Evaluation of Myocardial Viability and Stem Cell Survival
- Cardiac Regeneration -

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CIRM Roundtable with FDA

October 16th, 2012

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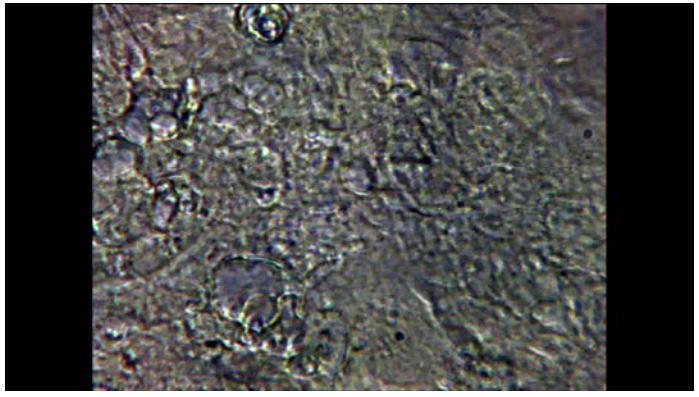
OVERVIEW



I. Myocardial viability
   - Tissue characterization
   - Clinical end-point

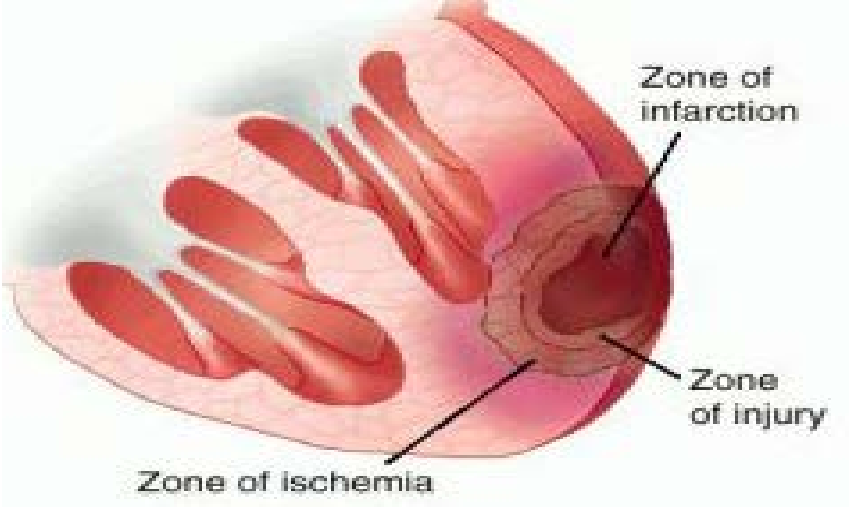
II. Stem cell survival and engraftment
   - Fundamental mechanism for myocardial restoration
   - Myocardial regeneration
   - Optimal cell population for clinical translation

I. Myocardial Viability

- Coronary artery disease: #1 killer in the US
  - 1.1 million new or recurrent MI and 500,000 deaths
  - Improved acute MI therapy shift in the landscape
- Heart failure (HF): #1 cause of hospital admission
  - Prevalence: 5 million patients
  - Incidence: 300,000 patients/year
  - Advanced therapy: 5-year survival ~50%
  - Heart transplant: 1,000 pt/year
- Accurate diagnosis and optimal therapeutic intervention

Clinical Issues

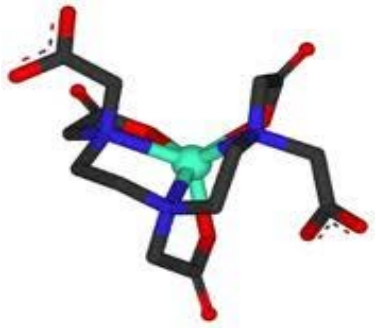
- Challenge: dysfunctional myocardium
  - Dead, viable, or viable but injured myocardium
  - Sufficient viability to salvage the injured myocardium
  - Revascularization, device, and/or medicine
- Mandate: regenerate the myocardium
  - Permanent and sustained restoration of the myocardium
  - Increase myocardial viability ⟺ survival

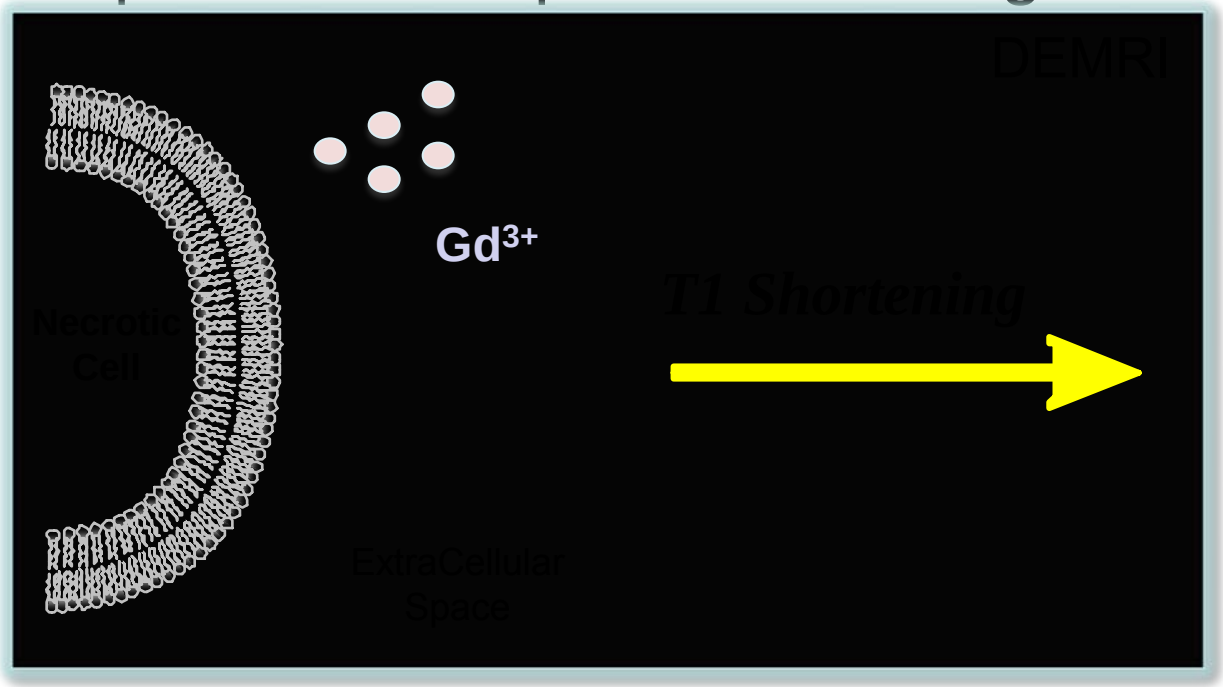


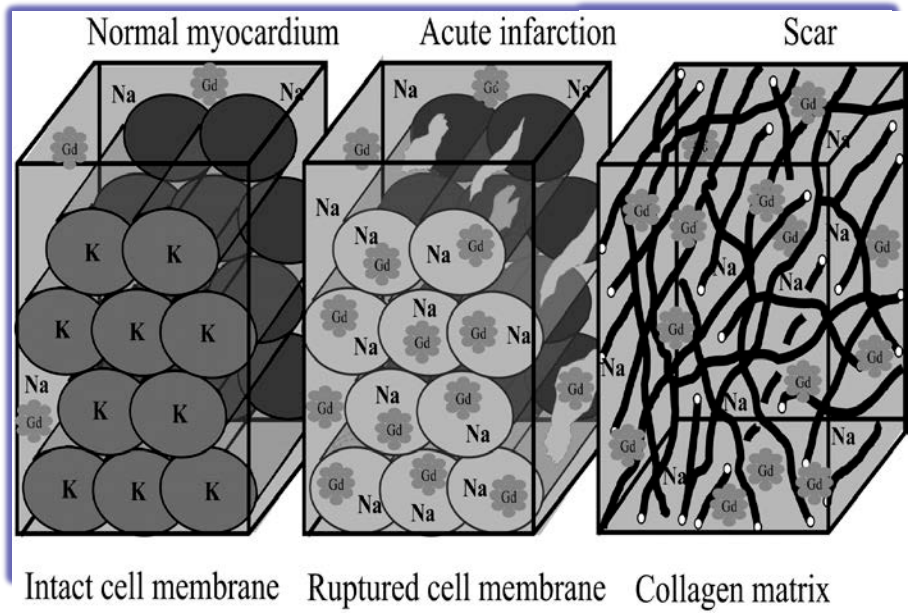
MRI: Gold Standard

Delayed Gadolinium (Gd) Enhanced MRI (DEMRI)

- Relies on non-specific distribution of Gd into extracellular space
- Delayed Gd clearance from infarcted myocardium/scar produces T1 positive MRI signal







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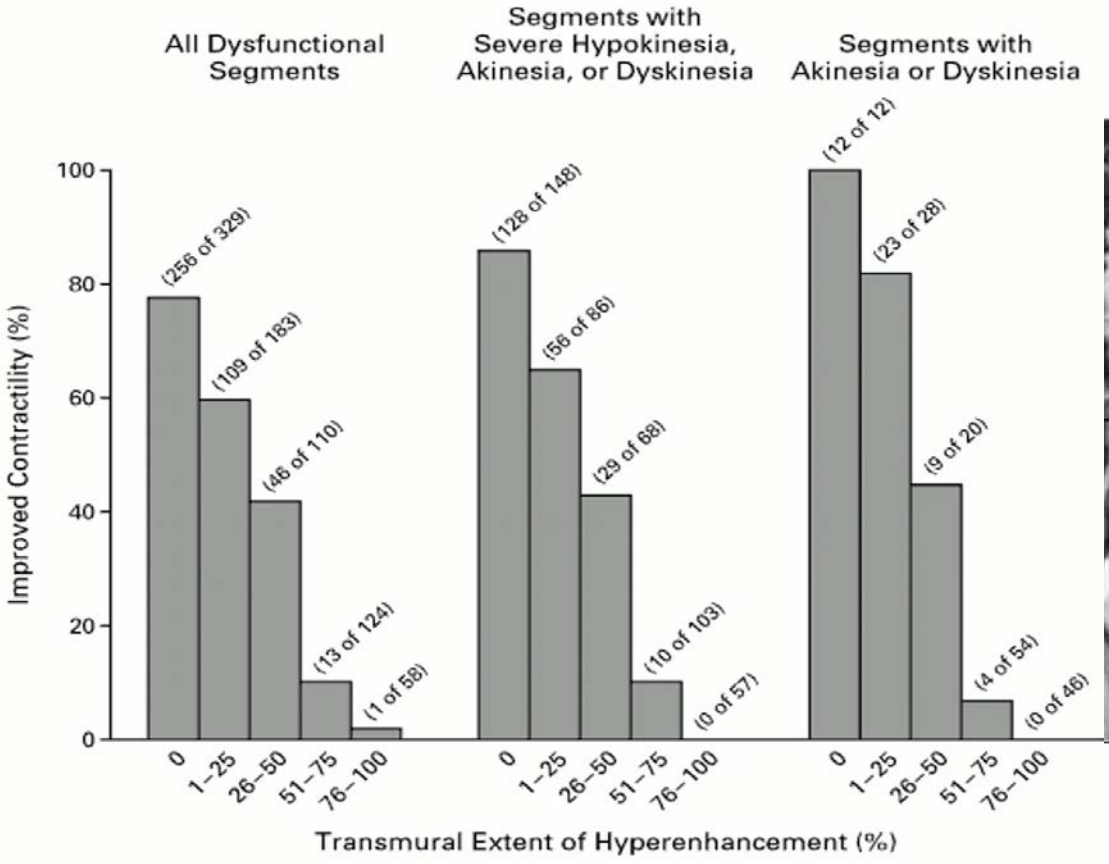
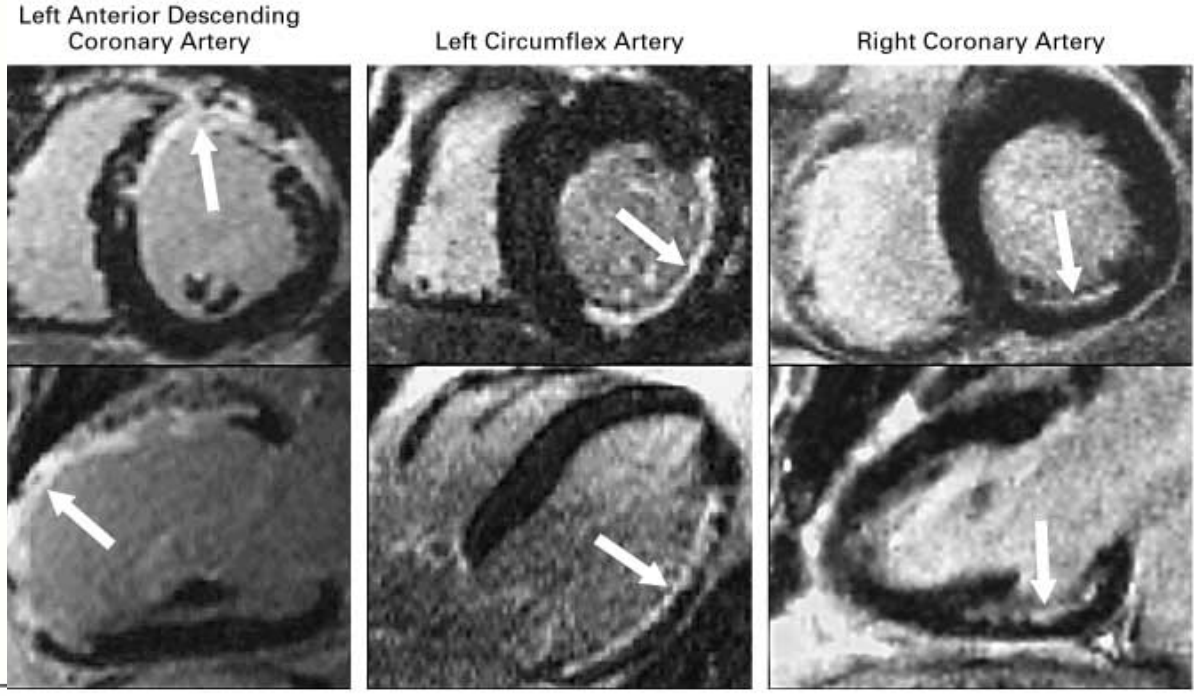
Recovery of Regional Contractility





Kim R, et al. NEJM ‘00

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Clinical End-Point

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informa
healthcare

Peri-Infarct Ischemia Determined by Cardiovascular Magnetic Resonance Evaluation of Myocardial Viability and Stress Perfusion Predicts Future Cardiovascular Events in Patients with Severe Ischemic Cardiomyopathy

Miwako Tsukiji, MD,[1] Patricia Nguyen, MD,[1] Girish Narayan, MD,[1] Jeffrey Hellinger, MD,[2] Frandics Chan, MD, PhD,[2] Robert Herfkens, MD,[2] John M. Pauly, PhD,[3] Michael V. McConnell, MD,[1] and Phillip C. Yang, MD[1]

Hypothesis

Presence of peri-infarct ischemia predicts future CVE in patients with severe ischemic cardiomyopathy
(CVE: death, MI, stroke, CHF, ventricular arrhythmia, syncope).

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Results: Peri-infarct Ischemia

	CVE (+) (n=6)	CVE (-) (n=17)	*p*
Peri-infarct ischemia, n (%)	4/6 (67)	2/17 (12)	0.03
Scar volume, cm^3	20±15	21±18	NS
Scar percentage of LV volume, %	9±7	15±12	NS
Ejection fraction, %	29±10	23±13	NS
Age, years	51±12	54±11	NS
Coronary anatomy, n (%) **2 vessel (include P-LAD or LMT)**	2/6 (33)	8/17 (47)	NS
3 vessel disease	4/6 (67)	9/17 (53)	NS

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ues are expressed as a mean ±SD.

Journal of Cardiovascular Magnetic Resonance

BioMed Central

Research

Open Access

Quantitative characterization of myocardial infarction by cardiovascular magnetic resonance predicts future cardiovascular events in patients with ischemic cardiomyopathy

Hajime Yokota[1], Shahriar Heidary[1], Chandra K Katikireddy[1], Patricia Nguyen[1], John M Pauly[2], Michael V McConnell[1] and Phillip C Yang*[1]

Hypothesis

Quantitative characterization of myocardial scar by CMRI can predict cardiovascular events in patients with severe ischemic cardiomyopathy.

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Results: Myocardial Scar

	CVE (+) n=33	CVE (-) n=53	*p* - value
Scar volume (cm3)	**16.8 ± 12.4**	**11.7 ± 12.6**	**0.023**
Scar % of the myocardium	**10.2 ± 6.9**	**7.2 ± 6.7**	**0.037**

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Results: Transmurality

	CVE (+)	CVE (-)	*p* - value
Non-transmural MI (1- 75% scar of myocardium)	18.4 ± 14.0%	13.8 ± 11.2%	0.049
1 – 25%	9.2 ±11.0%	6.7 ± 9.3%	0.12
26 – 50%	9.2 ± 10.6%	3.2 ± 3.6%	0.03
51 – 75%	3.5 ± 4.2%	4.0 ± 4.5%	0.30
Transmural MI (76 – 100% scar of myocardium)	5.8 ± 10.2%	7.2 ± 11.4%	0.28

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Quantitative Tissue Characterization of Infarct Core and Border Zone in Patients With Ischemic Cardiomyopathy by Magnetic Resonance Is Associated With Future Cardiovascular Events

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Stanford, California; and Niskayuna, New York

Hypothesis

Evaluation of infarct heterogeneity in the peri-infarct region may be a stronger predictor of CVE than the traditional measurements.

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Results: Heterogeneity Analysis

Significant predictors for CVE

	CVE (+) n=29	CVE (-) n=41	*p*-value
Total Scar Mass (g)	36.7 ± 22.2	27.0 ± 21.0	0.03
Peri-Infarct Scar Mass (g)	17.0 ± 13.1	11.2 ± 11.0	0.02
Peri-Infarct Scar % of the Myocardium (%)	10.6 ± 7.9	7.3 ± 7.7	0.04

Medicine vs. revascularization

- Medicine: Peri-infarct zone
- Revascularization: Total scar and core zone

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Results: LVEF and Volume

	CVE (+)	CVE (-)	*p* - value
LVEF (%)	25 ± 10	27 ± 13	0.26
LVEDV (ml)	234 ± 76	230 ± 88	0.41
LVESV (ml)	180 ± 73	175 ± 90	0.40

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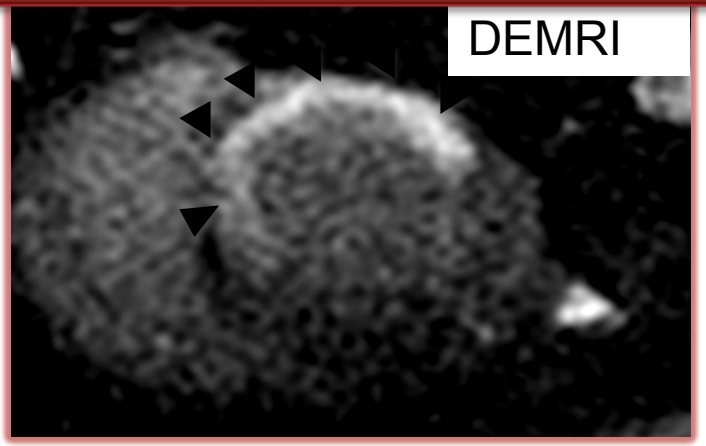
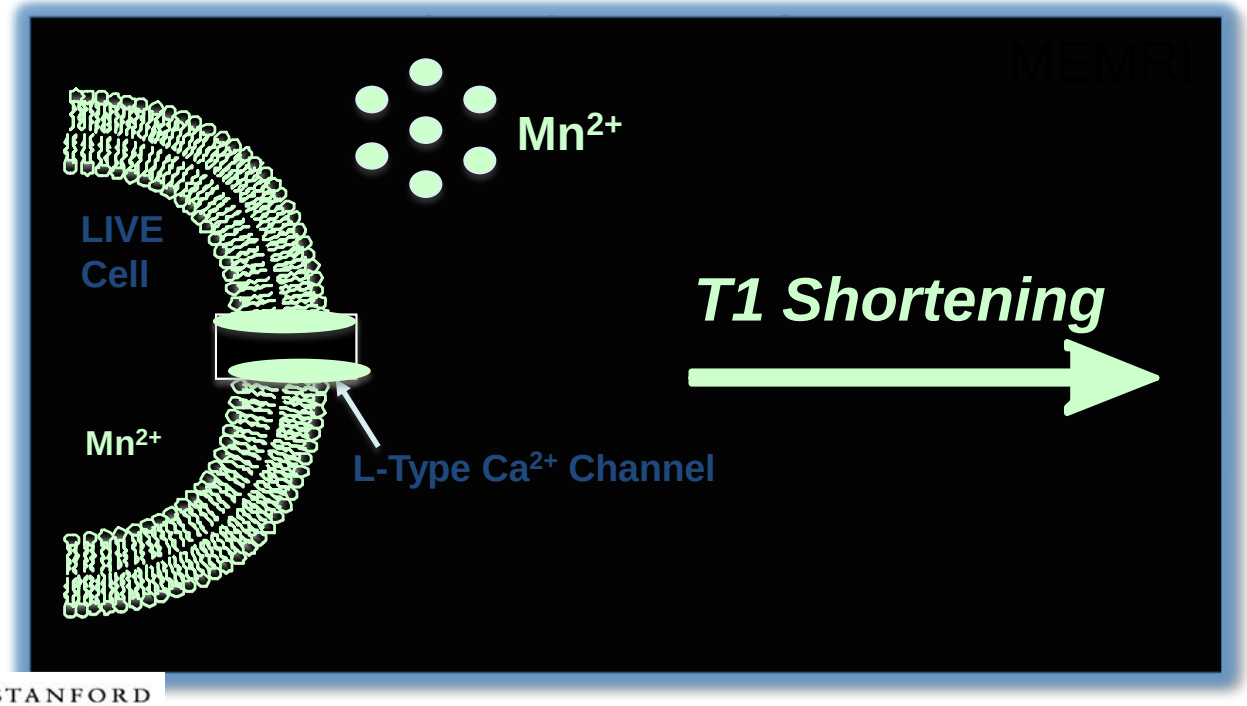
In Vivo Manganese-enhanced MRI (MEMRI)

Manganese-Enhanced MRI (MEMRI)

- Manganese (Mn^{2+}) produces T1 shortening
- Enters **live** cells via L-type Ca^{2+} channels
- Uptake is specific for live cardiomyocytes





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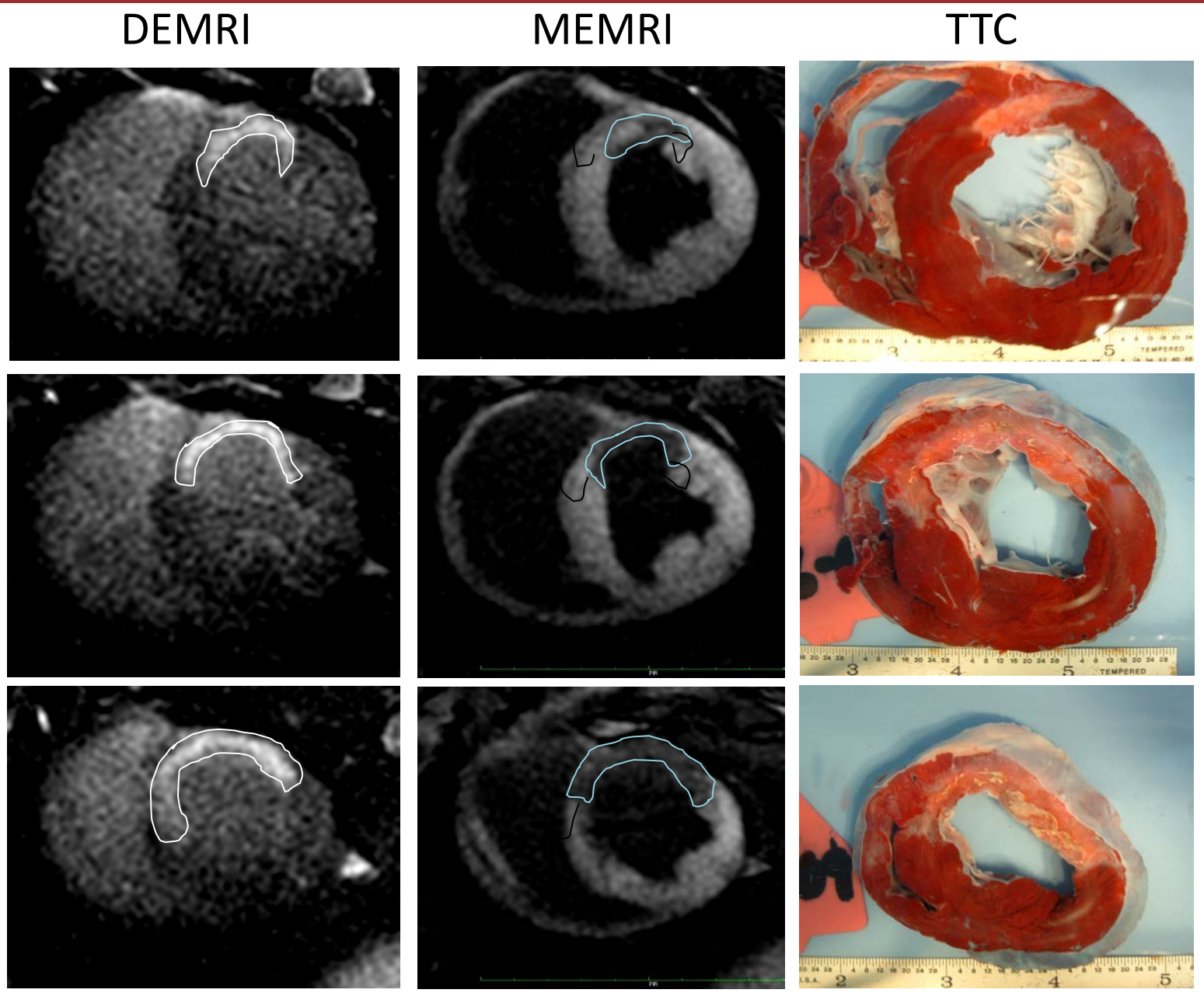
Dash et al, *Circulation: Cardiovasc Imaging*, 2011

MEMRI-DEMRI of Peri-Infarct Region



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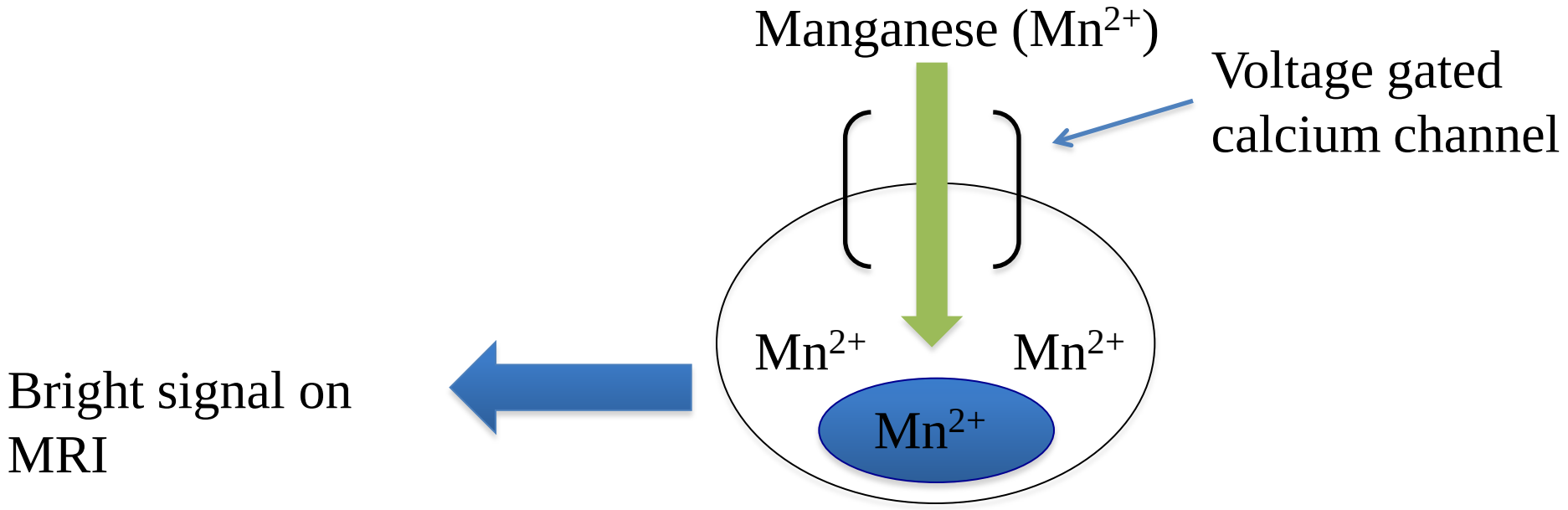
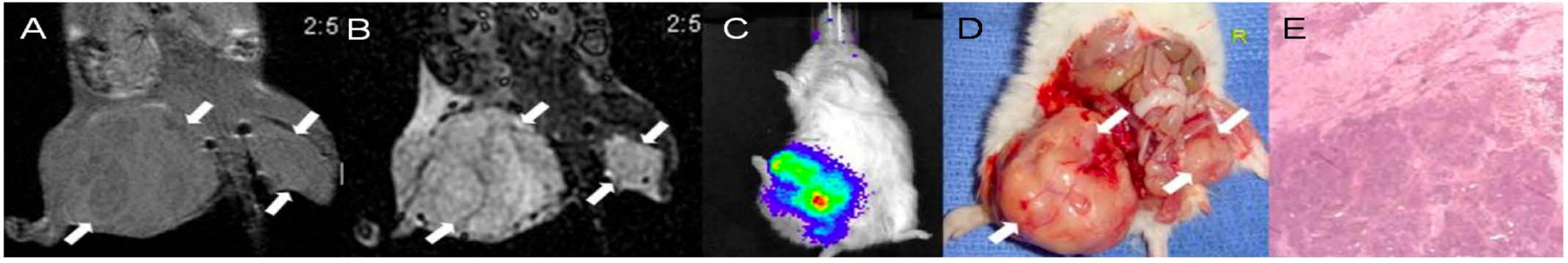
Dash et al, *Circulation: Cardiovasc Imaging*, 2011

II. In Vivo MEMRI of Stem Cell Survival





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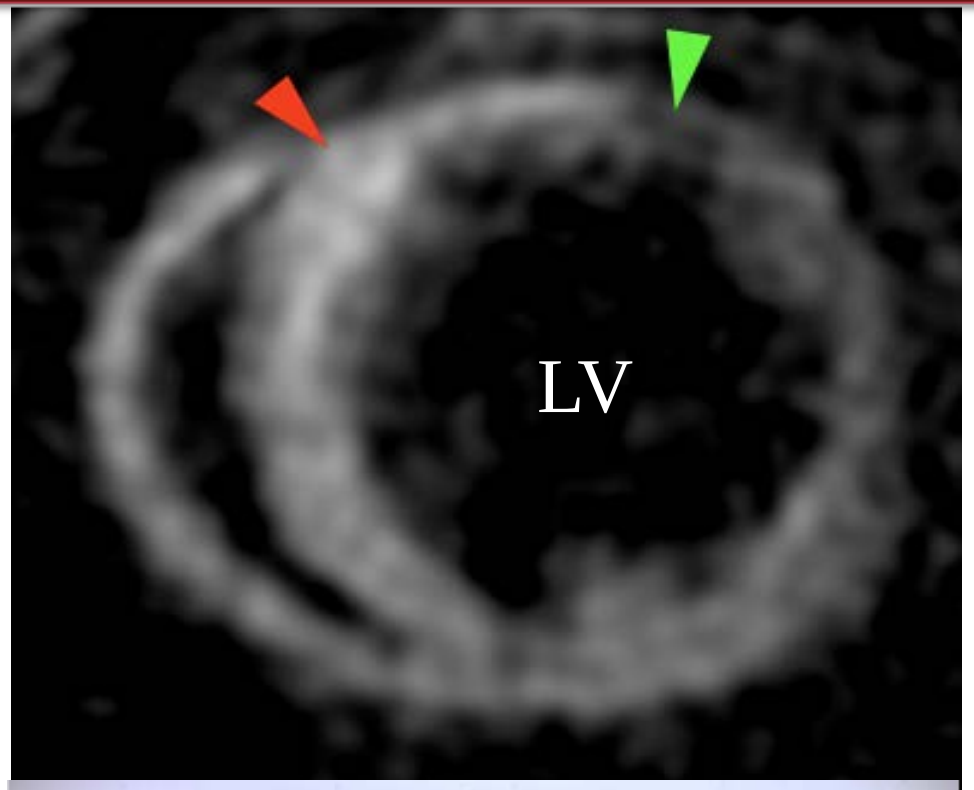
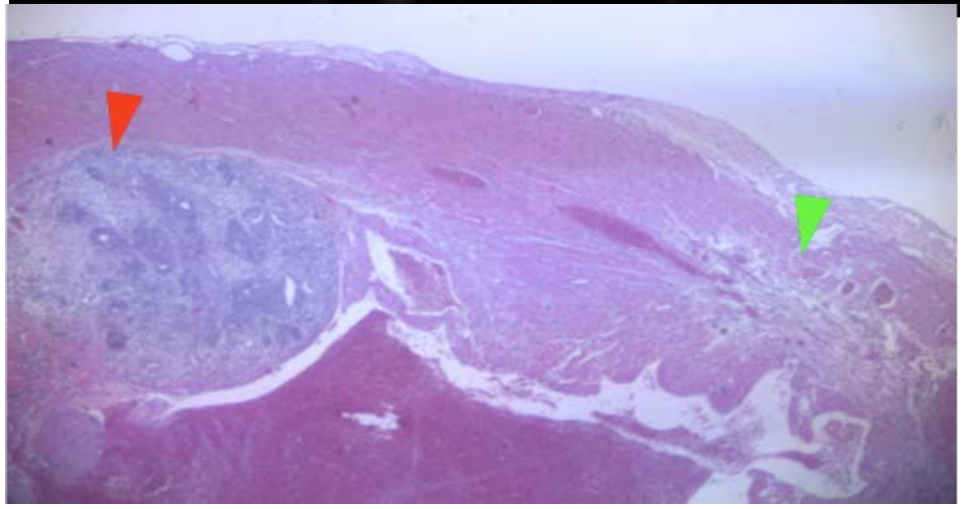
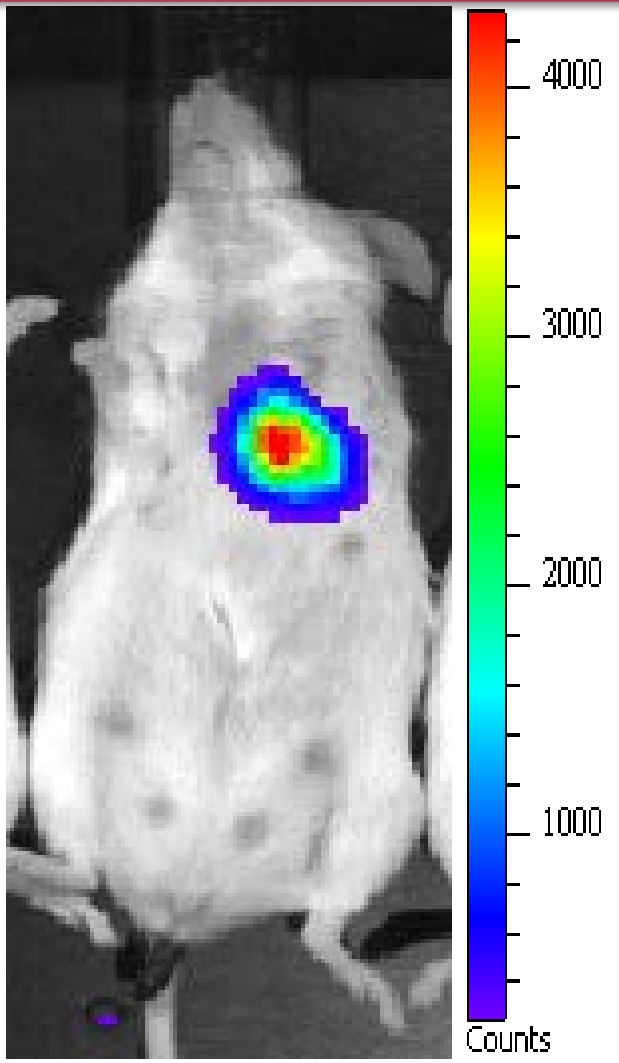
Yamada et al, *Magn Reson Med*, 2009

In Vivo MEMRI in Murine Myocardium







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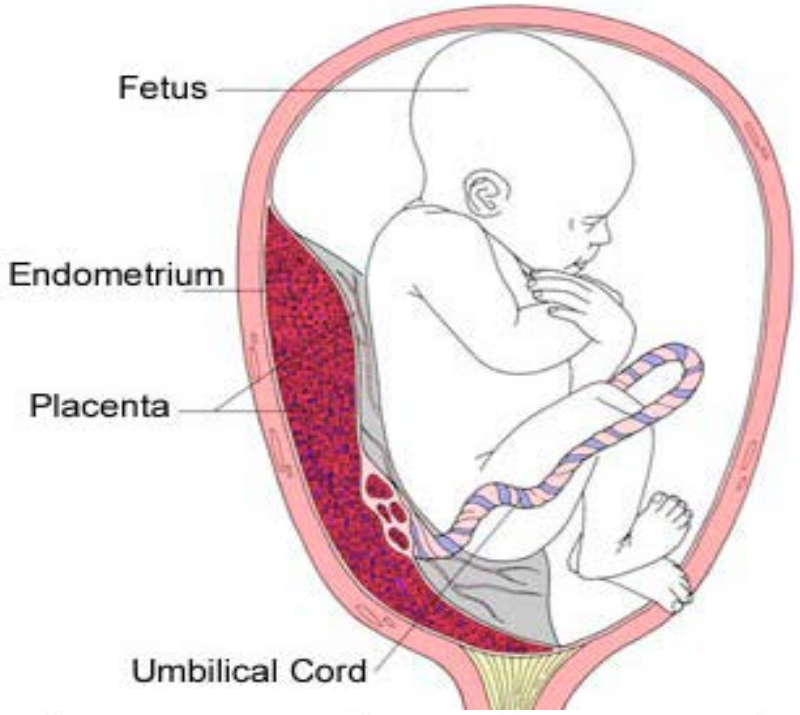
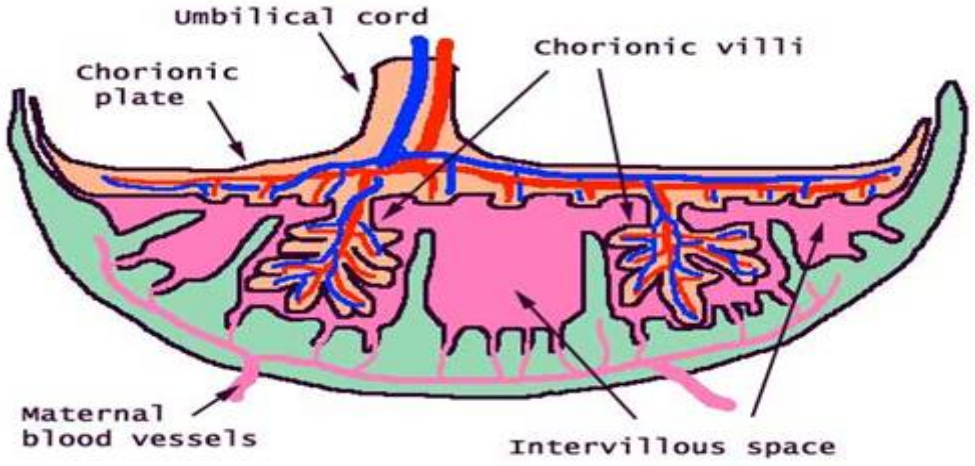
Kim PJ et al, *Circulation,* 2012, YIA

Myocardial Regeneration: Human Amniotic Mesenchymal Stem Cells

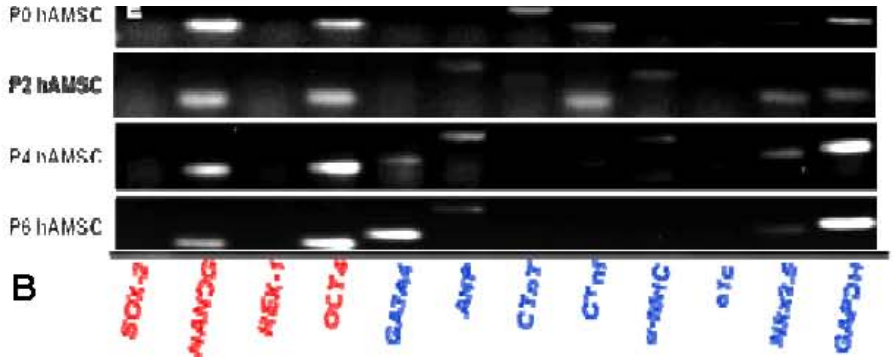




A

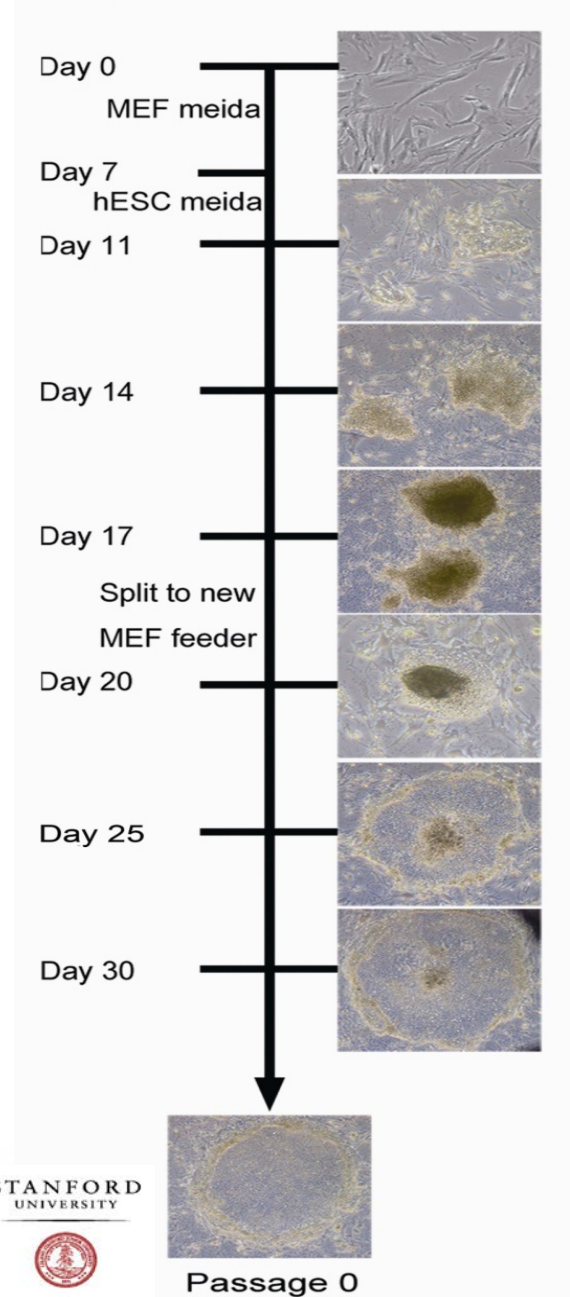
Stem Cell Profile		Immune Profile		Heamotopoetic Profile	
SSEA-3	+	HLA-DR	–	CD34	–
SSEA-4	+	HLA-G	+	CD45	–
TRA 1-81	+	CD59	+	Cardiovascular Profile	
Thy-1 (CD-90)	+			c-kit (CD117)	+

B

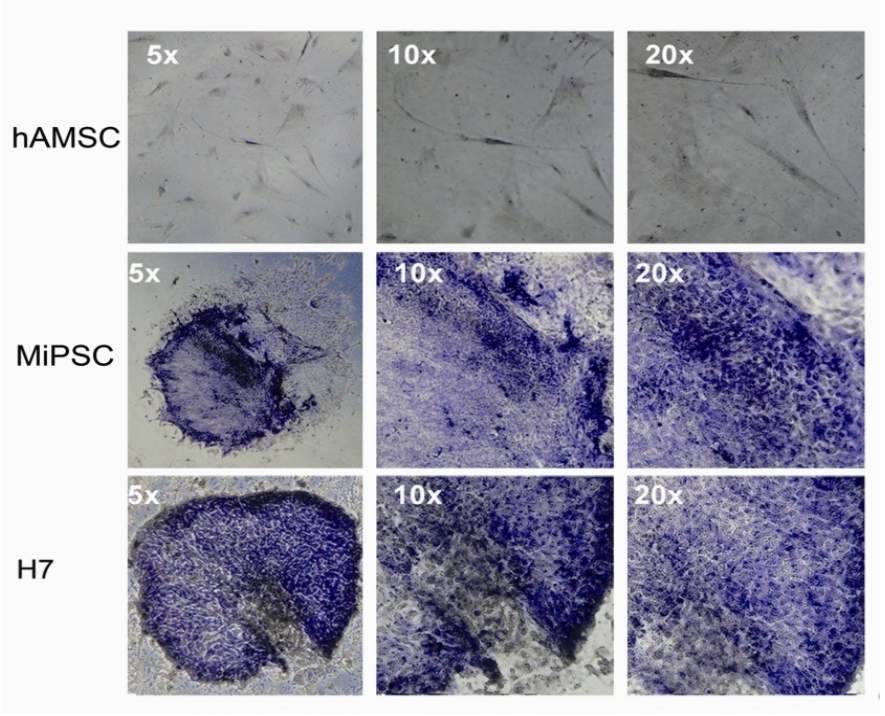


Ge et al, *Stem Cells Dev,* 2012

iPSC Generation



- **Around day 10: colony formation**
  - **At least ~20 hESC-like colonies were found from 20,000 cells (more than 0.1%)**
- **At day 20, the characteristic iPSC colonies were observed**



- **high activity of alkaline phosphatases (ALP) was observed.**

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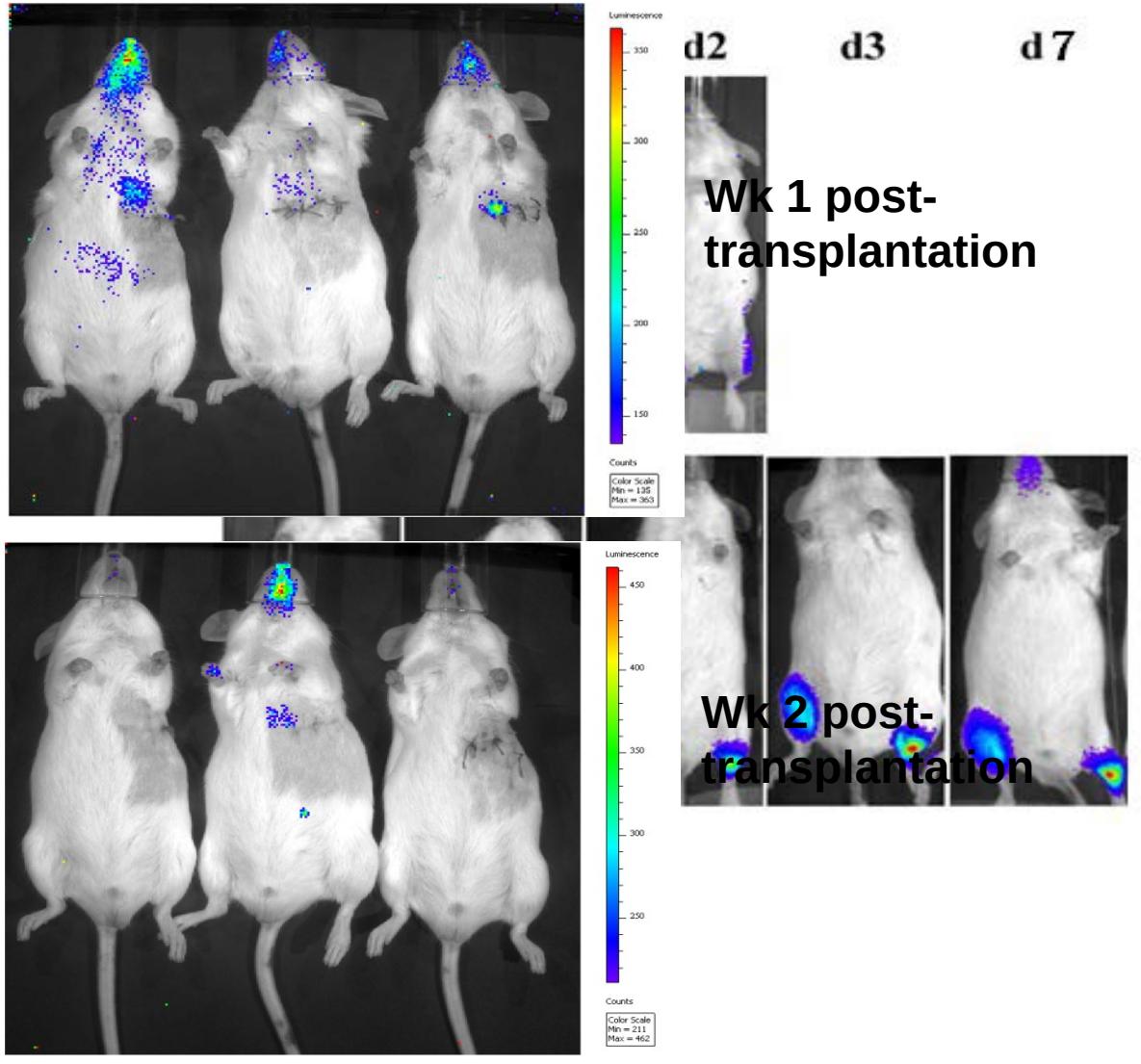
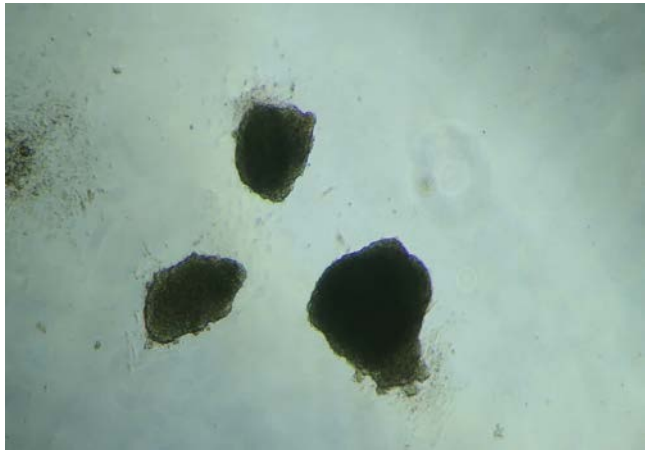
Immunomodulation *in vivo*

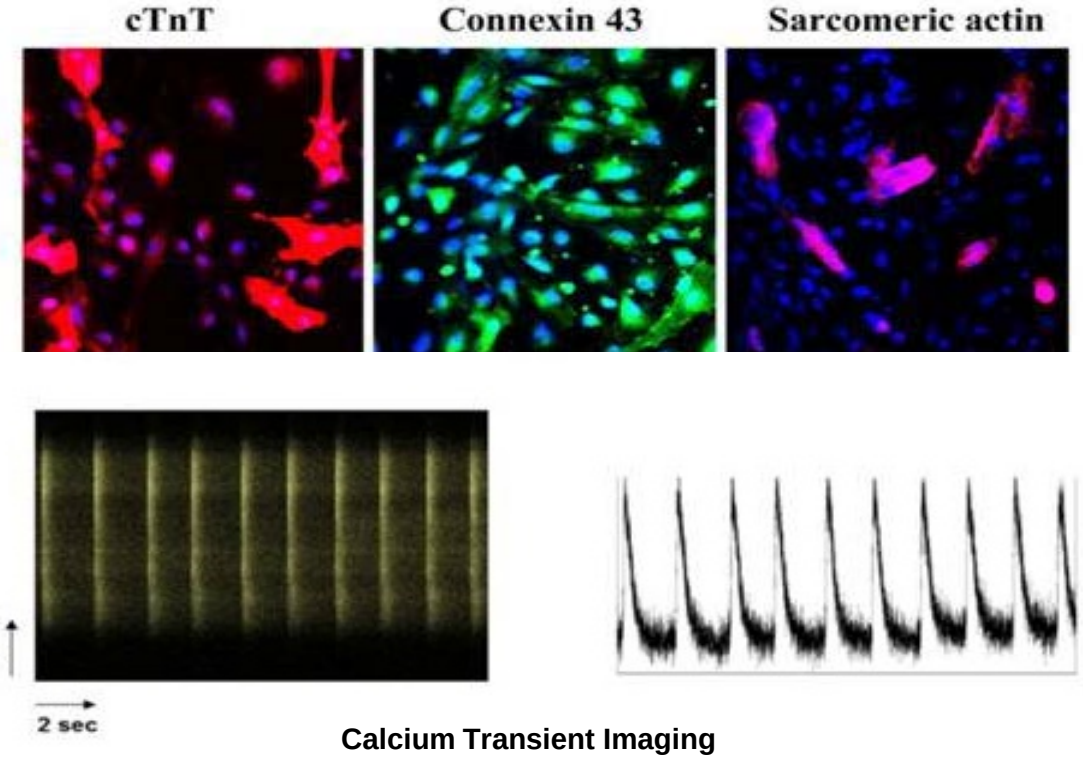
- 250K MiPSCs were injected into hind limbs of immunocompetent FVB mice;
- MiPSCs survived in the myocardium ~2 weeks;
- MiPSCs survived in FVB mice 7days.



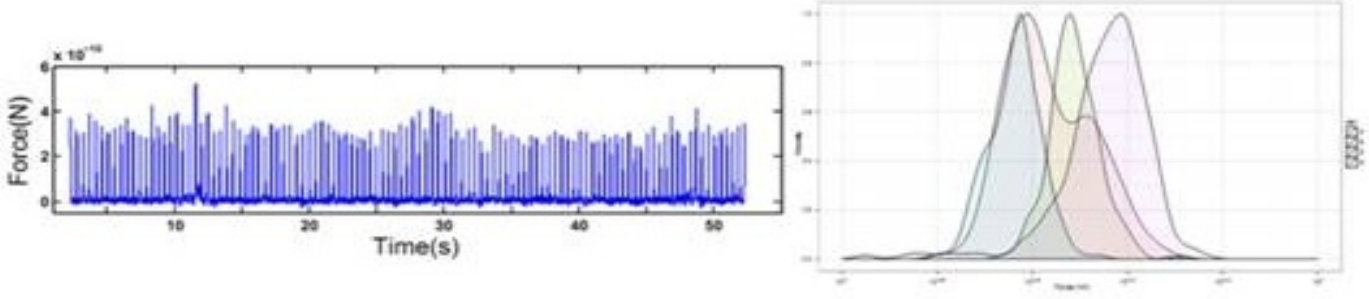
Cardiac Differentiation - Phenotype

- **High levels of mature cardiac marker expression: cTNT, connexin 43 and sarcomeric actin**
- **Calcium transient through L-type calcium channel and contractile force**





Calcium Transient Imaging

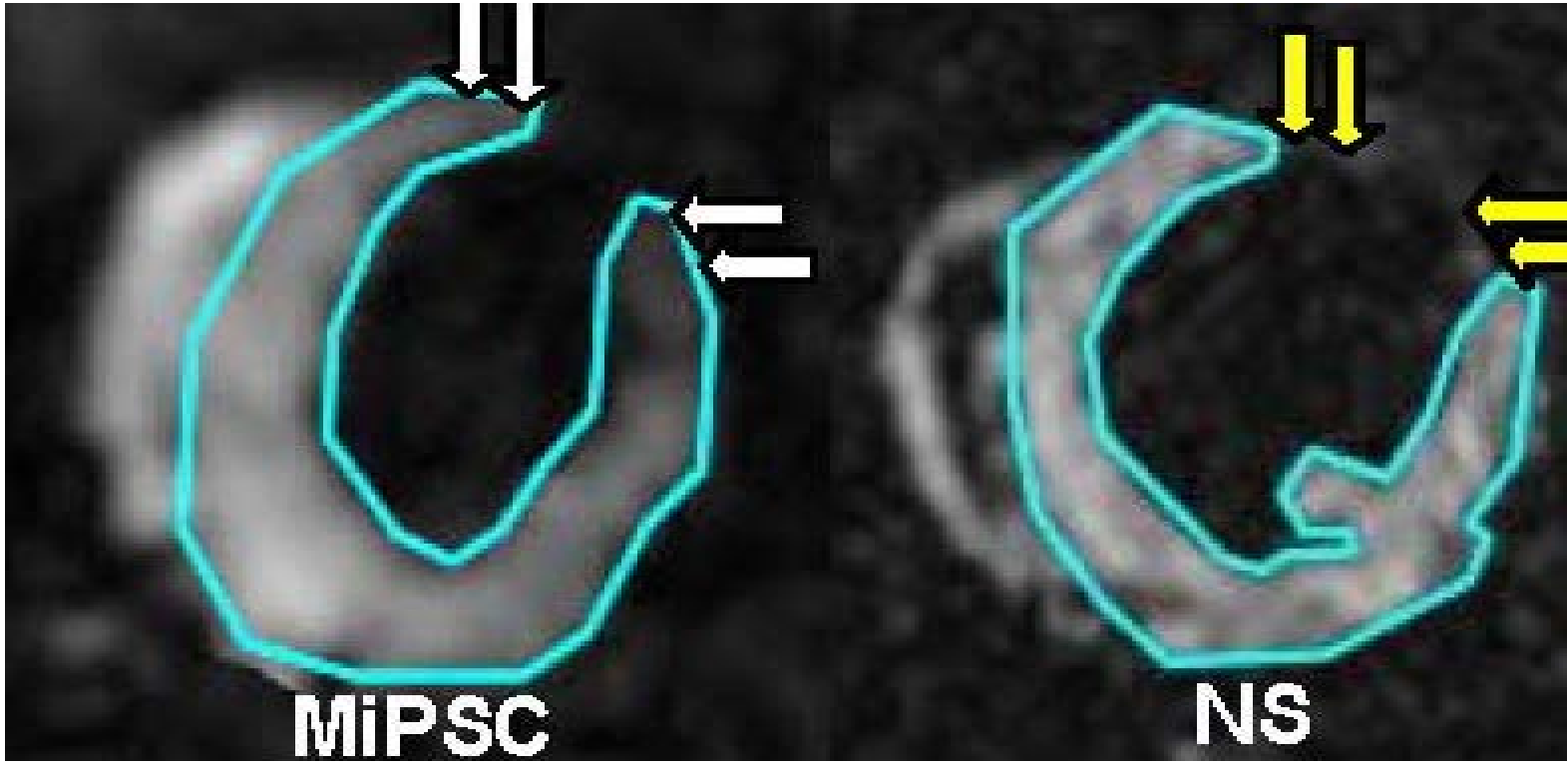


Atomic Force Microscpy

Wang et al, *PlosOne*, 2012

Functional restoration

- **The EF improved by MiPSCs (control NS LVEF 15-20%)**
- **The myocardial viability was increased significantly (control NS 75%).**



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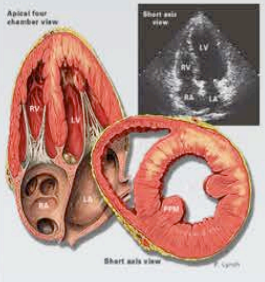
Kim PJ et al, *Circulation,* 2012, YIA

Porcine Myocardial Injury Model

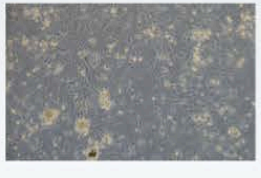
8 Adult Farm Pigs

DAY #0: I/R Injury, 1 hr

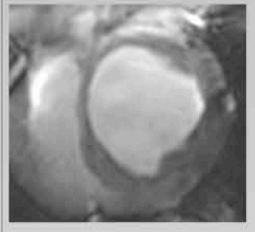


hAMSC Isolation

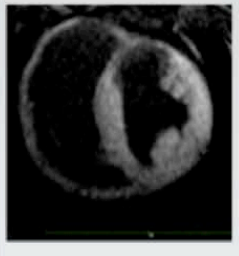
hAMSC Preparation



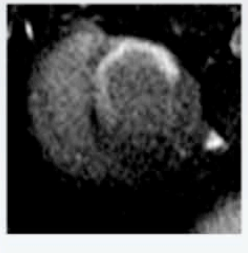
Day 7 Post-IR / Pre-hAMSC



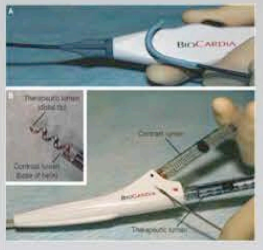
MEMRI: 25-40min



DEMRI 70-80min



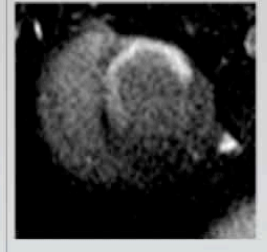
Day 0 Intramyocardial hAMSC



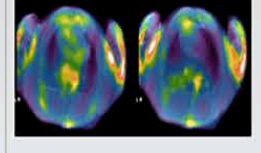
~80 million hAMSCs

Helical Catheter

Serial Cardiac MRI at d7, 14, 21 post-HAMSC

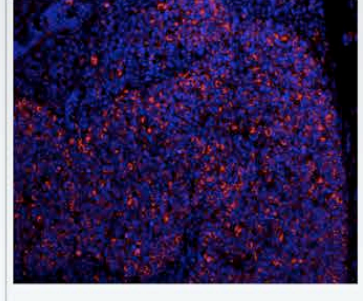


Cardiac PET-CT Imaging



Subpopulation of cells (10-15 million hAMSCs) transduced with HSV-tk PET reporter gene

Immunohistochemistry

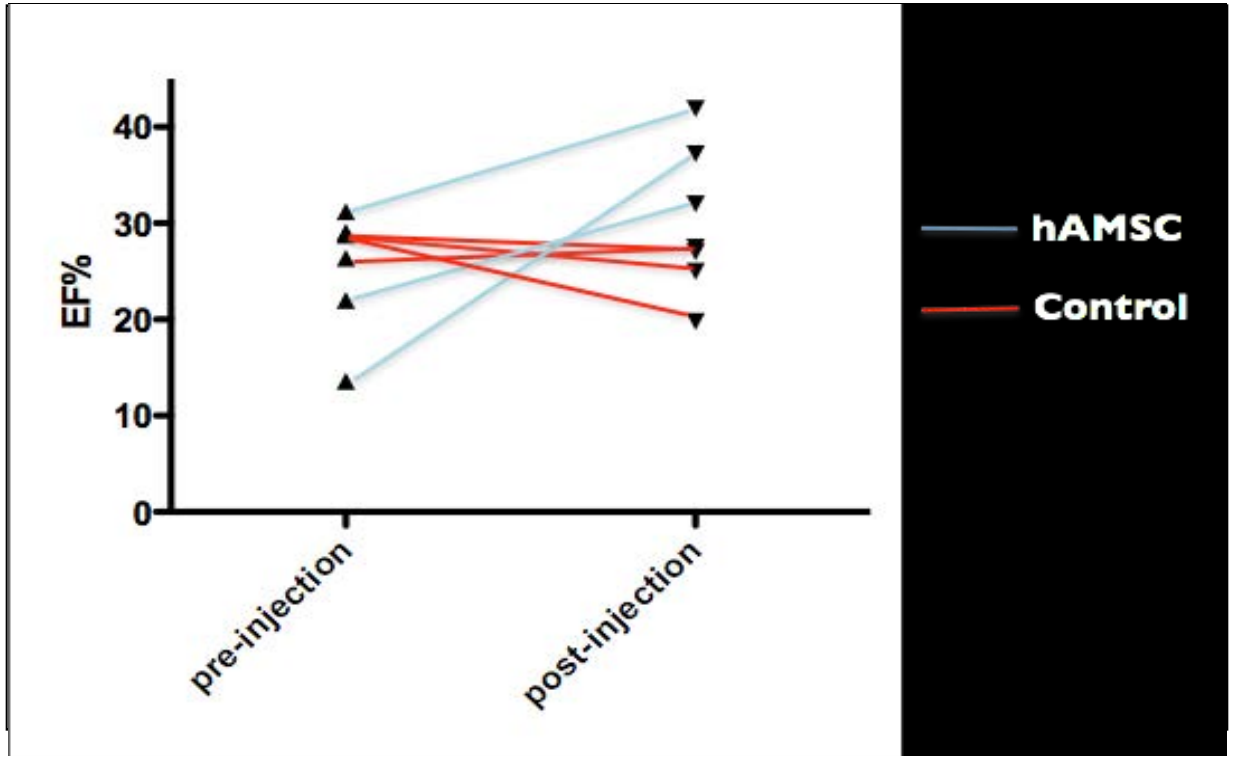


Human Anti-Mitochondrial Ab

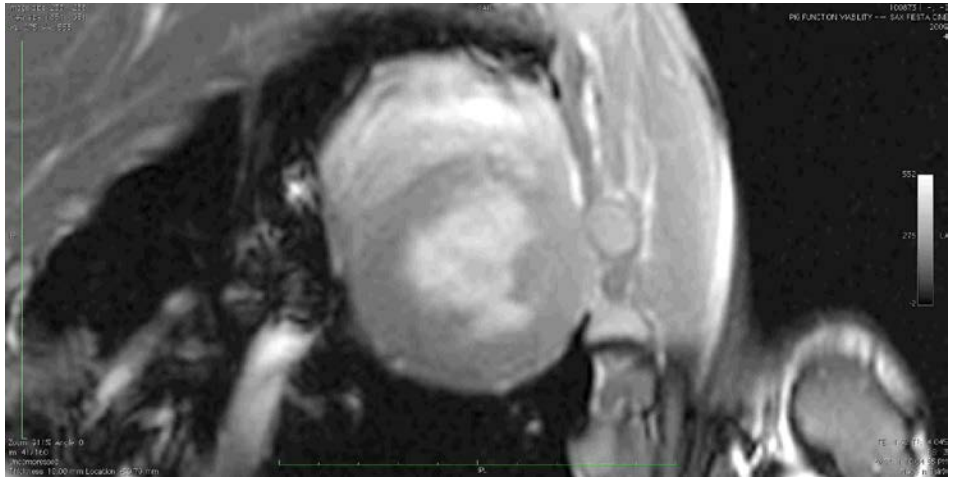
hAMSCs Improve LV Function

Porcine Ischemia-Reperfusion Model

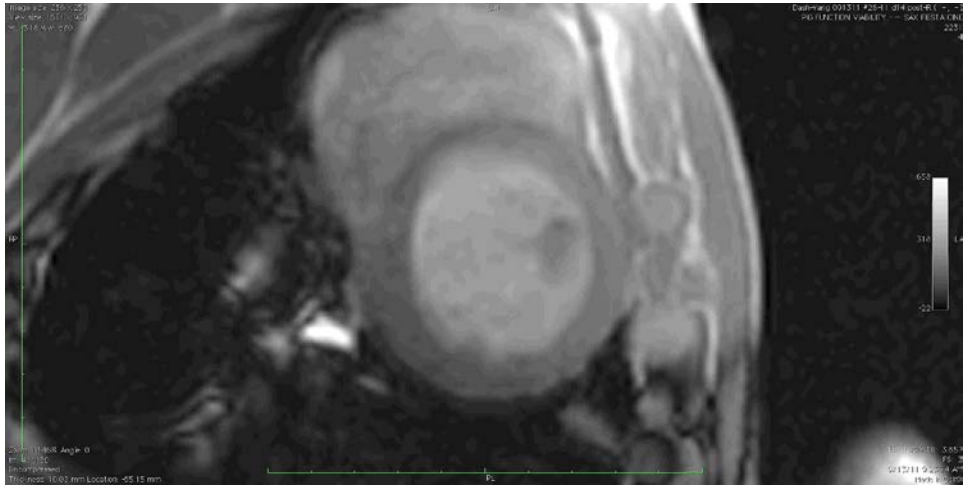
- **hAMSCs**
- **Increased LVEF**



Control - 14 d



hAMSC - 14 d



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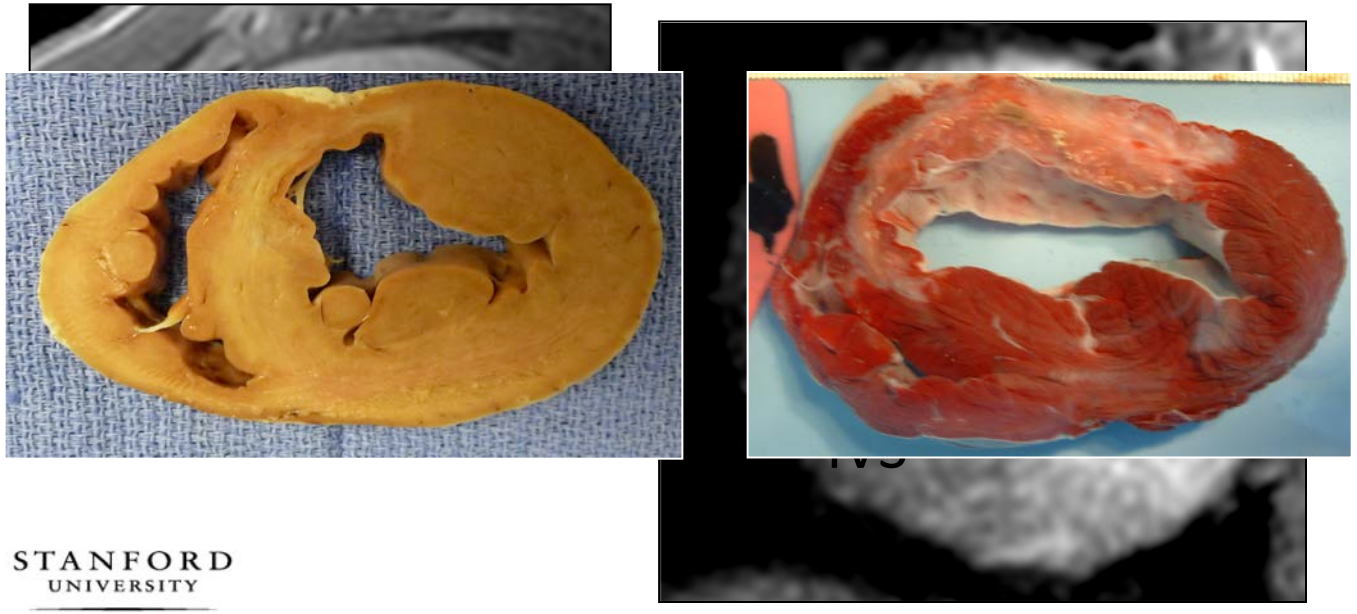
hAMSCs Exhibit Prolonged Survival

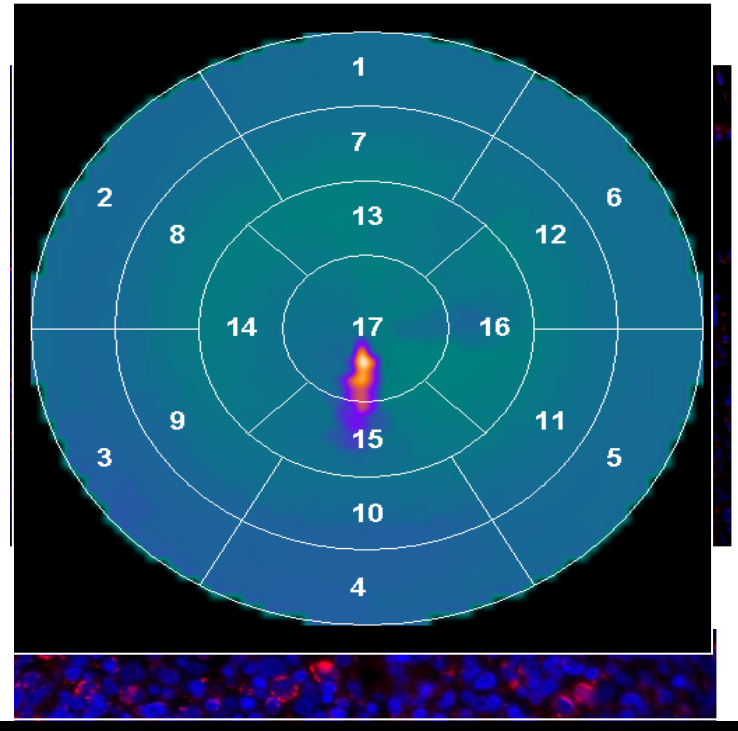
Prolonged hAMSC Survival:

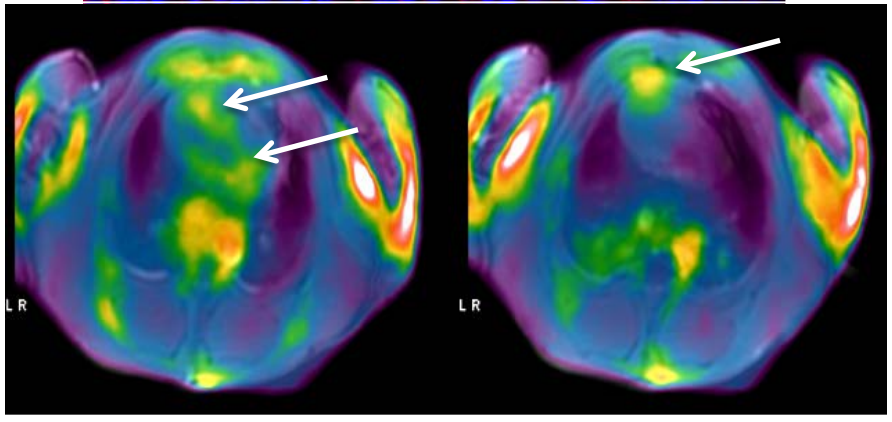
- Transduced w/ HSV-tk PET reporter gene
- PET-CT positive for live cell populations within apex and septum, corresponding to hAMSC injection sites
- IHC positive for human Anti-Mitochondrial antibody

d66 post-IR; d38 post-hAMSC



Anti-human Mitochondrial Ab





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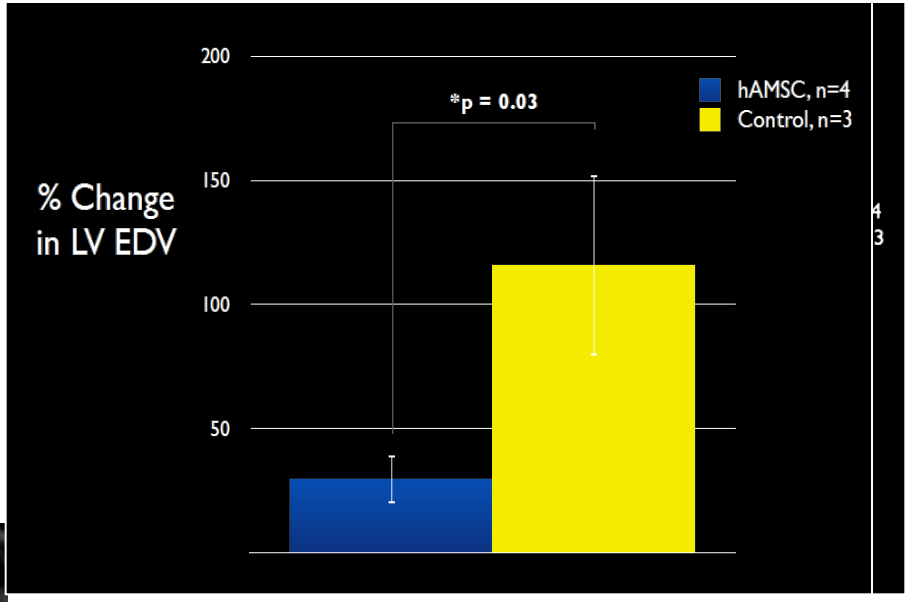
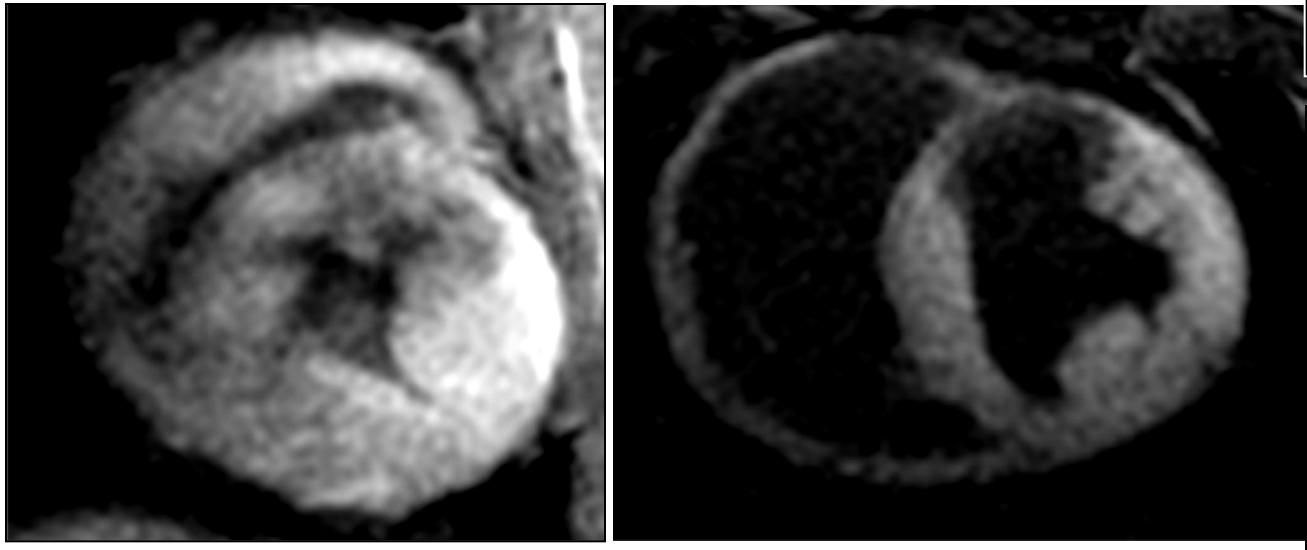
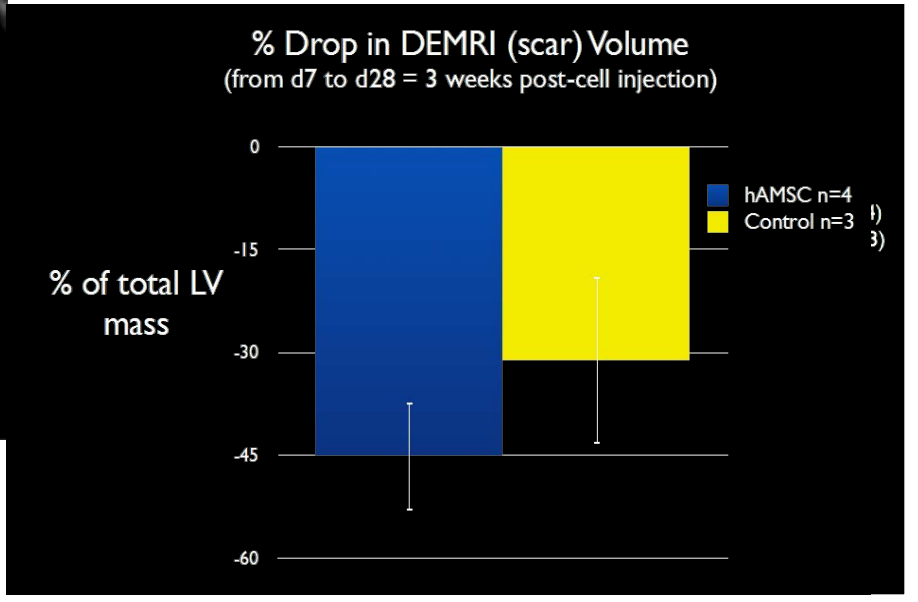
hAMSCs Increase Viable Myocardium

- **hAMSCs generated higher MEMRI signal within the infarct zone: viable hAMSCs**
- **hAMSCs generated smaller infarct zones**
- **hAMSCs decreased remodeling**







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Cardiovascular Stem Cell Imaging

- Evaluation of myocardial viability: **clinical end-point using MEMRI and DEMRI**
  - **IND approval for MEMRI**

- Translational imaging technique will visualize: **survival of cardiovascular stem cell**

- Permanent and sustained restoration of the injured heart: **myocardial regeneration by optimal cell population**

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Laboratory for Cellular and Molecular MRI of Cardiovascular Stem Cells

Yang Laboratory

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Dwight Nishimura
John Pauly
Joe Wu

CT Surgery

Michael Fischbein
Robert Robbins

CV Intervention

Todd Brinton
Alan Yeung

Stem Cell Biology

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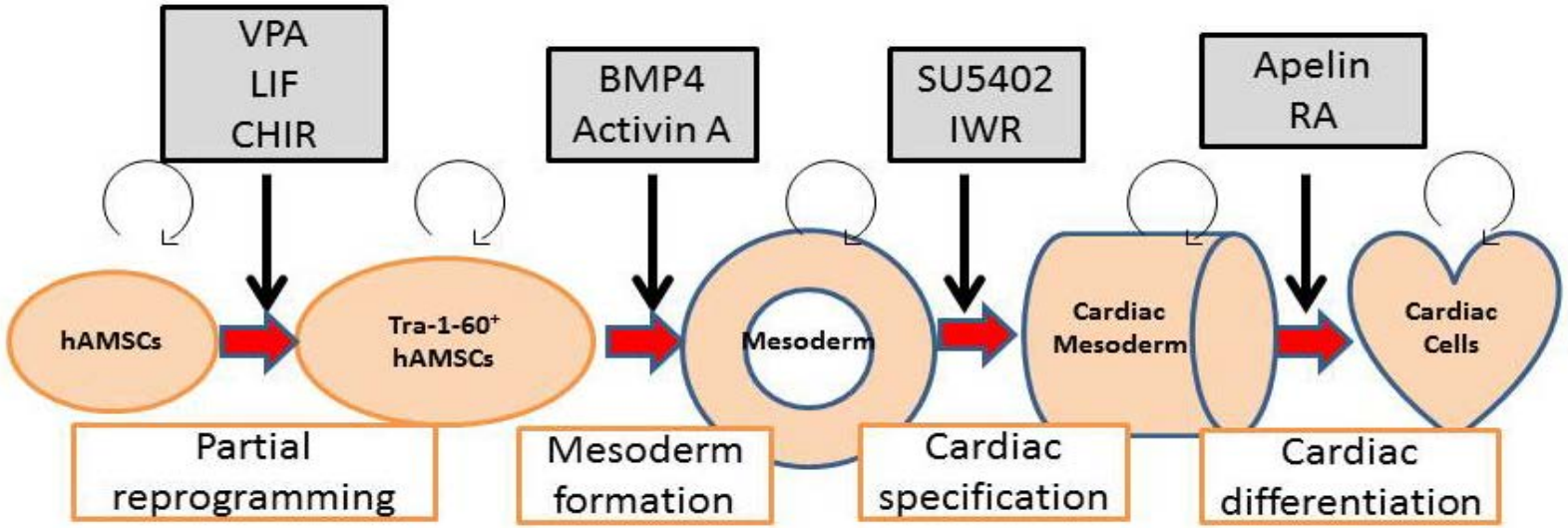
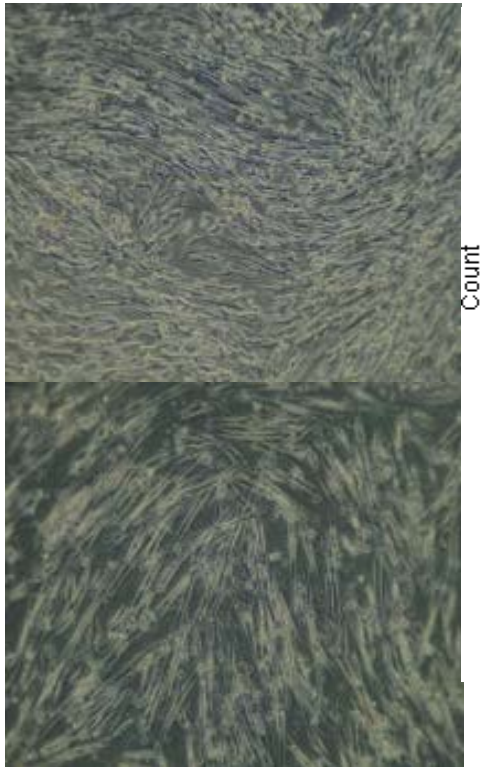
Renee Reijo-Pera
Chelsey Simmons
Irv Weissman

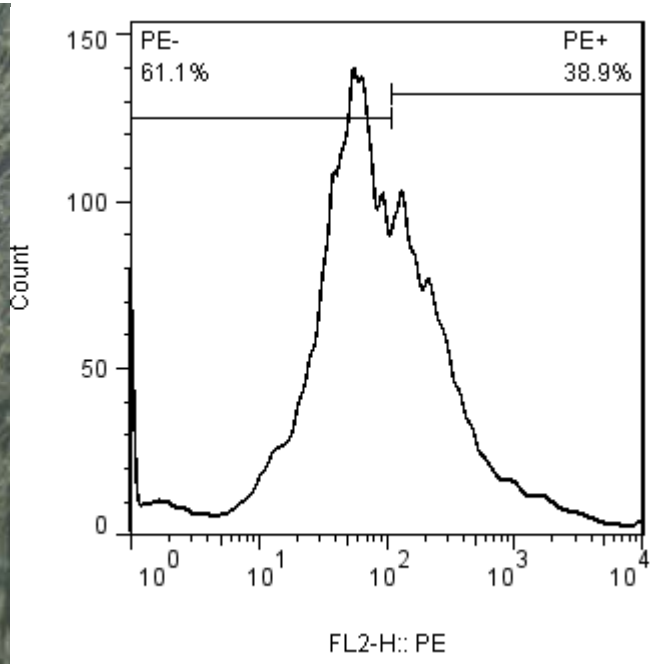
Funding Source

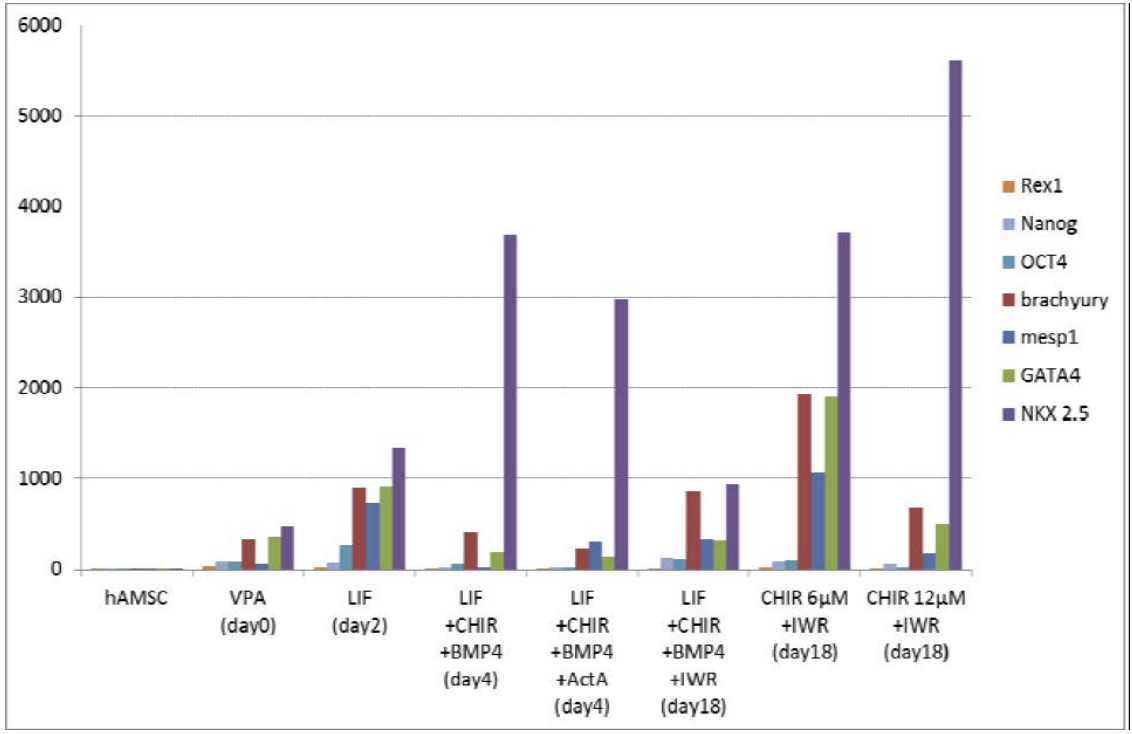
NIH R01-HL097516-01
R01-HS019738-01
UM1-HL-12026
R01-HL39297
CIRM, CVI SEED, AHA

Direct cardiac transdifferentiation of hAMSCs









VI. Current and Future Clinical Studies

- Direct cardiac transdifferentiation
- Simulation of durable myocardial tissue
- Clinical trial
  - MEMRI FOR MYOCARDIAL VIABILITY: FDA IND
  - NIH Cardiovascular Cell Therapy Research Network
- Disease Modeling of Congenital Heart Disease

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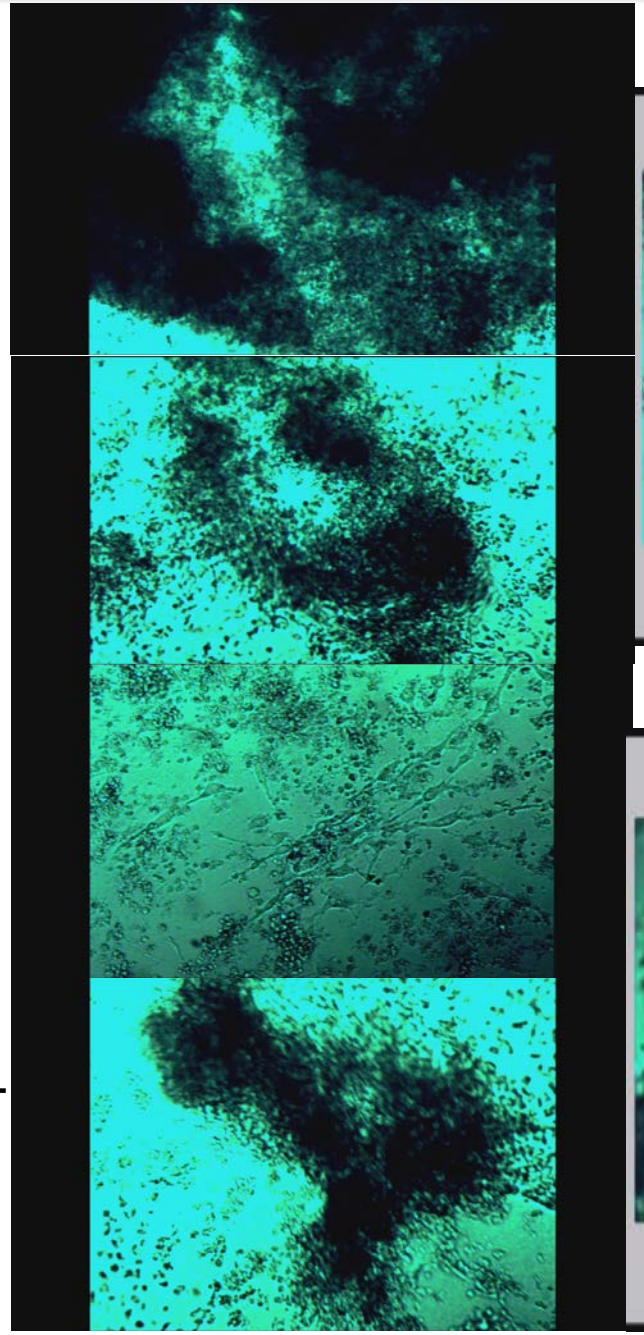
Durable Myocardial Tissue

hCMs only

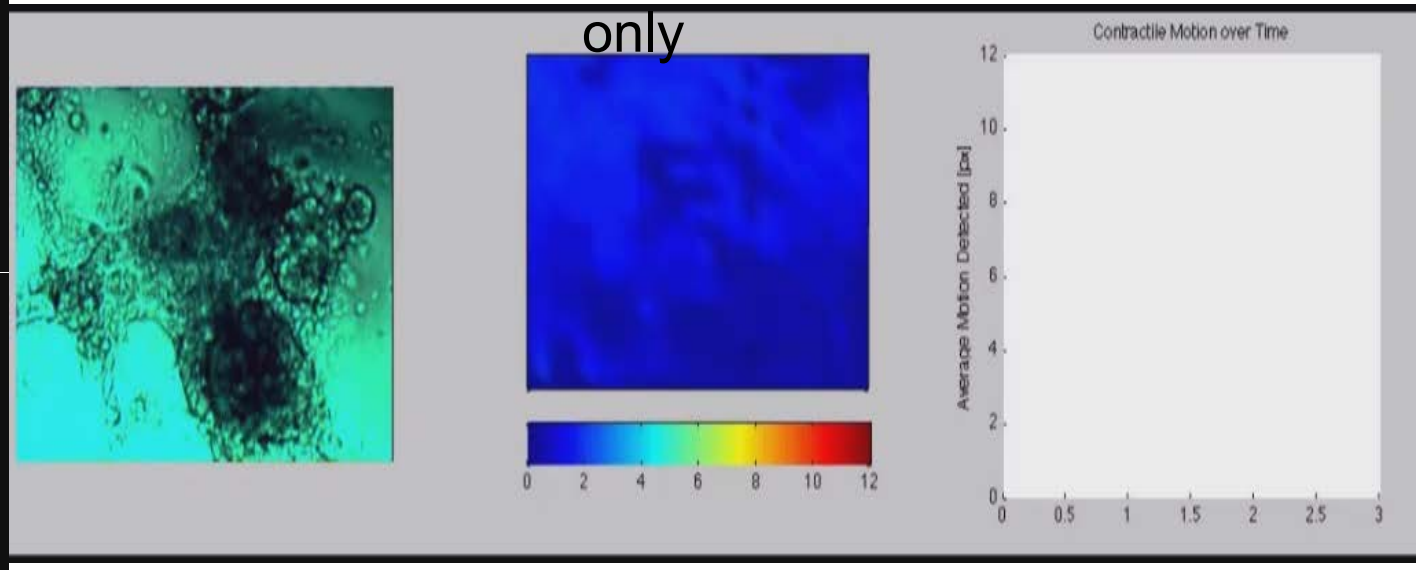
hCMs + hAMSCs

hCMs + hECs

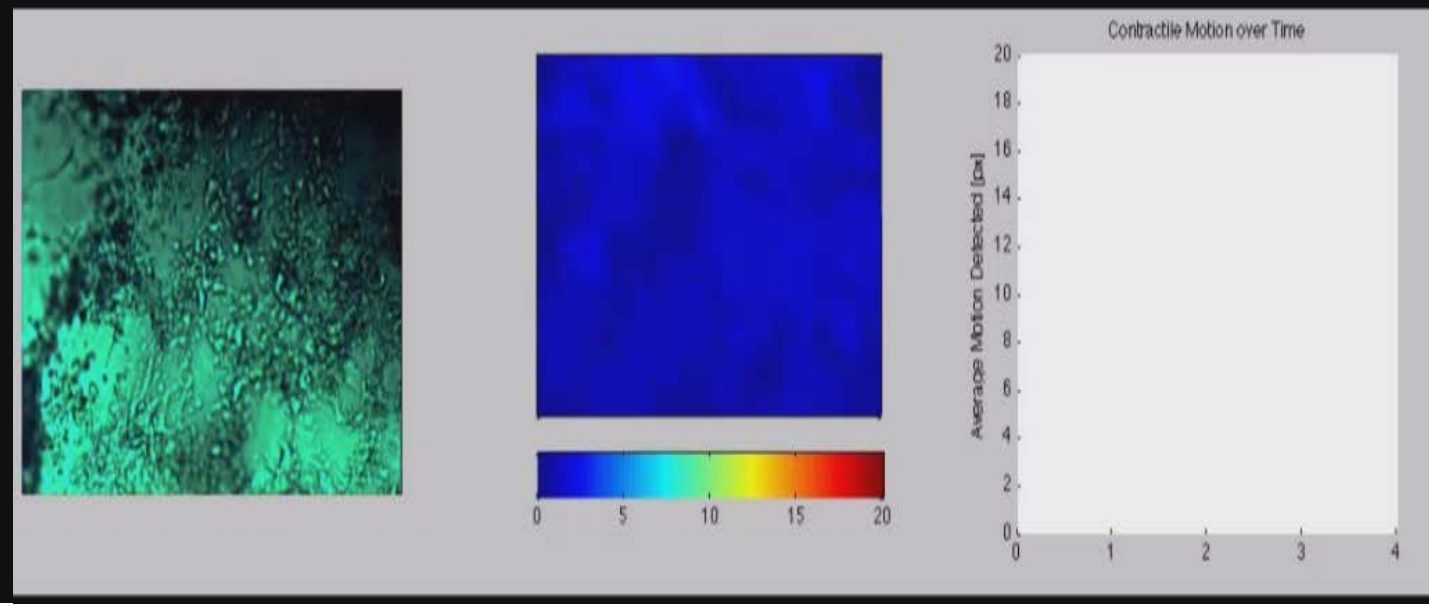
hCMs + hAMSCs+ hECs



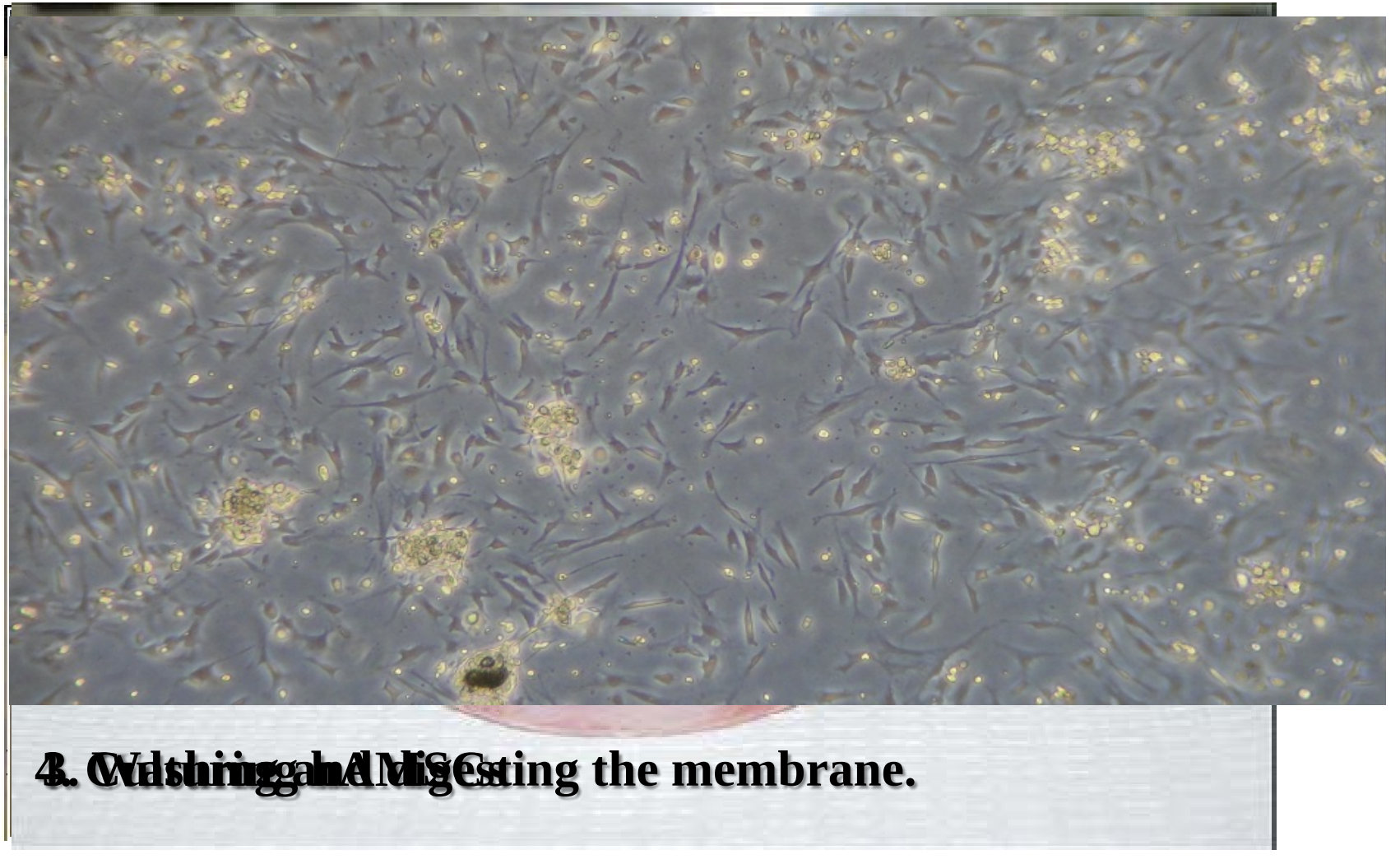
hCMs only



hCMs + hECs



hAMSC isolation



4. Washing hAMSC
4. Culturing hAMSC
4. Digesting the membrane.

Reference: Fabio Marongiu et al. Isolation of Amniotic Mesenchymal Stem Cells. Current Protocols in Stem Cell Biology 1E.5.1-1E.5.11

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Literature

REVIEW

THE AMERICAN JOURNAL of MEDICINE®

Clinical Relevance of Hibernating Myocardium in Ischemic Left Ventricular Dysfunction

Nov 2010

Arend F.L. Schinkel, MD,[a,b] Jeroen J. Bax, MD,[c] Victoria Delgado, MD,[c] Don Poldermans, MD,[d] Shahbudin H. Rahimtoola, MD[e]

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Learn and Live℠

Predicting Benefit From Revascularization in Patients With Ischemic Heart Failure : Imaging of Myocardial Ischemia and Viability

Feb 2011

Orla Buckley and Marcelo Di Carli

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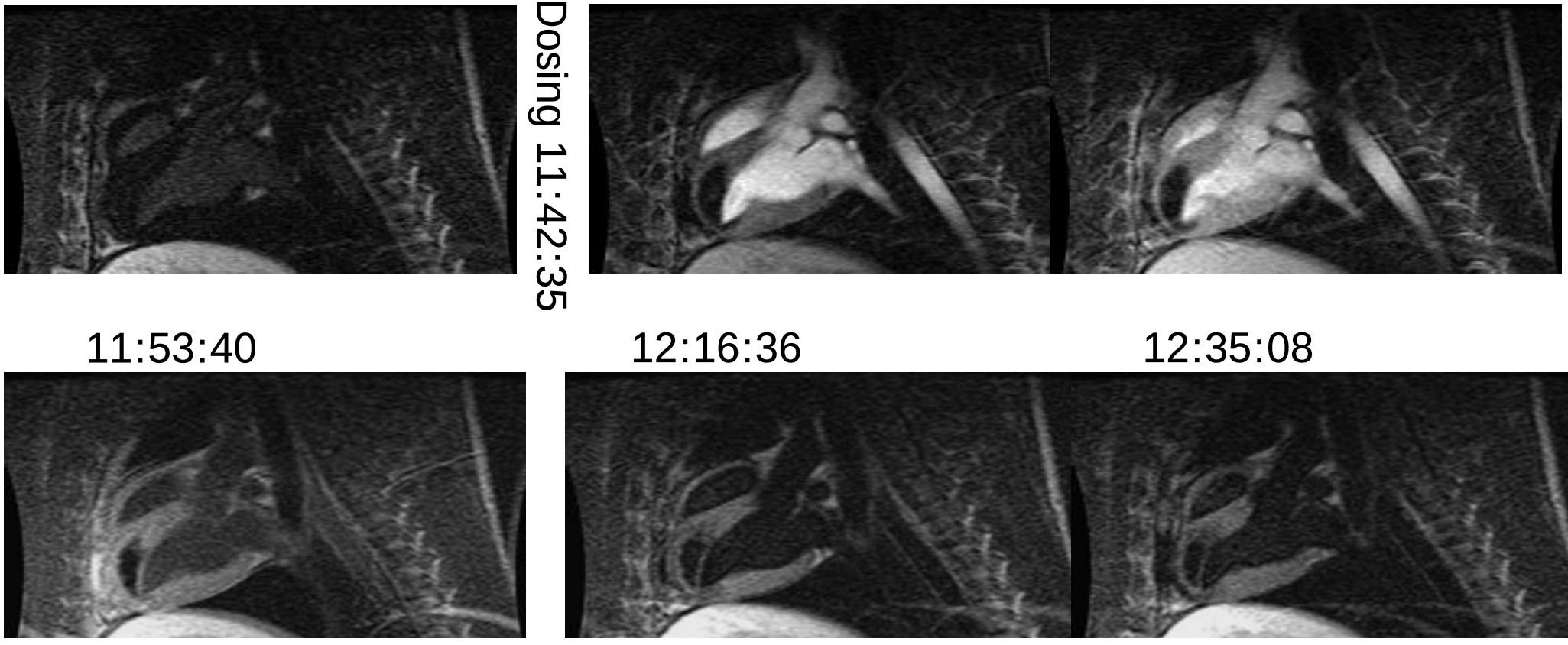
MEMRI of Myocardial Viability



- Rapid assessment of myocardial viability
  - Tissue enhancement pattern established within 30 seconds
  - Persists for at least one hour and blood signal gone by 10 minutes
  - Viable cell specific

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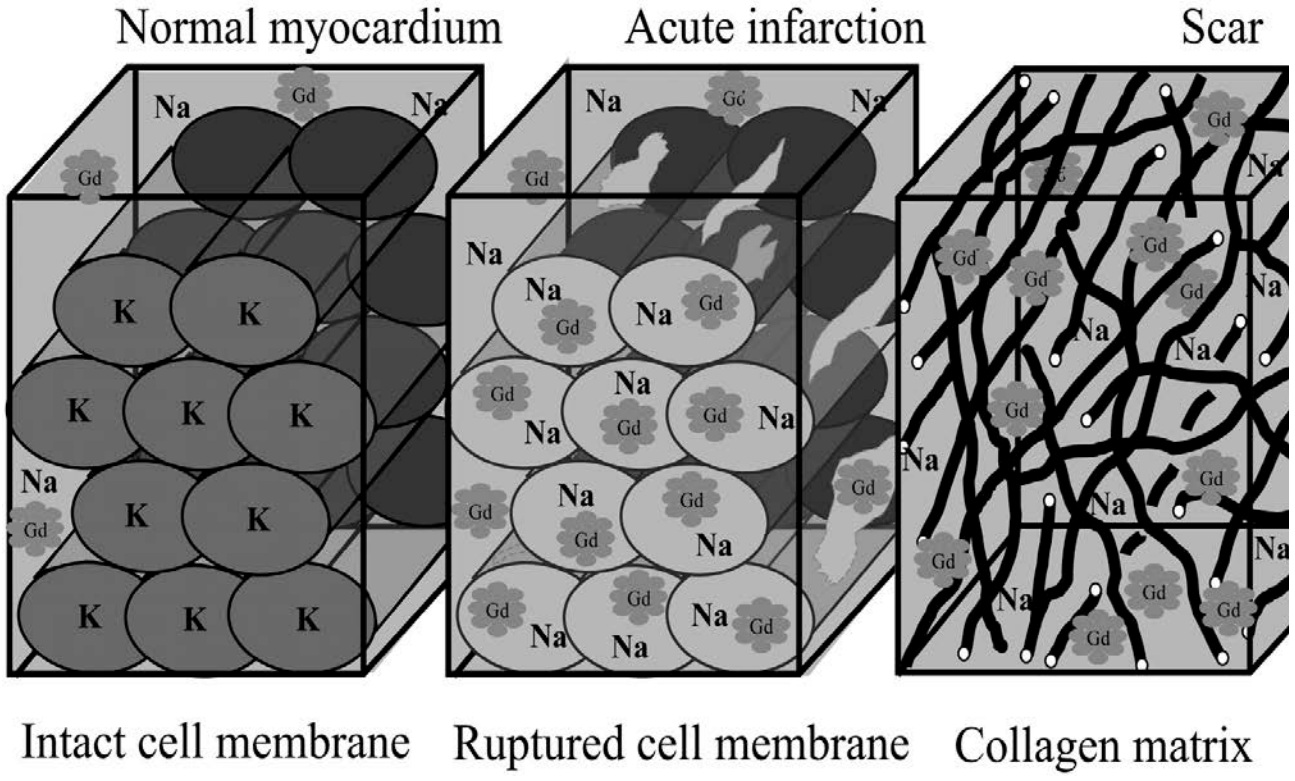
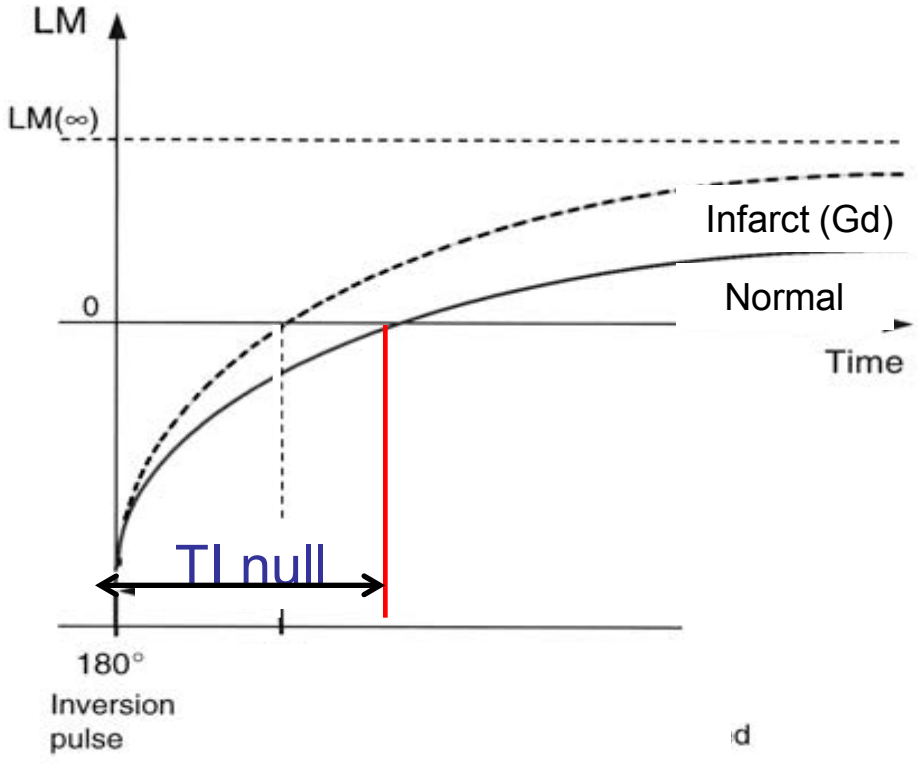
Mechanism of Delayed Enhancement



Mahrholdt, H. et al. Eur Heart J 2005 26:1461-1474



- Gd is injected & wait 10-20 min
- Gd accumulates in injured tissue
- 180˚ RF pulse inverts all the spins
- Tissues return to nl at different rates
- At time TI, image acquisition begins

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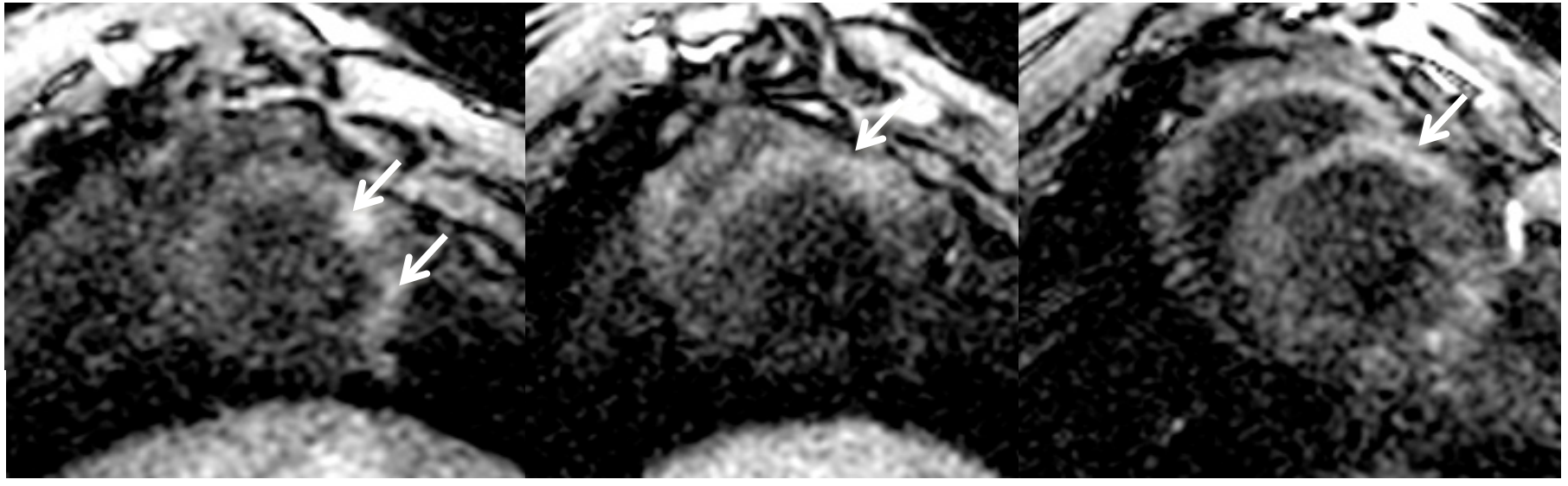
In vivo MEMRI of viable hAMSCs

- hAMSCs survived over 5 weeks (and beyond?) in porcine heart with minimal cyclosporine immunosuppression
- hAMSCs improved cardiac function predictably and durably in subacute and chronic infarction model
- hAMSCs reduced infarct size and LV dilatation
- MEMRI tracked LIVE stem cells with no prior cell modification



D17
Post-Delivery

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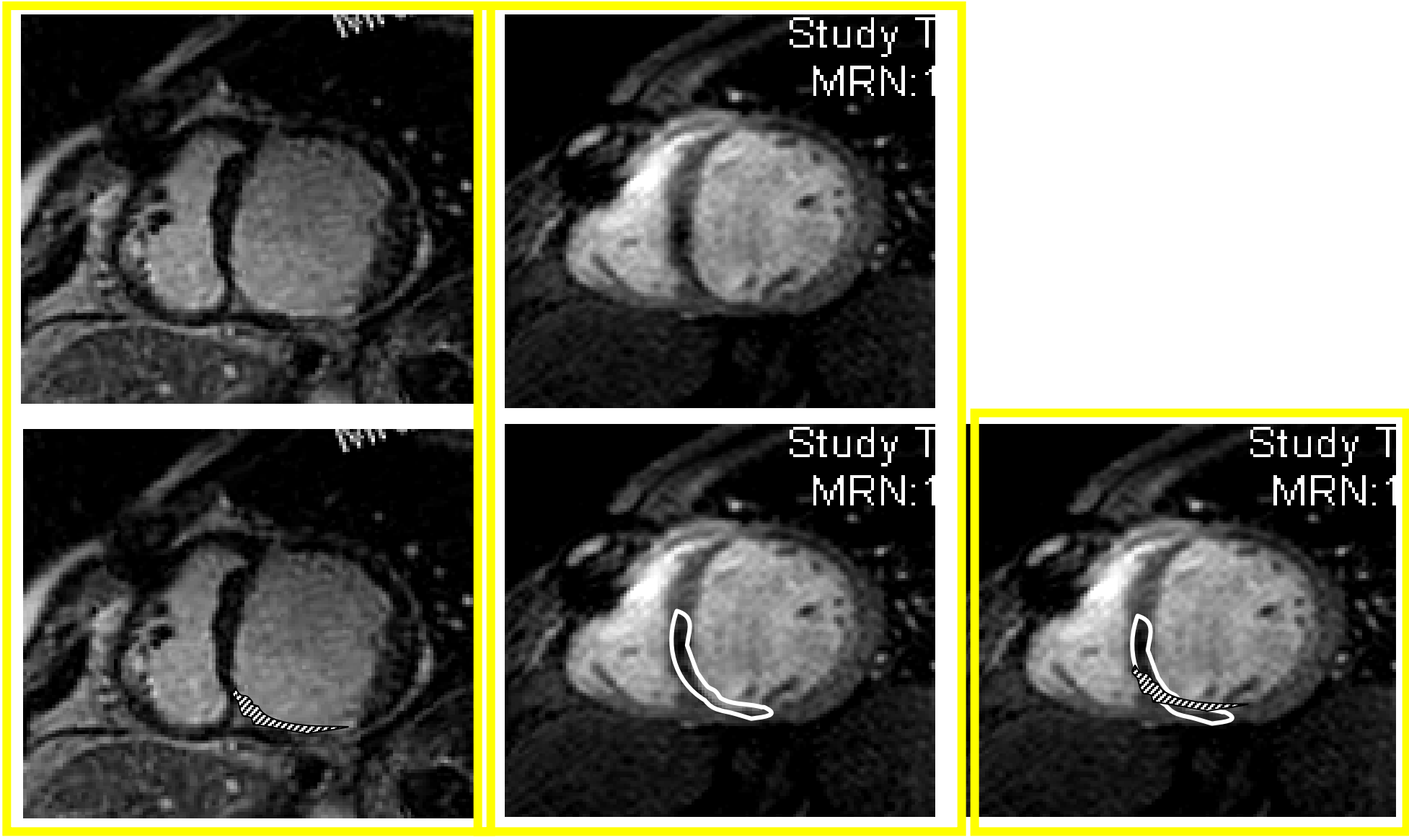
Peri-infarct ischemia



Delayed-enhanced **Stress Perfusion Image** **Peri-infarct Ischemia**

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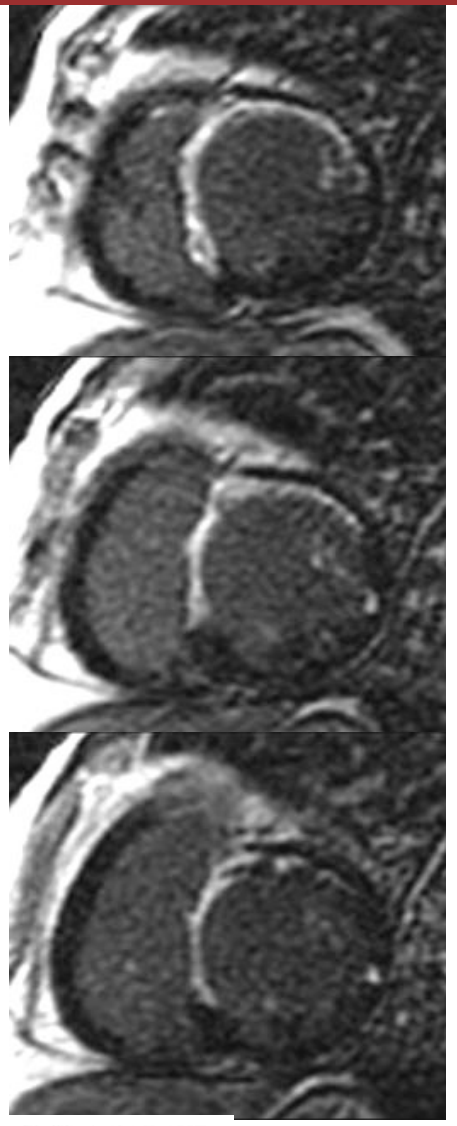
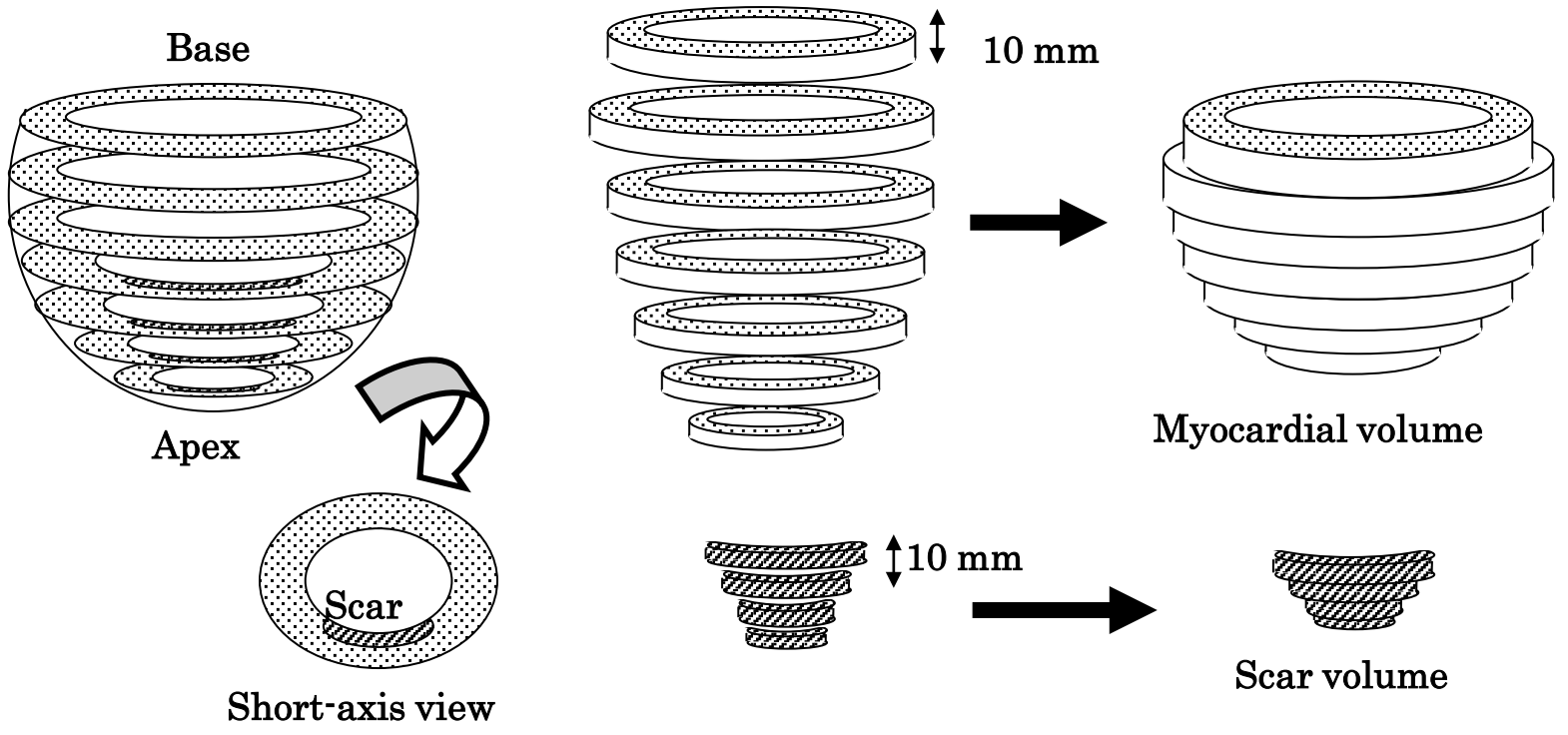
Measurement of Myocardial Scar





Scar percentage of the myocardium (%) = Scar volume / myocardial volume

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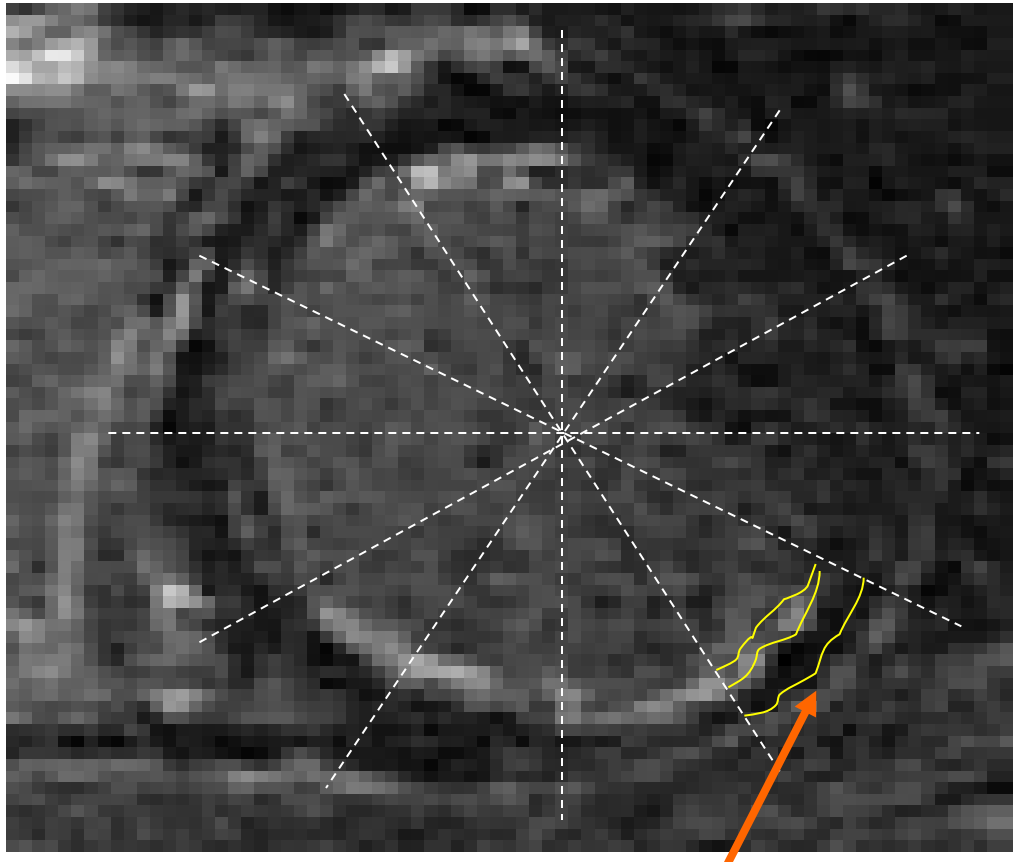
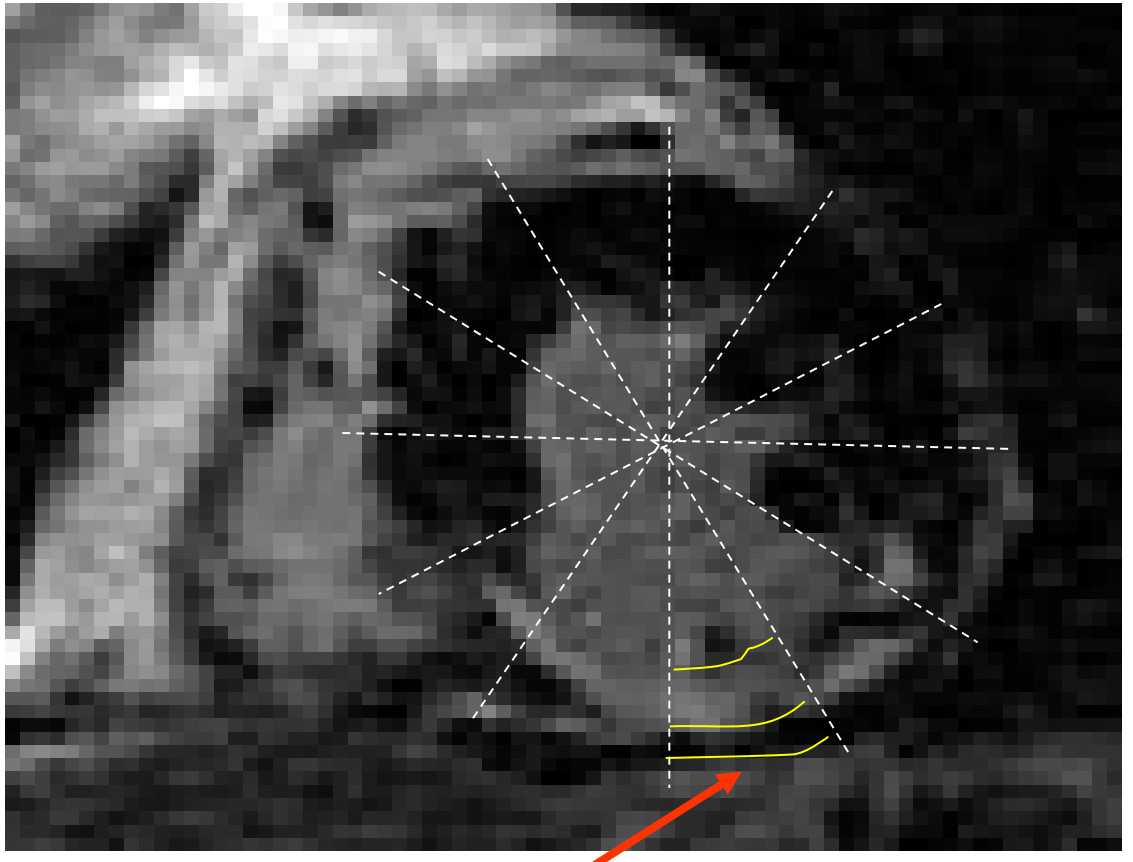
Analysis of Transmurality





1 - 50% (Non transmural)

76 - 100% (Transmural)

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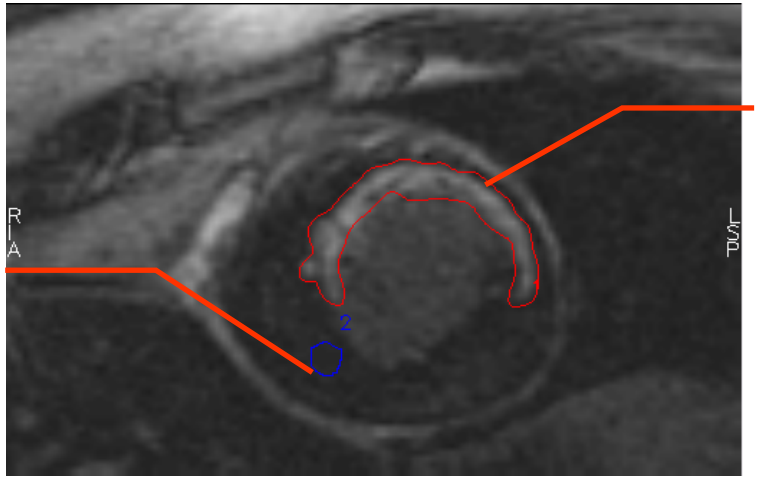
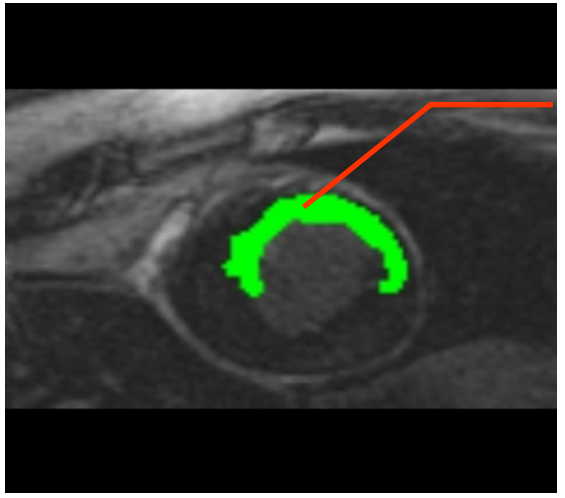
Tissue Heterogeneity Quantification

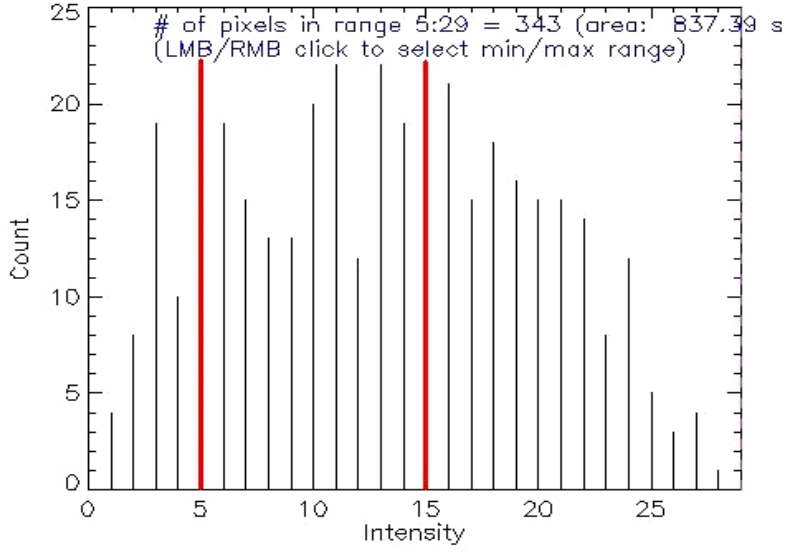


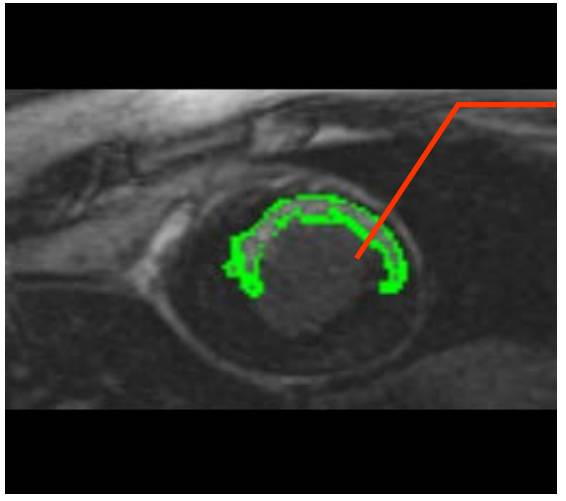
ROI

Infarct Outline



Infarct Zone





Peri-Infarct Zone

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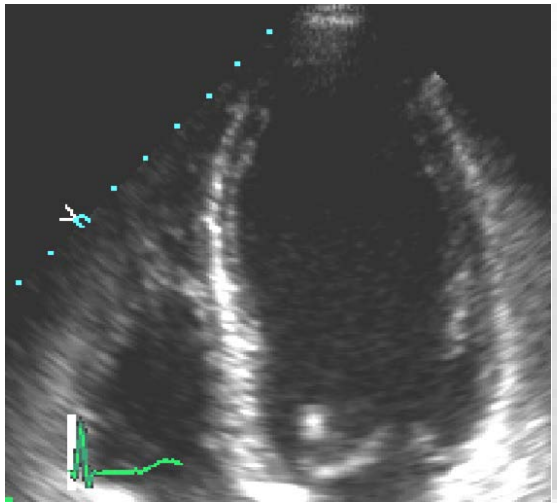
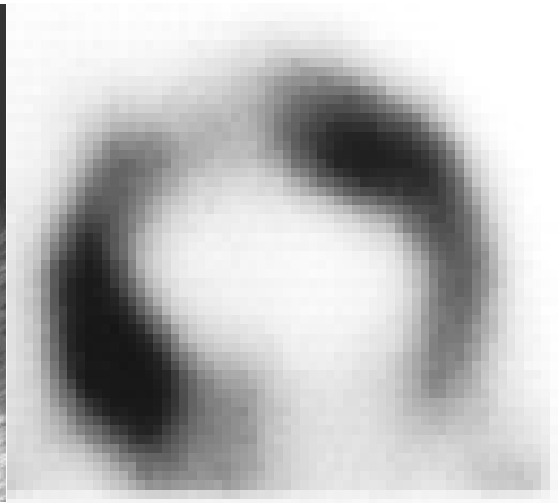
Tissue heterogeneity analysis performed with Cinetool version 7.1.2, GE Healthcare.

Evaluation Myocardial Viability

- Myocardial Function
  - Echo: safe vs. qualitative and acoustic window
- Cellular Metabolism
  - SPECT and PET: quantitative vs. ionizing radiation and low image quality
- Myocardial Scar and Infarct: gold standard
  - MRI: image quality and quantitative vs. Gd-contrast agent





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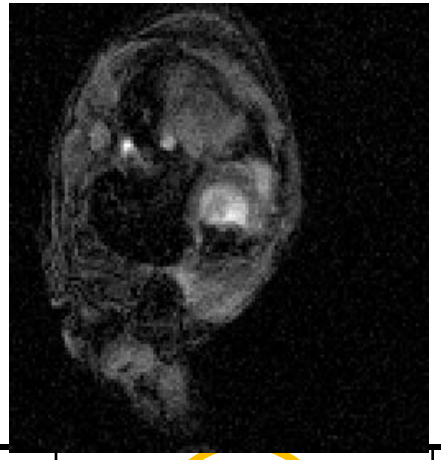
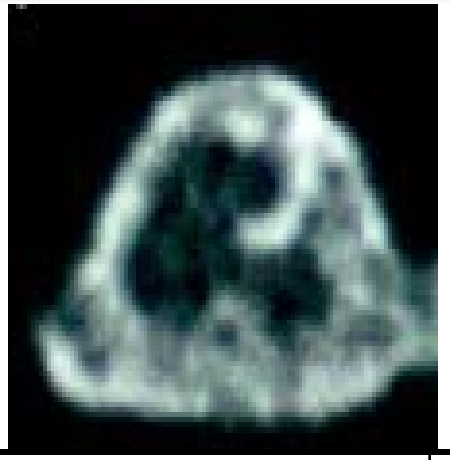
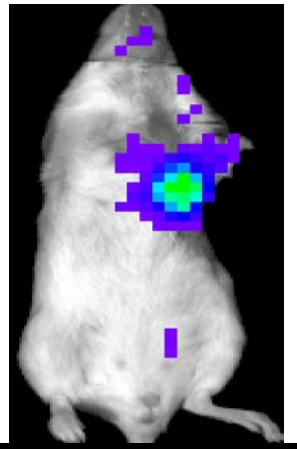
II. *In Vivo* Evaluation of Stem Cell Survival







	MRI	RN	BLI
Sensitivity	10^{-7} - 10^{-9} Mole/L	10^{-11} -10^{-12} Mole/L	10^{-15} - 10^{-17} Mole/L
Spatial resolution	500 µm	3-5 mm	3-5mm
Temporal resolution	10 ms	seconds	seconds

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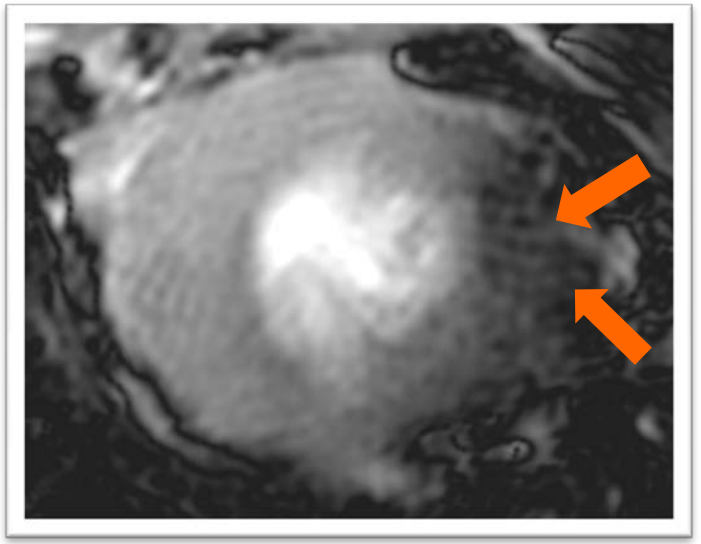
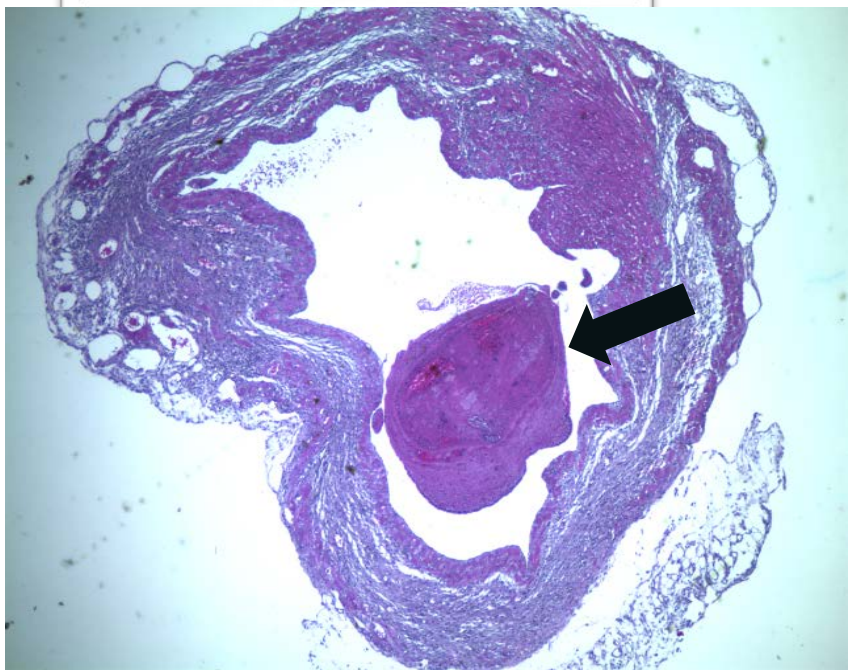
In Vivo Molecular MRI - Teratoma





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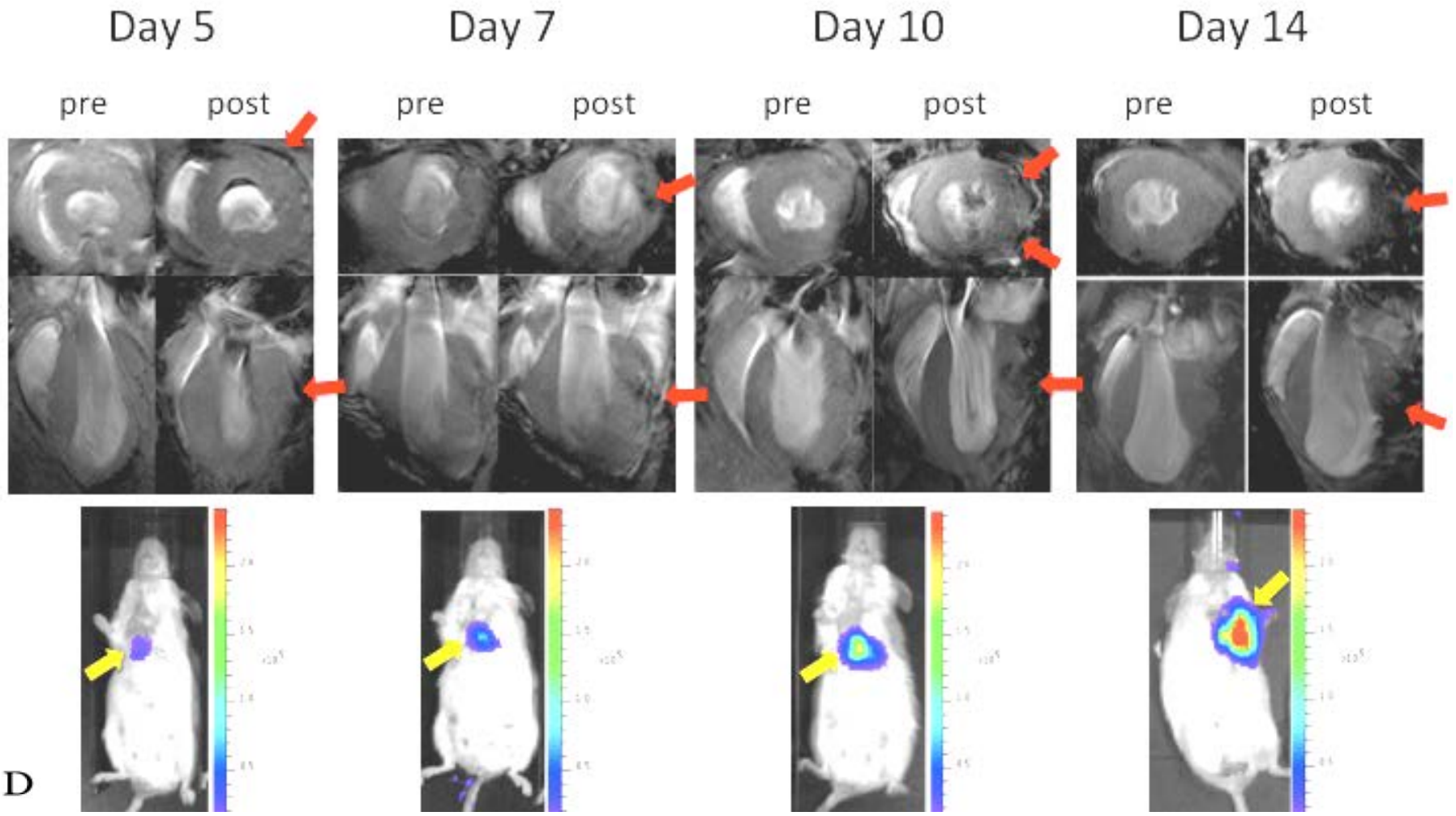
In Vivo Molecular MRI of Cell Survival



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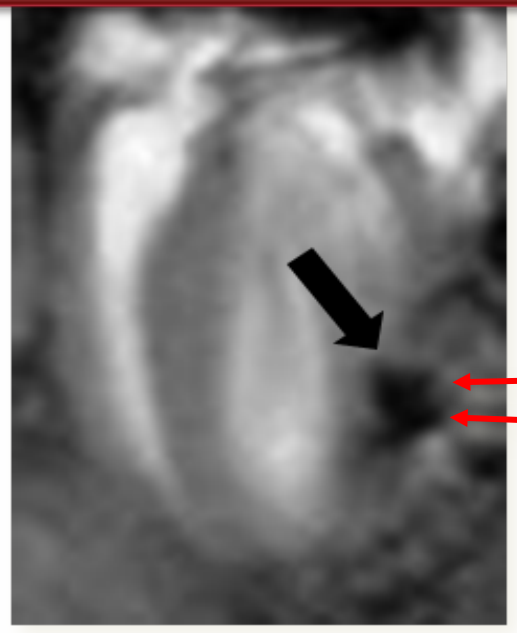
Chung et al, *Magn Reson Med*, 2011

In Vivo Molecular MRI of Cell Survival



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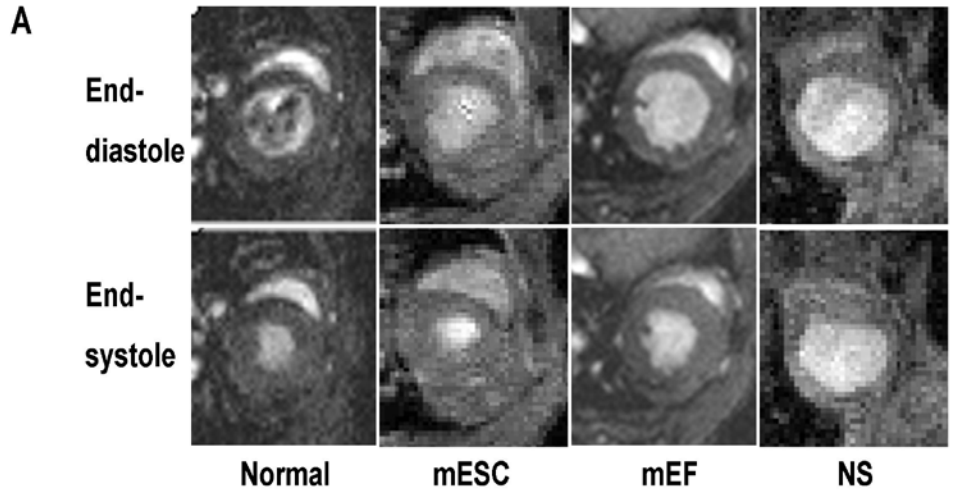
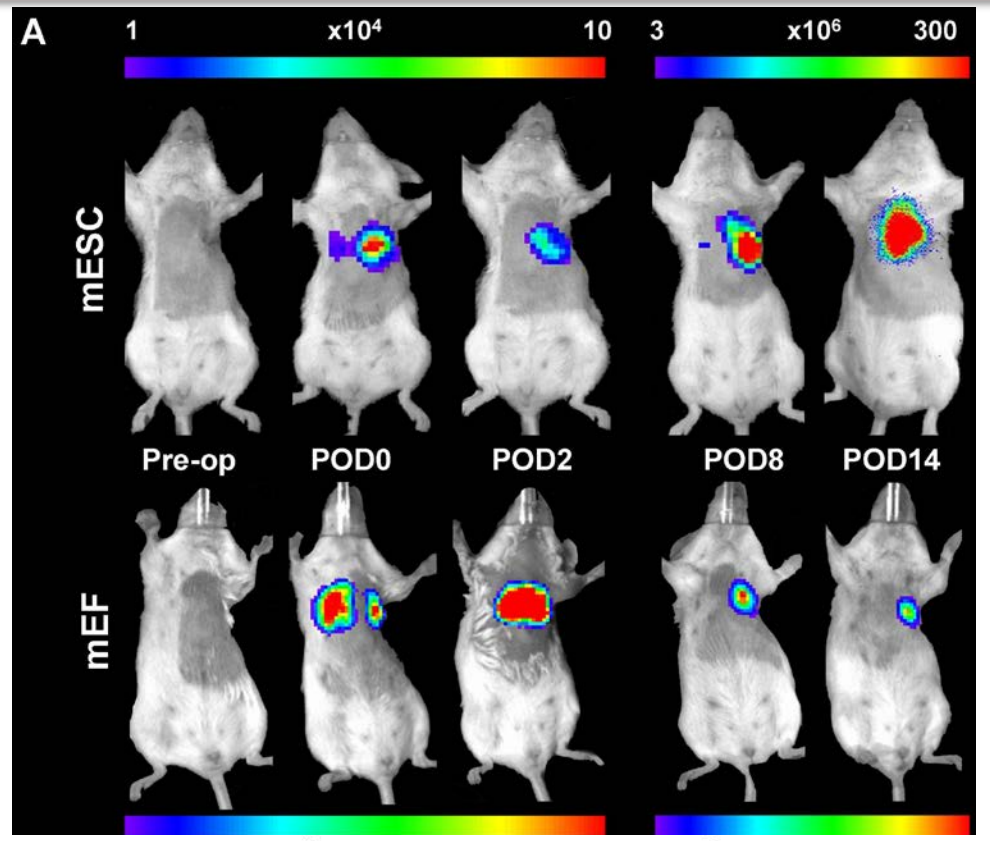
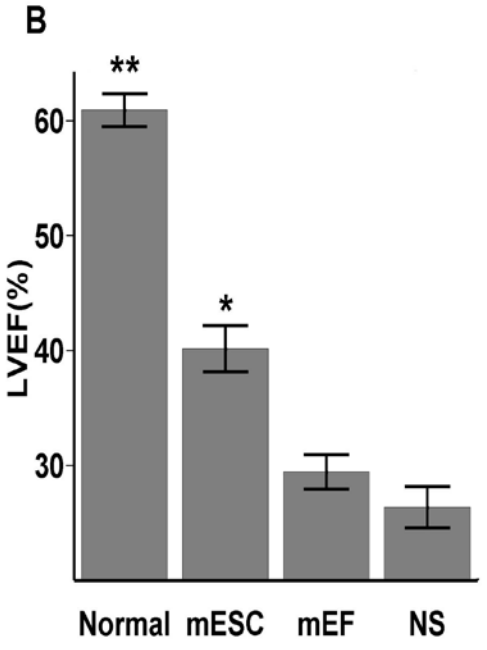
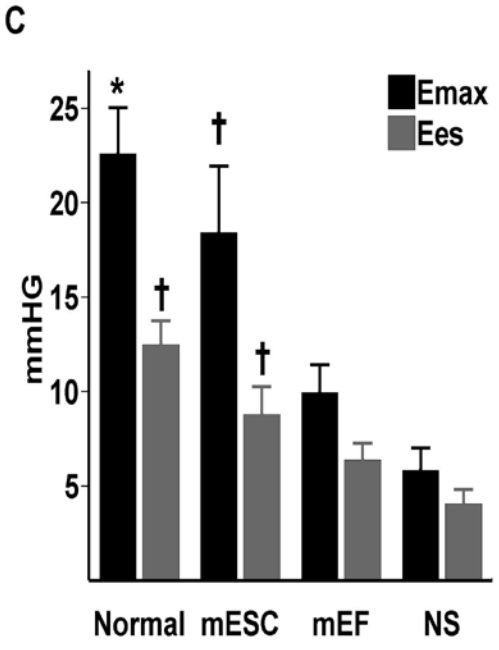
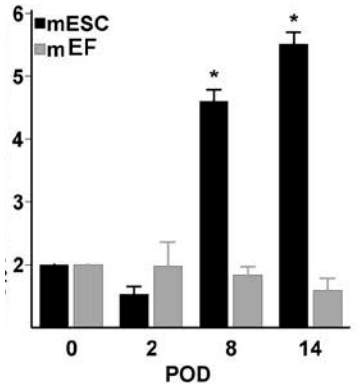
II. Stem Cell Survival in the Myocardium

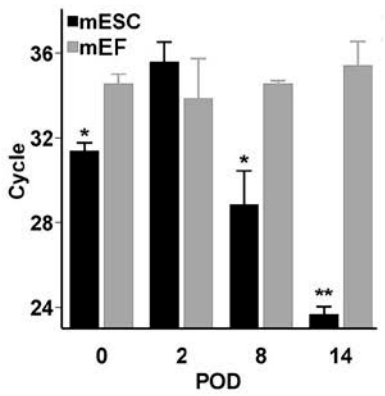


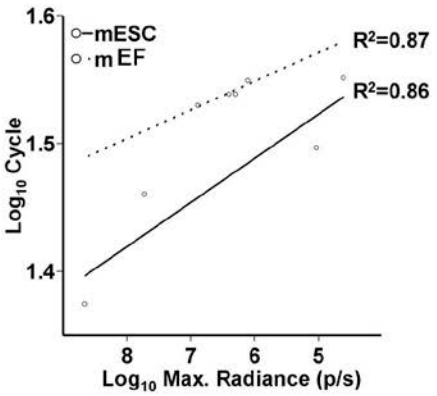






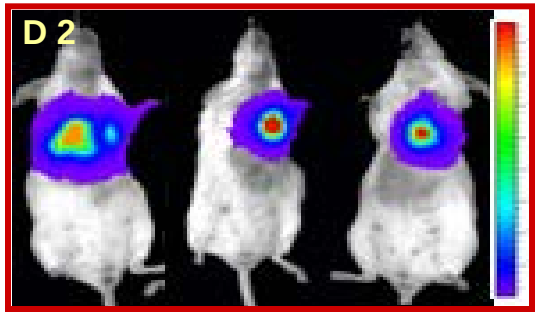


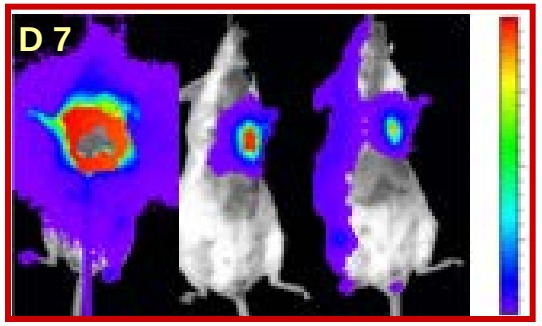


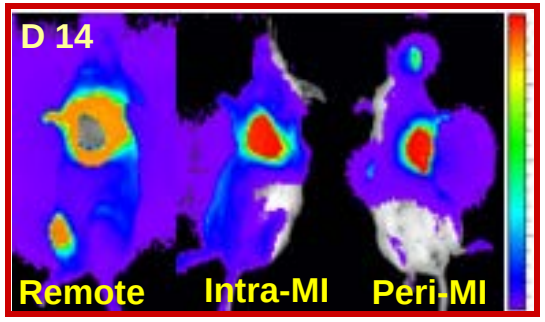


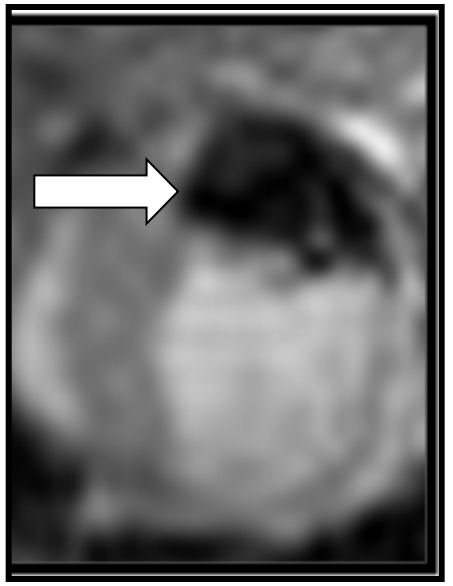
Hendry et al, *JTCS* 2008

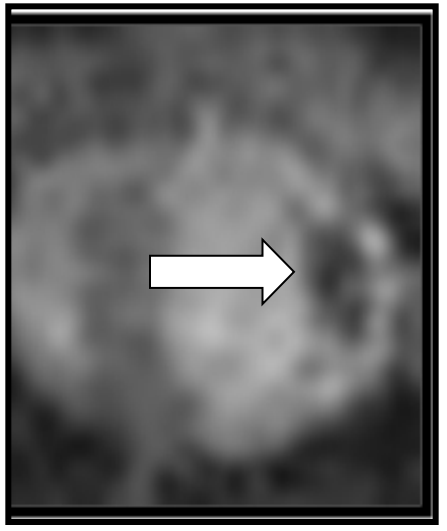
Mechanism of Myocardial Restoration

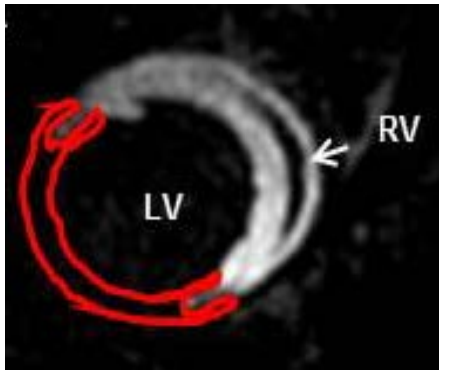


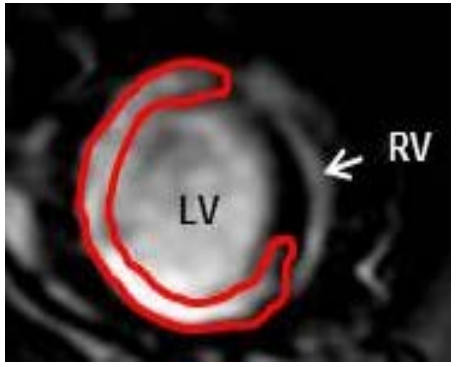


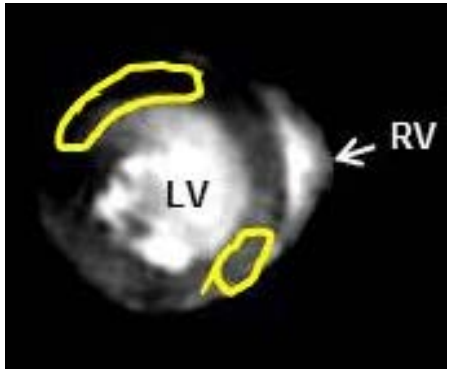


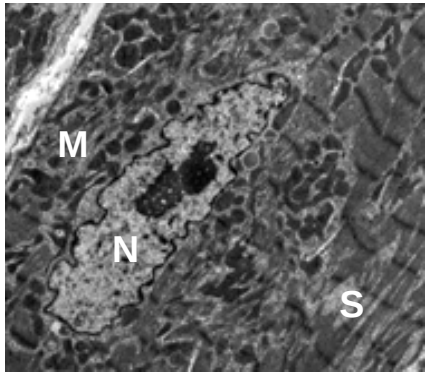


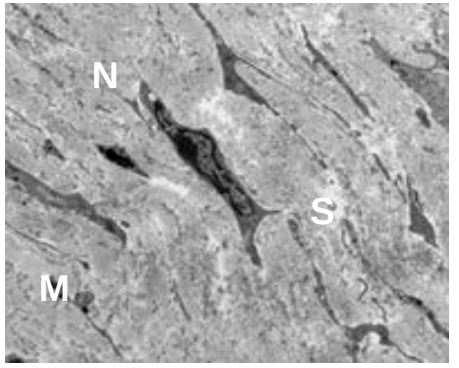


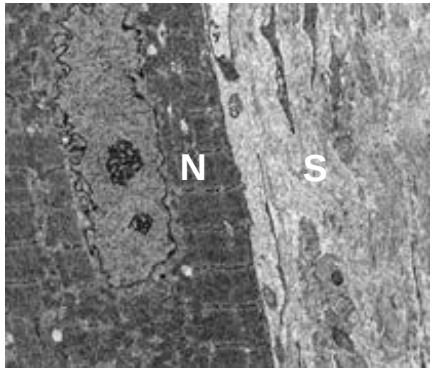












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Hung et al, *Circulation: Cardiovasc Imaging*, 2008