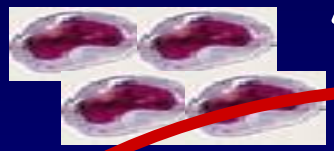
The Present and Future for Biopacemaker

Hung-Fat Tse, MD
Department of Medicine
The University of Hong Kong
Queen Mary Hospital
Hong Kong

Korean Society of Circulation 2007

Strategies for Cell Therapy in Cardiac Regeneration

Embryonic Stem Cell



Adult Stem Cell

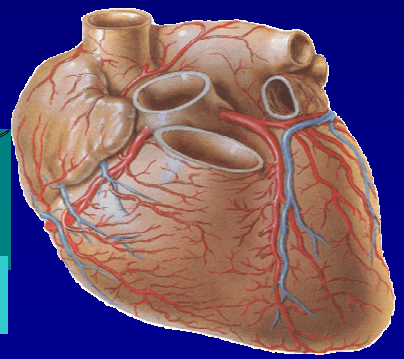
Stem cells

In Vivo Reprogramming

Gene

In Vitro Reprogramming

Delivery



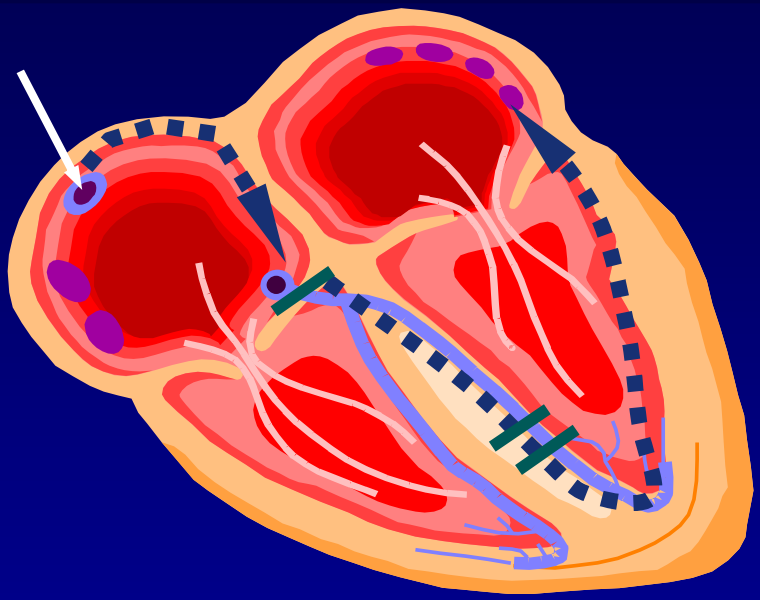
Pacemaker cells
Cardiomyocytes
Endothelial cells

Biological pacemaker
Angiogenesis
Cellular cardiomyoplasty

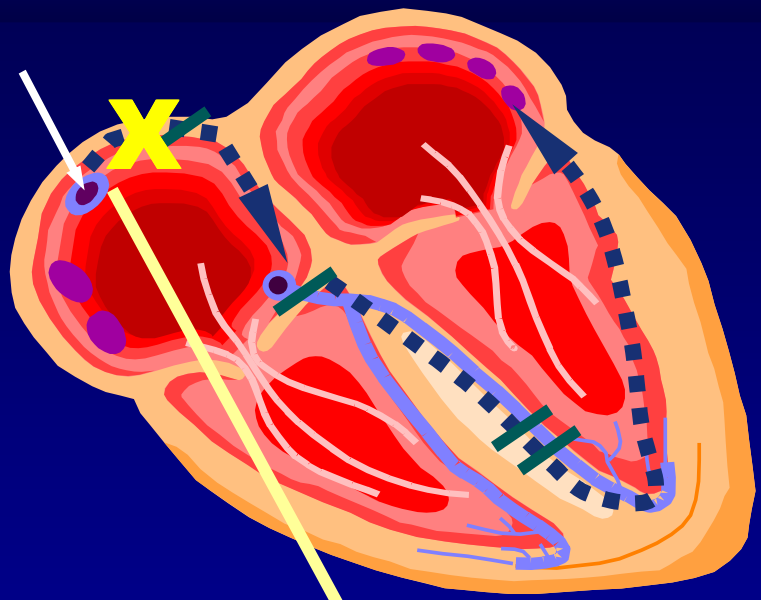
Korean Society of Circul

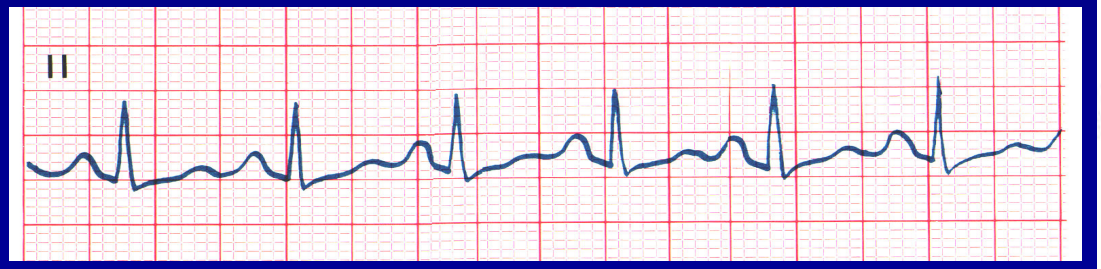
Native Biological Pacemaker

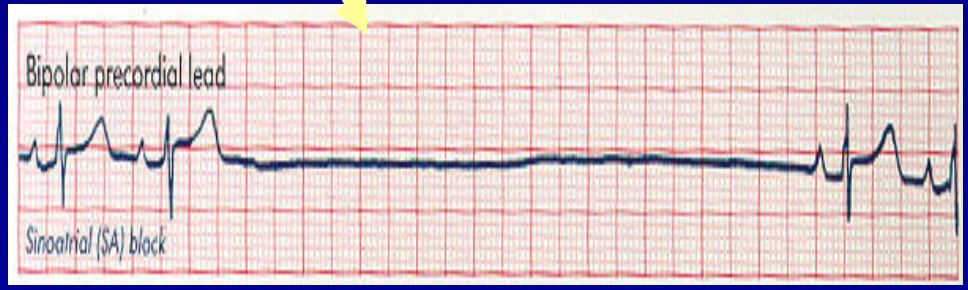
Sinus node



Sinus node

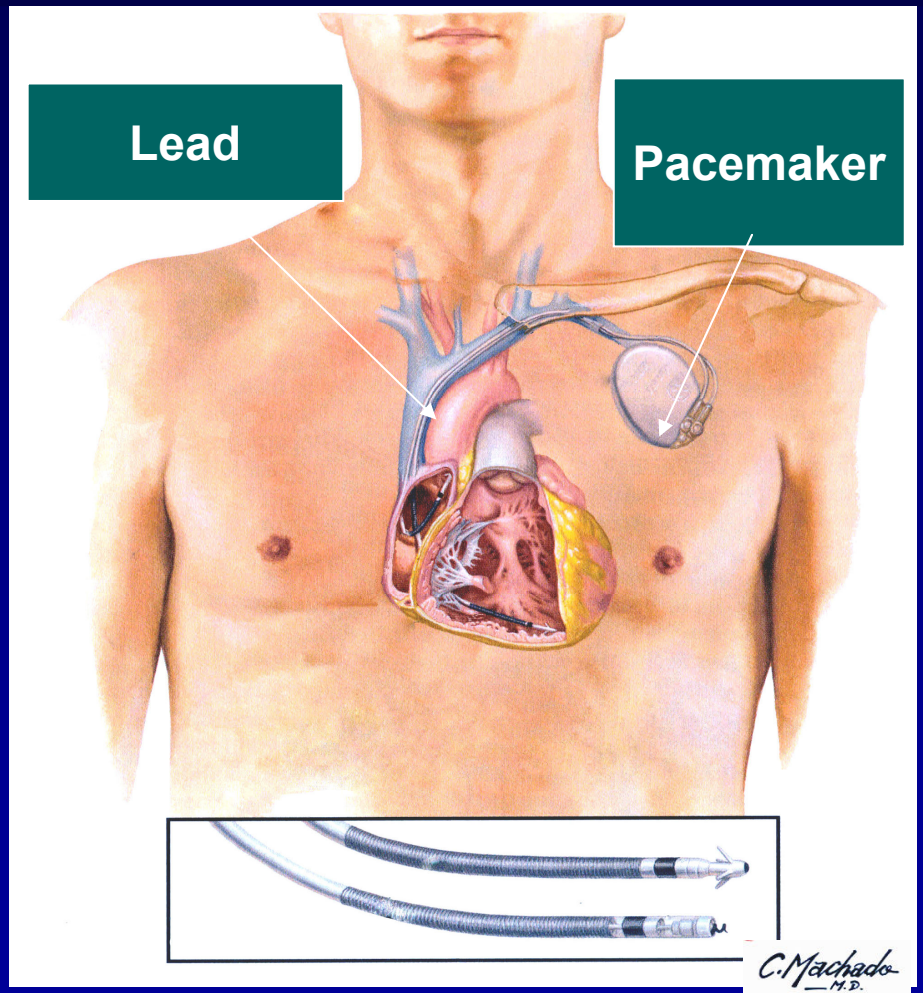




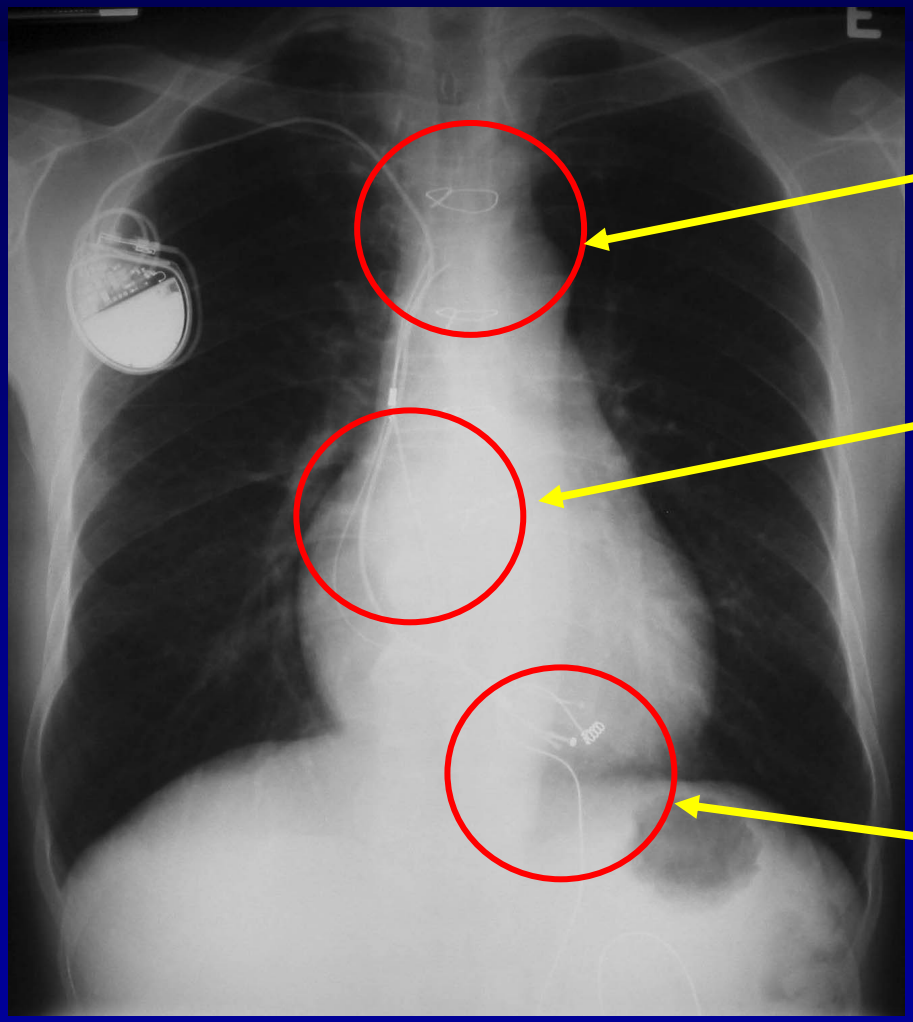


- The heart beats 2-3 billion times during the lifespan
- Malfunction of pacemaker cells due to aging leads to slow heart rate

Conventional Treatment: Electronic Pacemaker



Disadvantages of Electronic Pacemaker



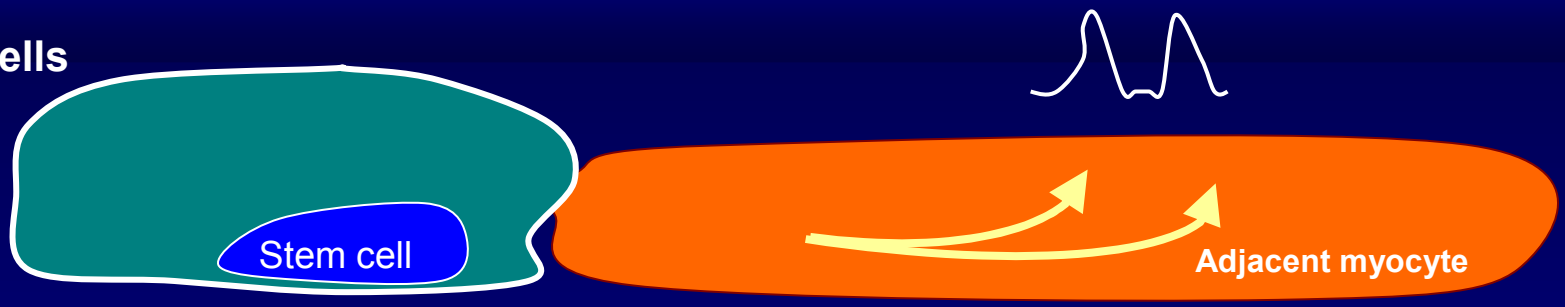
- Infection

- Replacement of malfunction leads and pacemaker

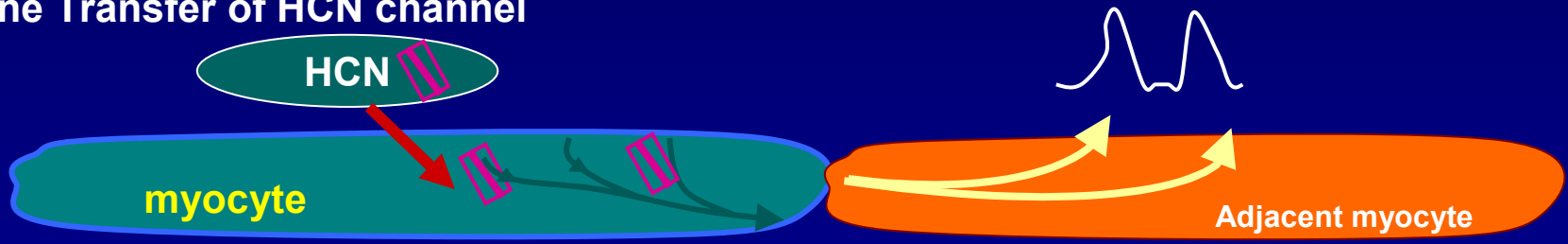
-Unable to meet the patients needs

Approaches for Biological Pacemaker

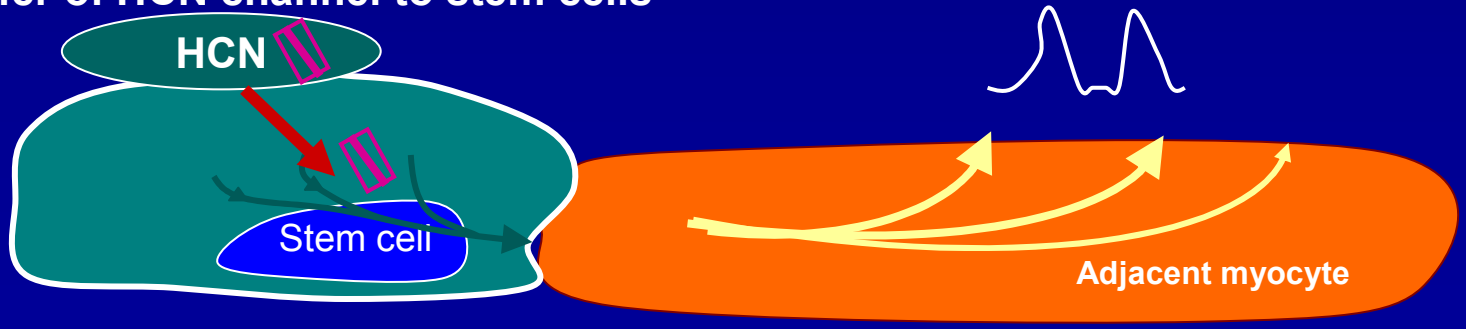
Stem Cells



Gene Transfer of HCN channel



Gene Transfer of HCN channel to stem cells

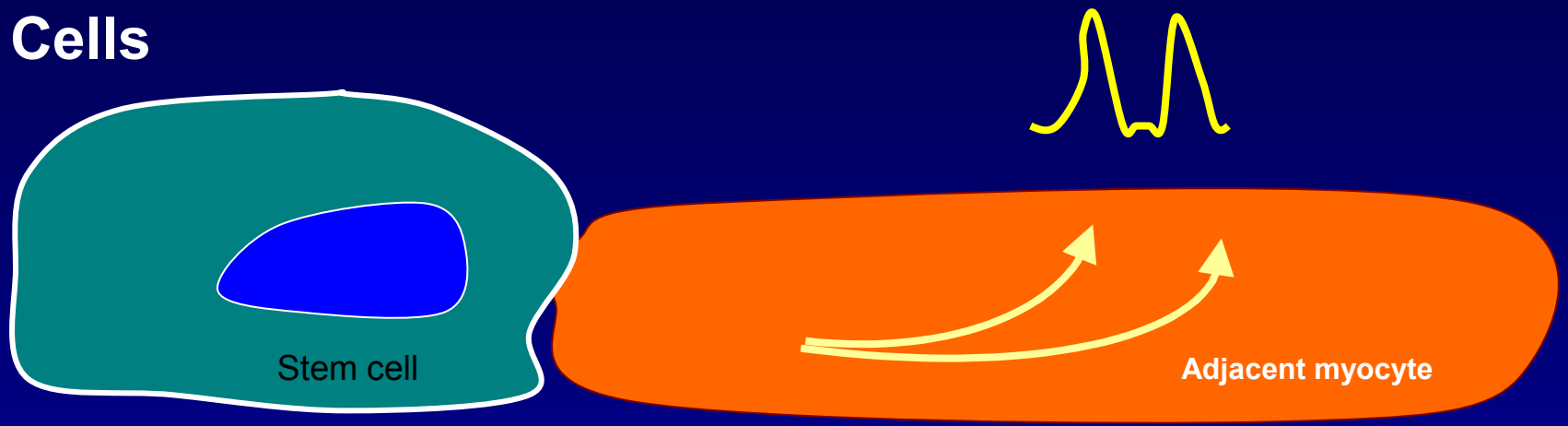


Korean Society of Circulation 2007

Modified from Rosen M et al, Cardiovasc Res: 2004:64:12-23

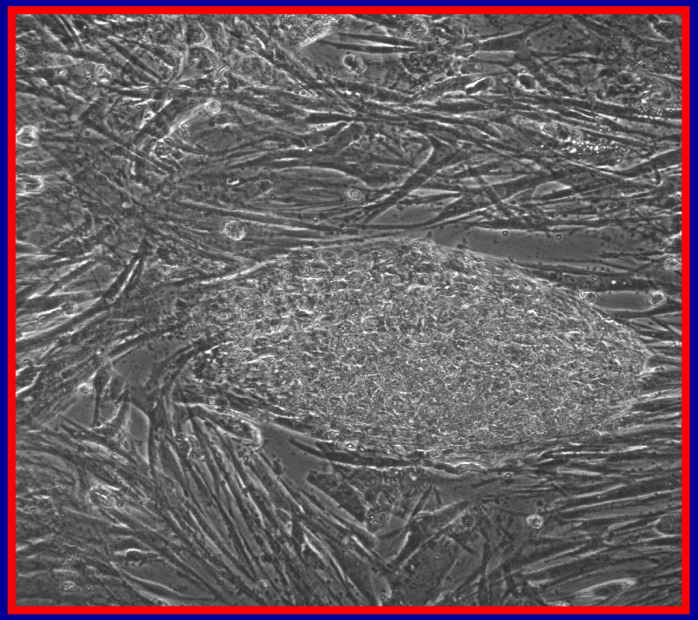
Stem Cells Therapy as Biological Pacemaker

Stem Cells

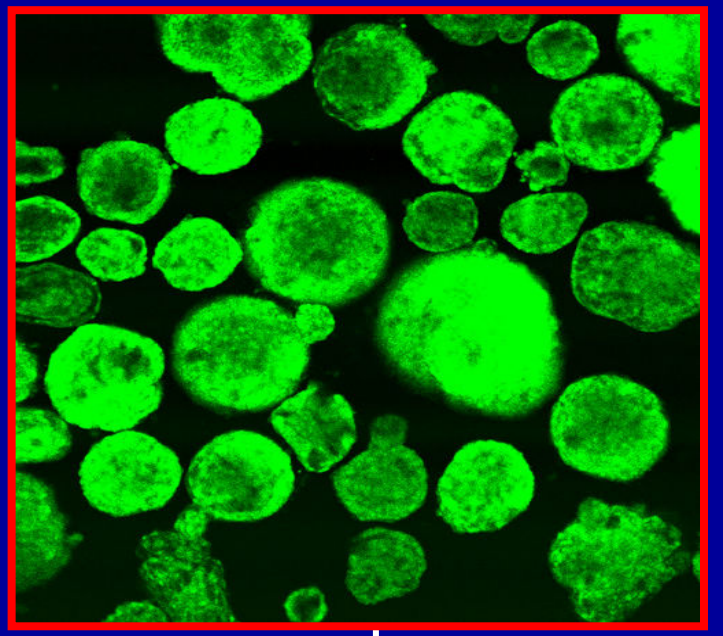


- Which cell?
- Undifferentiated vs. Differentiated Stem Cells ?
- Engraftment ?
- Mechanisms of Automaticity in Cardiomyocyte ?

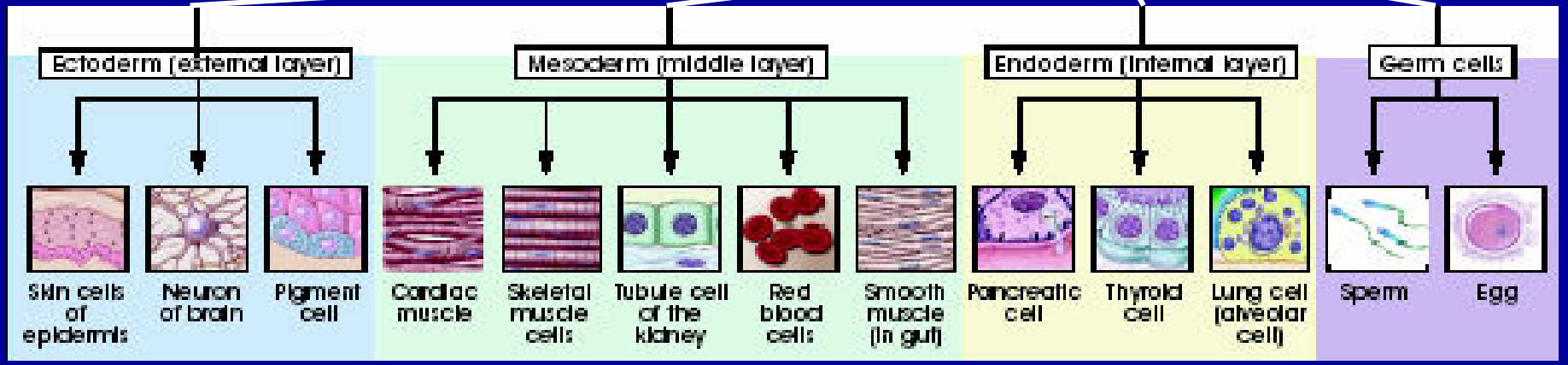
hESC COLONY



EMBRYOID BODIES

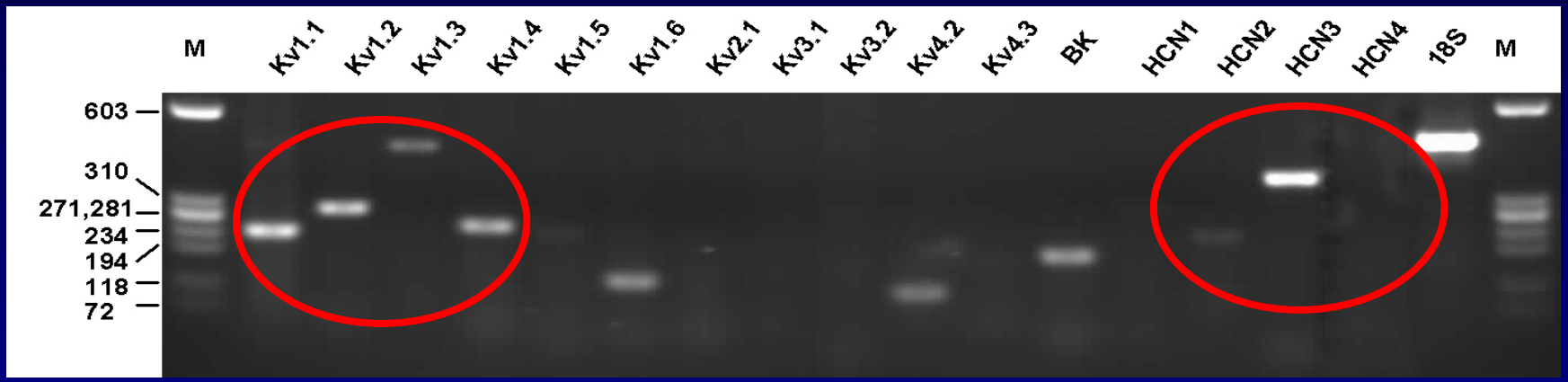


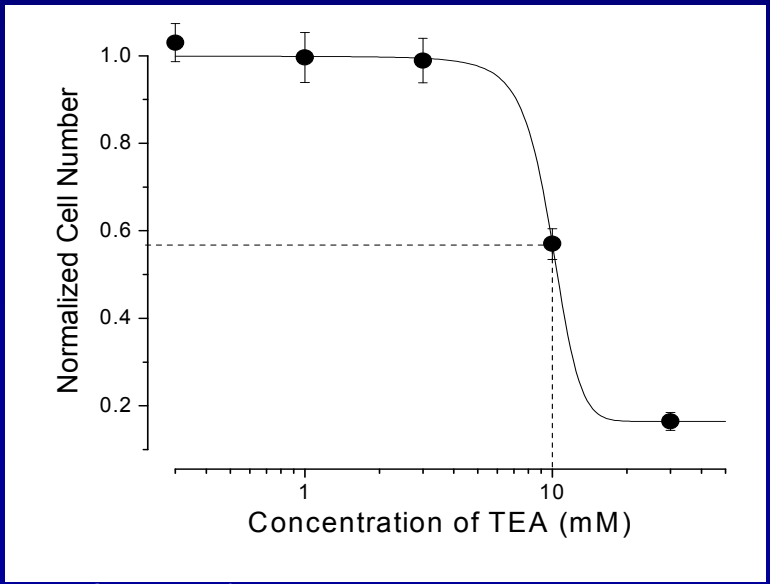
GFP : track human cells after transplantation into animals

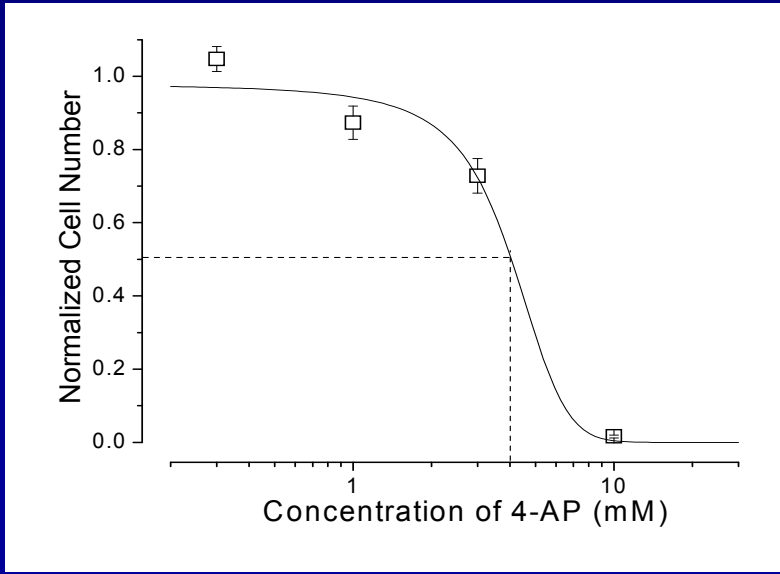


Tian Xue & Ronald Li *unpublished*

Ionic Channel Expression in Mouse and Human ESC



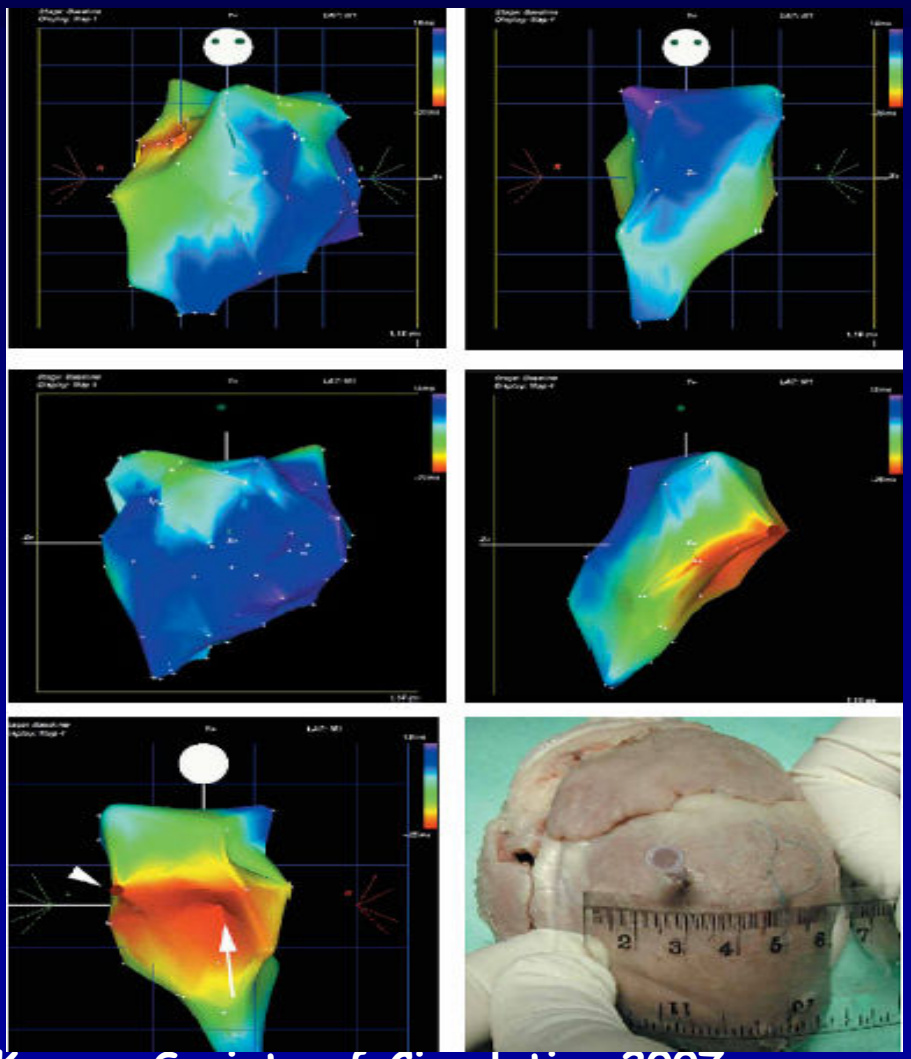




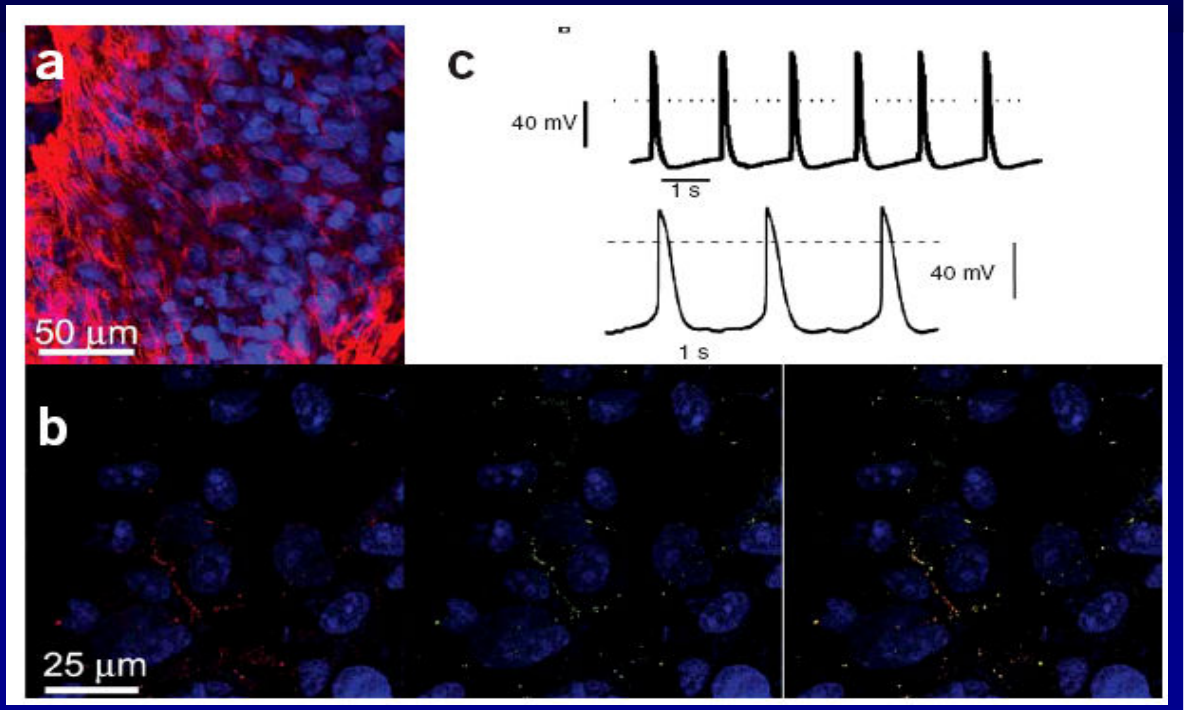
Korean Society of Circulation 2007

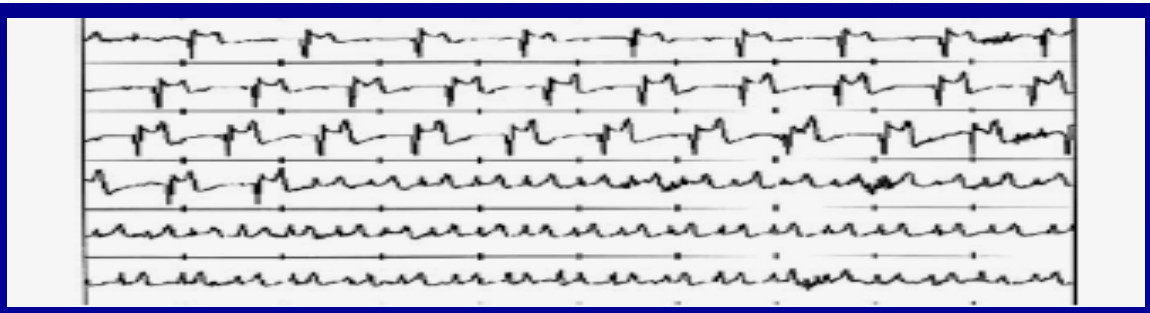
Wang G, Tse HF, Li RA. Stem Cells 2005

hESC-Biological Pacemaker



Korean Society of Circulation 2007

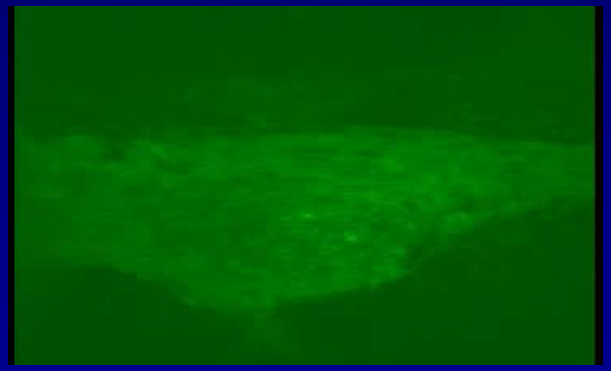




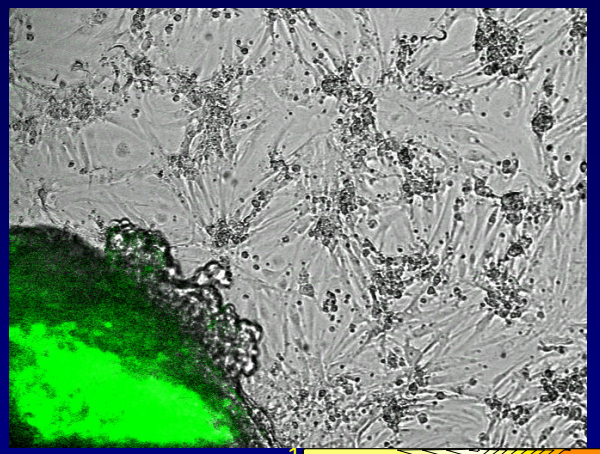
Kehat I, et al. Nat. Biotech 2004

hESC Derived CMC-Biological Pacemaker

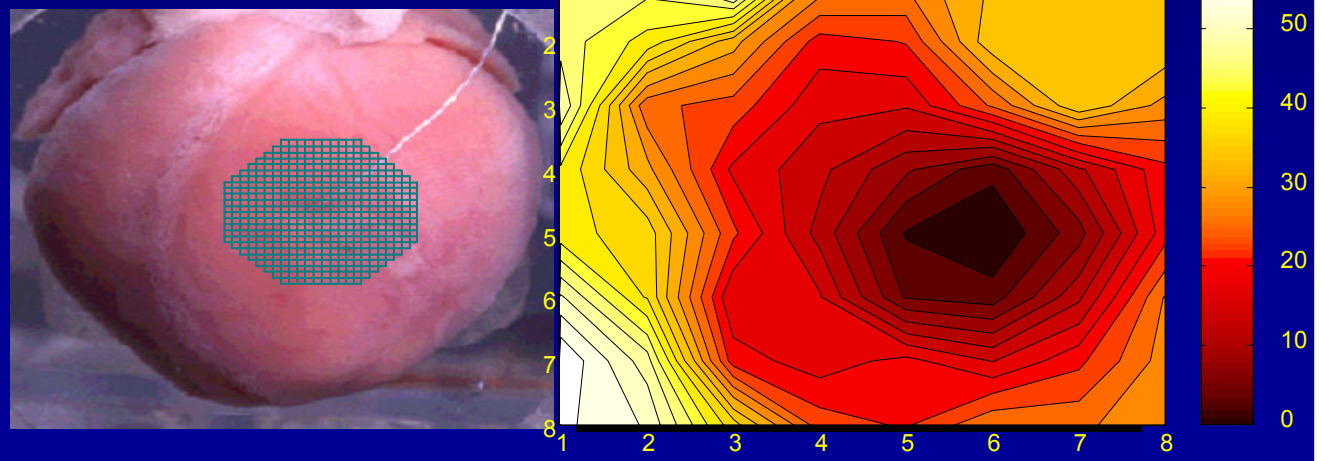
hESC-derived beating cardiomyocytes



In vitro



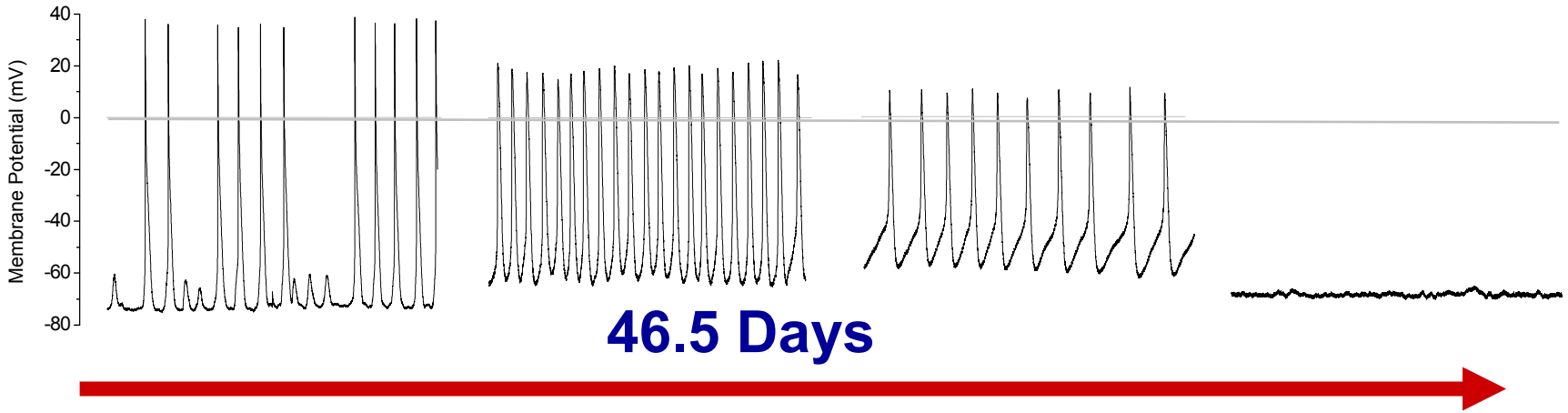
In vivo



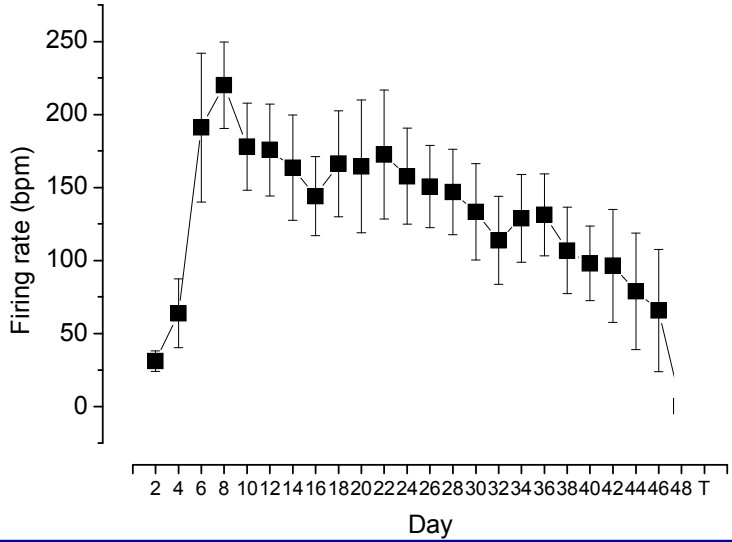
Korean Society of Circulation 2007

Xue T, Li RA, et al 2004 Circulation

Automaticity of Cardiomyocytes



Neonatal Cardiomyocytes



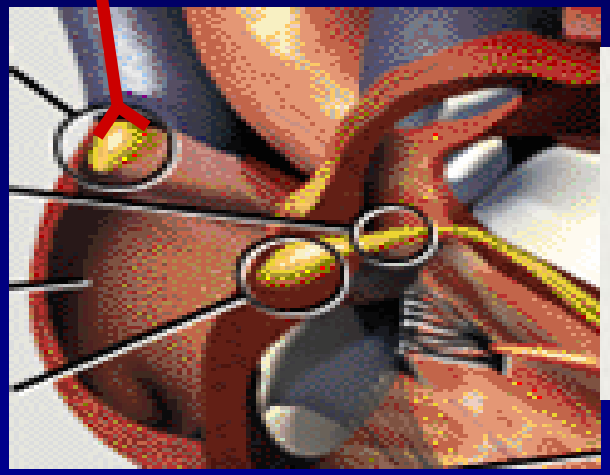
Korean Society of Circulation 2007

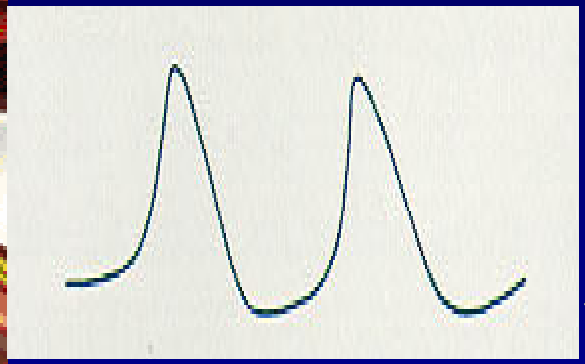
Wang G, Li R, Tse HF, et al. (unpublished data)

Mechanism of Biological Pacemaker

ANS

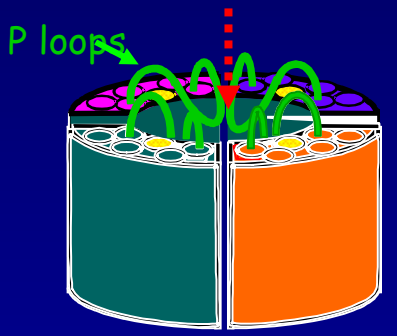
βAR+





Pacemaker current: I_f

\+



\+

\+

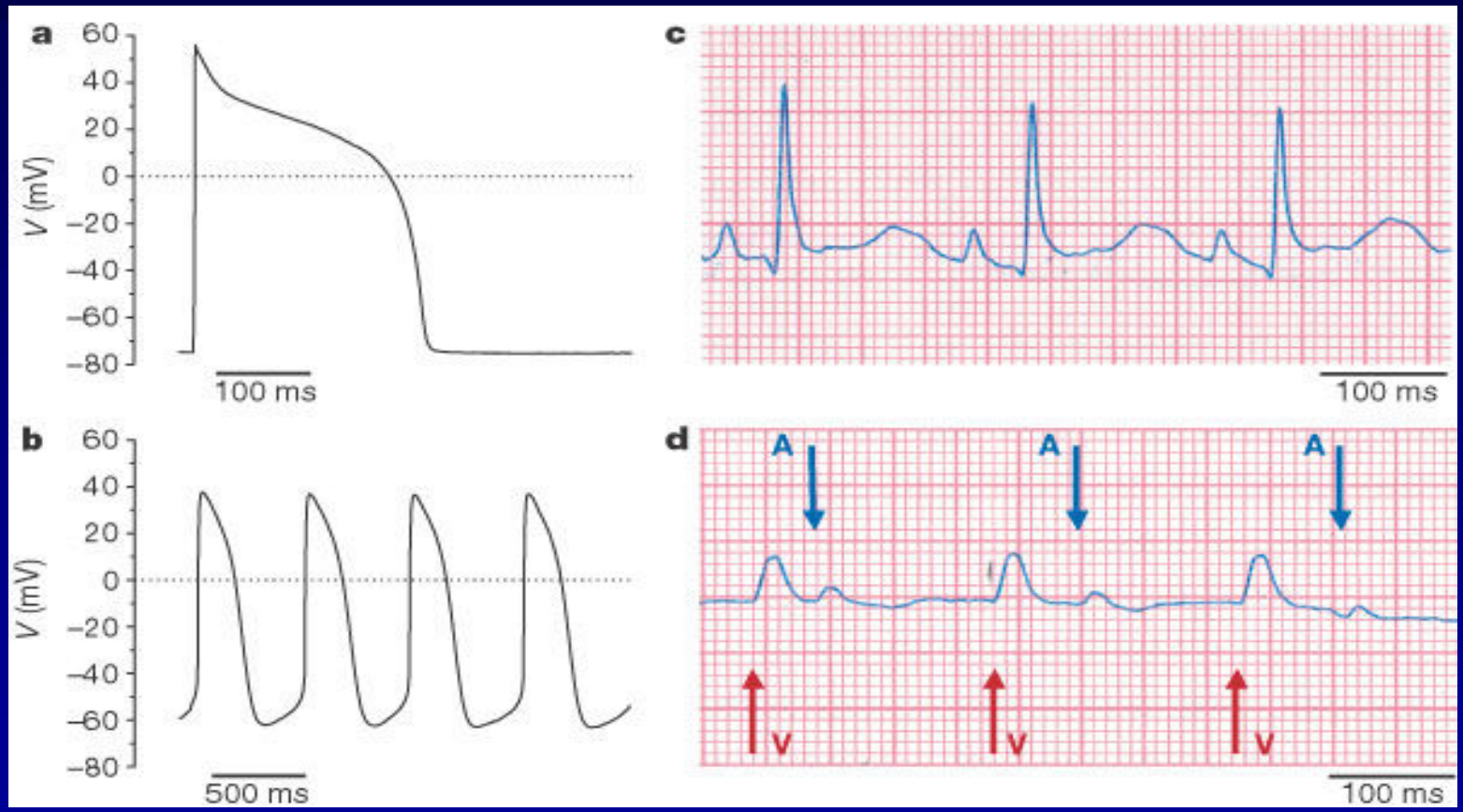
\-

Na+-dependent background current: I_{Na}

T-type Ca2+ current: I_{Ca}

Inward-rectifier current: I_{KI}

Gene Therapy to Suppress Kir2.1 for "Biological Pacemaker"

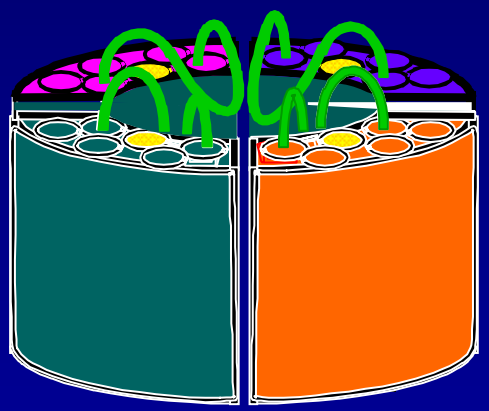


Suppression of Kir2.1 channels (I_{KI}) unleashes pacemaker activity in venticular myocytes

Korean Society of Circulation 2007

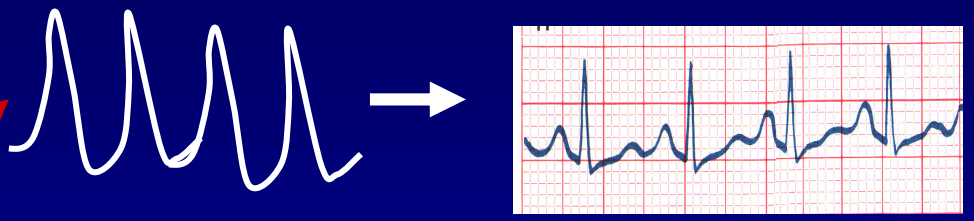
Miake J et al Nature 2002

Functional Roles of Pacemaker current?

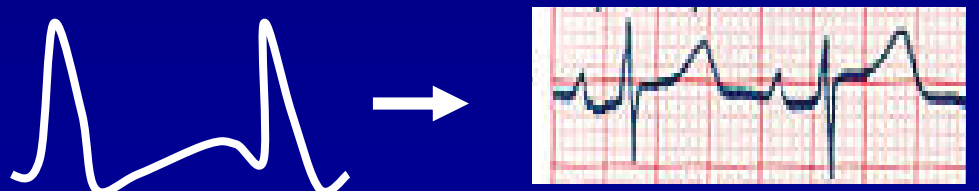


HCN Channels

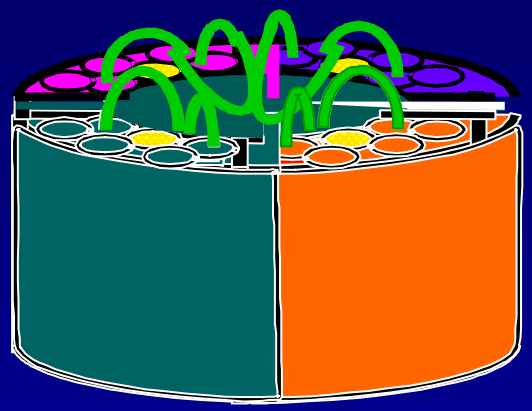
\+



\-



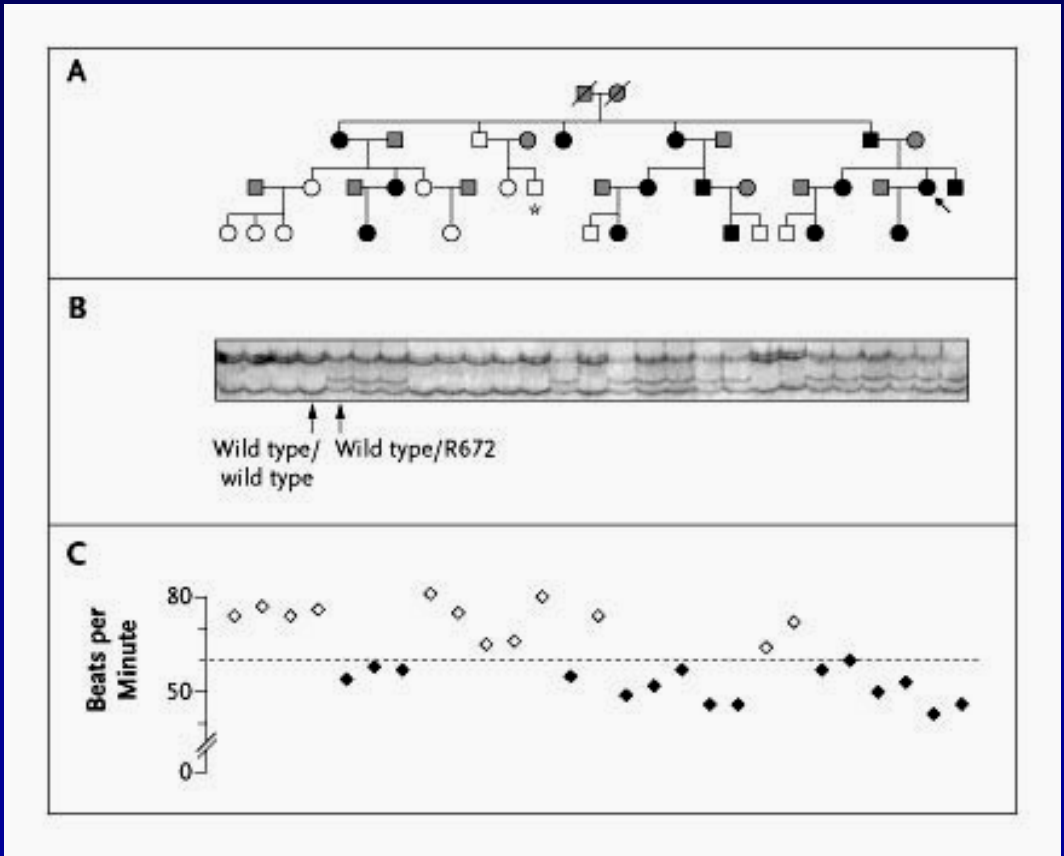
Functional Roles of Pacemaker current?



HCN Channels

- 4 isoforms, namely HCN1-4, each with a distinct pattern of tissue distribution and biophysical profiles
- Of the two predominant isoforms in the SA node, time-dependent HCN1 currents open ~40 times faster than those of HCN4 channels
- the fastest isoform HCN1 activates at ~-80mV with opening time constants in the range of seconds.

Familial Sinus Bradycardia: HCN4 Mutation



HCN4 mutation at cyclic nucleotide-binding domain region

↓

Affect cAMP binding

↓

Modify channel kinetics by shifting the current activation range to hyperpolarized voltages & slowing current deactivation: mimic vagal stimulation

↓

↓inward current during diastolic depolarization

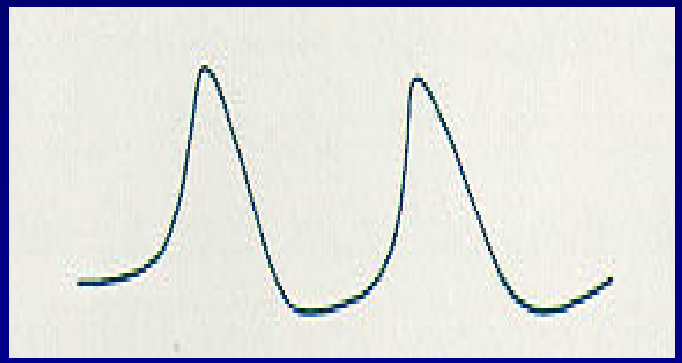
↓

↓heart rate.

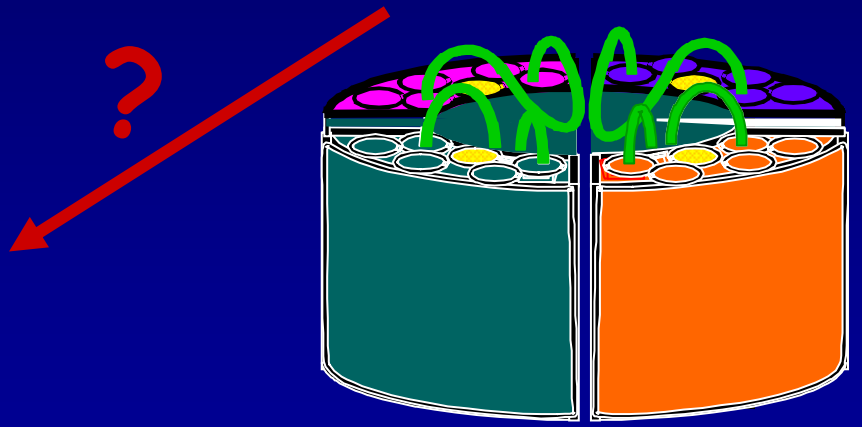
Korean Society of Circulation 2007

Milanesi et al. NEJM 2006;354:151-7

Possible Mechanisms of Automaticity of Cardiomyocytes



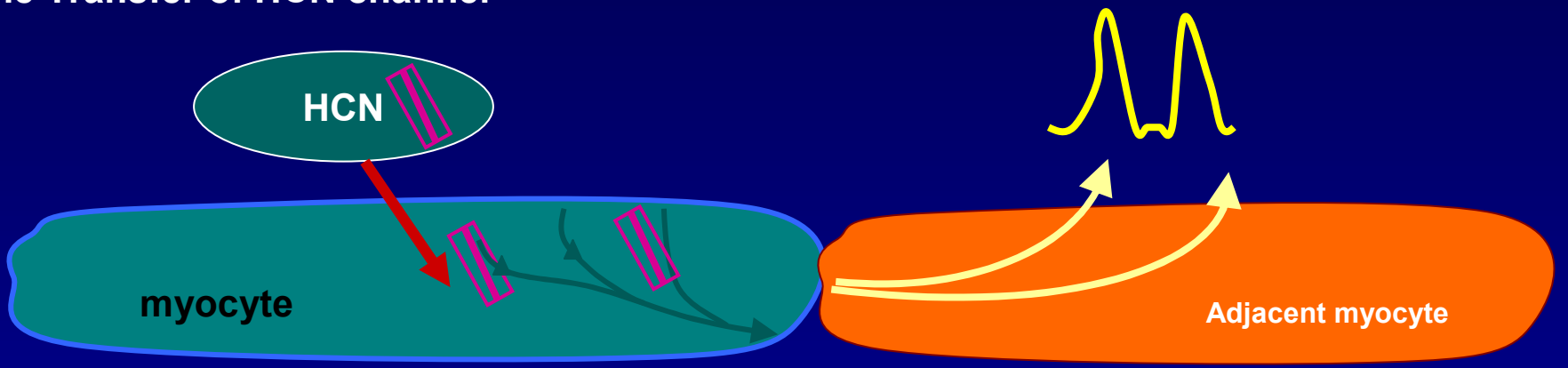
Pacemaker current: I_f



HCN Channel

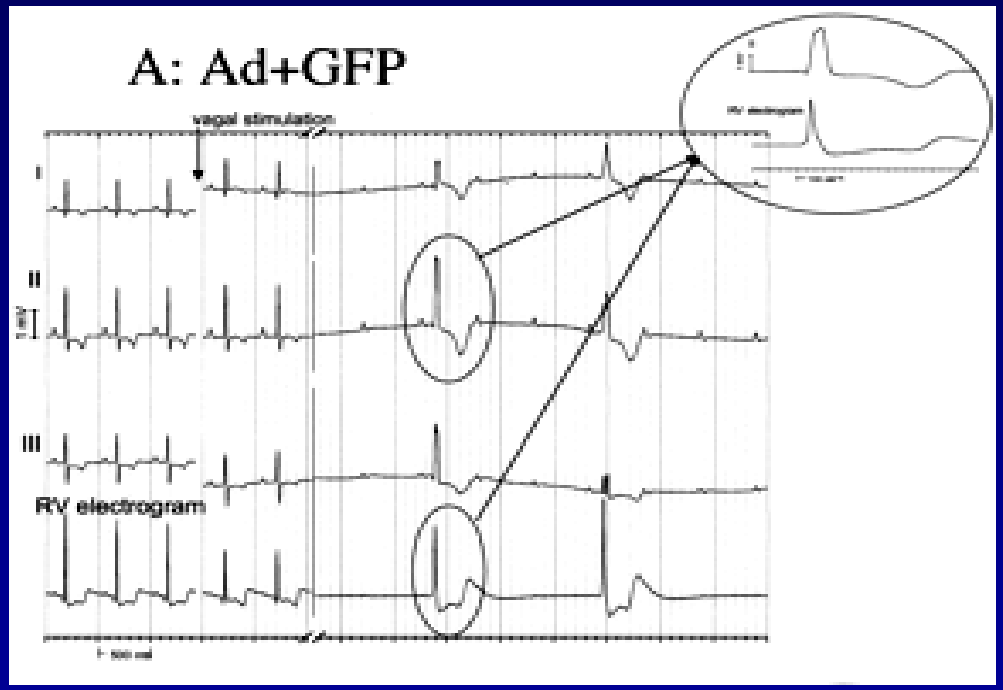
Gene Therapy for Biological Pacemaker

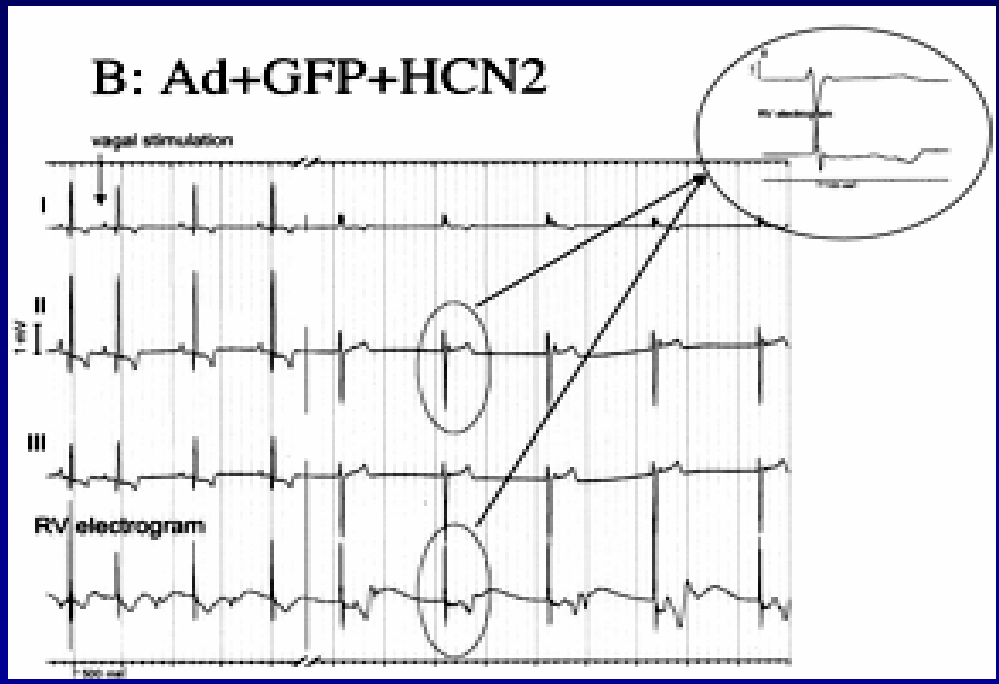
Gene Transfer of HCN channel



- Atrial vs. Ventricular CMC ?
- Kinetic of HCN Channels ?
- Integration and function?

HCN2 Gene Transfer to LV as Biological Pacemaker

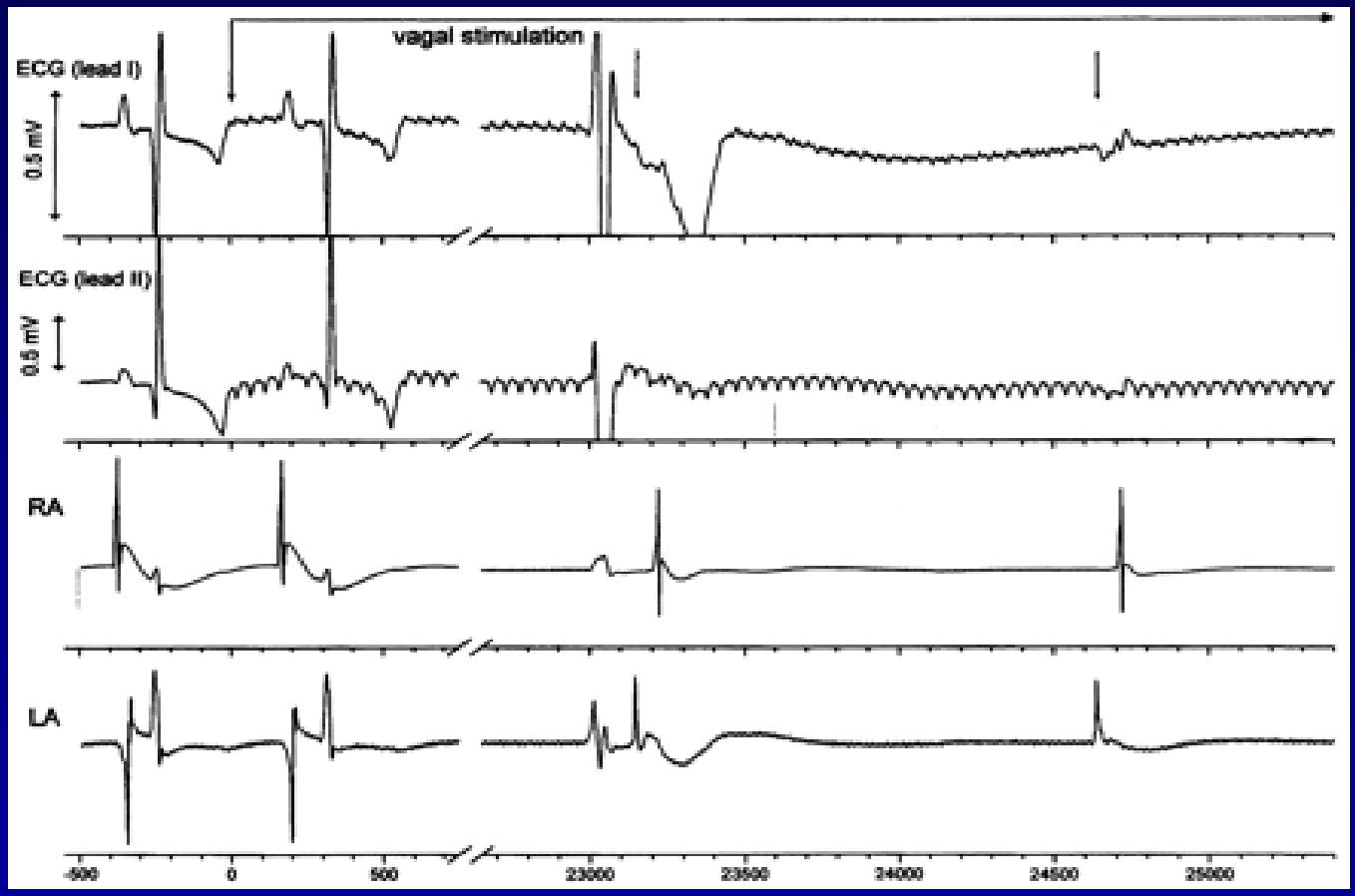




Korean Society of Circulation 2007

Plotnikov AN, et al. Circulation 2004;109:501

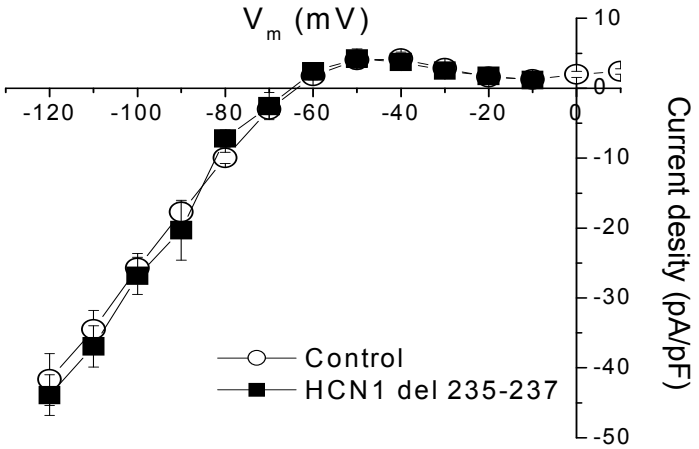
HCN2 Gene Transfer to LA as Biological Pacemaker

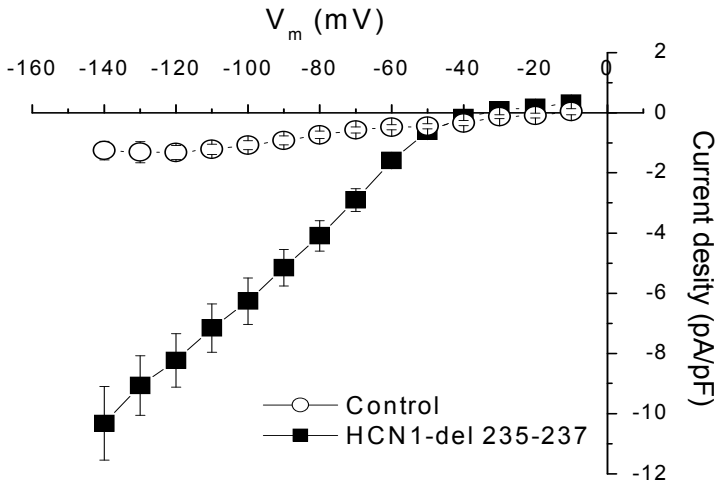


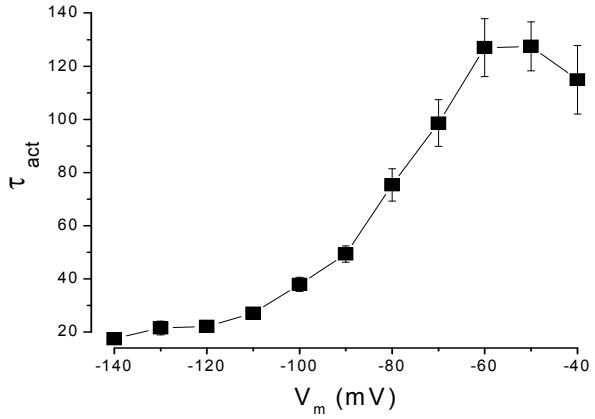
Korean Society of Circulation 2007

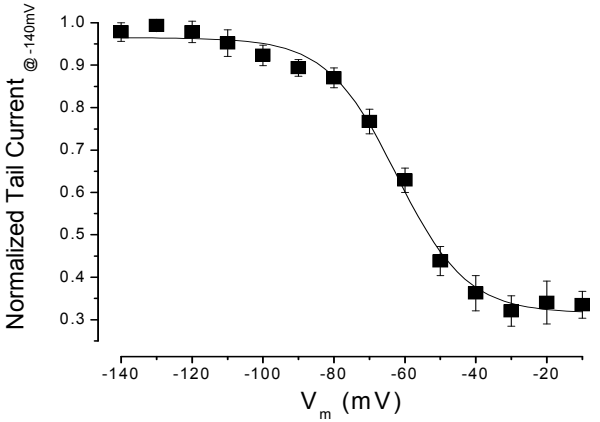
Qu, J. et al. Circulation 2003;107:1106-1109

Bio-engineering of HCN1 Channel









Tse HF, et al. Circulation 2006; 114:1000-1011

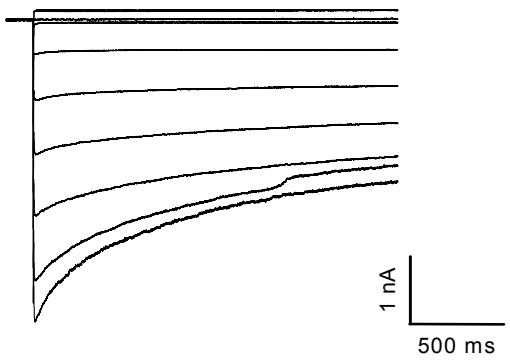
Korean Society of Circulation 2007 Yuan X, Siu CW, Tse HF, Li R, et al. Circulation (in press)

Bio-engineering of HCN1 Channel

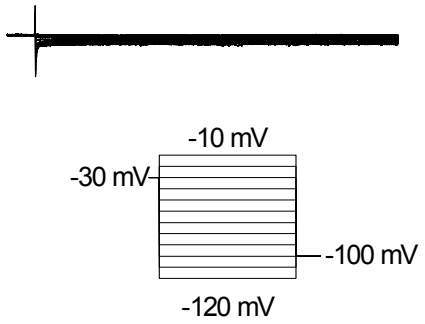
Gpig LVCM

HCN1ΔΔΔ

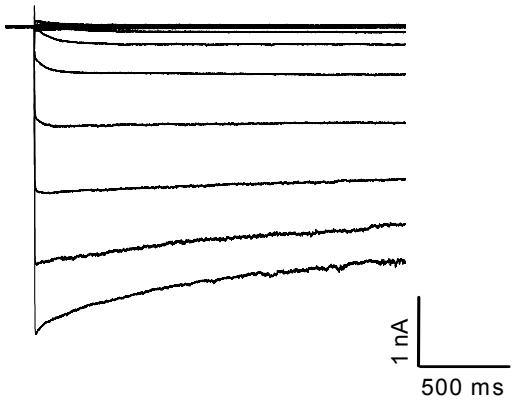
A



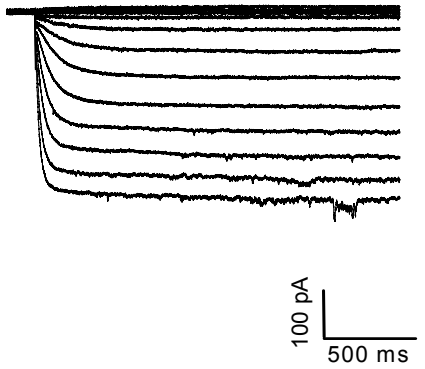
B



C

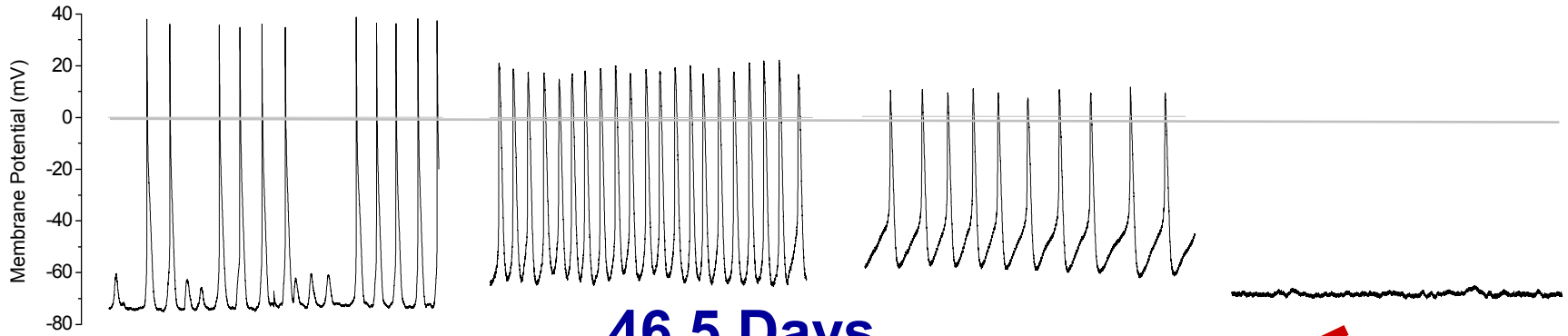


D



Tse HF, et al. Circulation 2006; 114:1000-1011
Tian X, Siu CW, Tse HF, Li R, et al. Circulation 2007

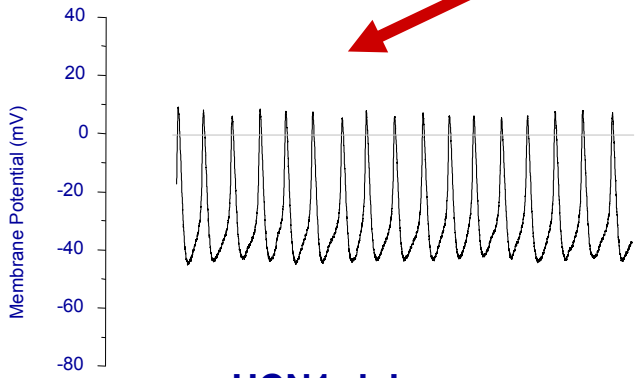
HCN Transfer on Automaticity of Cardiomyocytes



46.5 Days

Neonatal Cardiomyocytes

Transfection with Ad-CGI-HCN1-Δ Δ Δ

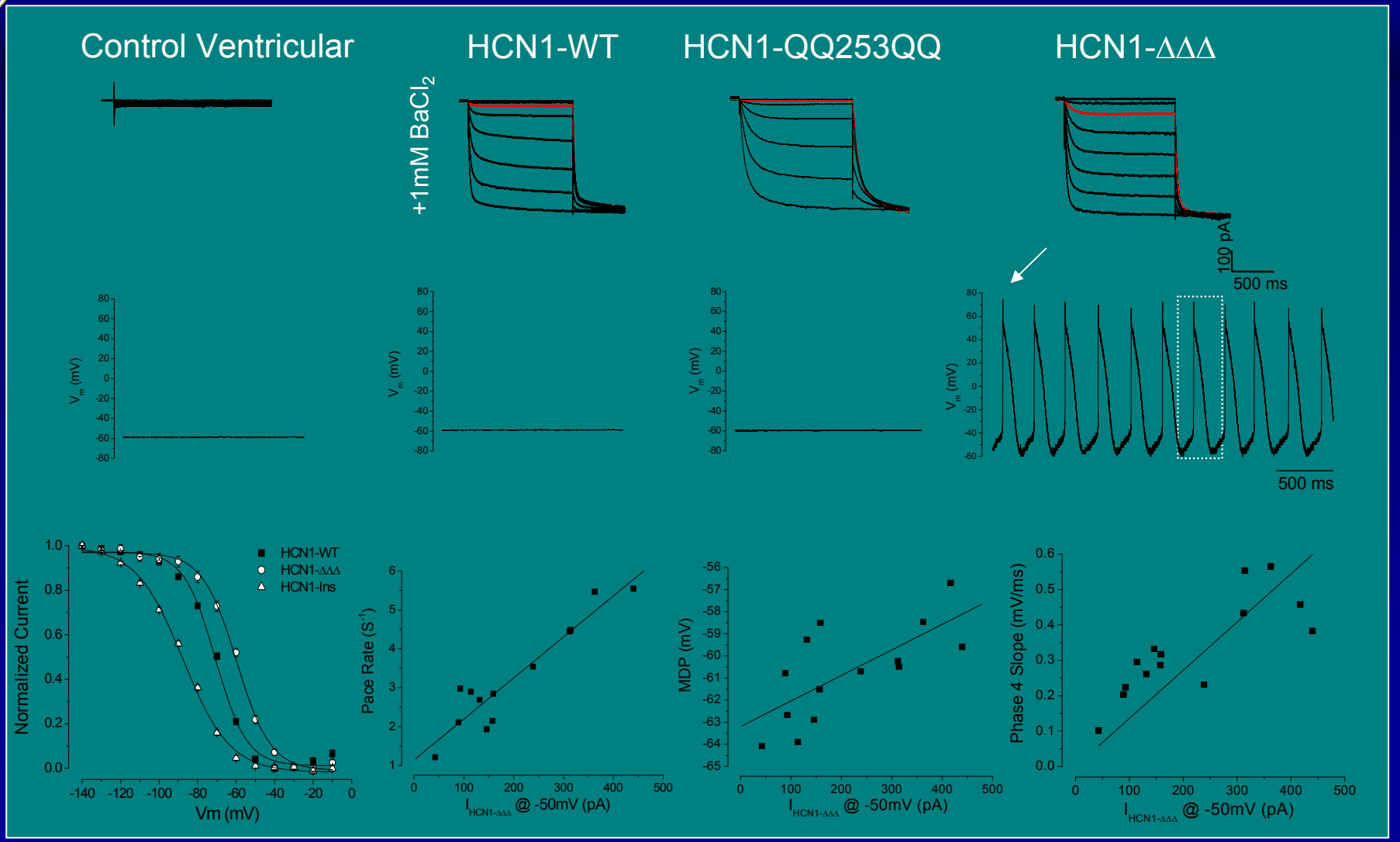


HCN1-del

Korean Society of Circulation 2007

Wang G, Li R, Tse HF, et al. (submitted)

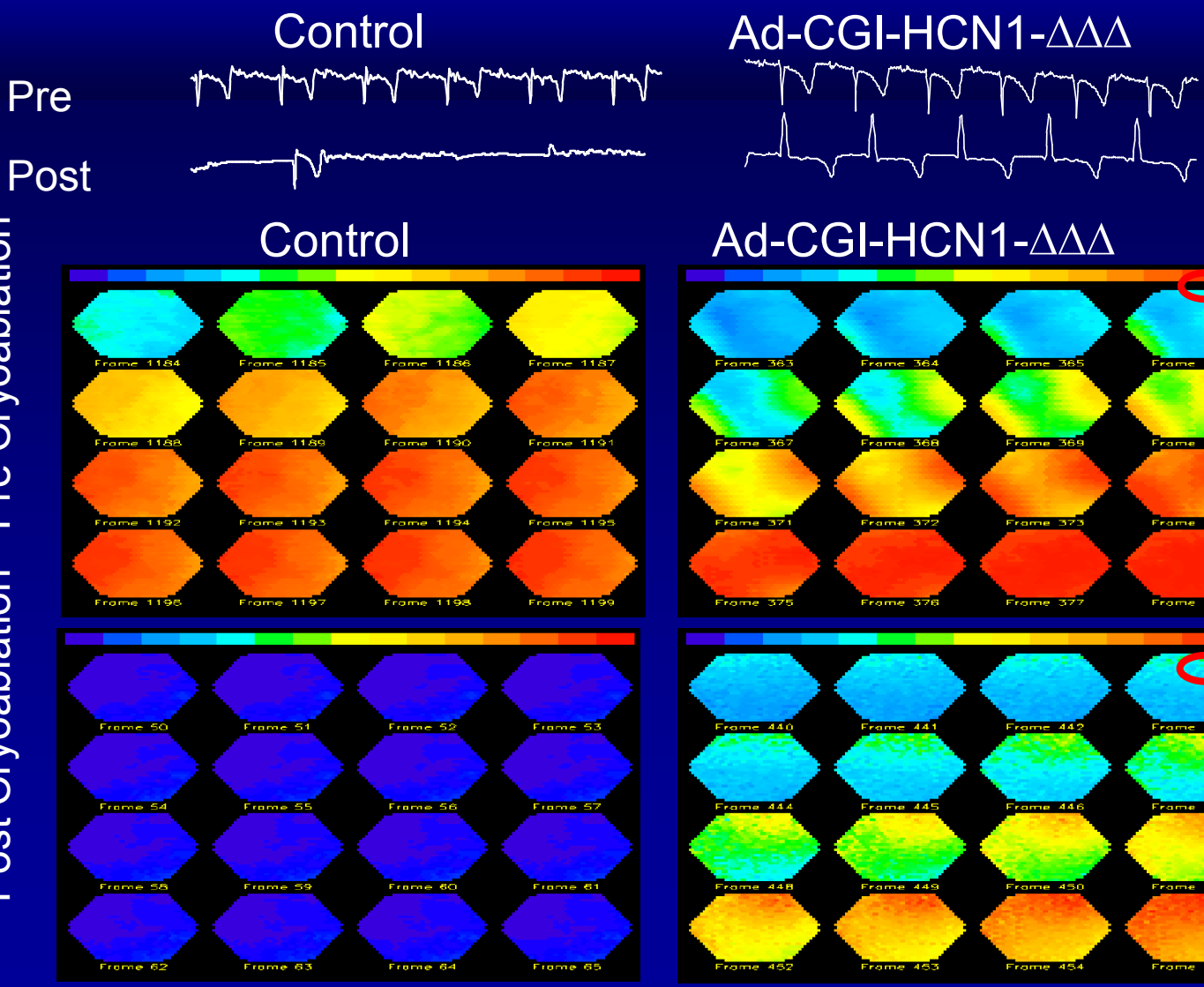
Transdifferentiation of Adult Atrial & Ventricular into Pacemaker cells



Korean Society of Circulation 2007

Xue T, CW Siu, Tse HF, Li R, et al 2007 *Circulation*

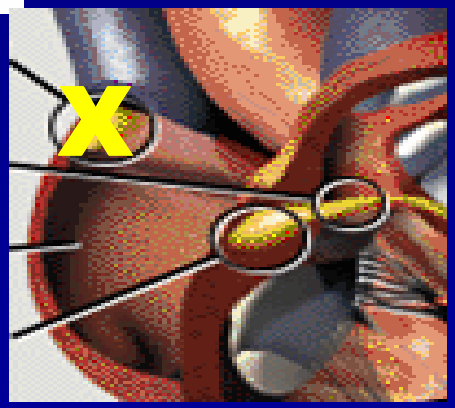
Optical Mapping of Guinea Pig LV



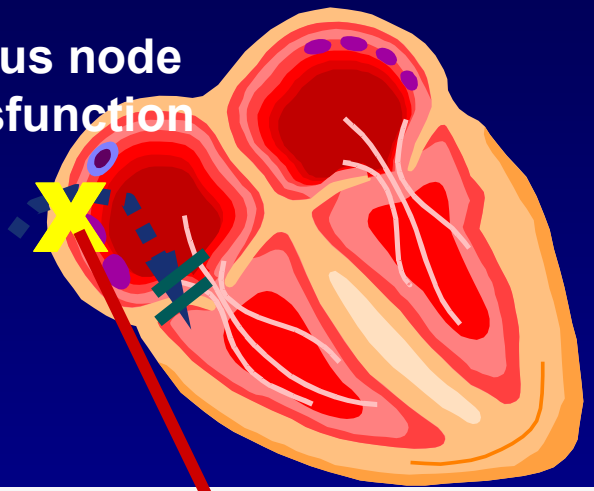
Korean Society of Circulation 2007

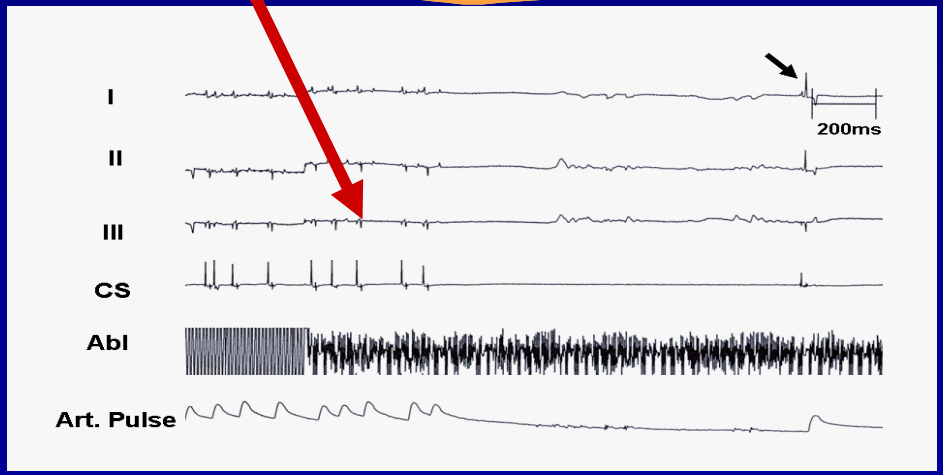
Tse HF, et al. Circulation 2006; 114:1000-1011

Animal Model of SA Node Dysfunction



Sinus node dysfunction

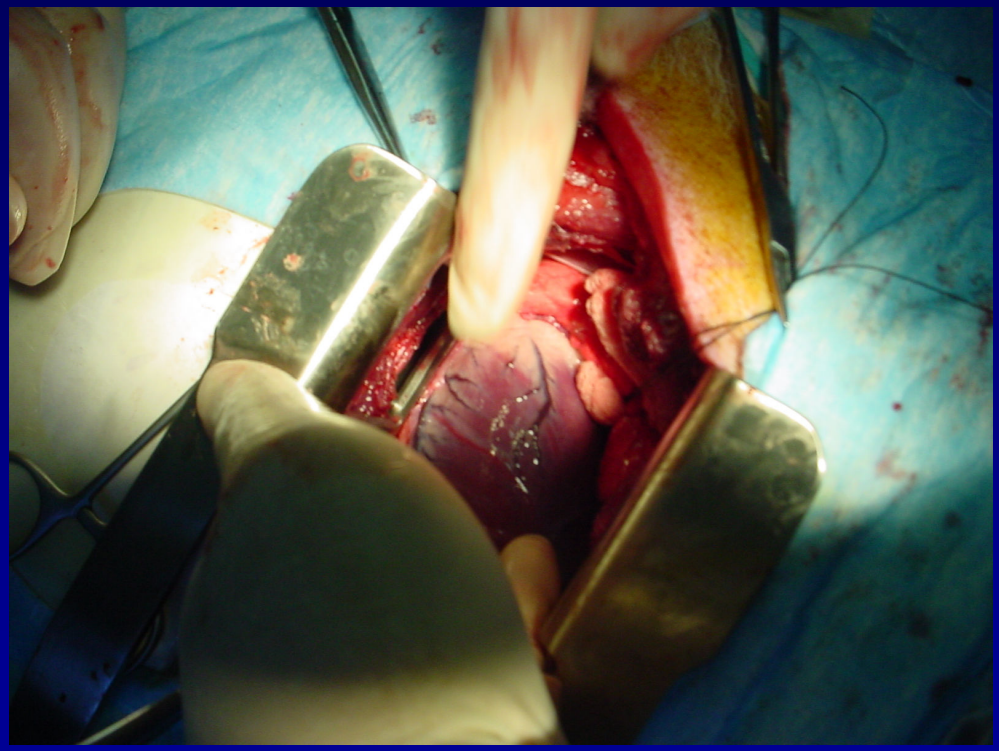




Korean Society of Circulation 2007

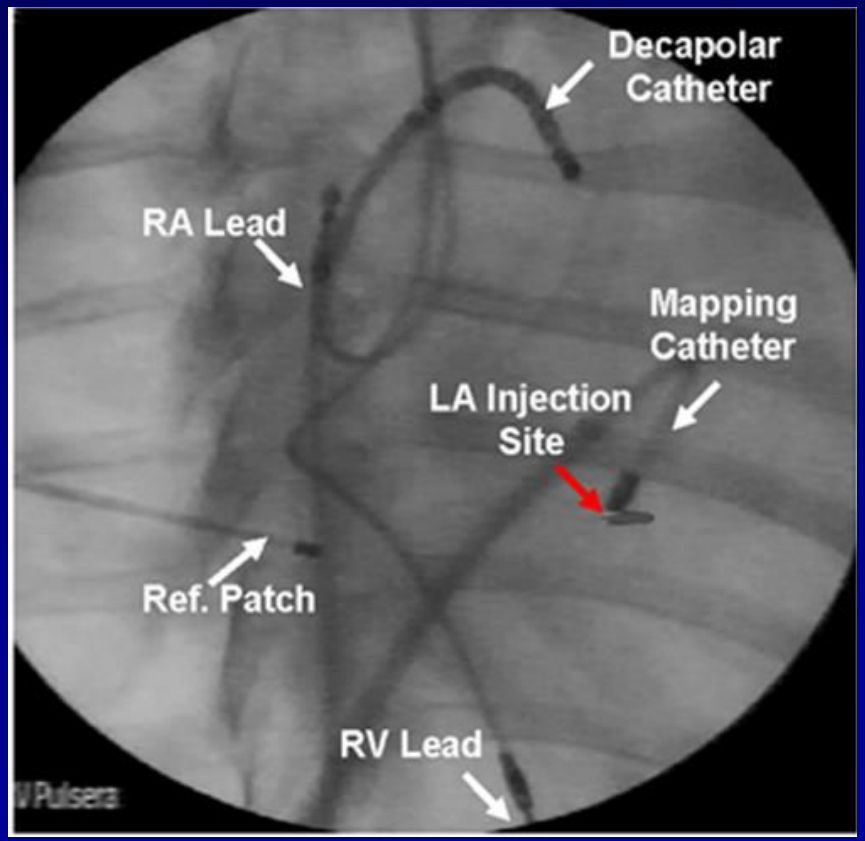
Tse HF, et al. Circulation 2006; 114:1000-1011

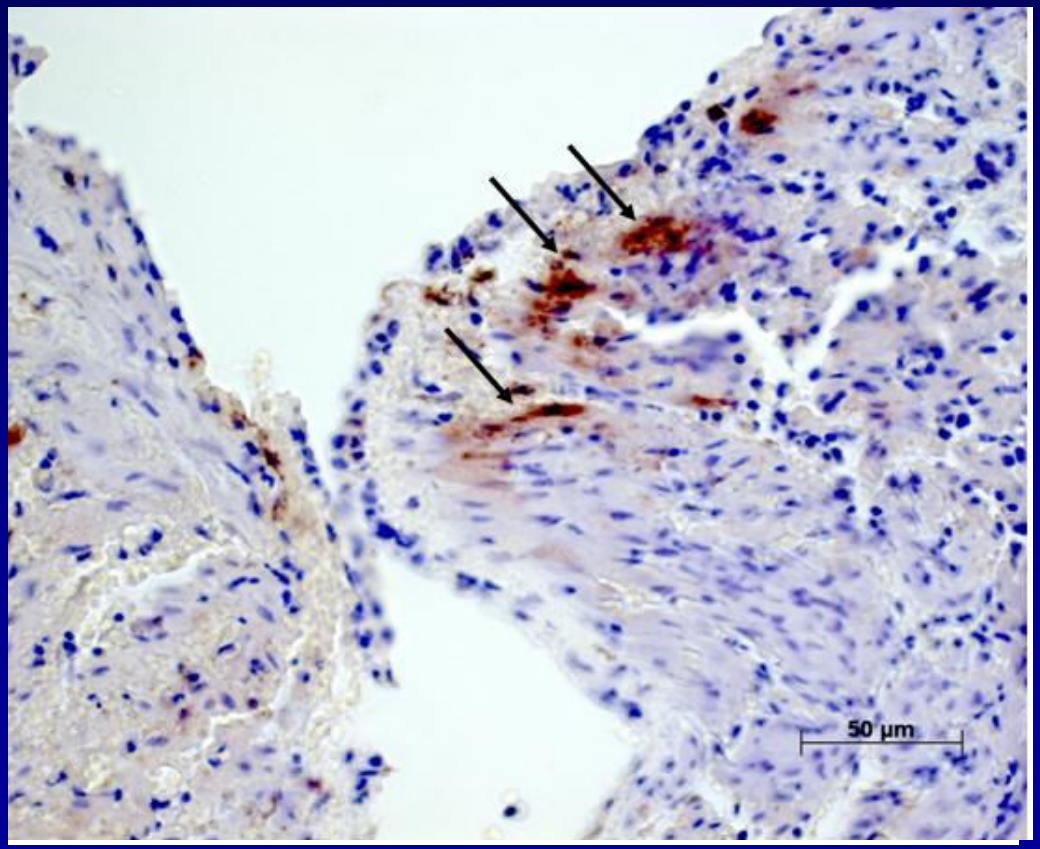
In-Vivo Mutant HCN Transfer



Korean Society of Circulation 2007

In-Vivo Mutant HCN Transfer



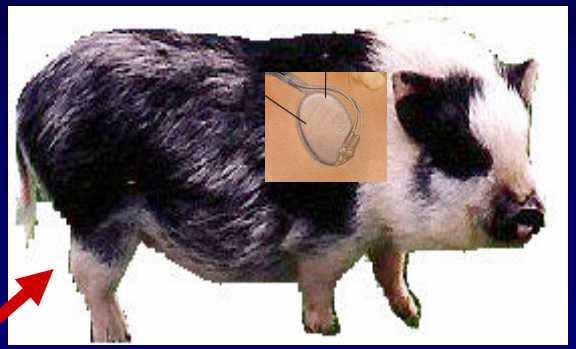


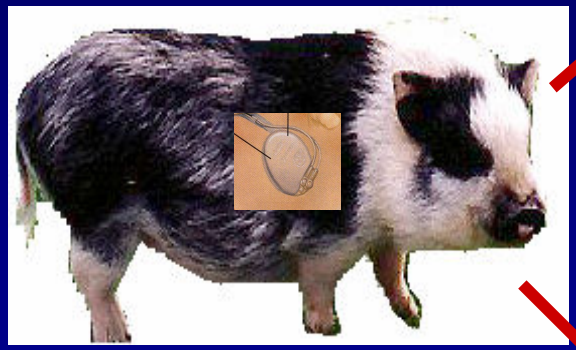
Korean Society of Circulation 2007

Tse HF, et al. Circulation 2006; 114:1000-1011

In-Vivo Mutant HCN Transfer

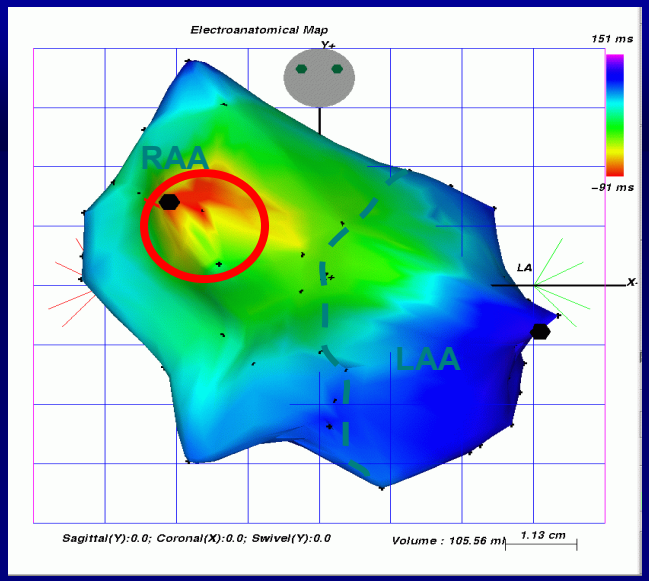
Saline (n=2) or GFP (n=2)

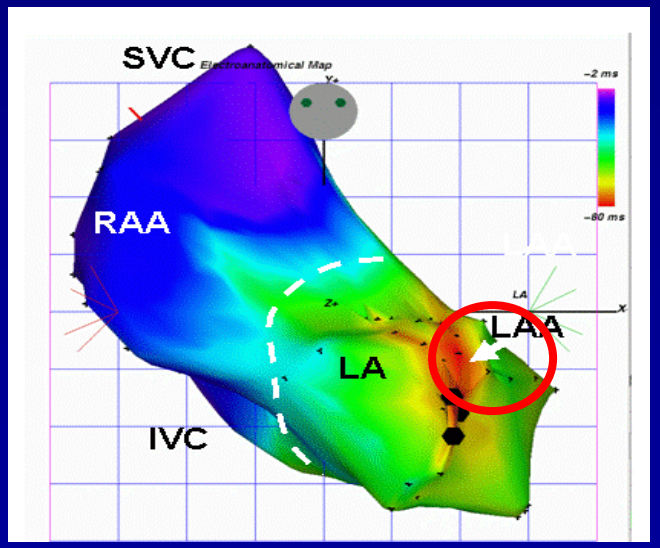




2 weeks

Ad-CGI-HCN1-ΔΔΔ (n=5)

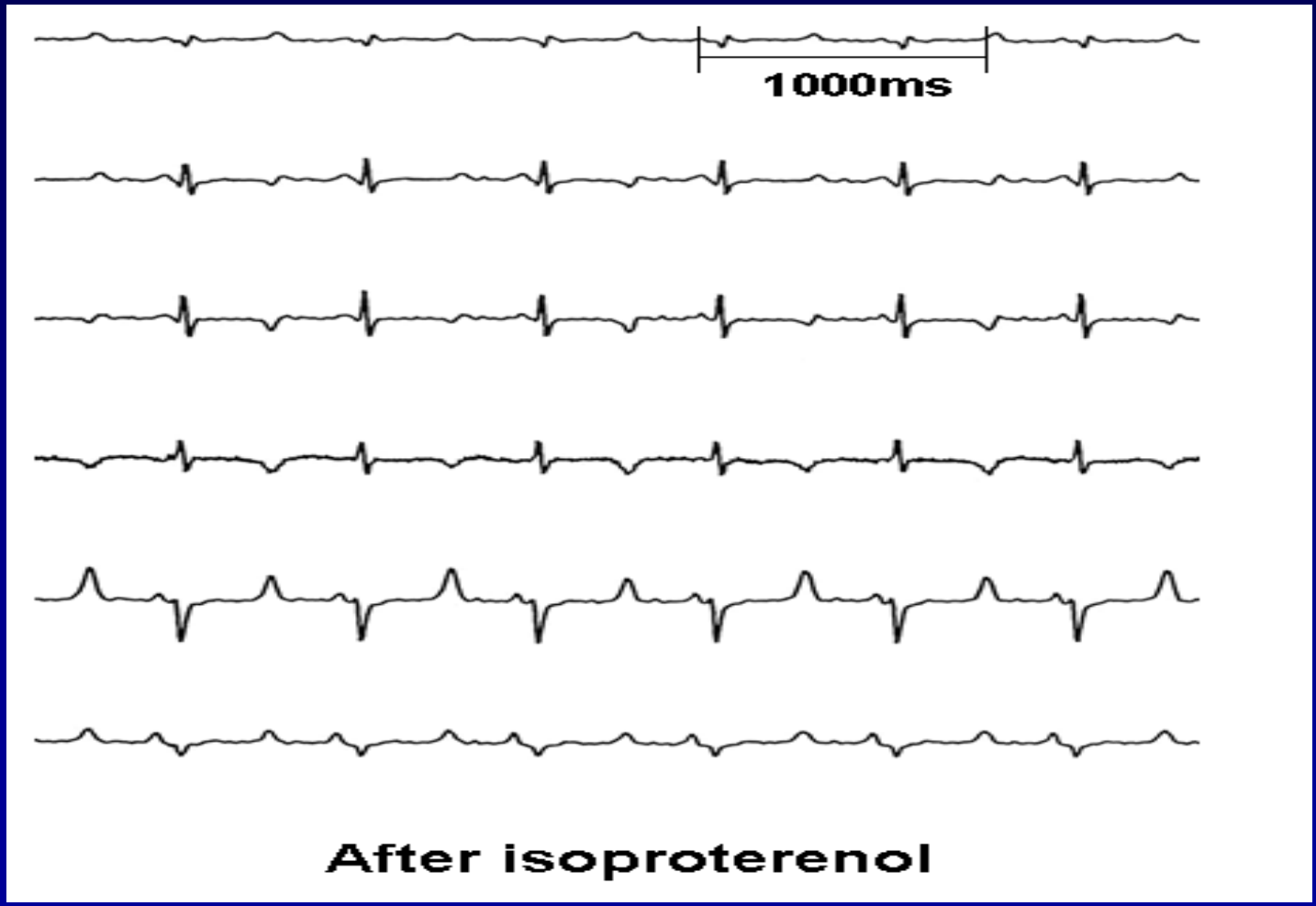




Korean Society of Circulation 2007

Tse HF, et al. Circulation 2006; 114:1000-1011

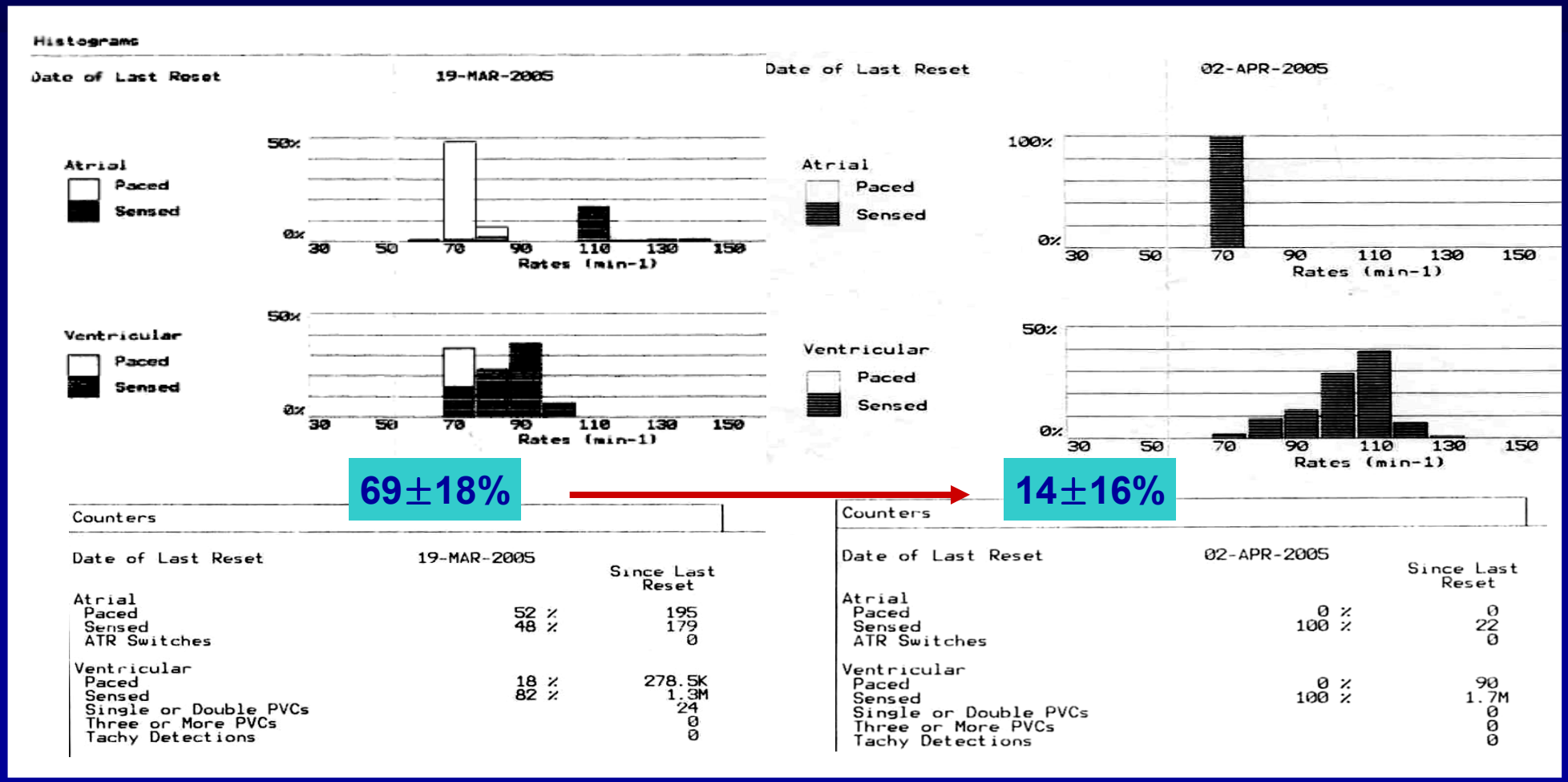
Spontaneous LA Rhythm after Mutant HCN1 Gene Transfer



Korean Society of Circulation 2007

Tse HF, et al. Circulation 2006; 114:1000-1011

Spontaneous LA Rhythm after Mutant HCN1 Gene Transfer



Before Ad-CGI-HCN1-ΔΔΔ

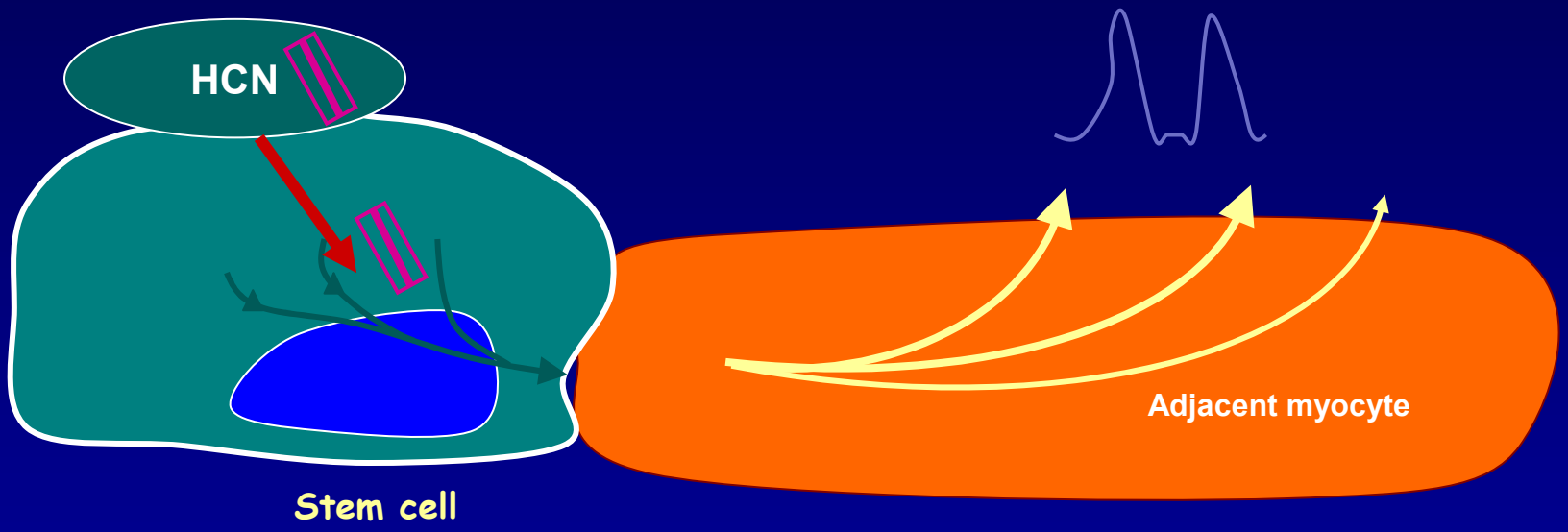
After Ad-CGI-HCN1-ΔΔΔ

Korean Society of Circulation 2007

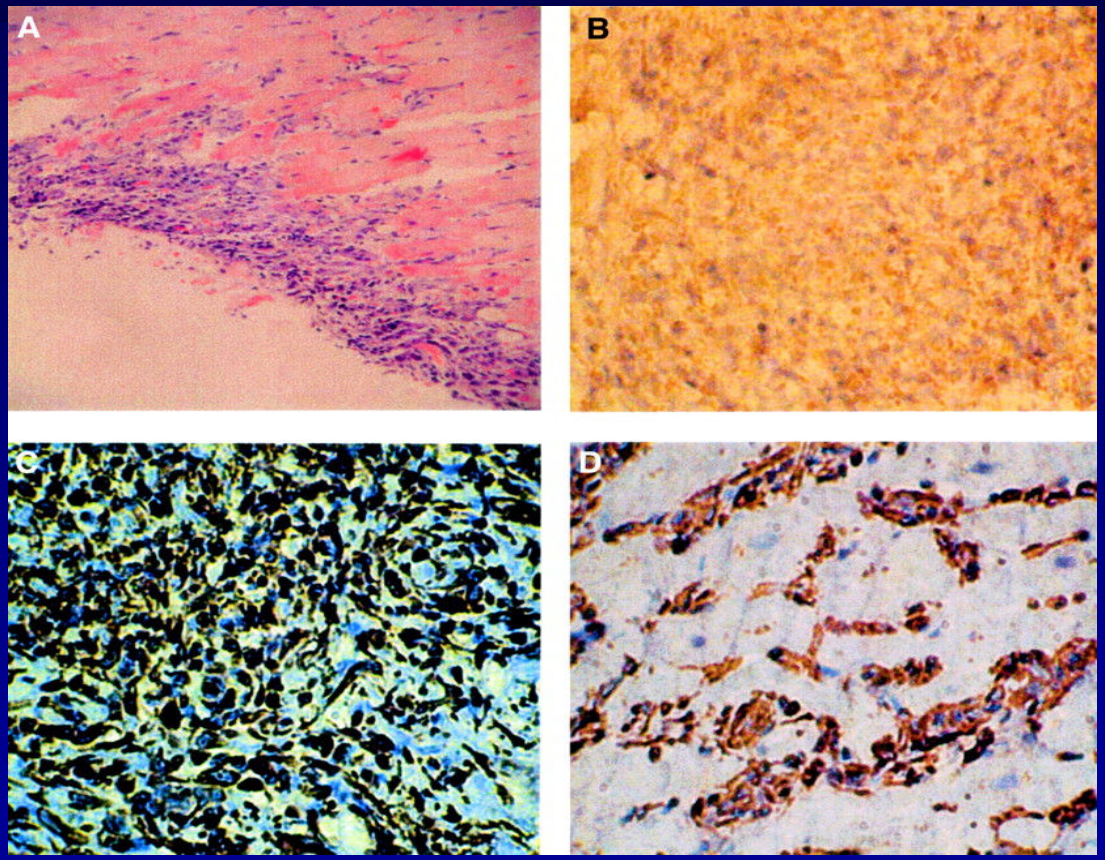
Tse HF, et al. Circulation 2006; 114:1000-1011

Hybrid (Cell + Gene) Approaches for Biological Pacemaker

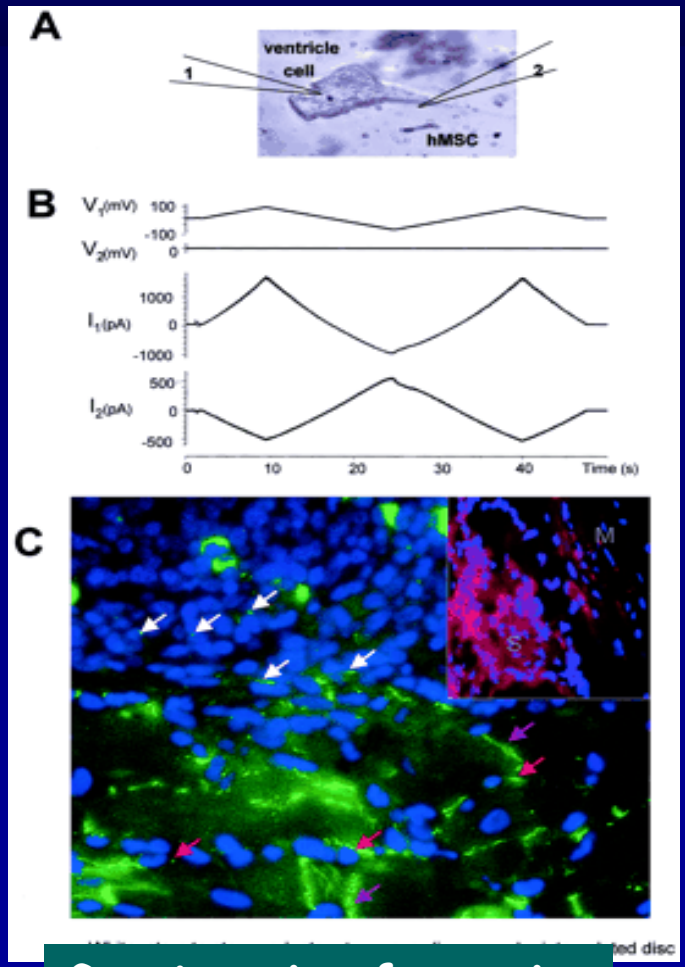
Gene Transfer of HCN channel to stem cells



HCN2 Gene Transfer to hMSC as Biological Pacemaker



Integration of hMSC to canine myocardium

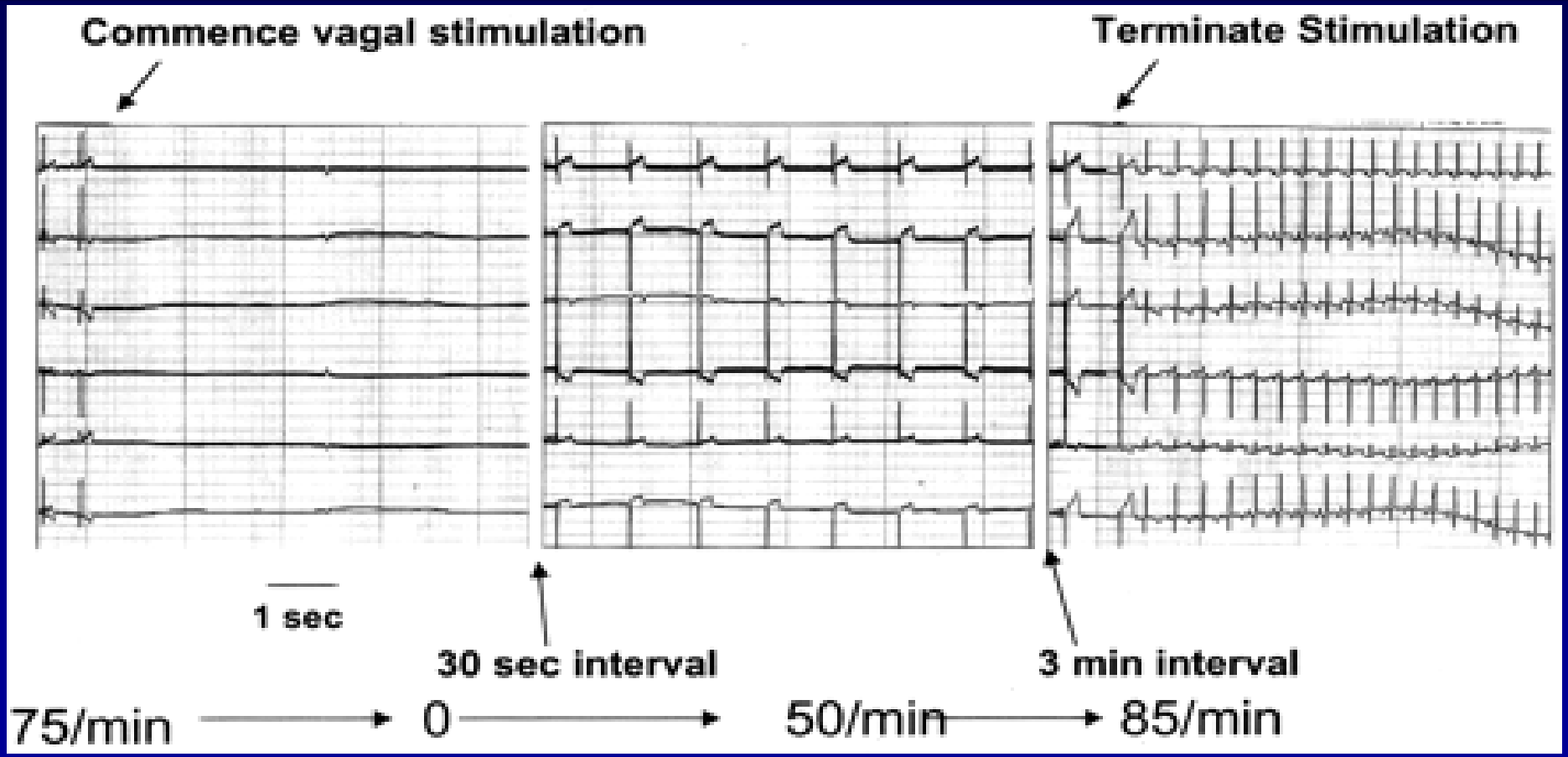


Gap junction formation

Korean Society of Circulation 2007

Potapova, I. et al. Circ Res 2004;94:952-959

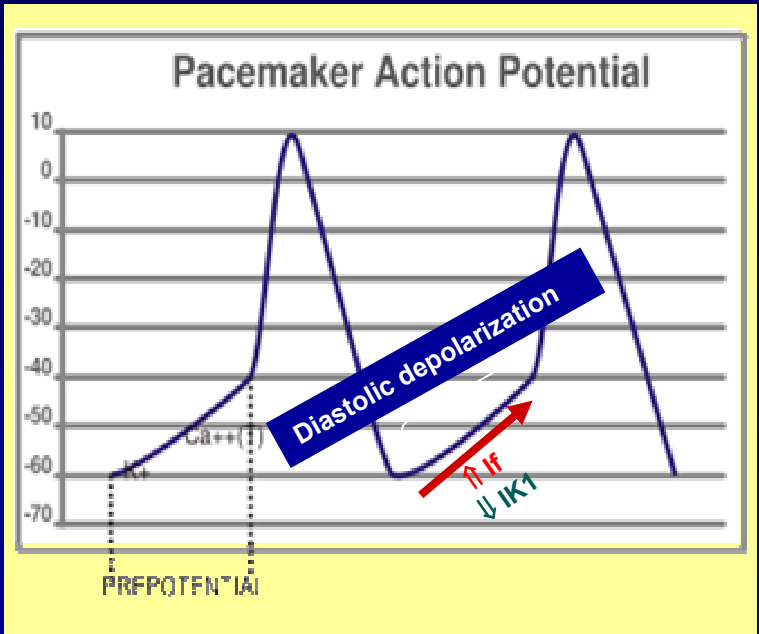
HCN2 Gene Transfer to hMSC as Biological Pacemaker



Korean Society of Circulation 2007

Potapova, I. et al. Circ Res 2004;94:952-959

Modulation of Biological Pacemaker



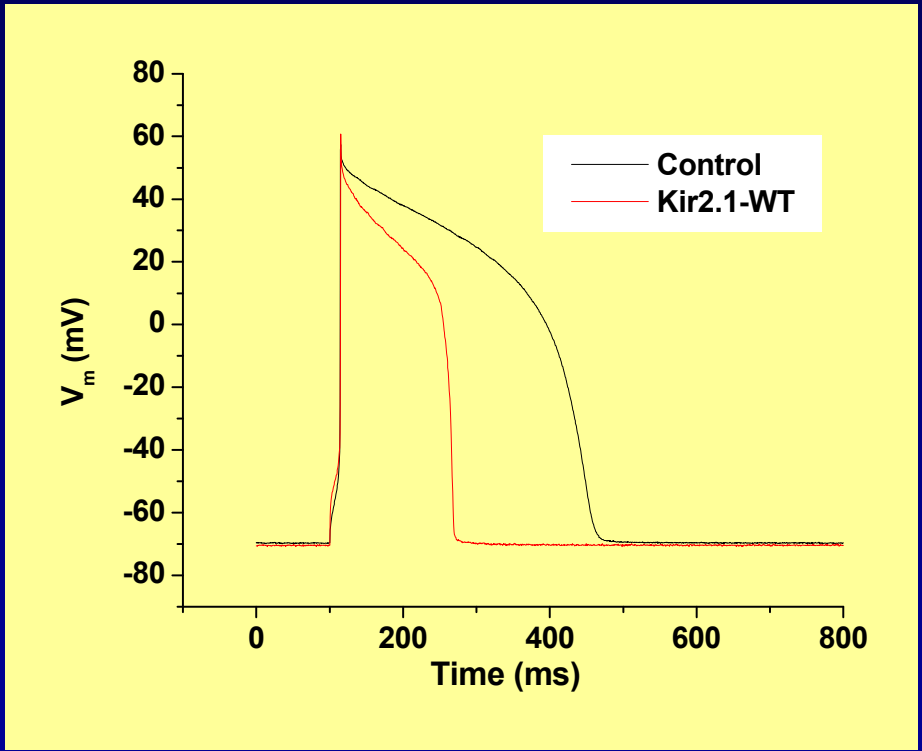
I_{K1}	I_f	τ_y	$V_{1/2}$
0.00	1.00	1.00	-70

I_{K1}	I_f	τ_y	$V_{1/2}$
1.00	1.00	1.00	-70

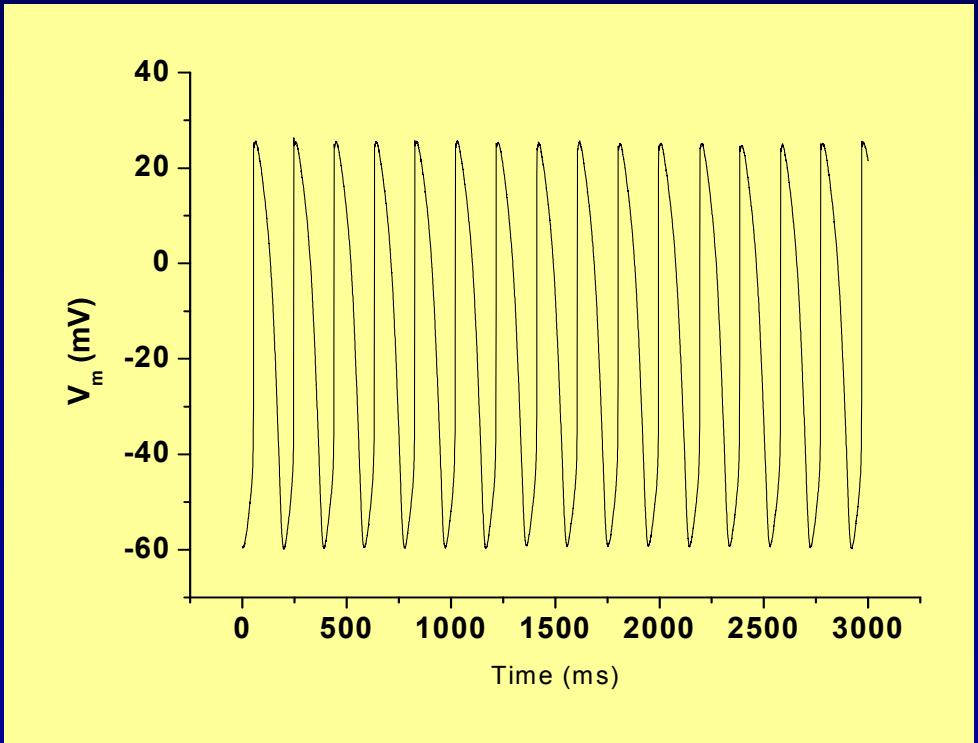
The strategies of genetic suppression of I_{k1} and overexpression of I_f for inducing pacemaker activities may not be necessary synergistic

Azene *et al.*, Cardiovas Res 2005

Modulation of Biological Pacemaker



Kir2.1-WT overexpressed GPLVCMs

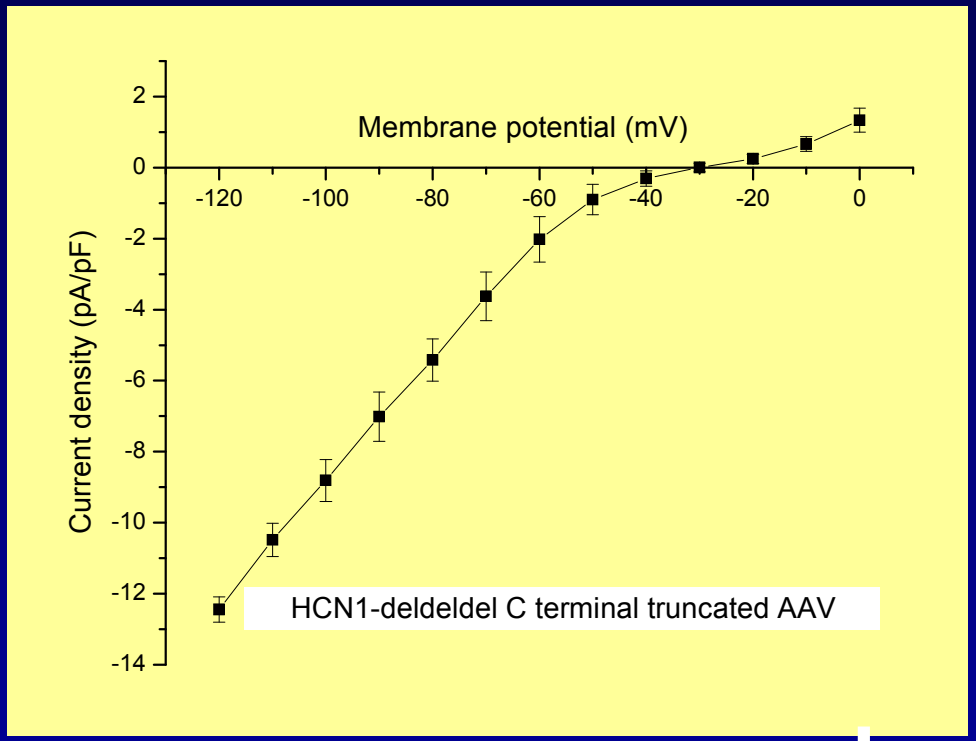


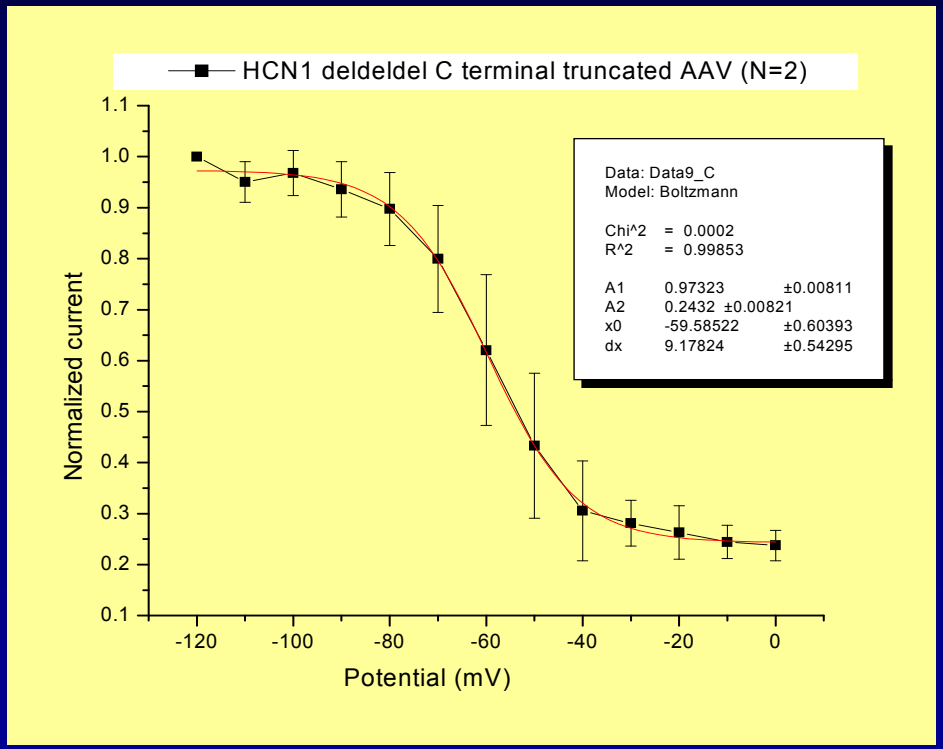
I_f and I_{K1} co-expression

Korean Society of Circulation 2007

Lau YM, Tse HF, Li RA, et al (submitted)

AAV Gene Vector for Biological Pacemaker





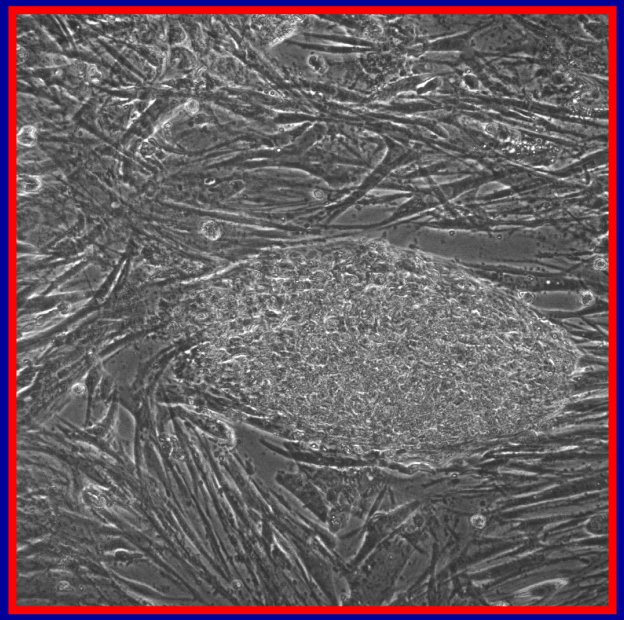
Cell sorting (GFP positive cells)

Cell culture (for 3 months)

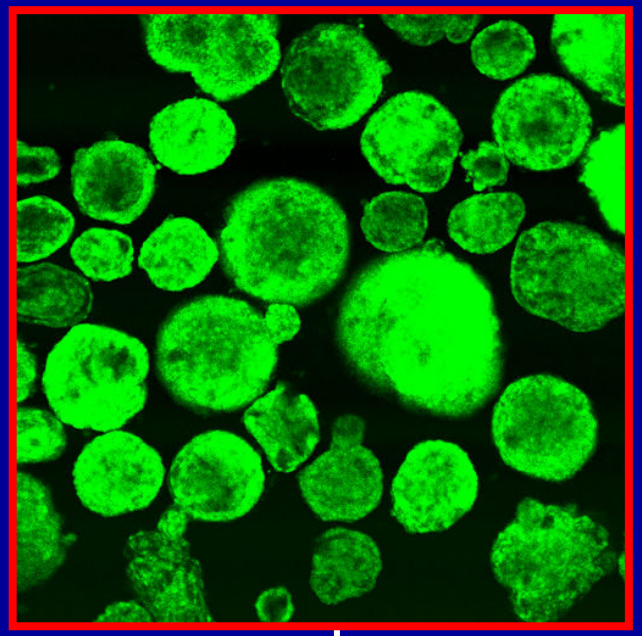
Korean Society of Circulation 2007

Lau YM, Tse HF, Li RA, et al (unpublished data)

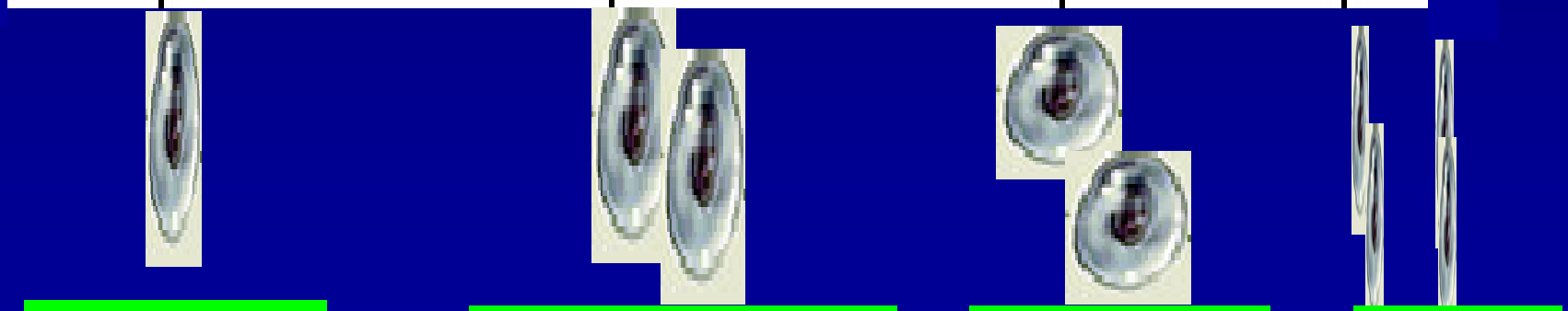
hESC COLONY



EMBRYOID BODIES



GFP : track human cells after transplantation into animals



Atrial Cells

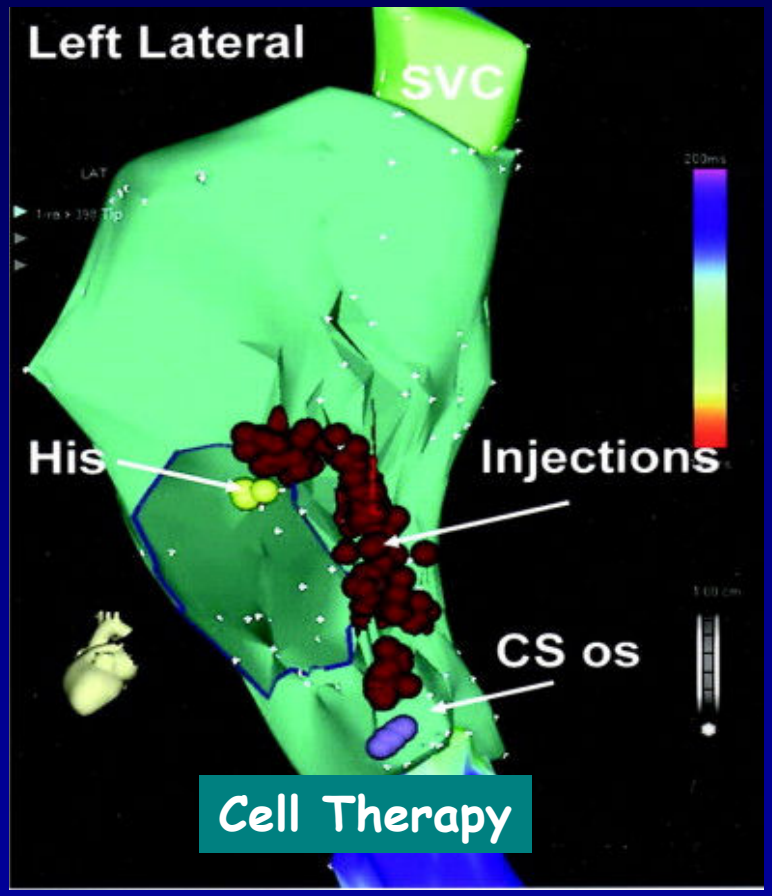
Ventricular Cells

Nodal Cells

Vessels

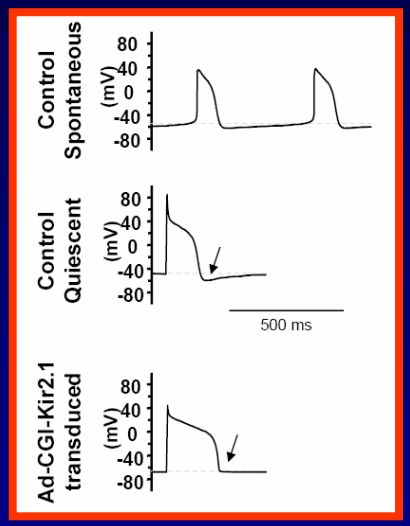
Korean Society of Circulation 2007

Novel Approaches: Biologic Lesions



Cell Therapy

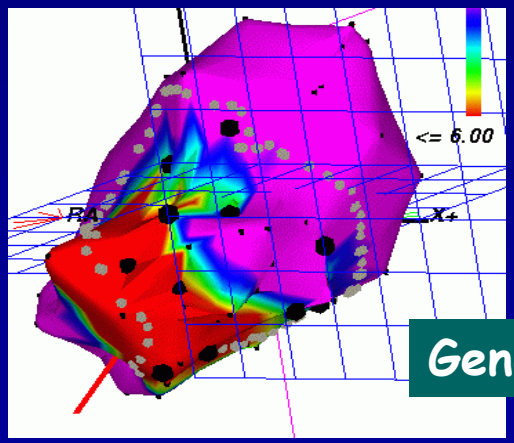
Bunch TJ, et al. Circulation 2006
Korean Society of Circulation 2007



Arrhythmogenic cells

Ad-CGI-Kir2.1

Non-Arrhythmogenic cells



Gene Therapy

Li RA, Tse HF *unpublished*

Conclusions

- The lack of optimal therapeutic options for treatment of organ failure motivates the pursuit for alternative biological therapeutic approach.
- Recent development in molecular and stem cells biology and tissue engineering have provided a new biological solution to replace or modify malfunctioning organ
- The heart represents an attractive target for different gene and cell therapies-
  - → **biological pacemaker**
  - → **cellular cardiomyoplasty**
  - → **angiogenesis**


Our Peoples


Basic and
Stem Cell
Laboratory
Large Animal
Laboratory
Cardiac MRI