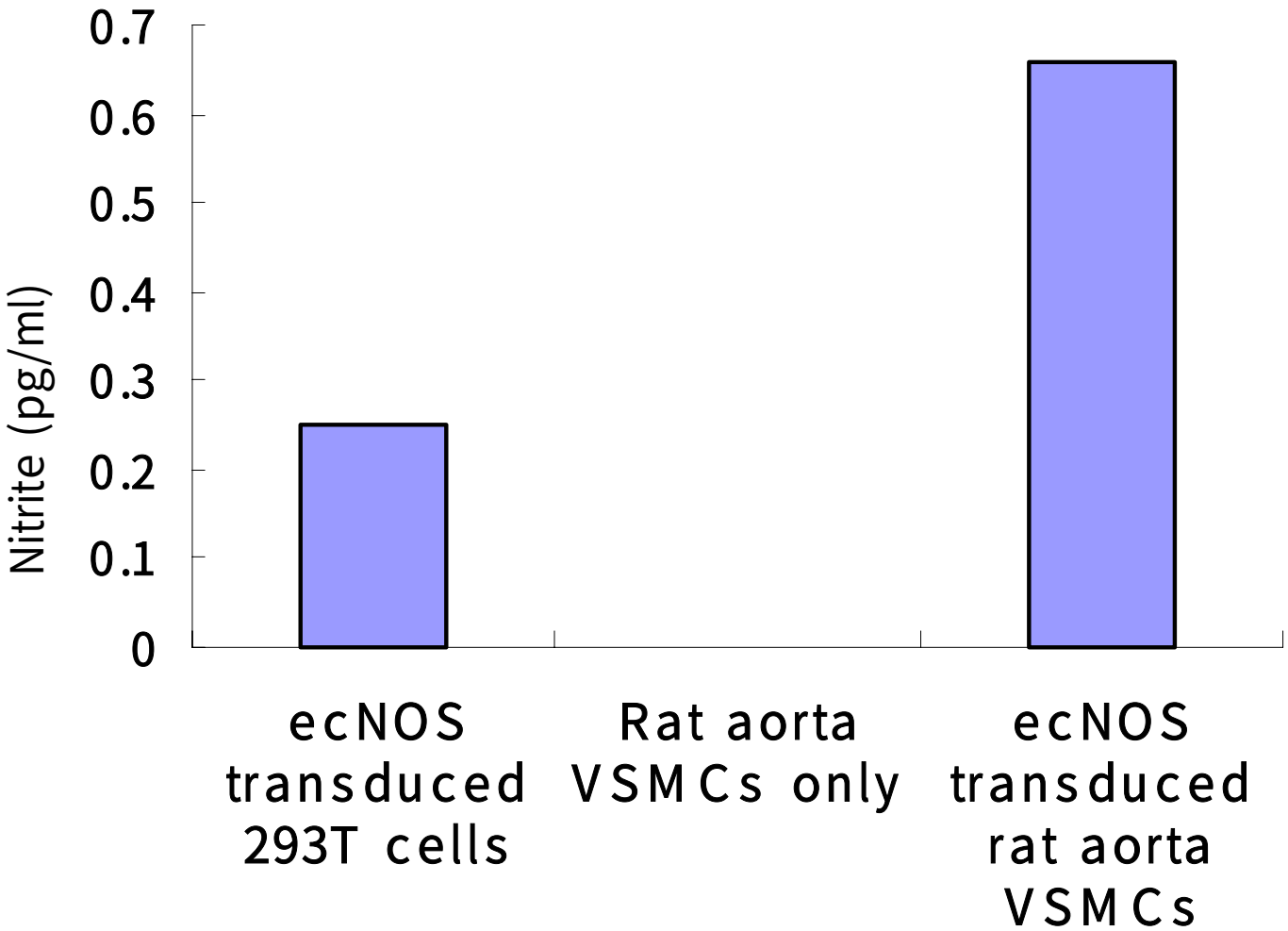
eNOS production at 293 T cells

Marker Control 293T

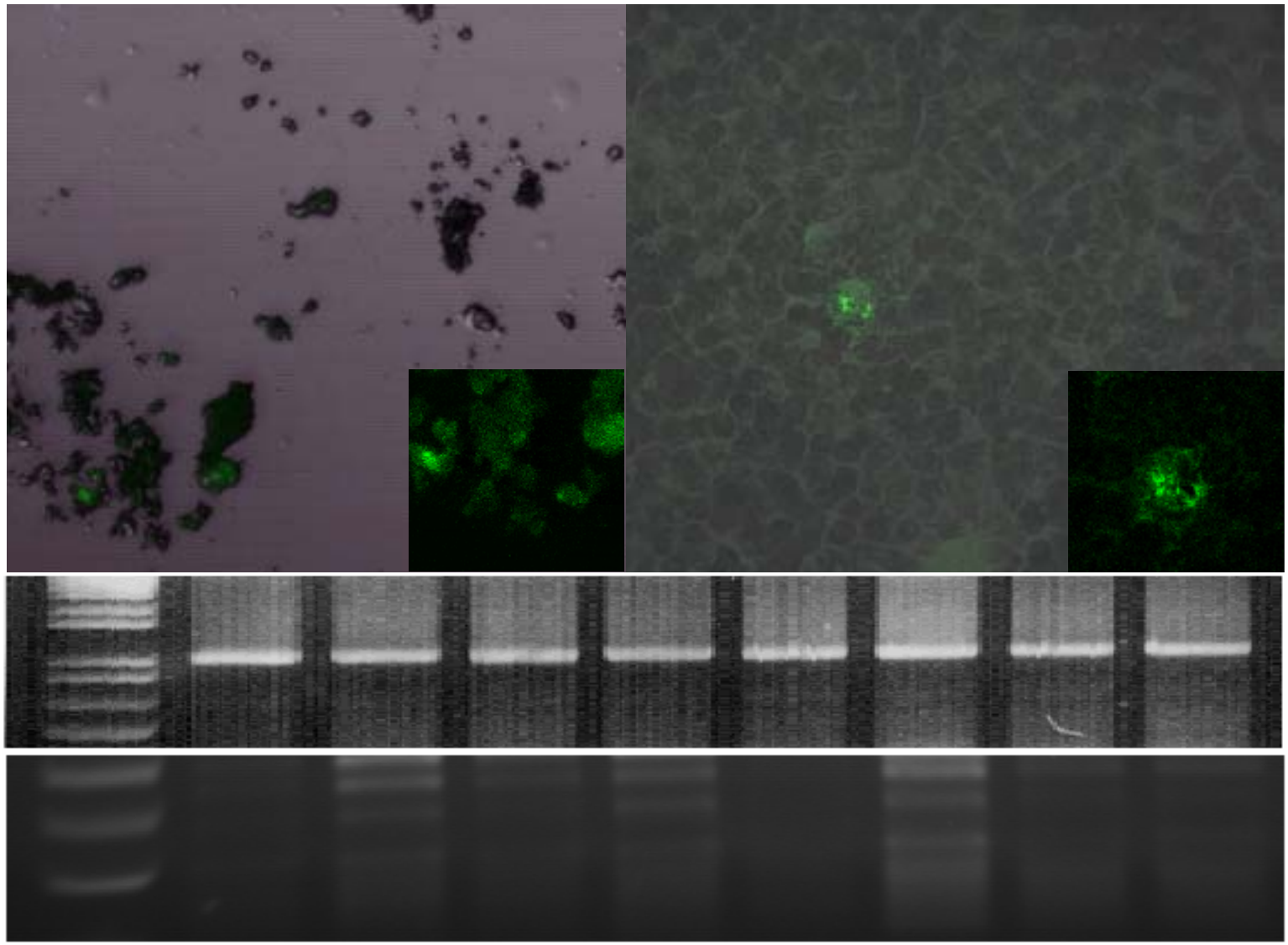
NO Concentration in supernatant

Rat aorta VSMCs only	0.00 pg/ml
ecNOS transduced 293T cells	0.25 pg/ml
ecNOS transduced rat aorta VSMCs	0.66 pg/ml

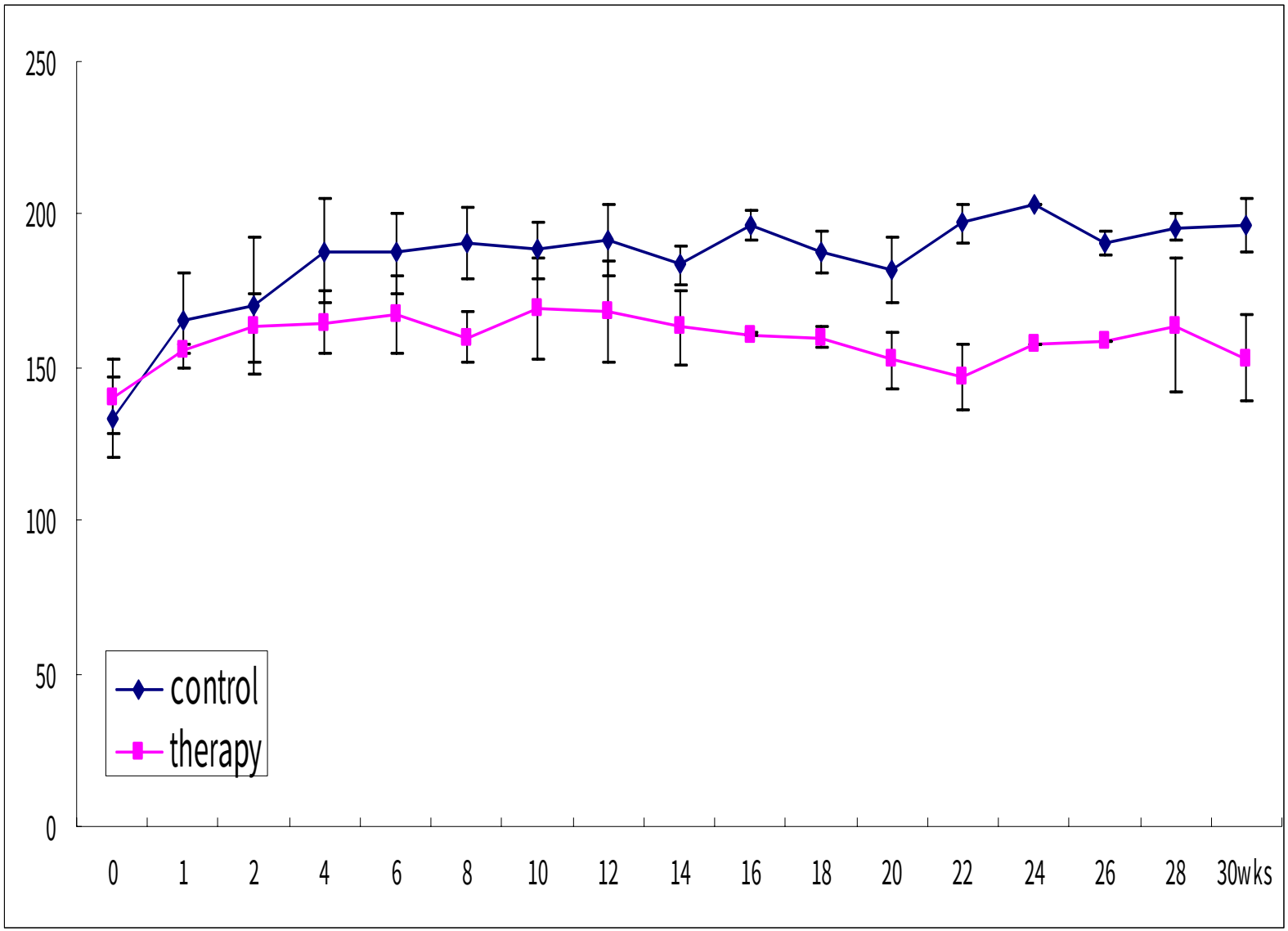
Serum NO levels after NOS gene therapy



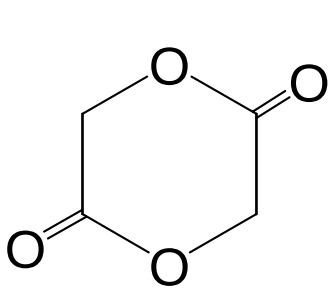
GFP expression (*in vivo*)



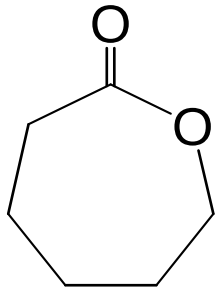
Systolic Blood Pressure (mmHg)



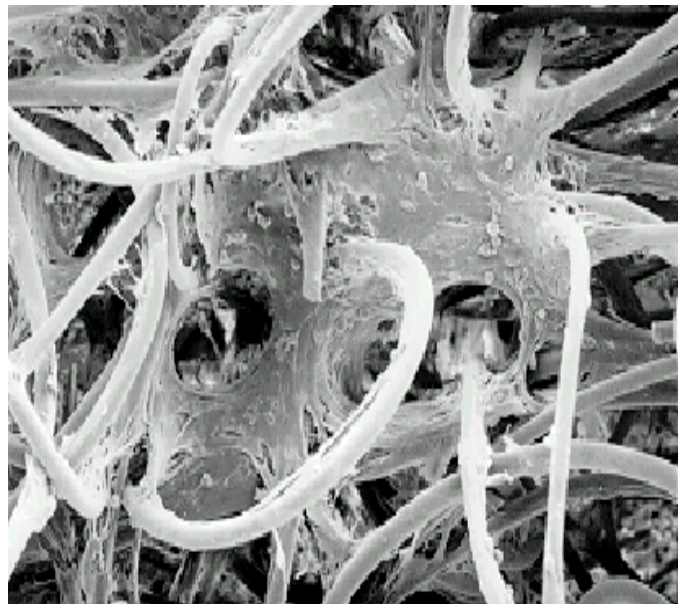
Co-polymerization of PGCL



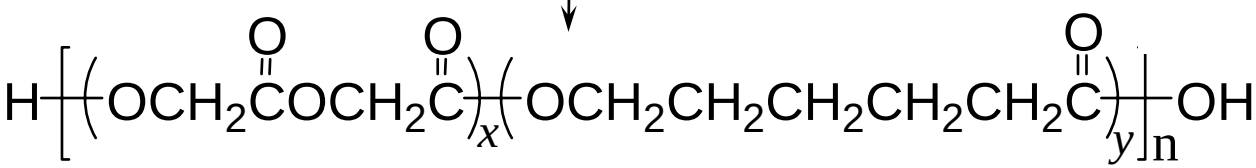
+



Glycolide (50%) : **e-Caprolactone (50%)**



Sn-oct **170 °C, 20 hrs**

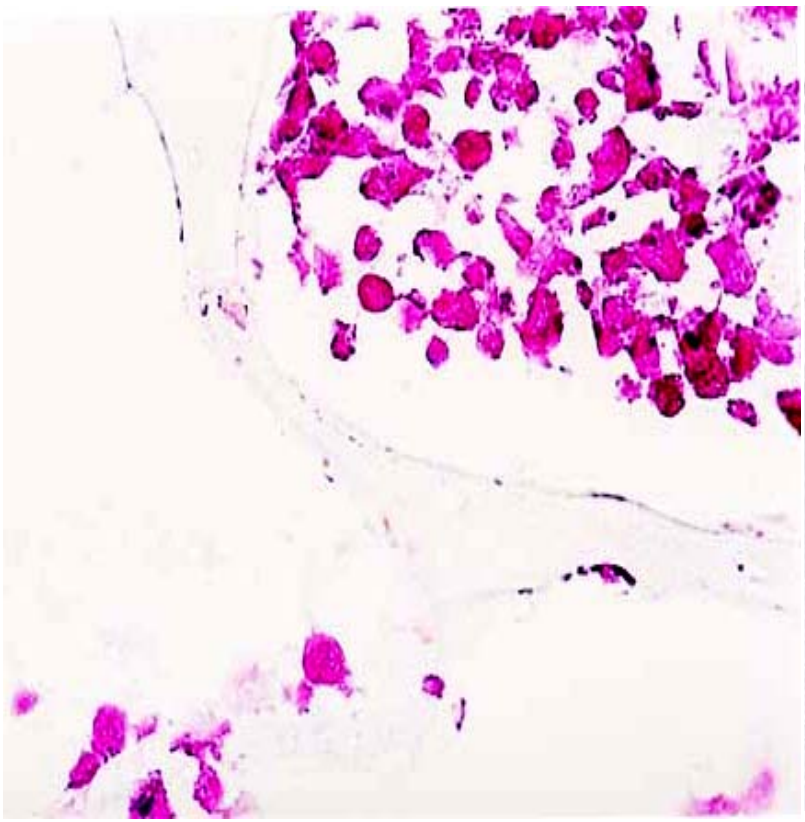


Glycolide/ e-Caprolactone Copolymer (PGCL)

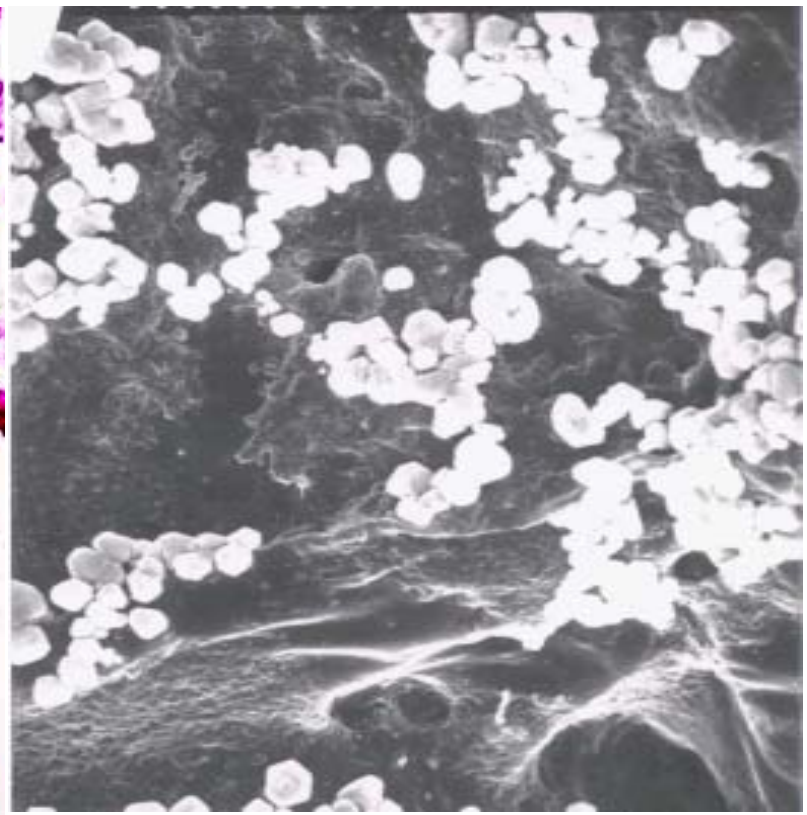
Absorbed within 2 months

rMSCs Seeded on a PGCL Scaffold

Culture for 48hrs

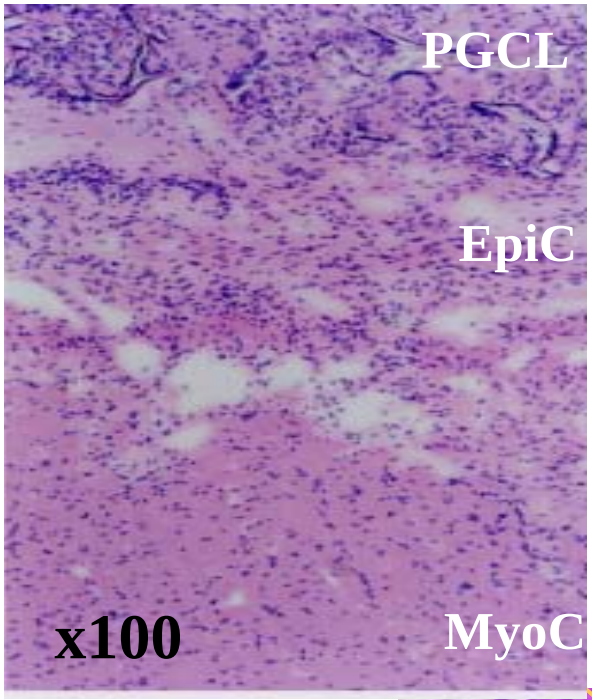


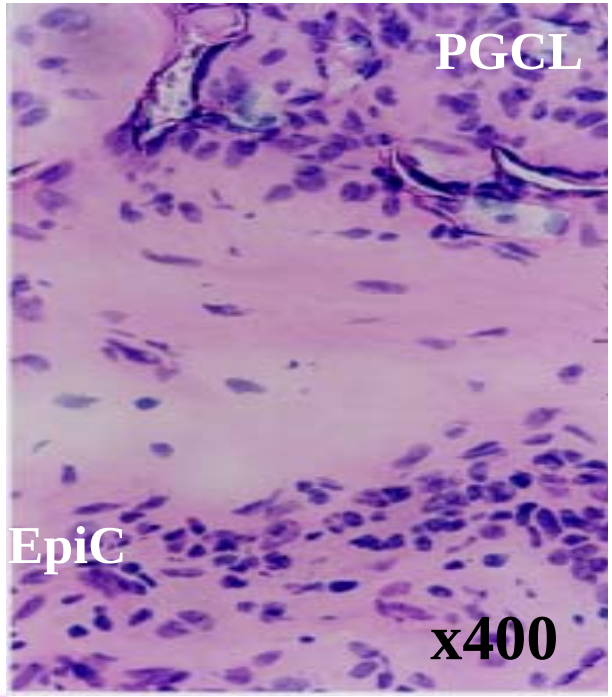
H&E x 200



SEM x 1,100

rMSCs-Seeded PGCL Patch Implanted to Normal Heart





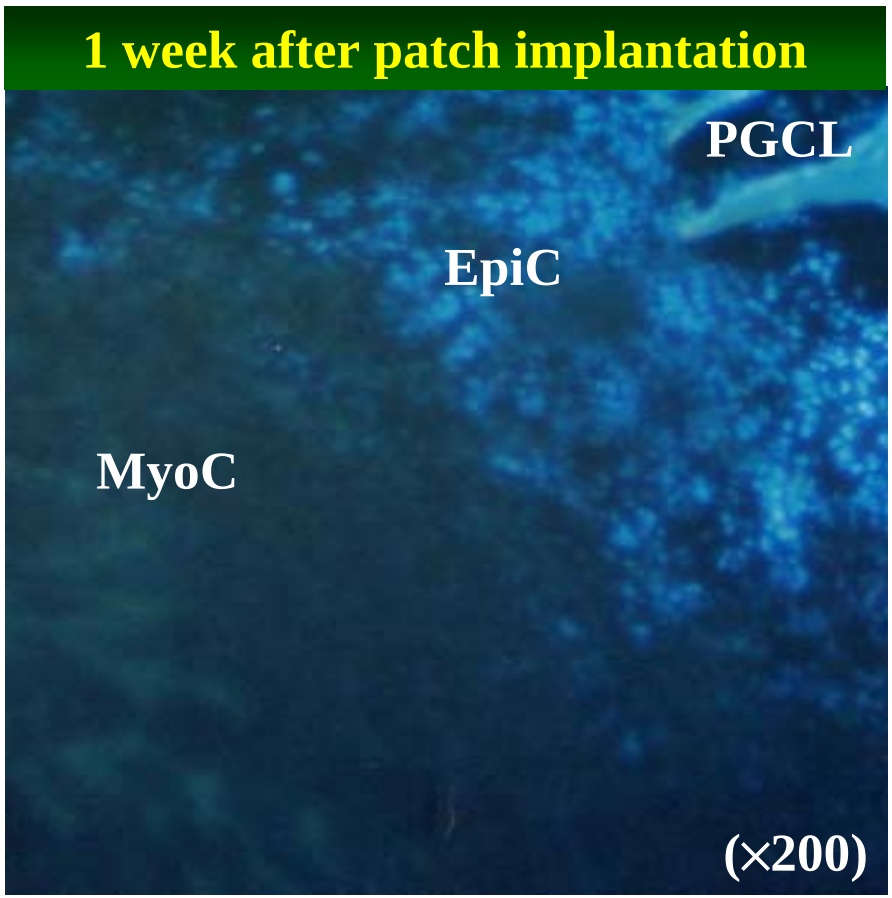
5 weeks after patch implantation

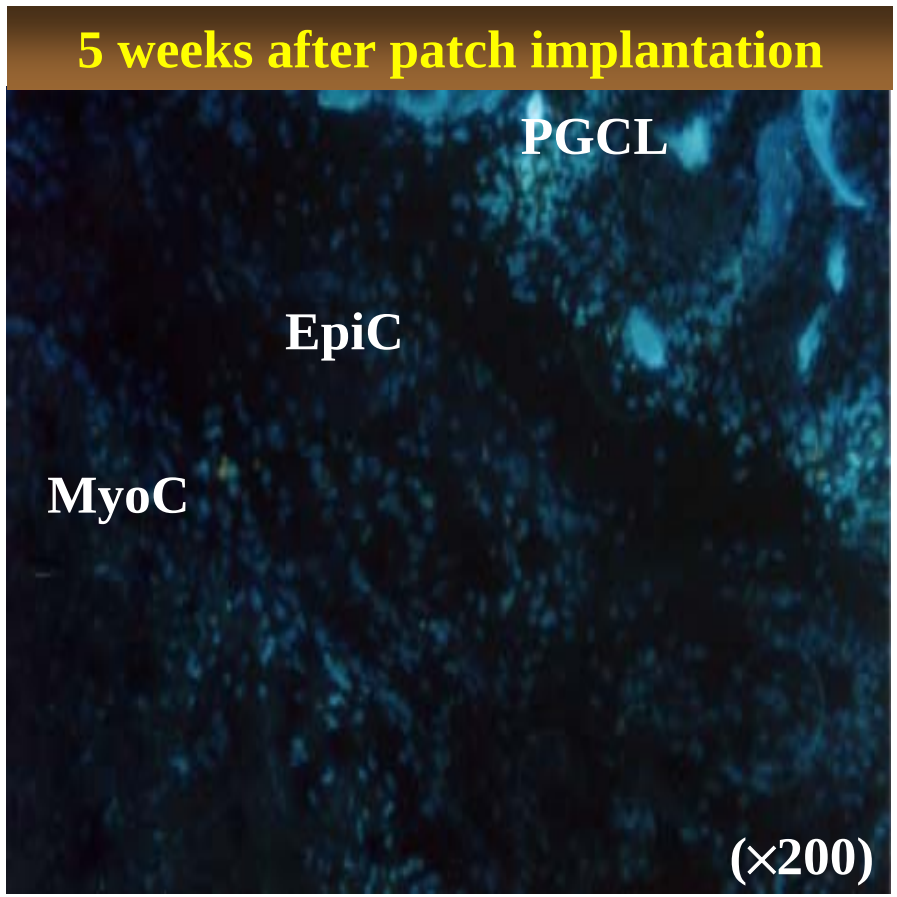
PGCL patch
Epicardium(EpiC)

Myocardium(MyoC)

rMSCs-Seeded PGCL Patch Implanted to Normal Heart

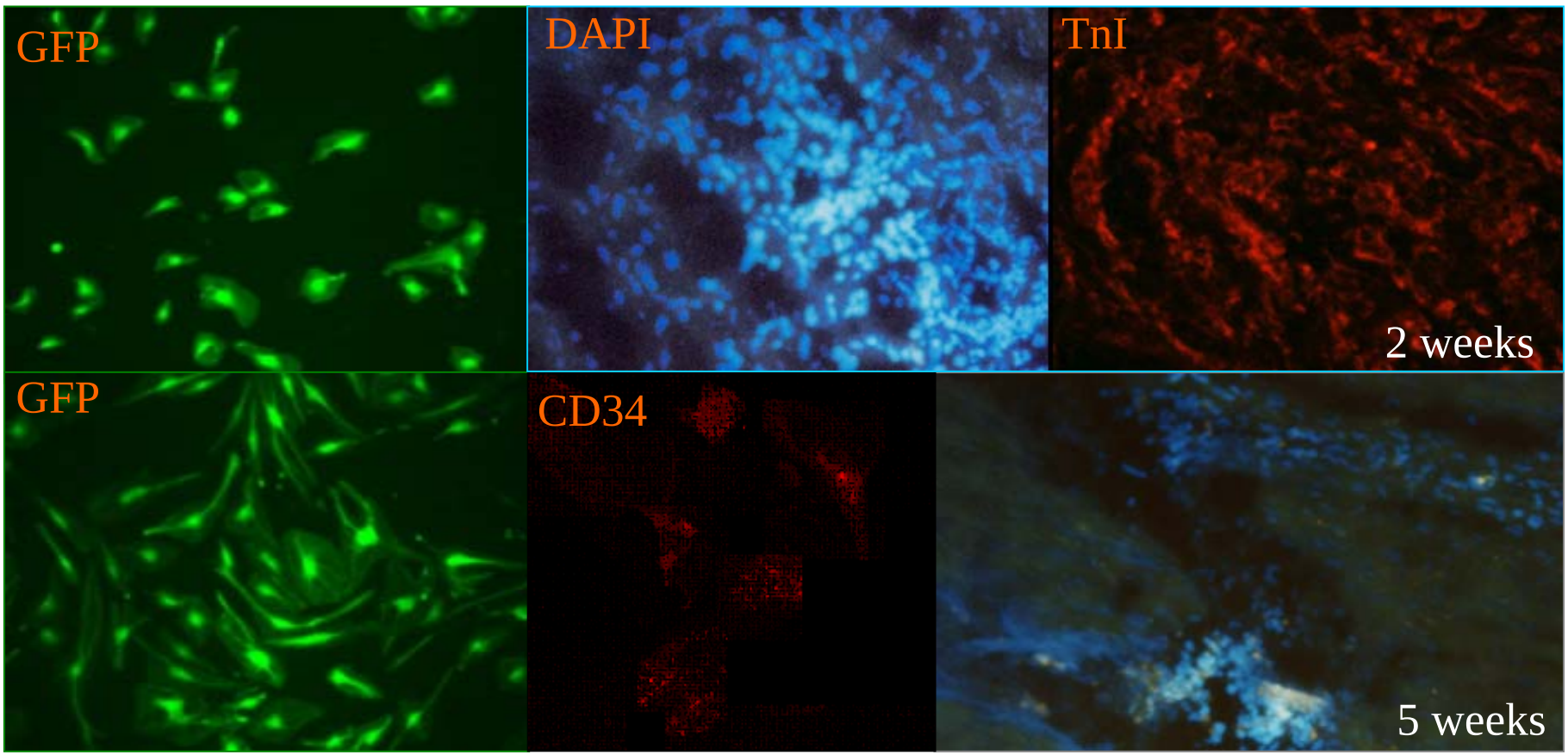
DAPI-Labeling





HUVECs and EPCs for Myocardial Regeneration

- Lentivirus-mediated GFP transfer to EPCs
- DAPI-labeled EPCs injection into normal myocardium

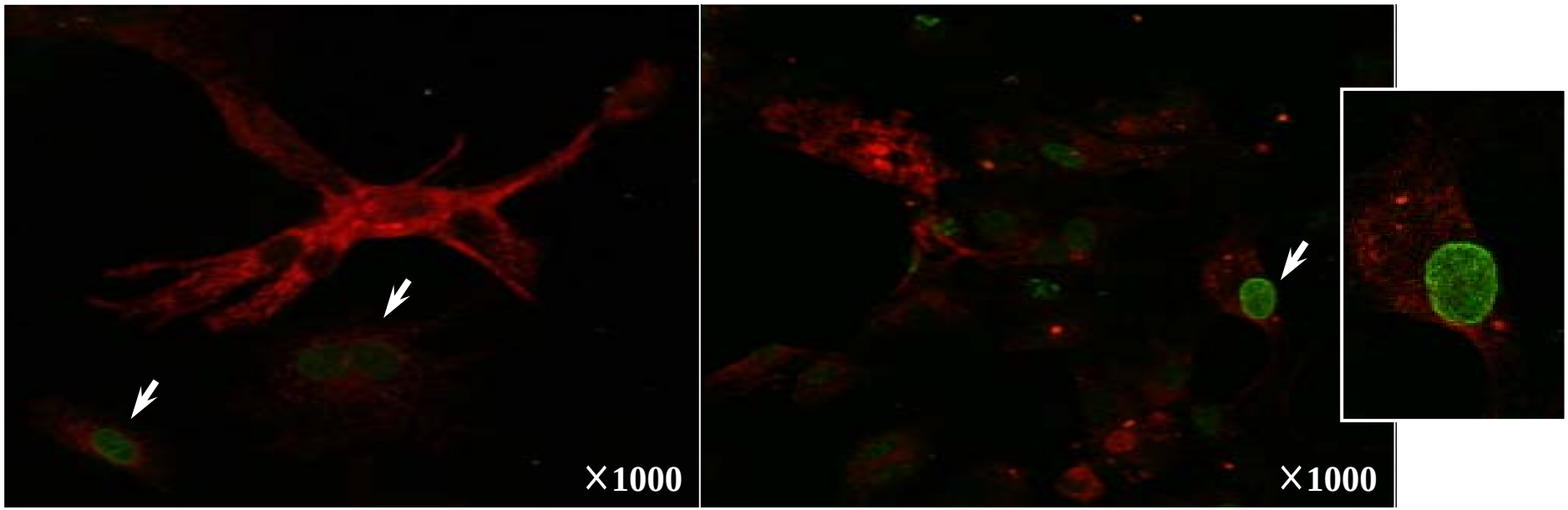


- Genetic makeup with VEGF, Akt, antiapoptotic genes

HUVEC: Co-culture with cardiomyocytes for 2 Weeks

Differentiation of HUVECs into a Cardiomyocyte Phenotype

BrdU (+) / Tn I (+) cell

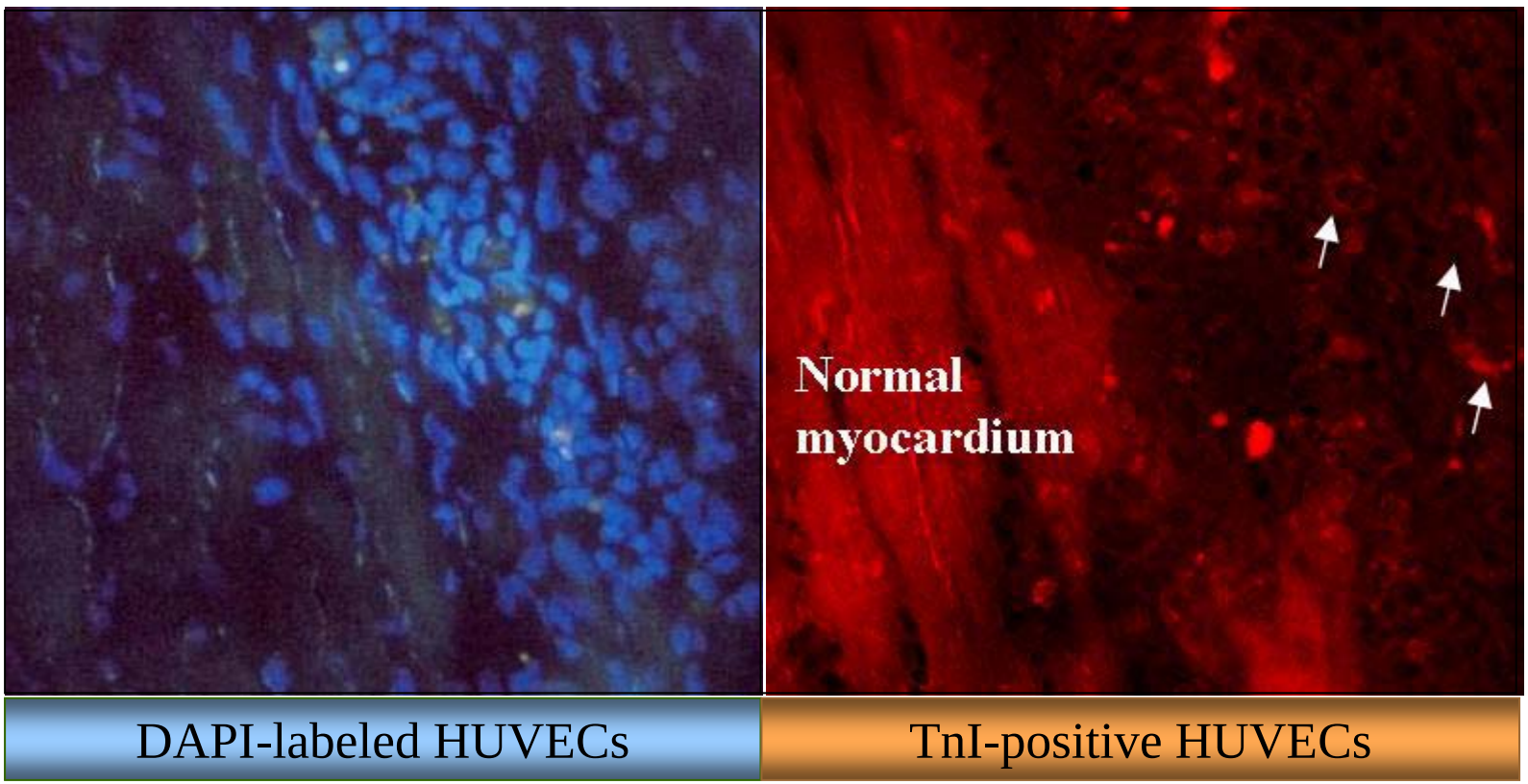


HUVECs: pre-labeled with BrdU

Green fluorescence : BrdU **Red flourescence : Troponin I (TnI)**

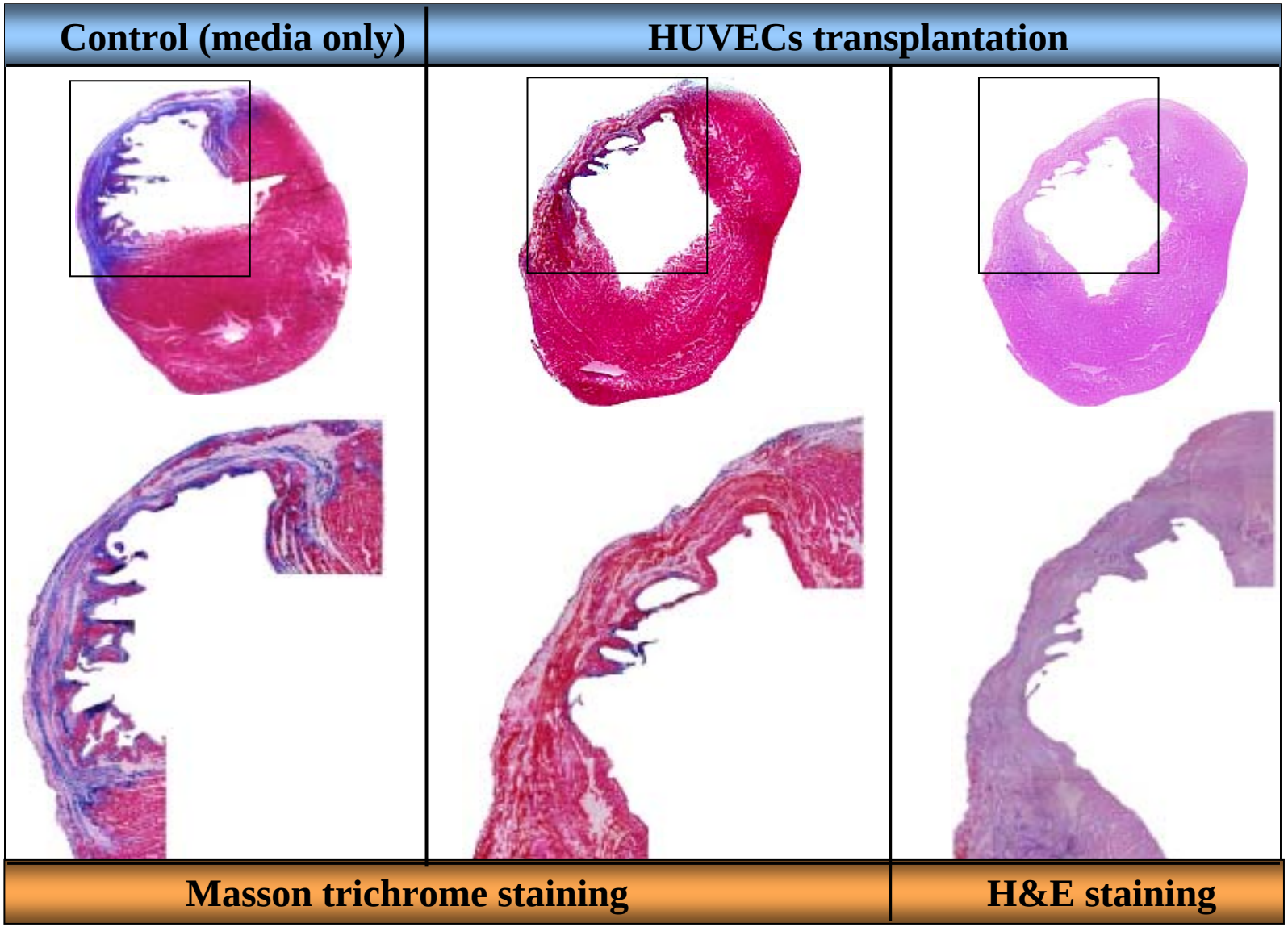
In Vivo Study at Normal Myocarium

4 weeks after HUVECs injection into normal myocardium



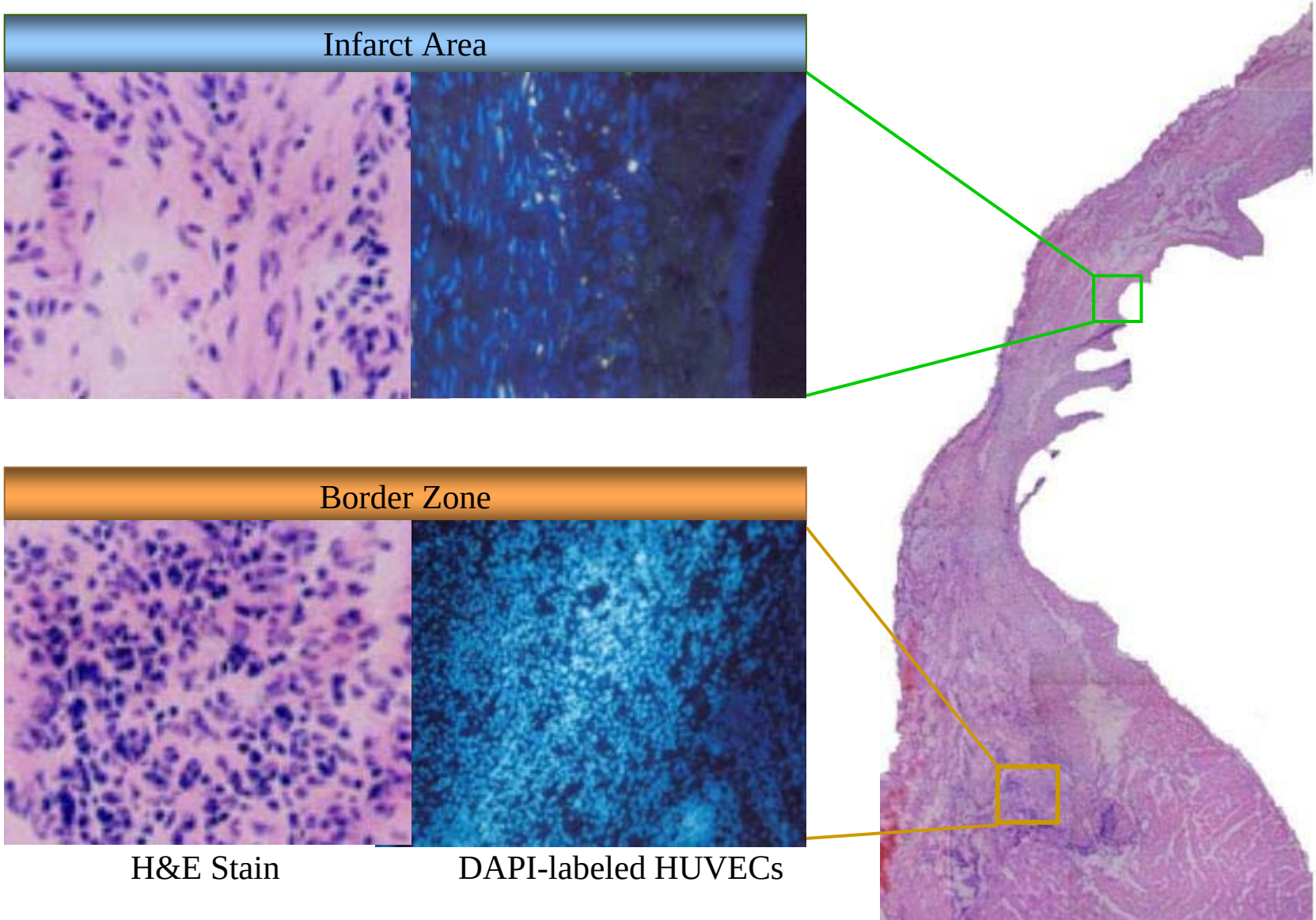
Gross Findings After HUVECs transplantation

- 2 weeks after HUVECs injection into border zone

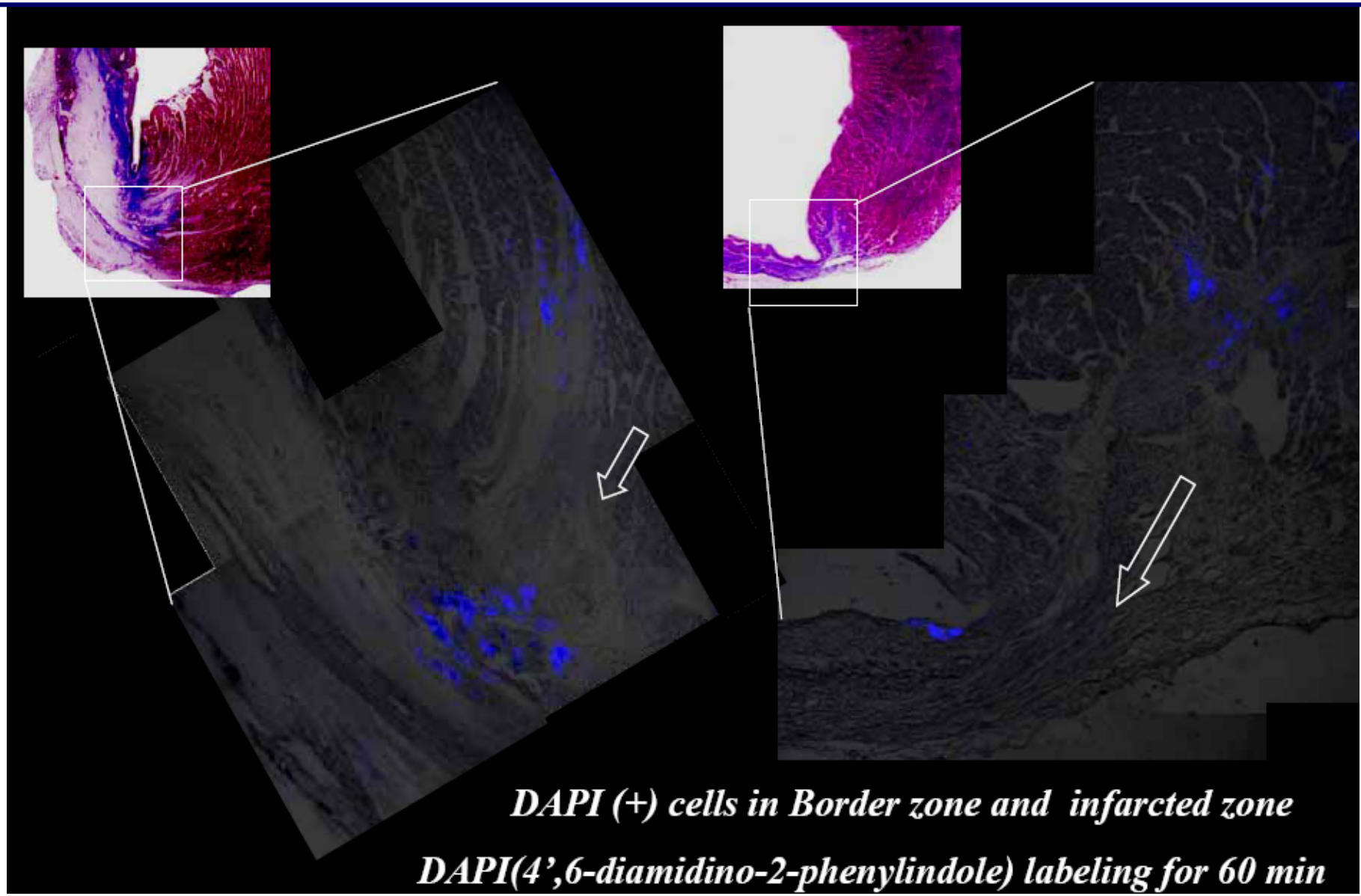


DAPI (+) HUVECs at Both Infarct & Border zones

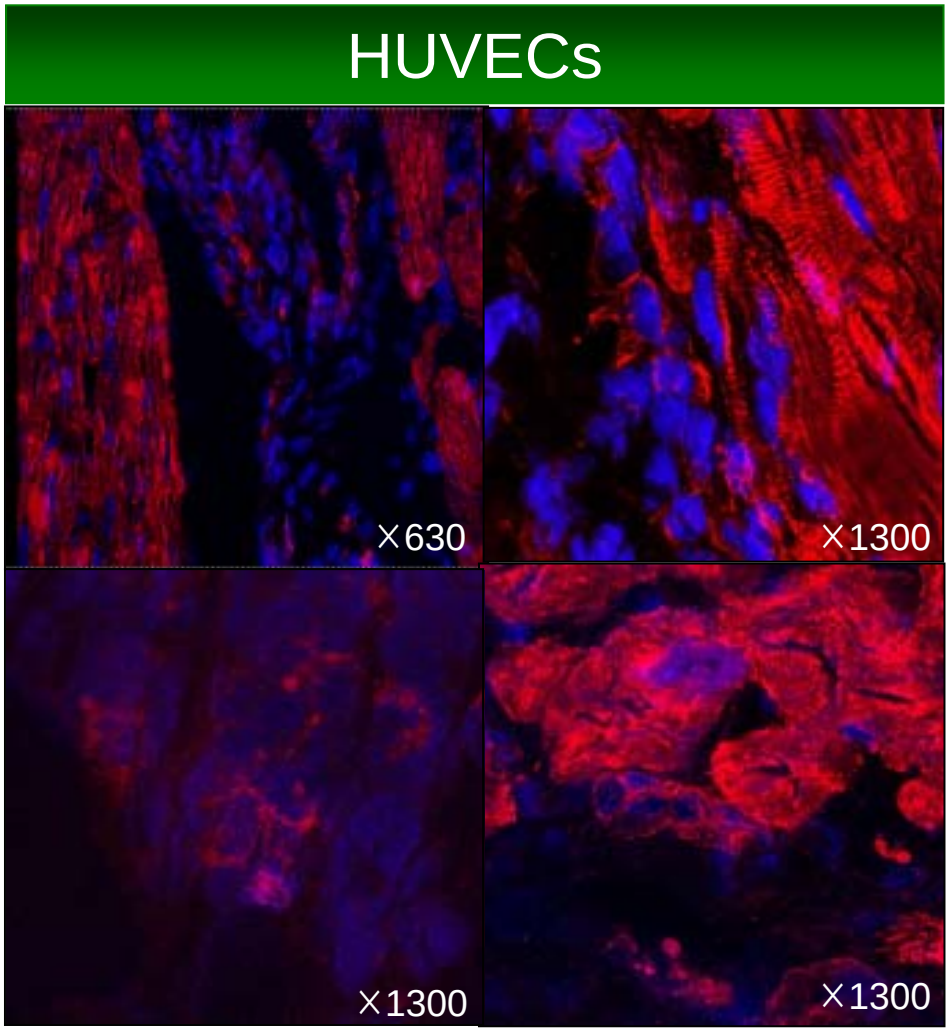
- 2 weeks after HUVECs injection into border zone
- DAPI labeled cells in border zone and infarct zone

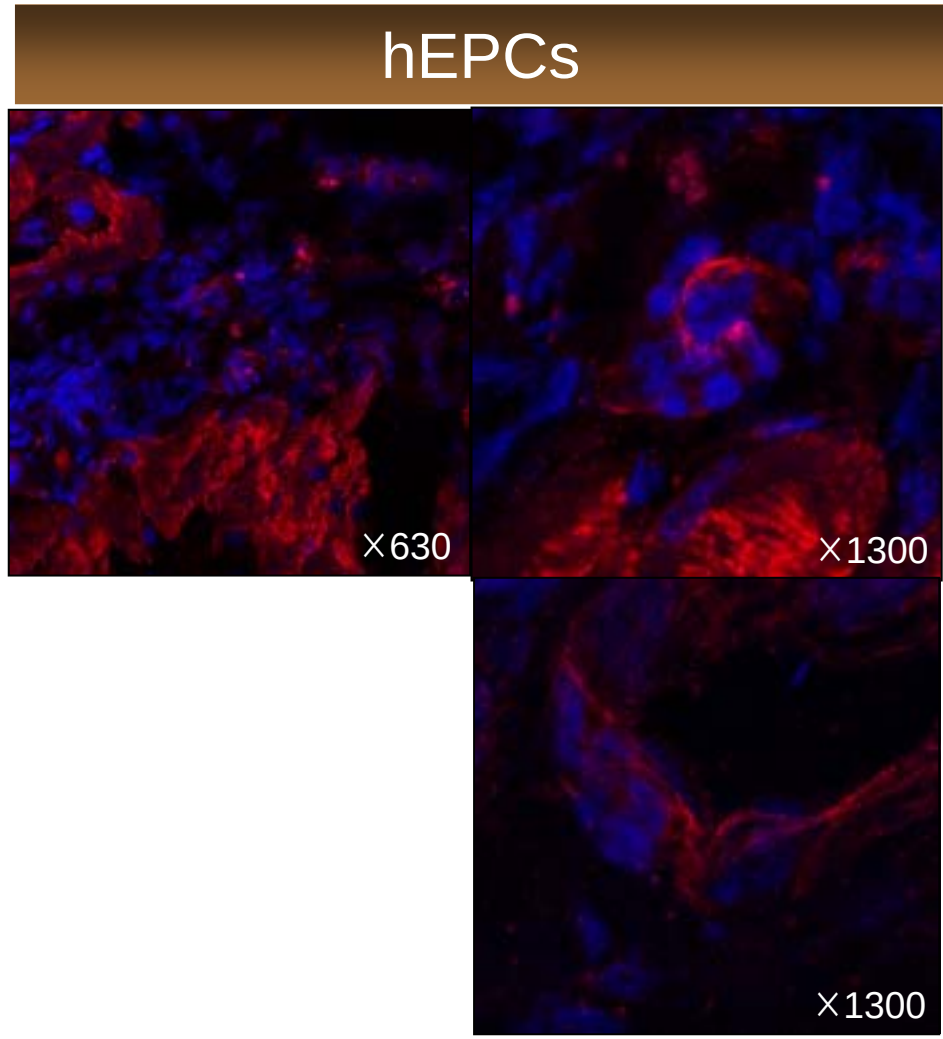


HUVECs at Both Infarct & Border zones



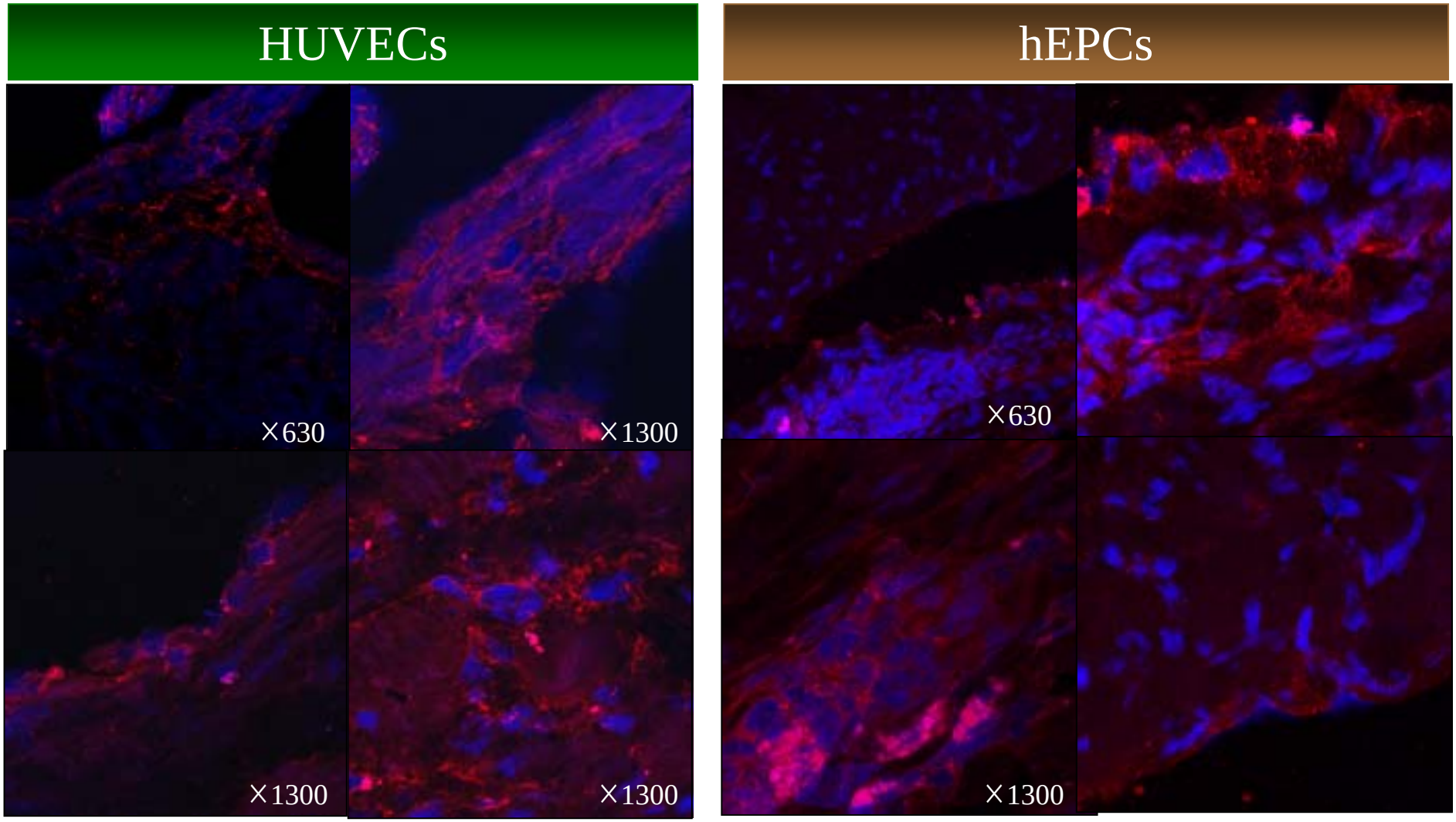
Cardiomyogenic Differentiation of Transplanted HUVECs or hEPCs into Infarct Border Zone





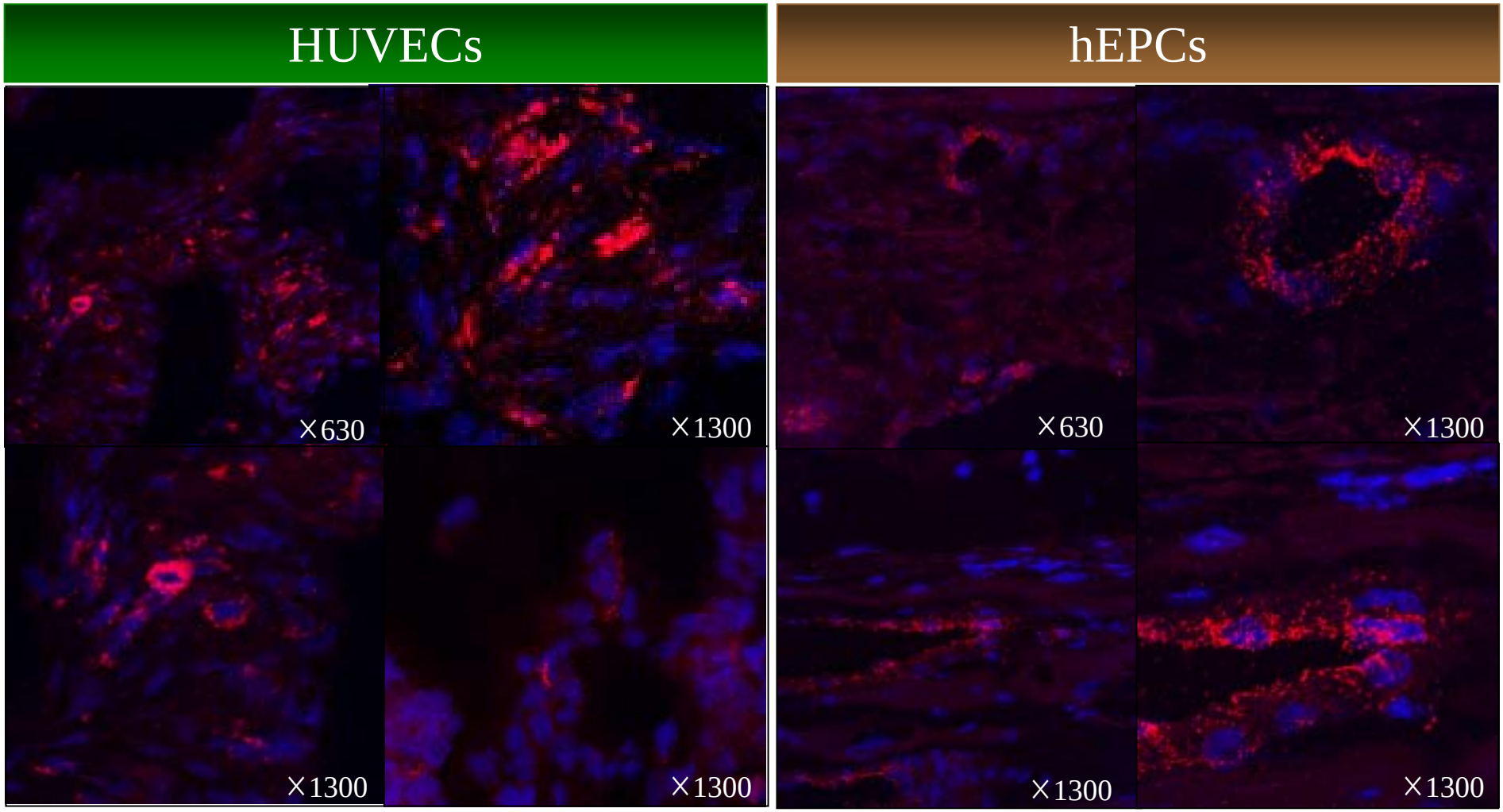
- **Red : Cardiomyocyte maker (cTnI)**
- **Blue : Nuclei of transplanted HUVECs or hEPCs (DAPI)**

Cardiomyogenic Differentiation of Transplanted HUVECs or hEPCs into Infarct Border Zone



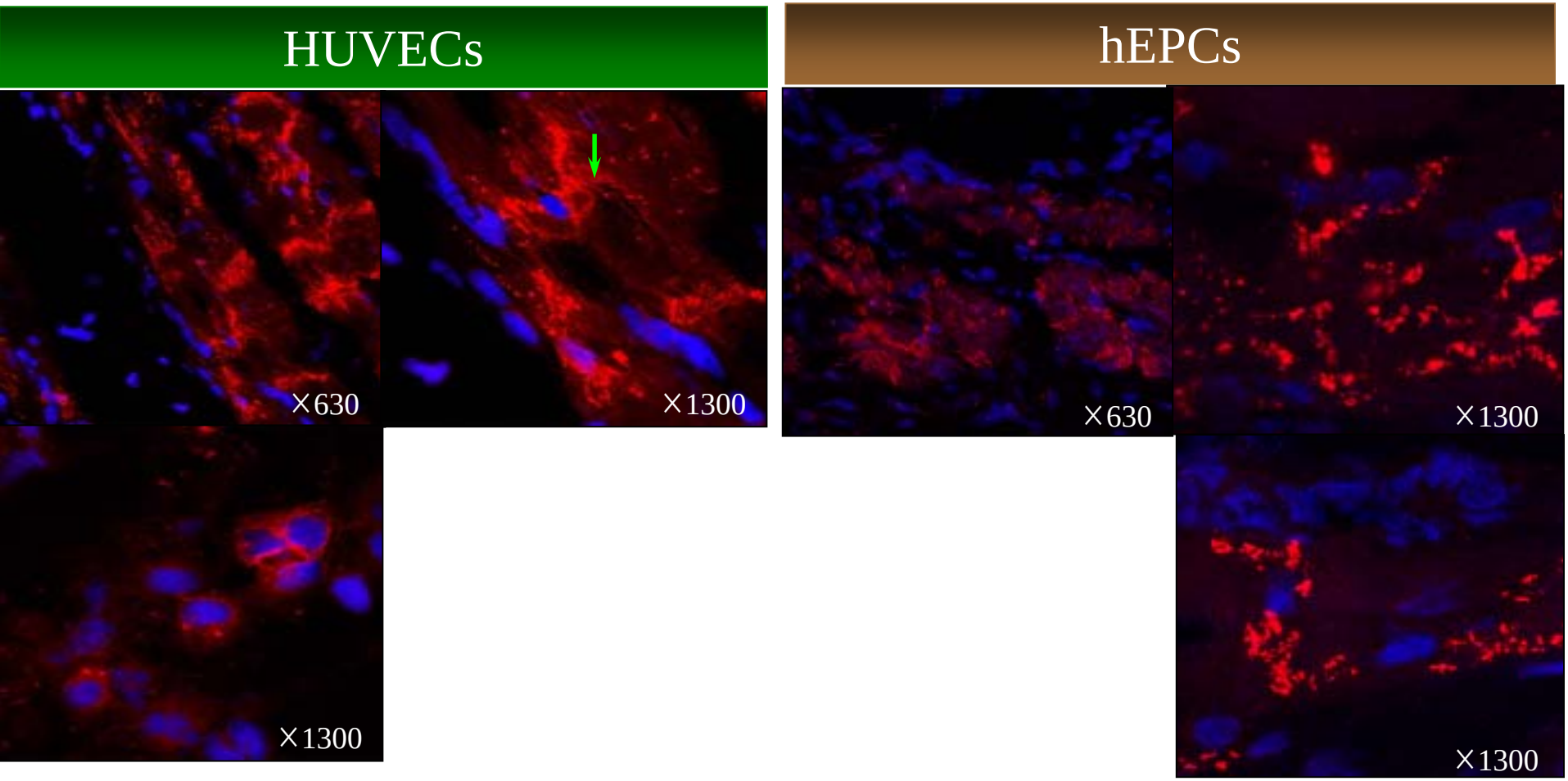
- **Red : Cardiomyocyte maker (Myosin heavy chain, MHC)**
- **Blue : Nuclei of transplanted HUVECs or hEPCs (DAPI)**

Primitive Vessel Formation or Angiogenesis of Transplanted HUVECs or hEPCs



- **Red : Endothelial marker (vWF)**
- **Blue : Nuclei of transplanted HUVECs or hEPCs (DAPI)**

Gap Junction Formation of Transplanted HUVECs or hEPCs with Native Cardiomyocytes



- **Red : Gap junction maker (Connexin 43)**
- **Blue : Nuclei of transplanted HUVECs or hEPCs (DAPI)**