

Cell Therapy Strategies to Mend a Broken Heart

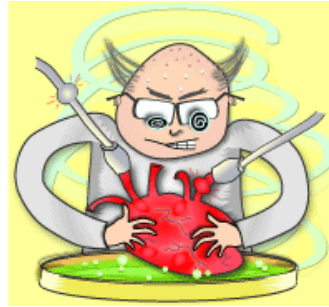
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www.incor.usp.br/genetica



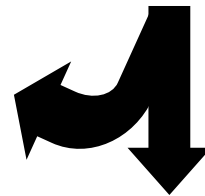
Simpósio Regional sobre Medicina Translacional

02/12/2011- FAPESP - São Paulo

Biological Cardiac Repair



- Clinic/Pharmacology
- Interventionist:
 - Angioplasty/Stents
 - Surgical Revascularization

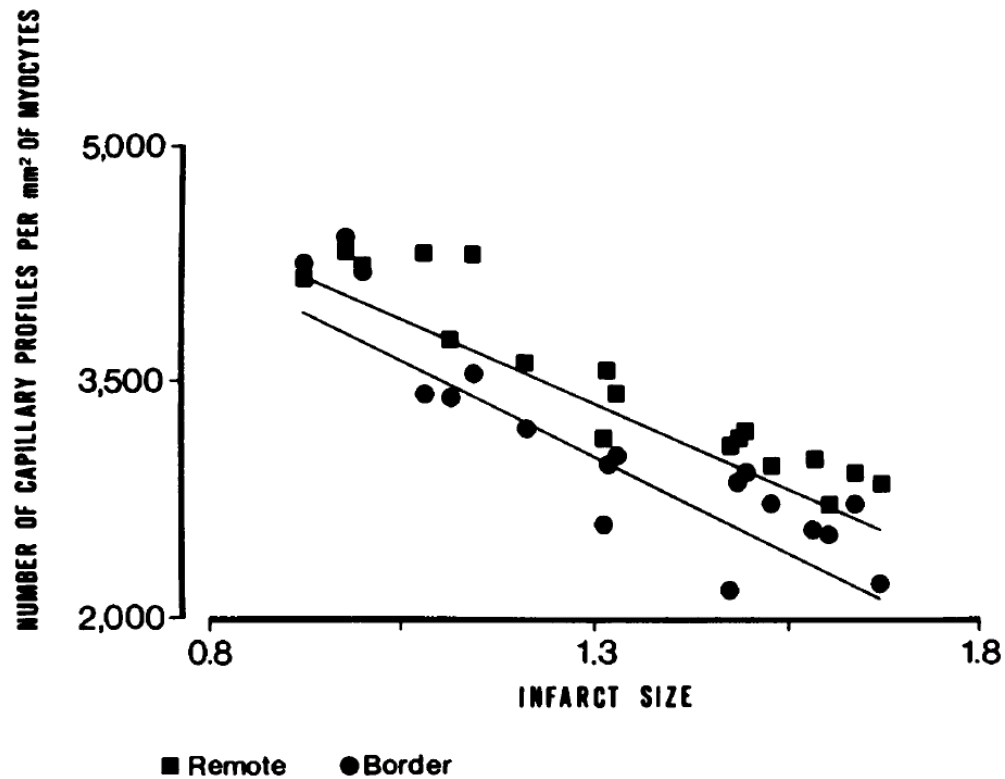


New &
Improved Approaches

Cell replacement - “biological cardiomyoplasty”
Angiogenesis

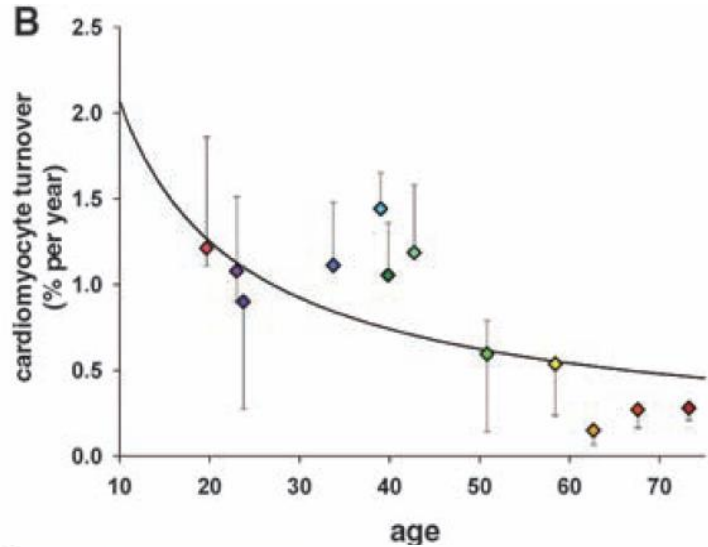
Targeting the Microvasculature for Ischemic Tissue Repair

Cappillary Vasculature in the Border Zone Post-MI

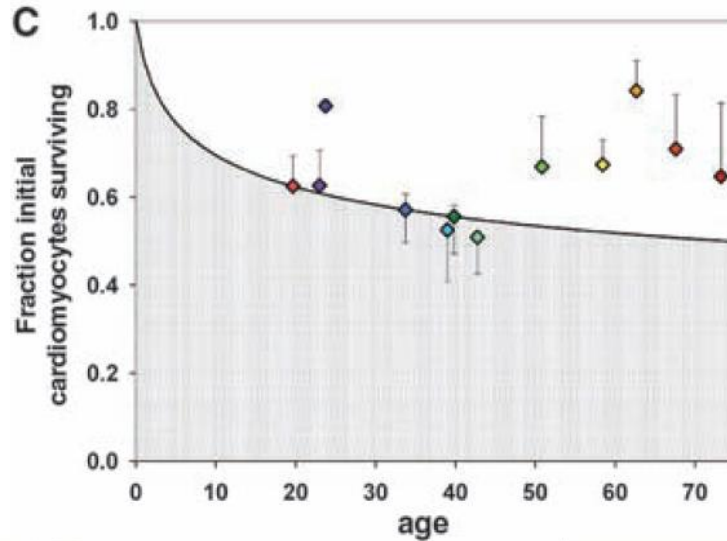


Cardiomyocyte Renewal in Humans

(Evidence from integration of ^{14}C from Nuclear Tests During the Cold War)

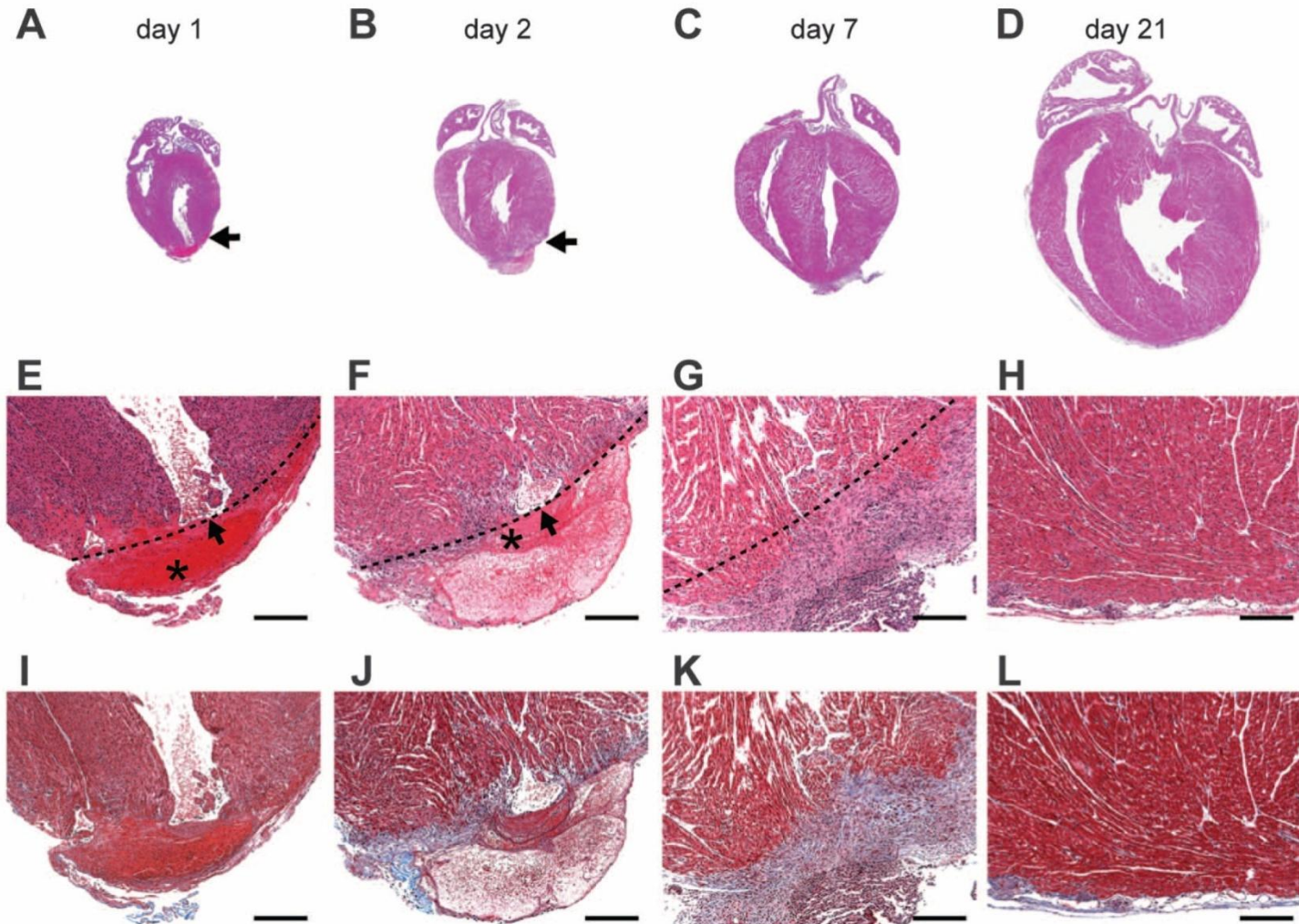


**Renewal Capacity:
2-0 – 0.45%/yr**

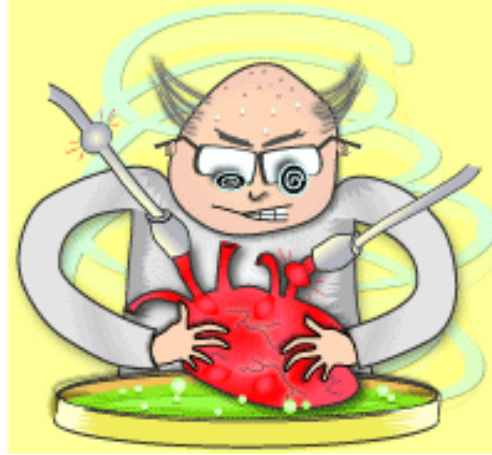


**Exchange Rate: Fewer Than
50% during normal life span**

Transient Regenerative Potential in Neonatal Mouse Heart



Biological Cardiac Repair

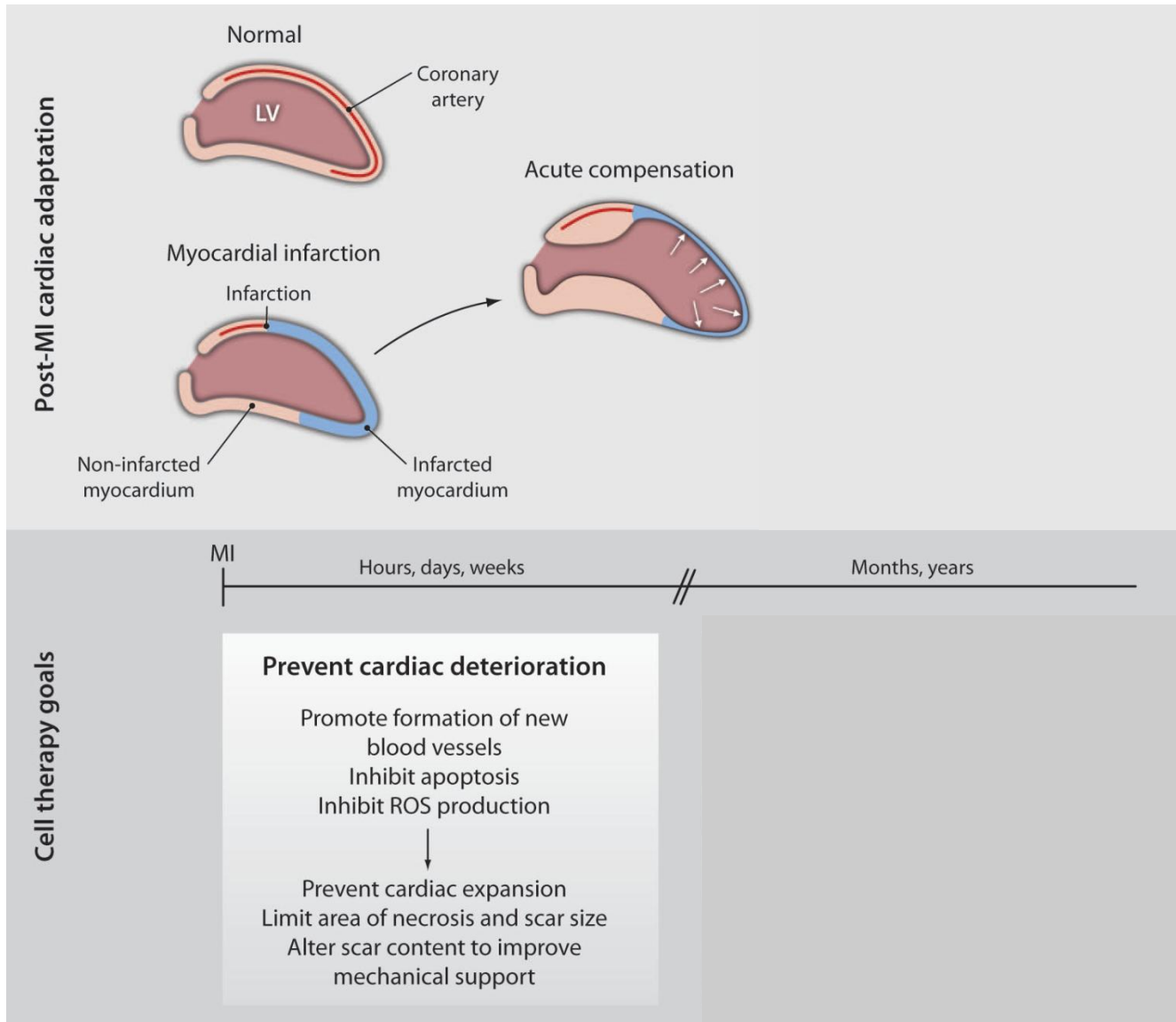


Cell replacement - “biological cardiomyoplasty”
Angiogenesis

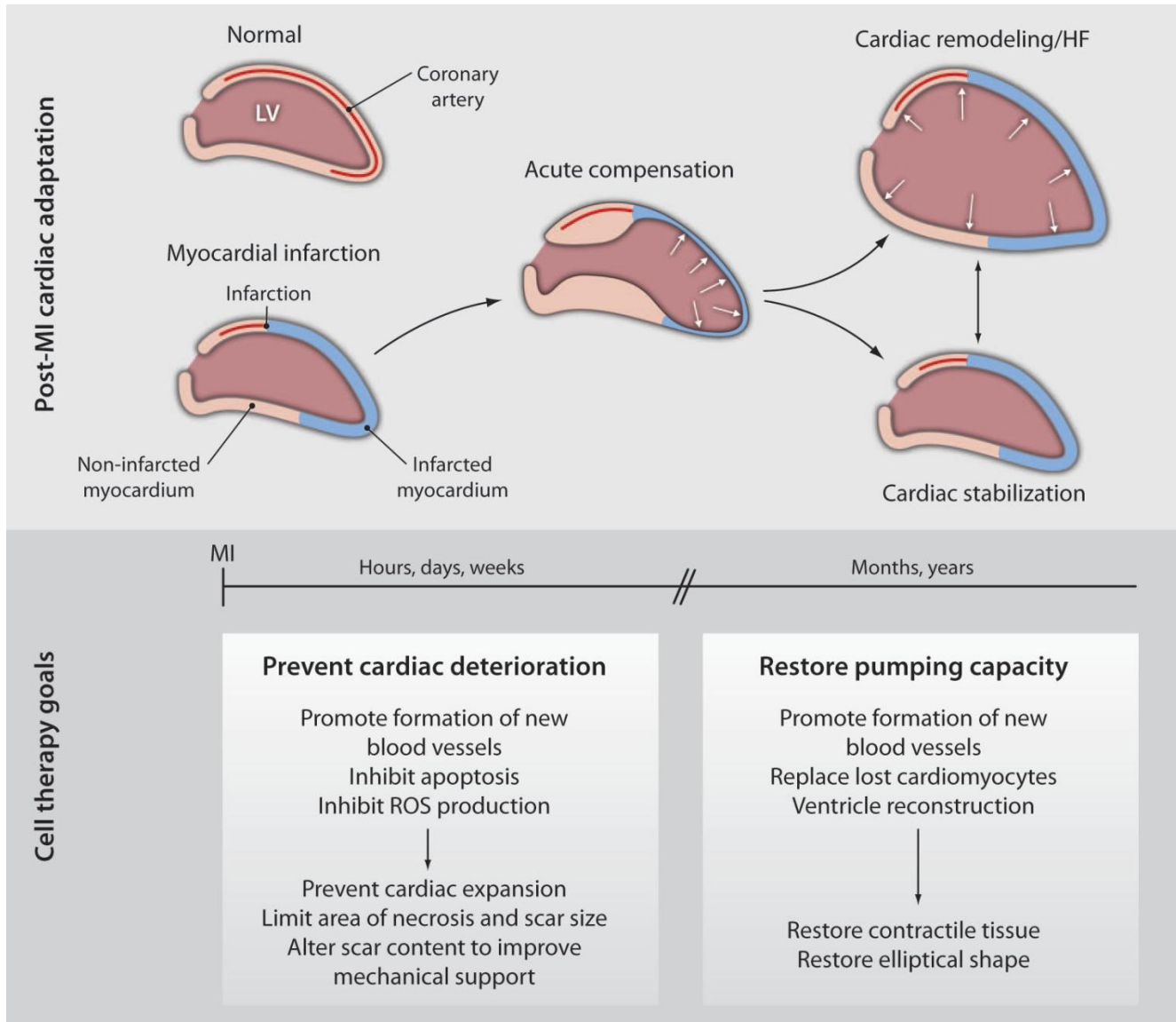


- Cell as a “vector”
- Embryonic/Adult Pluripotent & Reprogrammed Cells (iPS)
- Cardiac Tissue Engineering

Post-MI Adaptations & Goals For Cardiac Cell Repair (Complex Scenario)



Post-MI Adaptations & Goals For Cardiac Cell Repair (Complex Scenario)



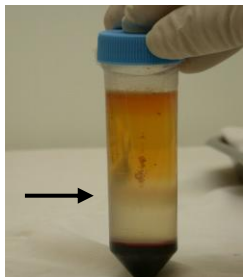
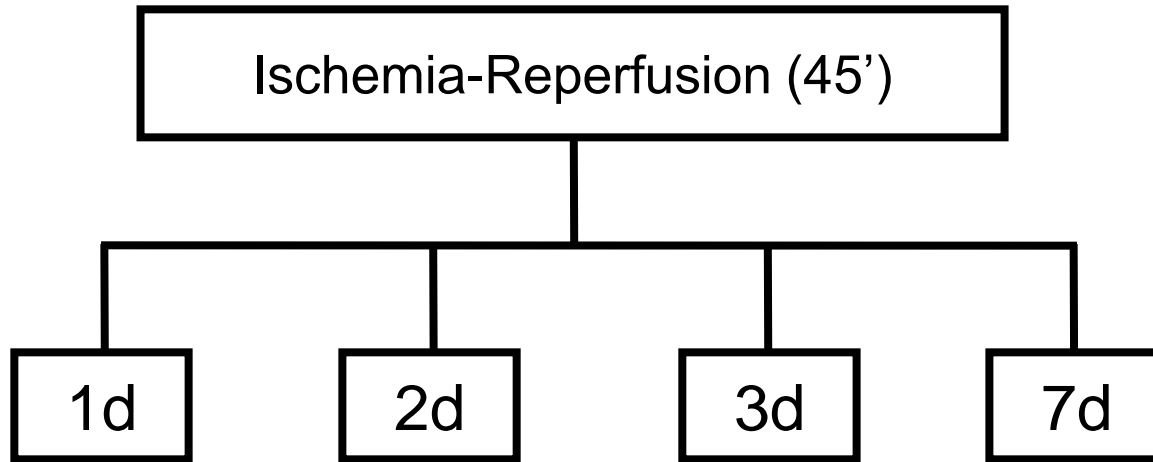
Cell Therapy in Ischemic Cardiac Disease

1. Routes & Timing for cell injection
2. BMC, Adipose Stem Cell (ASC) and genetic modified cells for cardiac repair
3. Combined use of CABG/TMLR & BMC for cardiac repair in humans

Cell Therapy in Ischemic Cardiac Disease

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Cell Retention Study

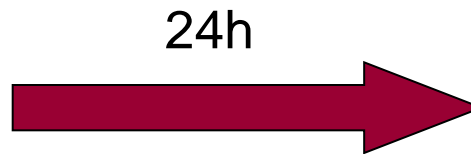


BMCs

(6×10^6)



Radiolabelig with
 ^{99m}Tc -Cerete^c®



After injection
IV LV LV+ IM

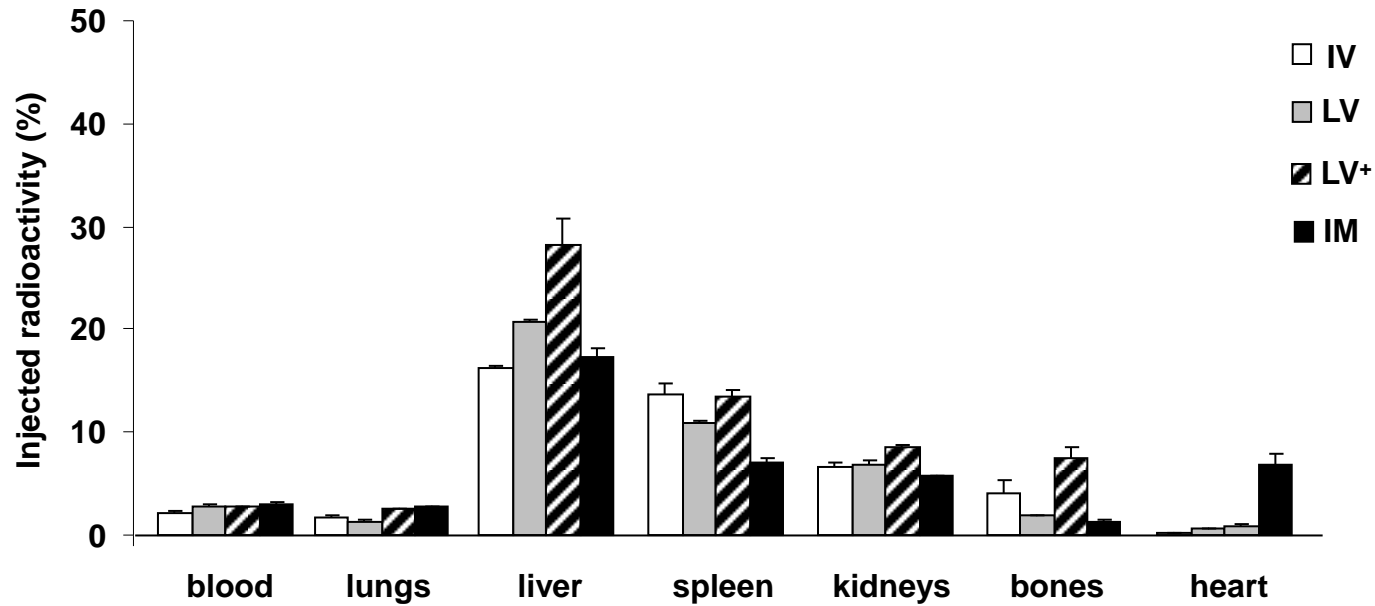
DMEM Fibrin

Sacrifice

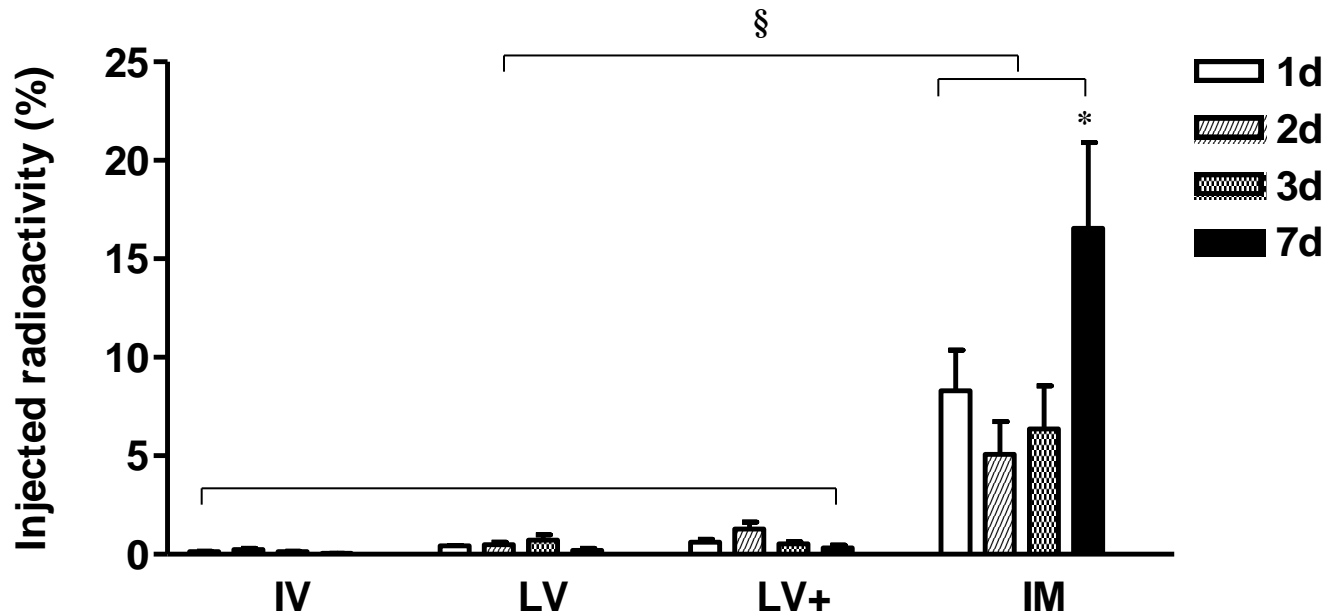


Radiometry

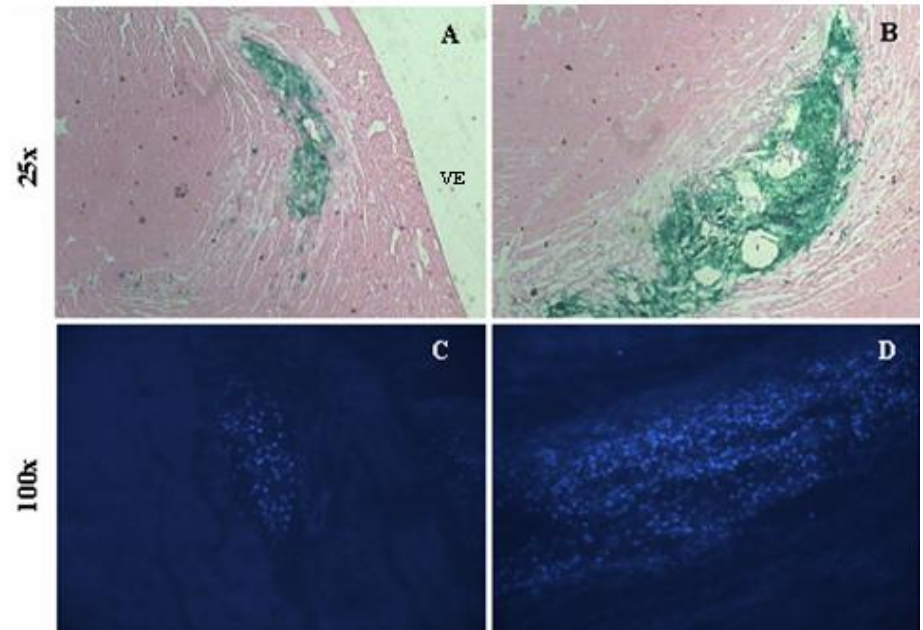
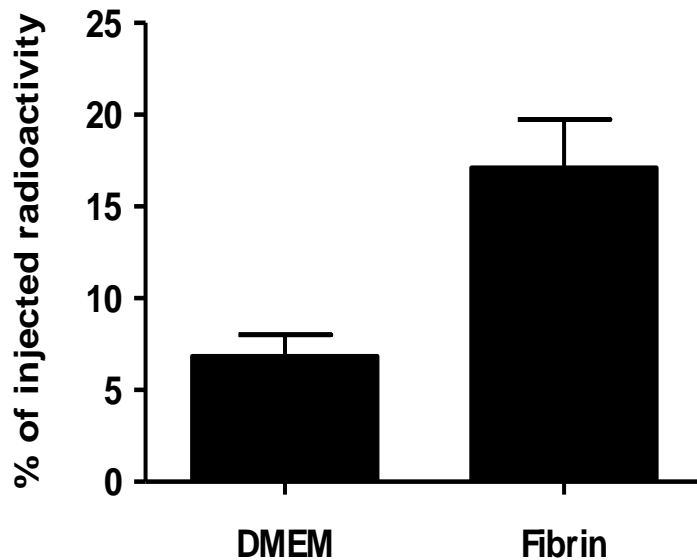
Tissue Body Biodistribution of BMCs



Cardiac Retention and Biodistribution of BMCs



Cardiac Retention of BMCs using fibrin as vehicle

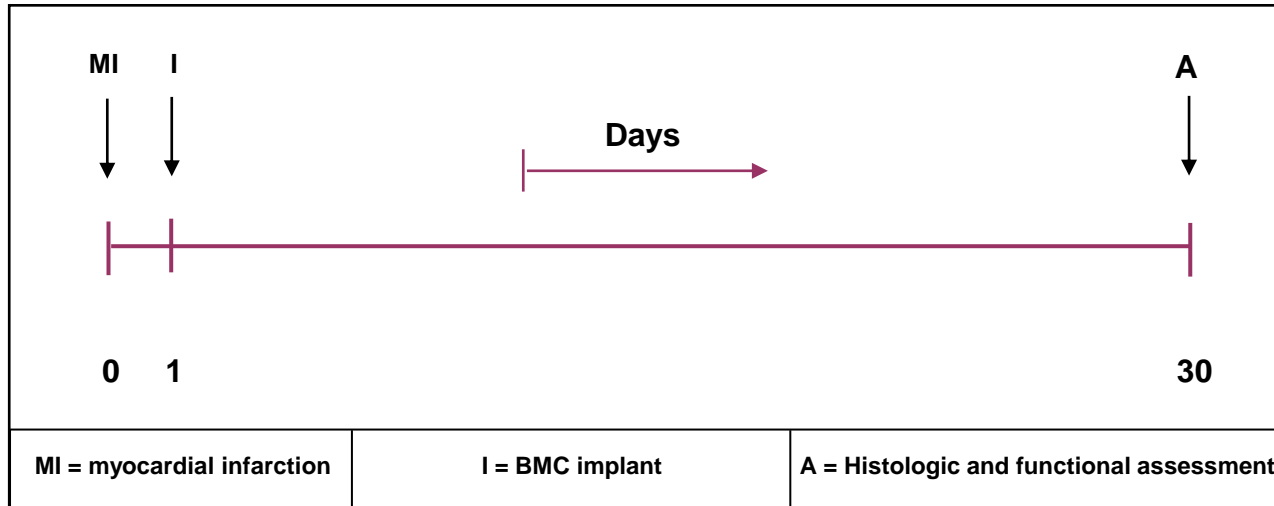


Histologic sections of hearts from animals that receive AdLacZ-fibroblast after b-gal assay (A e B) and visualized on fluorescence microscopy for DAPI nuclear stain (C e D). Left panels represents sections from animals transplanted with BMCs in medium DMEM and right panel that transplanted with BMCs in fibrin.

Cell Therapy in Ischemic Cardiac Disease

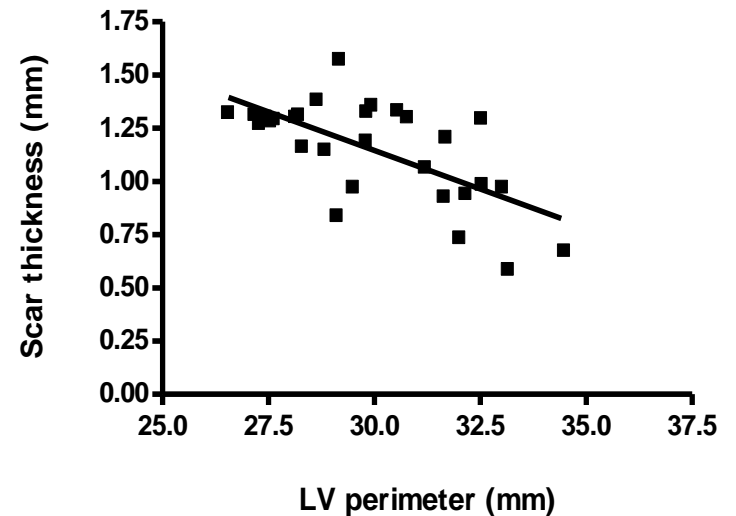
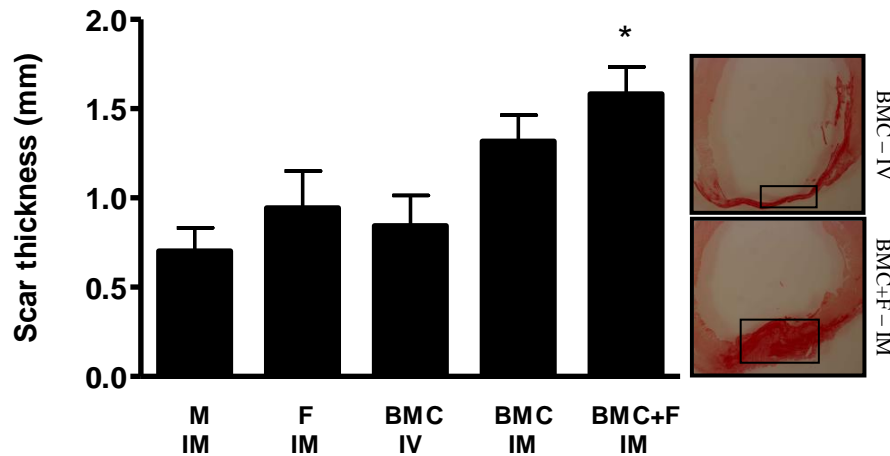
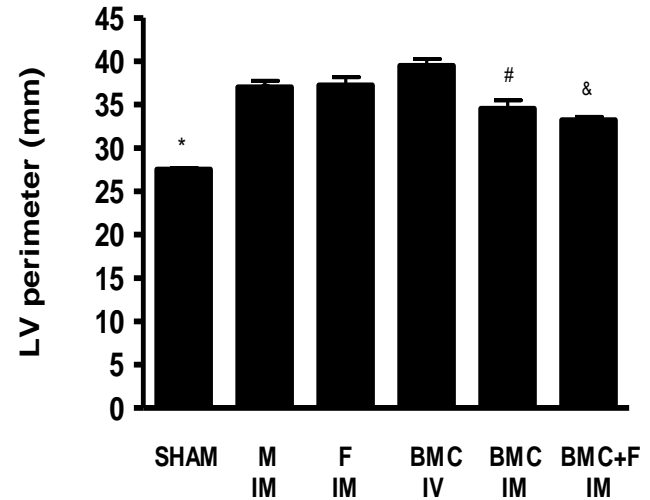
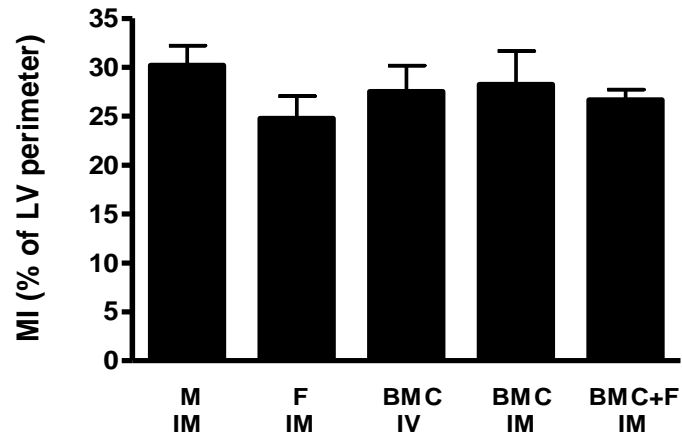
1. Routes & Timing for cell injection
2. BMC, Adipose Stem Cell (ASC) and genetic modified cells for cardiac repair
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BMC Transplantation & Cardiac Function Post-MI

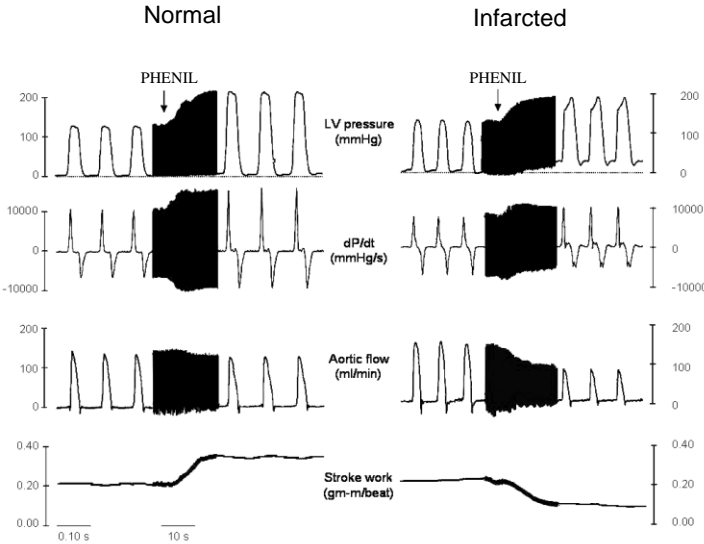


Group	MI	Content	Route
SHAM (N=5)	—	—	—
M – M (N=4)	+	culture medium	intramyocardial (IM)
F – IM (N=4)	+	fibrin	intramyocardial (IM)
BMC – IV (N=5)	+	BMC (1×10^6)	intravenous (IV)
BMC – IM (N=4)	+	BMC (1×10^6)	intramyocardial (IM)
BMC+F – IM (N=4)	+	BMC + fibrin (1×10^6)	intramyocardial (IM)

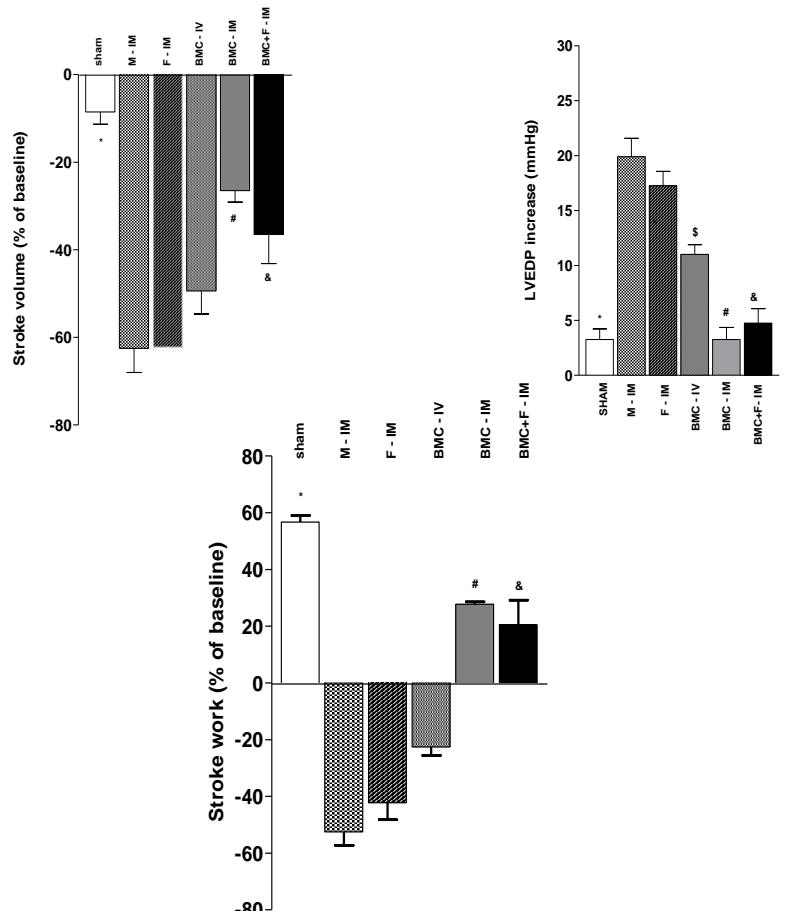
Cardiac Morphometry



Afterload Hemodynamic Stress to Assess Cardiac Performance



Santos et al, *Can J Physiol Pharmacol*, 2010



Nakamuta et al, *PLoS One*, 2009

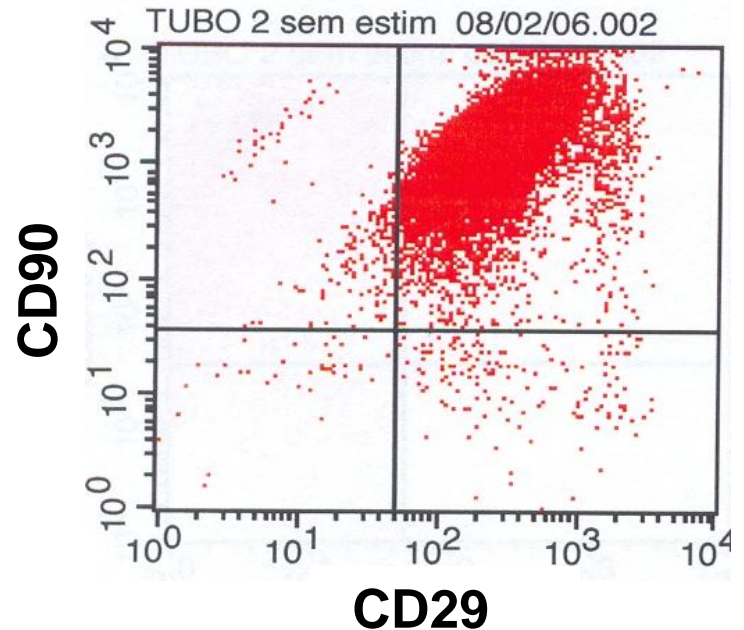
Liposuction



hASC Phenotype Homogeneity

- FACS analysis

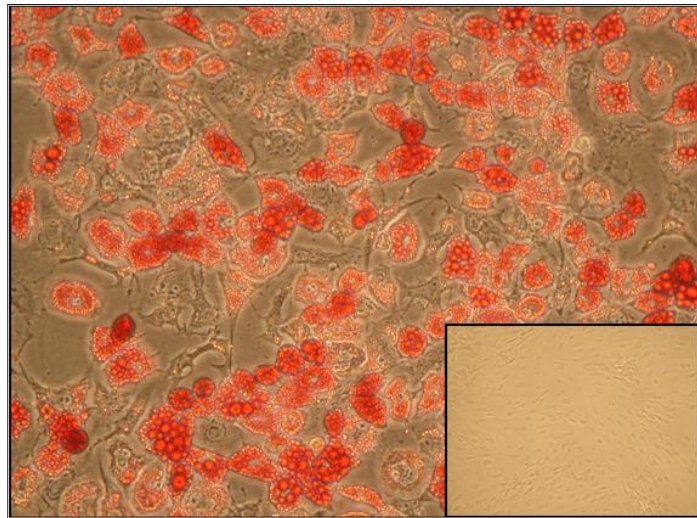
Marker	Average (%)	±	Stard. Deviation (%)	N	Phenotype
CD11	1.7	±	2.2	2	-
CD13	98.6	±	1.3	2	+
CD14	0.9	±	1.0	2	-
CD29	87.9	±	17.7	3	+
CD31	5.6	±	7.7	3	-
CD34	1.7	±	1.5	3	-
CD44	53.5	±	5.1	2	+
CD45	3.4	±	3.1	2	-
CD49	58.7	±	8.8	2	+
CD51/61	10.3	±	13.0	2	-
CD54	58.3	±	31.2	2	+
CD73	95.1	±	4.7	2	+
CD90	98.9	±	1.4	3	+
CD106	2.8	±	3.4	2	-
AC133	0.9	±	1.2	2	-
HLA-ABC	62.0	±	47.5	2	+
HLA-DR	1.6	±	1.9	2	-



hASC Selfrenewal & Pluripotency

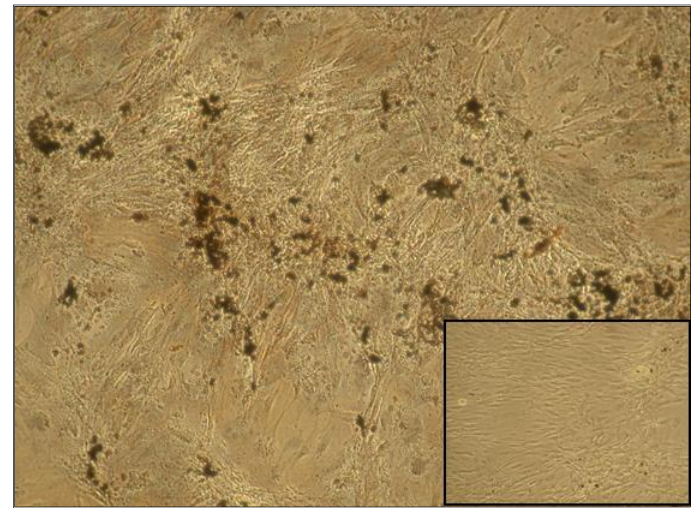
- hASC *in vitro* presents until 14th passage:
 - Doubling Time (94.1±18.3 hours, n=3-4)
 - Cumulative pop. Doubling (1.1+0.2 PD, n =4-9)
 - Cell Senescence (3.1±1.7 %, n=3-5)
- Differentiation potential:

Adipogenic



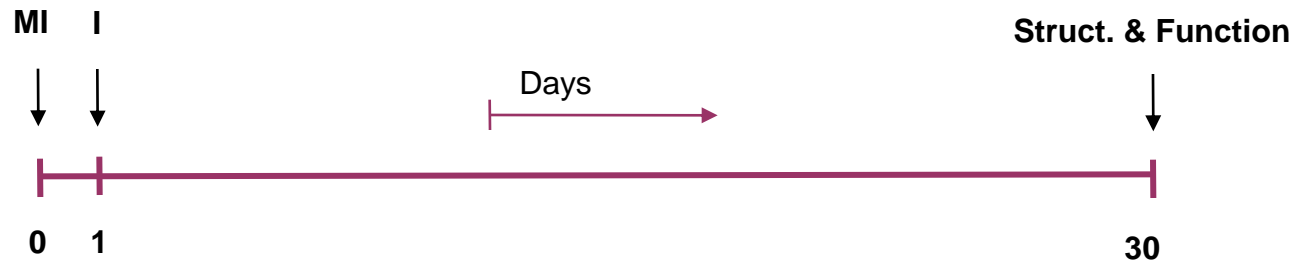
Oil red

Osteogenic



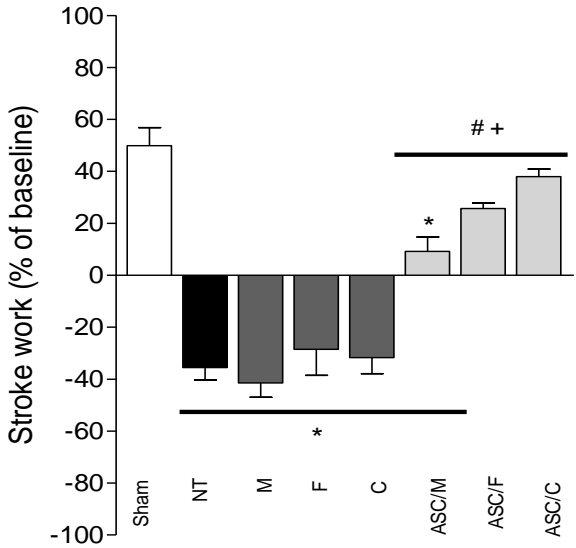
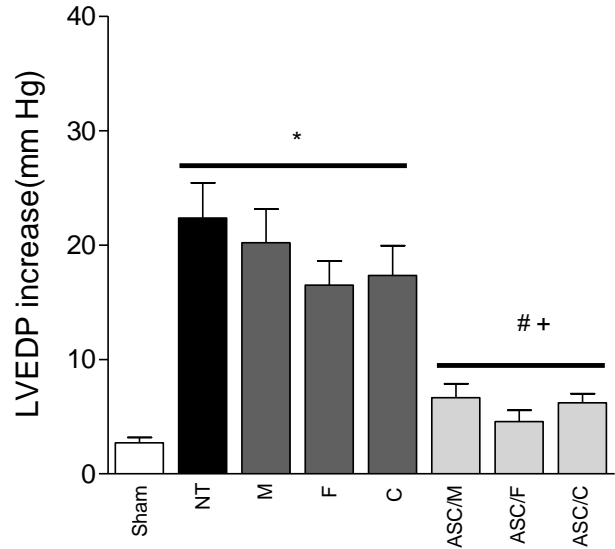
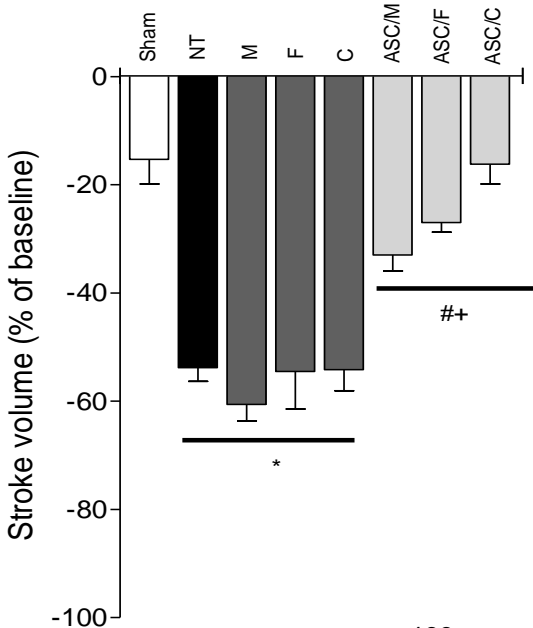
von Kossa

ASC Transplantation & Cardiac Function Post-MI

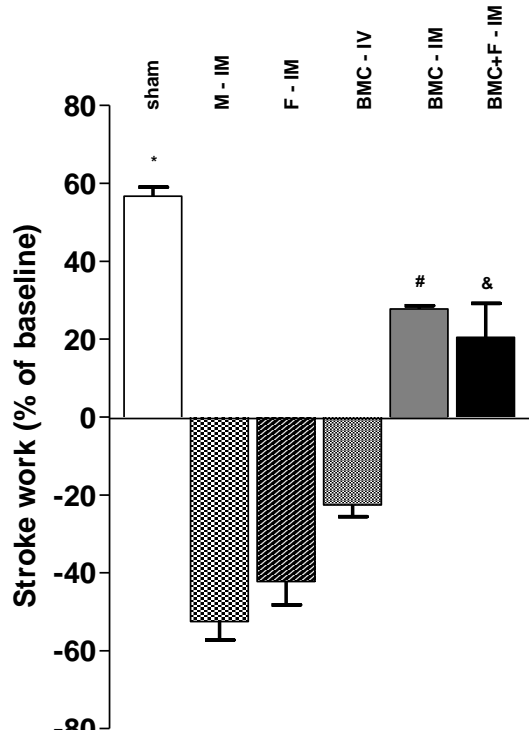


Group	MI	Content	N
SHAM	—	—	7
NT	+	—	7
M	+	Medium	5
F	+	Fibrin	6
C	+	Collagen	6
ASC/M	+	1x10 ⁶ ASCs + medium	5
ASC/F	+	1x10 ⁶ ASCs + fibrin	7
ASC/C	+	1x10 ⁶ ASCs + collagen	6

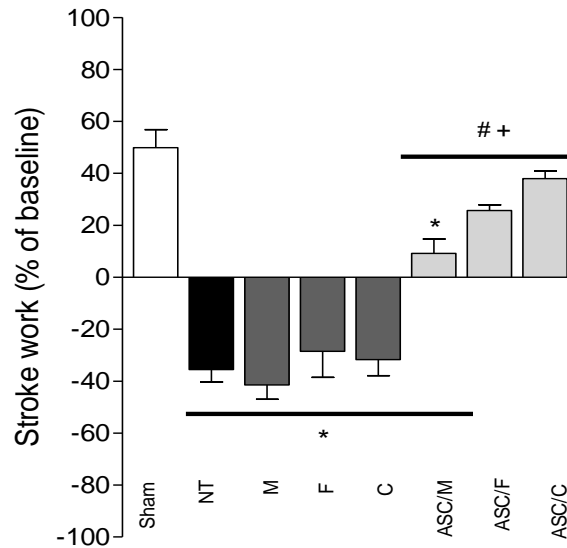
Hemodynamic Variables After Pressure Overload



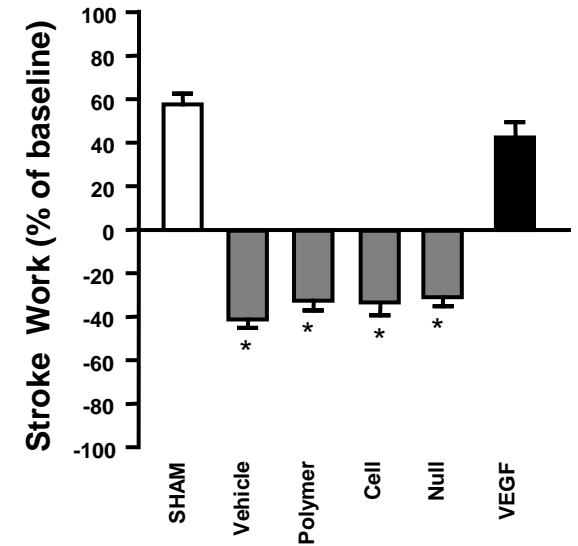
Prevention of Cardiac Function Deterioration Post-MI



Nakamuta et al, *PLoS One*, 2009

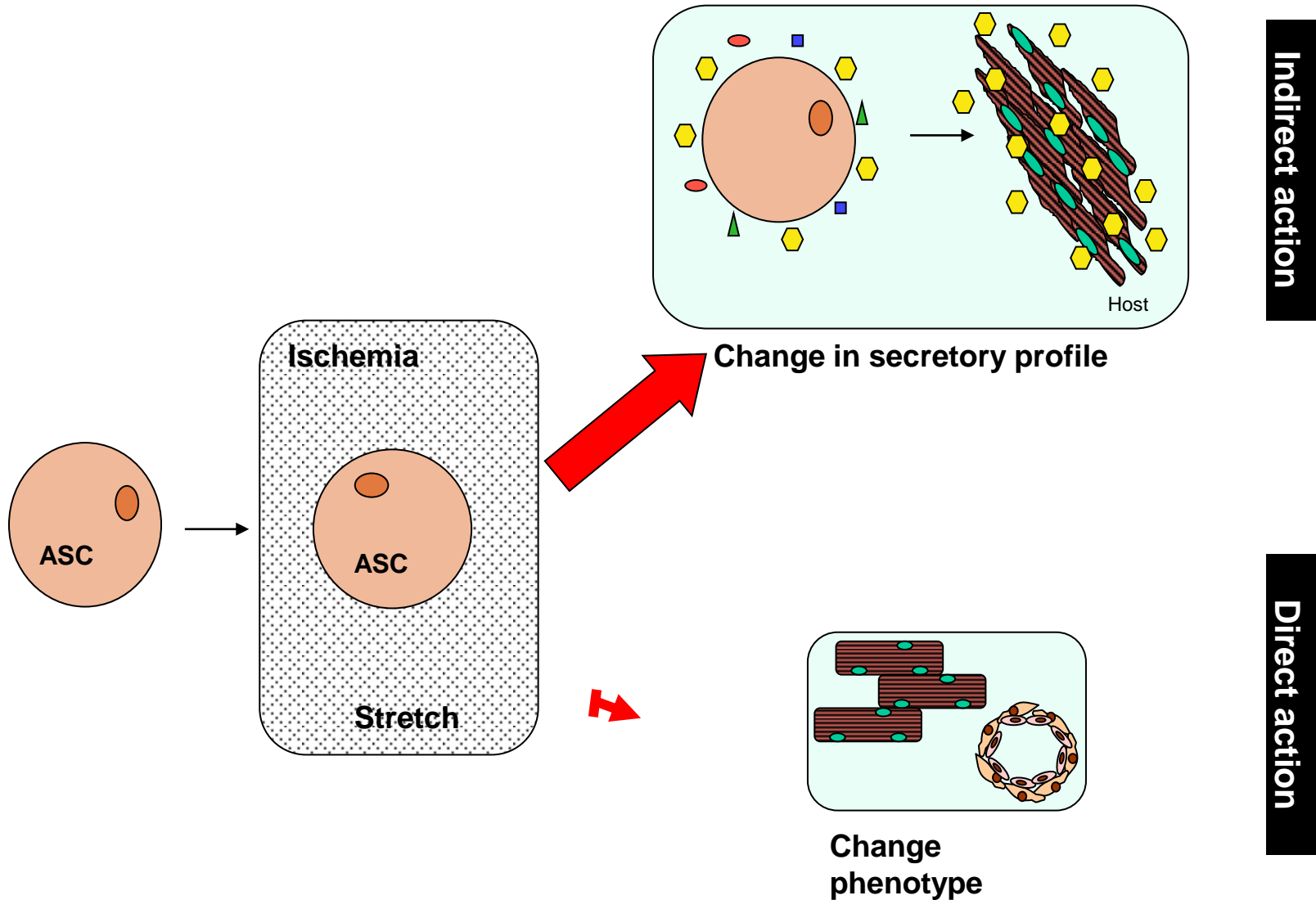


Danoviz et al, *PloS One*, 2010

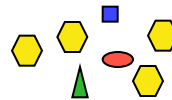
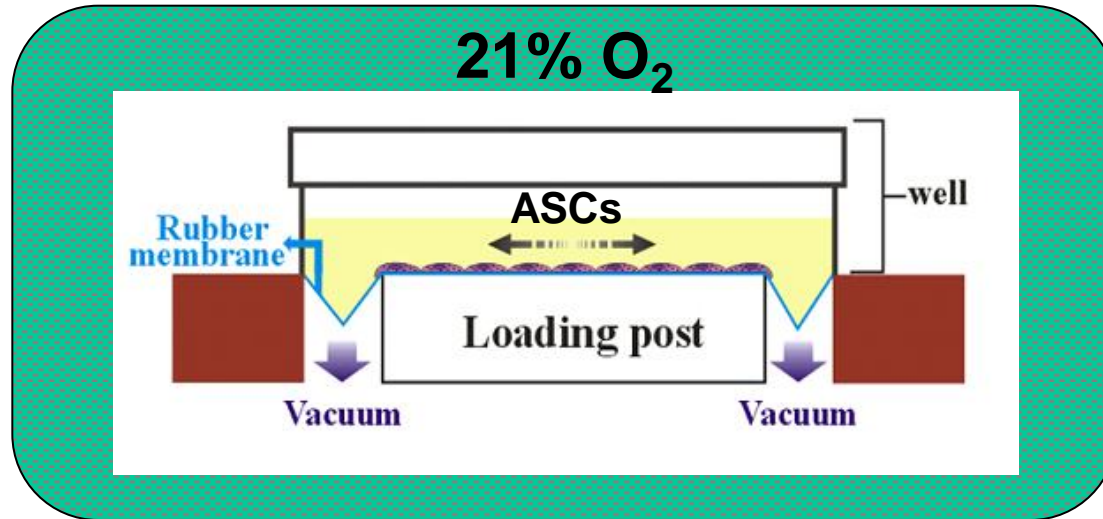


Goncalves et al, *Gene Ther*, 2009

Prevention of Cardiac Deterioration Post-MI: What are the Molecular Mechanisms?



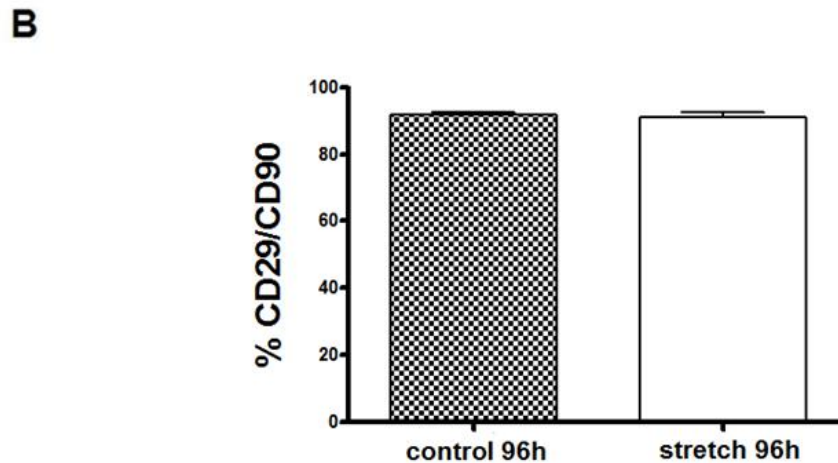
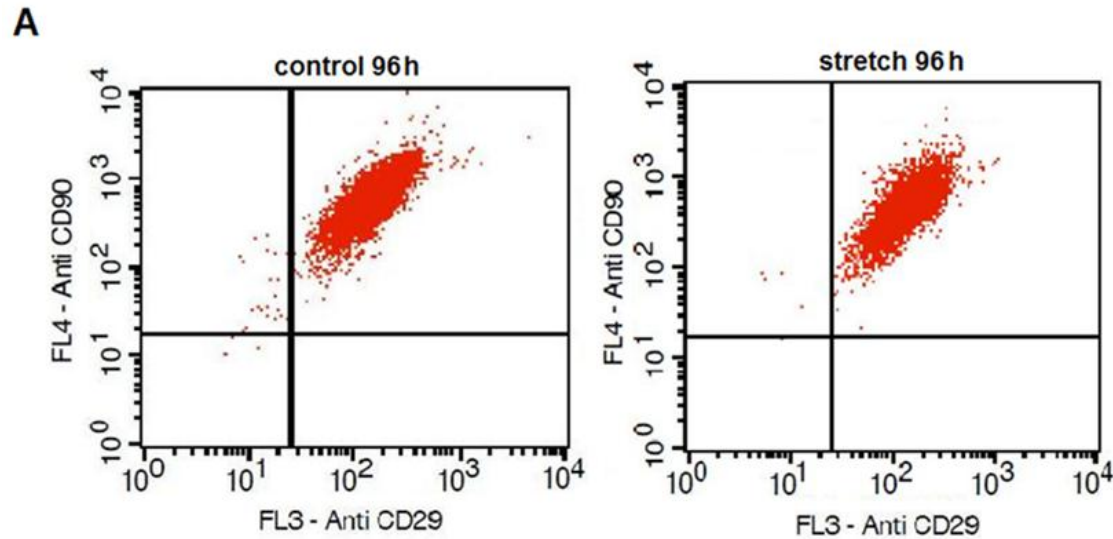
ASC Exposure to Controlled Stretch & Ischemia



Specific Markers
Unique Phenotypes
Secretory profile
(Protein Array & ESI Q-TOF)

Effect of Cyclic Mechanical Stretch on hASC

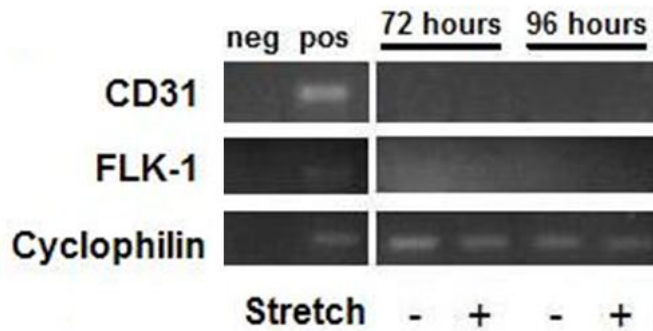
(12% amplitude & 1Hz)



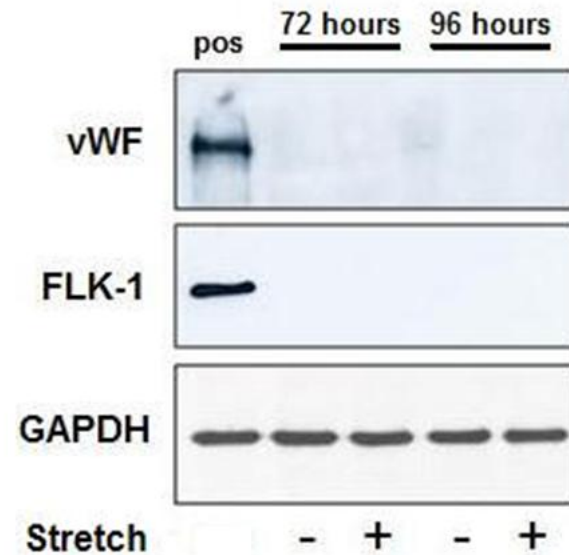
Endothelial Markers & Cyclic Stretch on hASC

(12% amplitude & 1Hz)

A



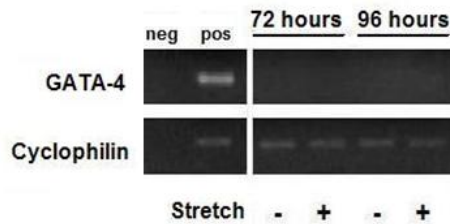
B



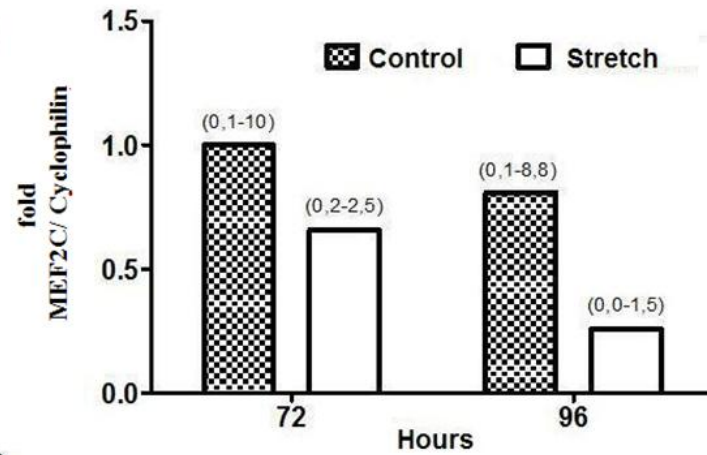
Cardiomyocytes Markers & Cyclic Stretch on hASC

(12% amplitude & 1Hz)

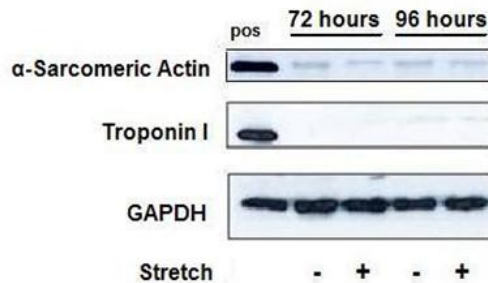
A



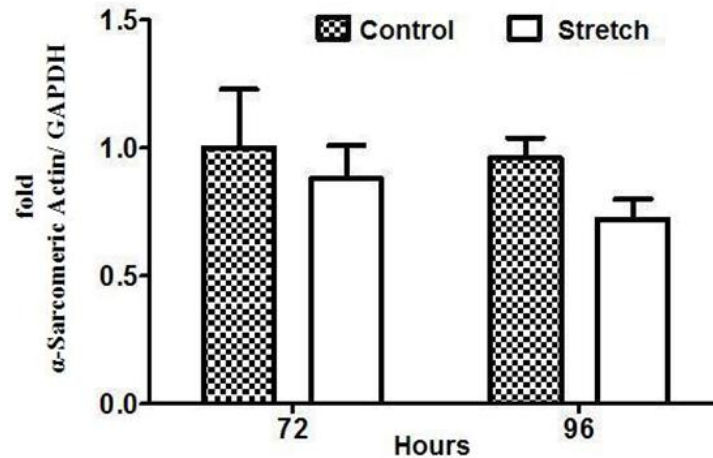
B



C

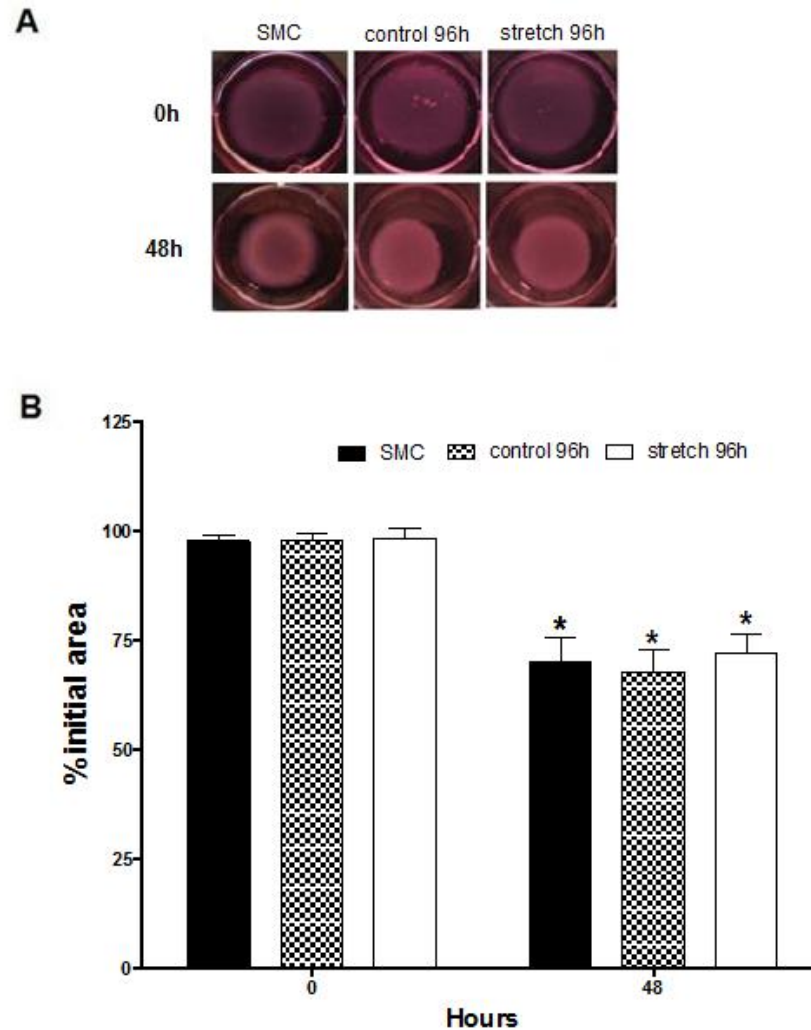


D

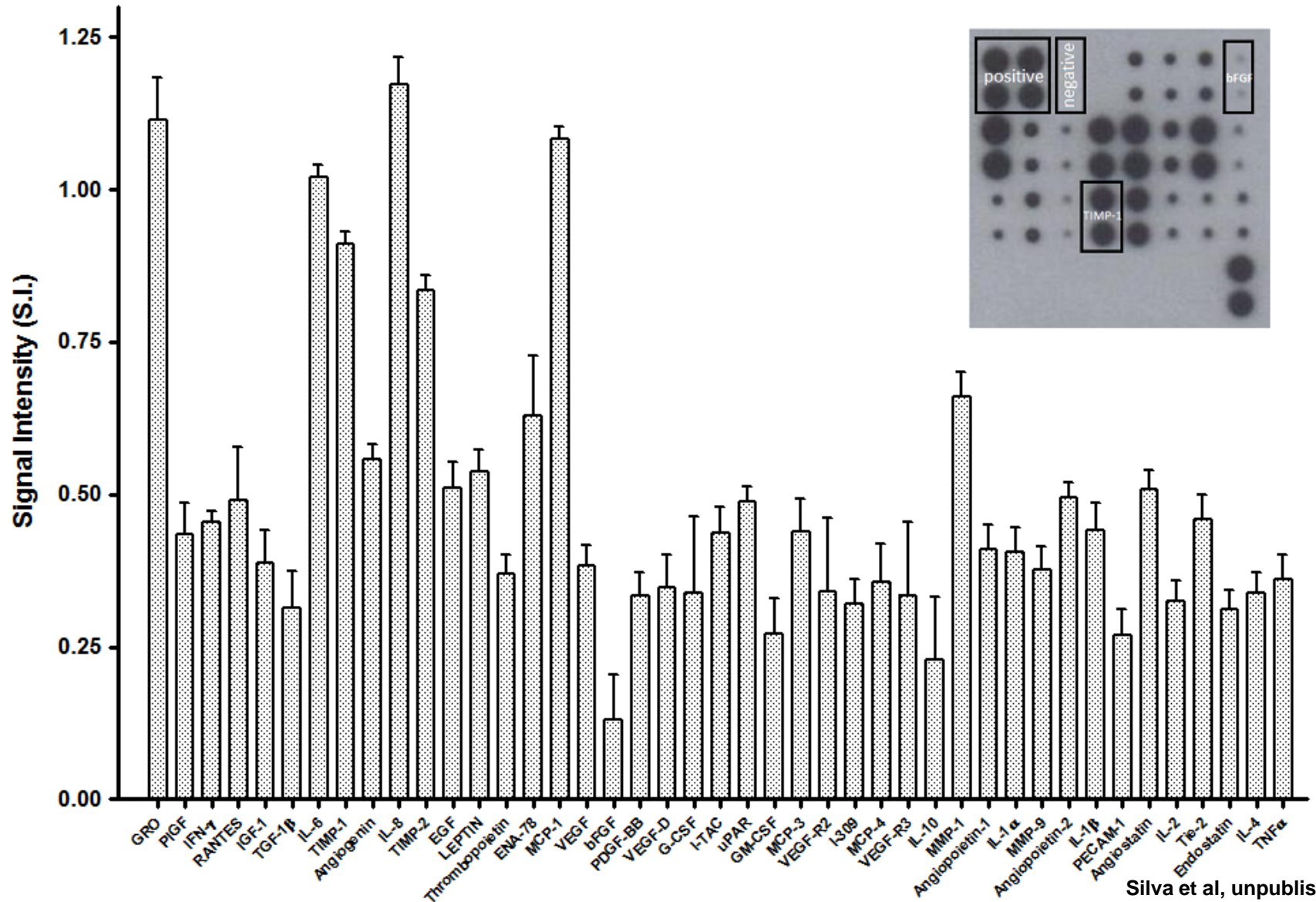


Contraction Assay & Cyclic Stretch on hASC

(12% amplitude & 1Hz)

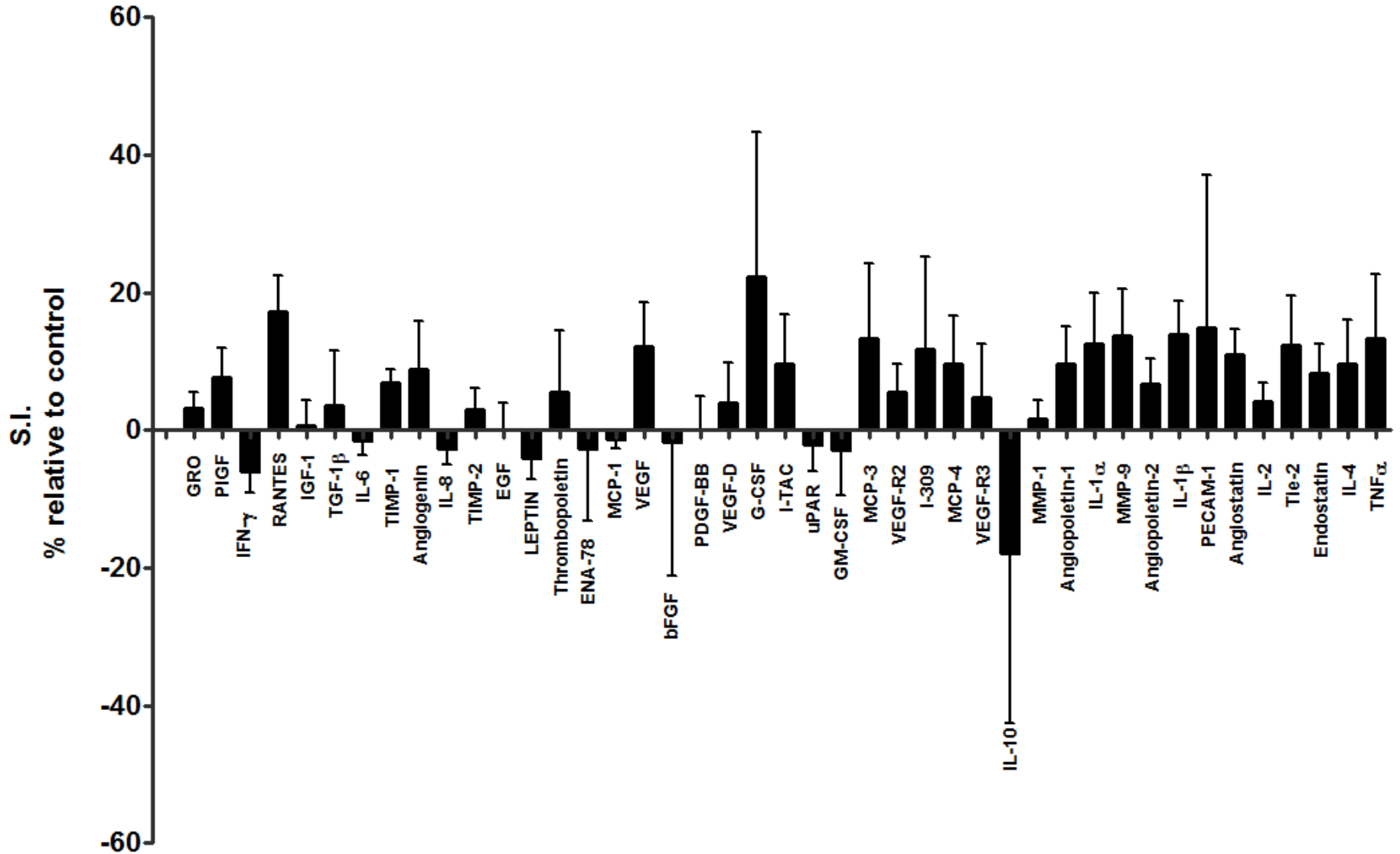


hASC & Expression of Angiogenic Cytokines

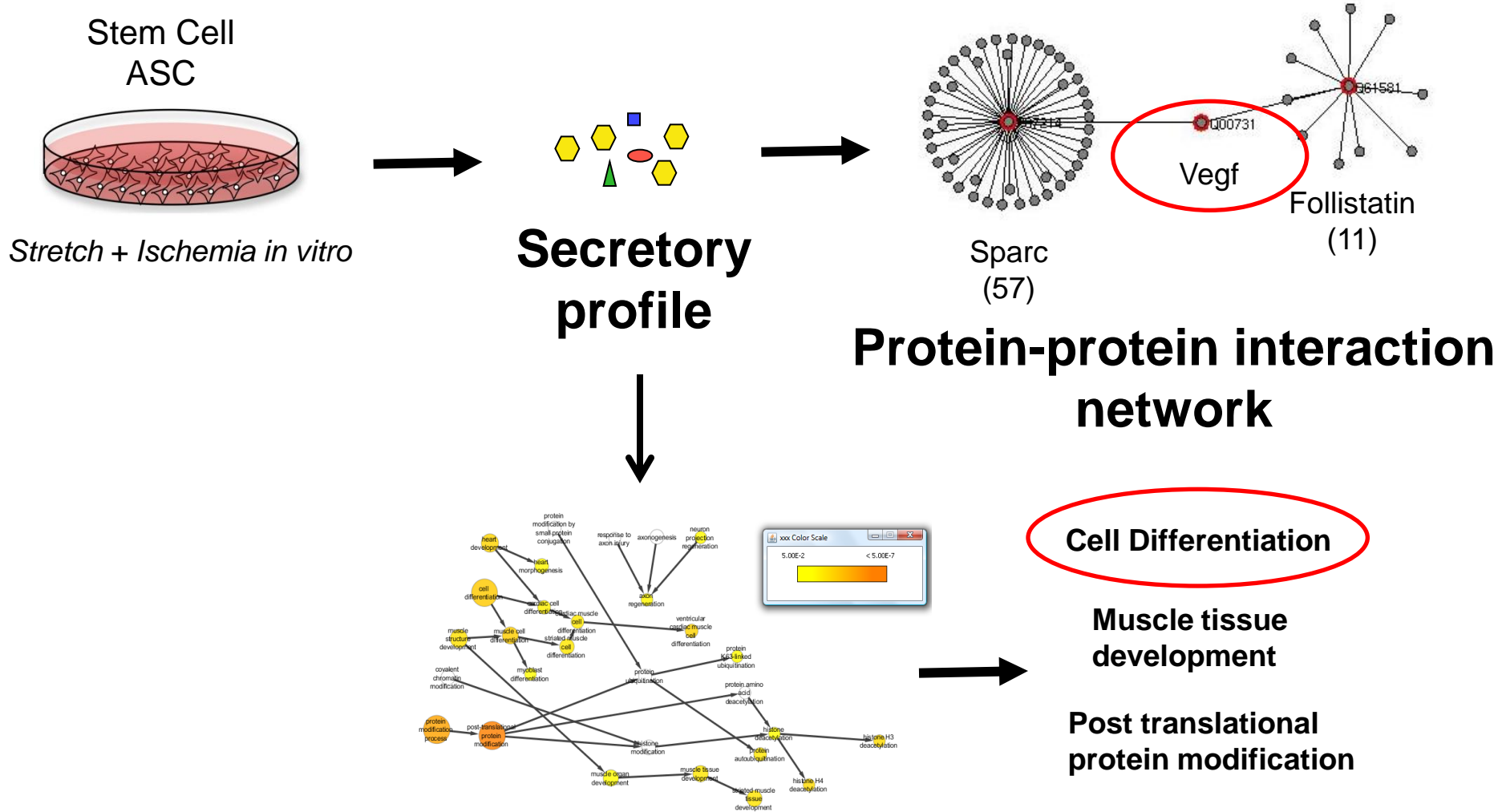


Angiogenic Cytokines & Cyclic Stretch on hASC

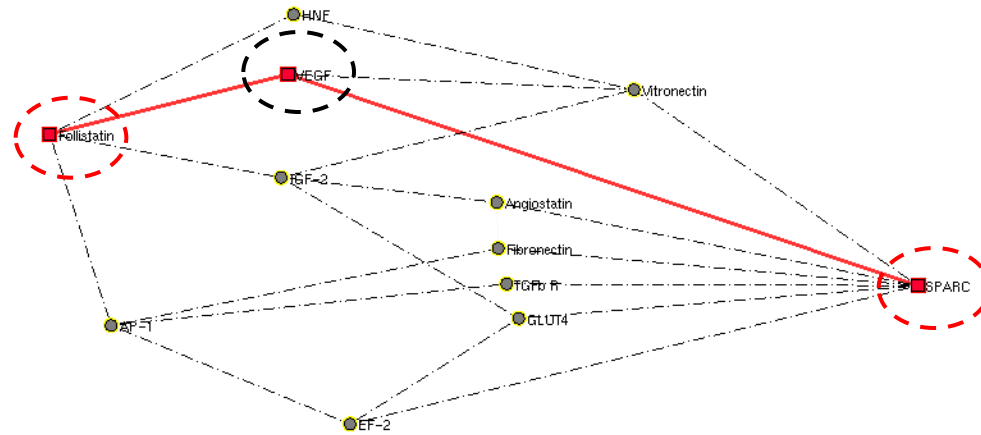
(12% amplitude & 1Hz)



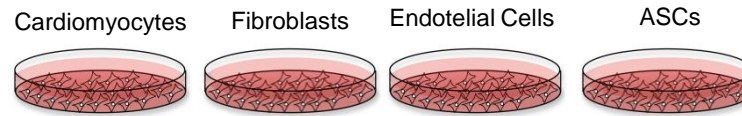
Myocardial Infarction Modulation by ASCs



Step 1



Step 2



VEGF stimulation/ inhibition

Step 3



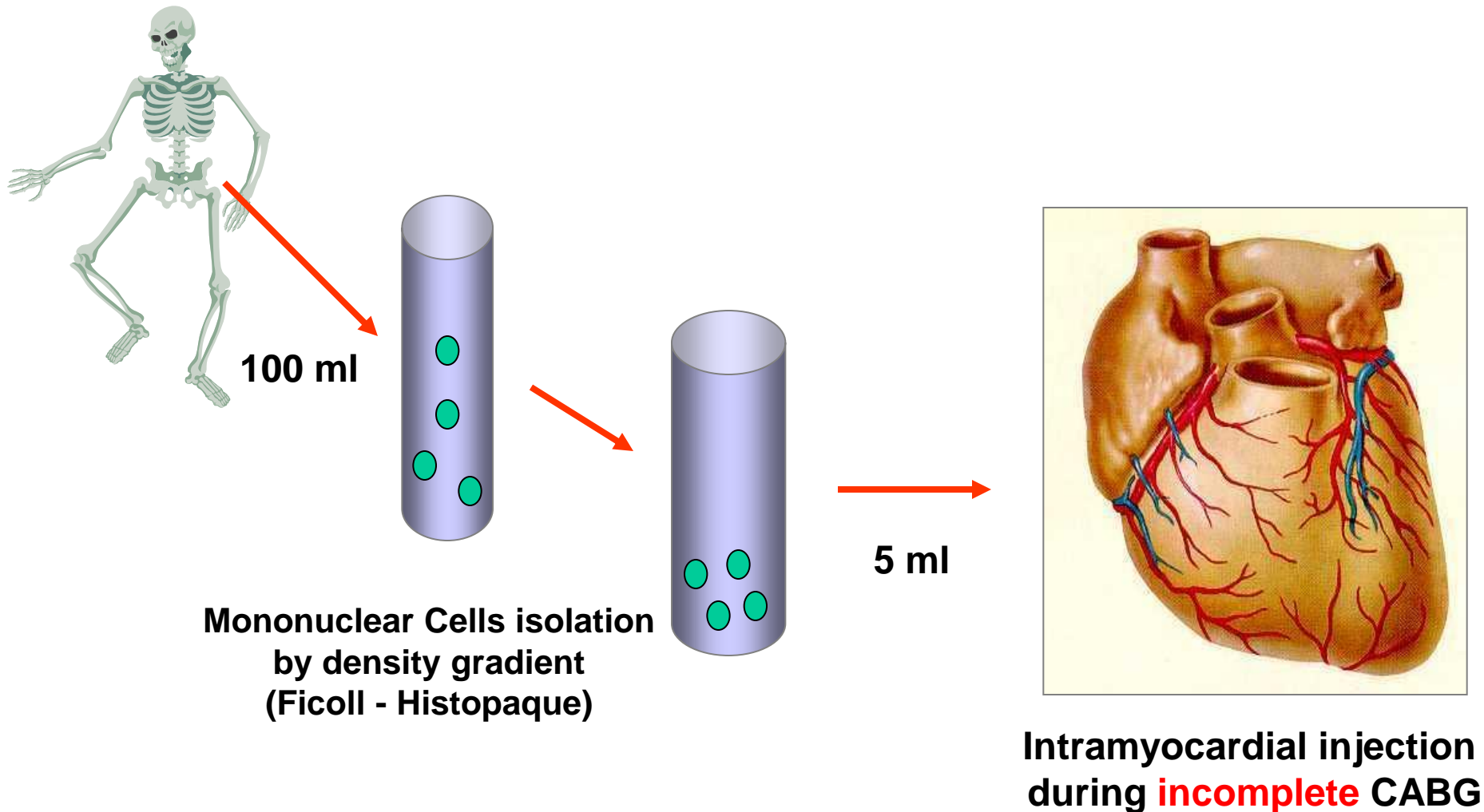
**Cardiac
Function
Assessment**

Cell Therapy in Ischemic Cardiac Disease

1. Routes & Timing for cell injection
2. BMC, Adipose Stem Cell (ASC) and genetic modified cells for cardiac repair
3. Combined use of CABG/TMLR & BMC for cardiac repair in humans

Bone Marrow Progenitor Cells

(Separation of lymphomonocitary cells)

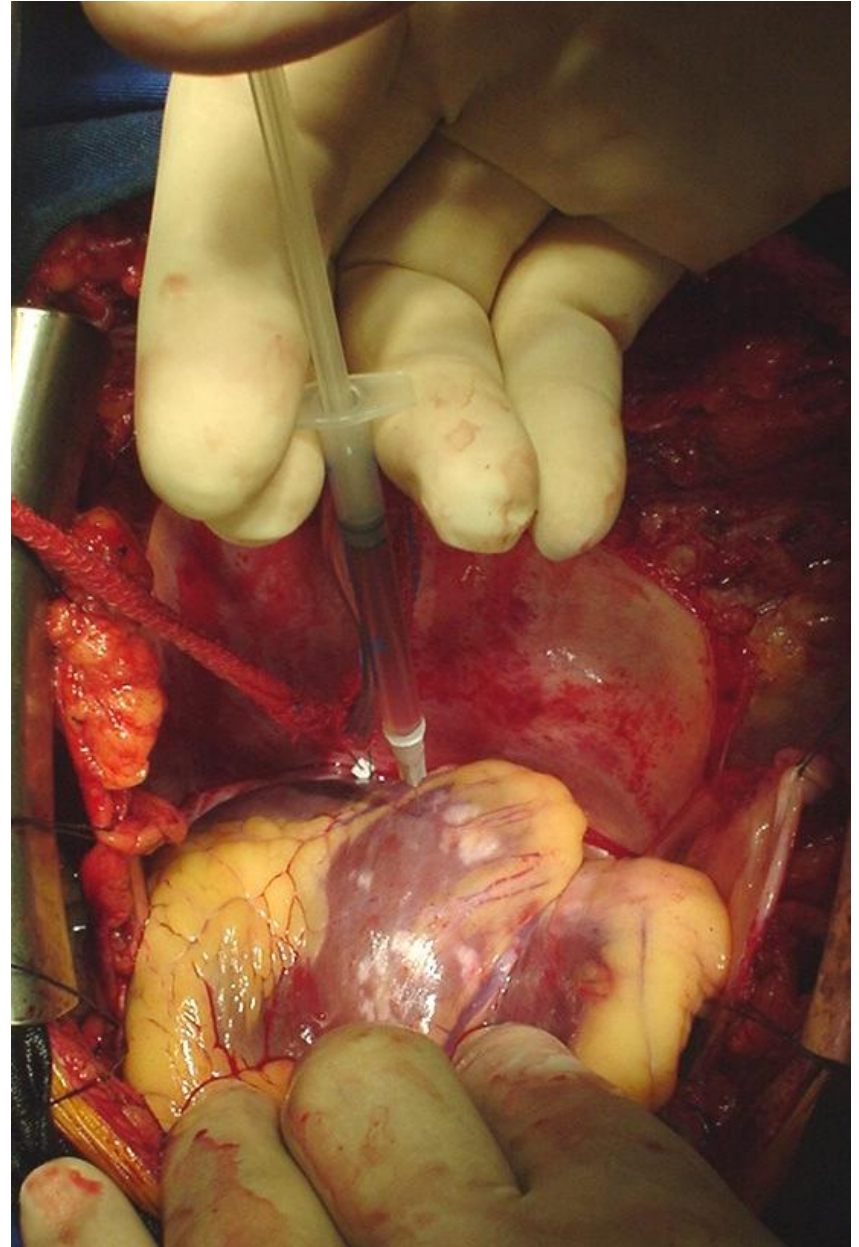


Safety Assessment

BMC Injection:

Total = $130 \pm 3 \times 10^6$ cels/pt

CD34+ = $1,30 \pm 0,40\%$



MRI Injected Area: Anterior Wall

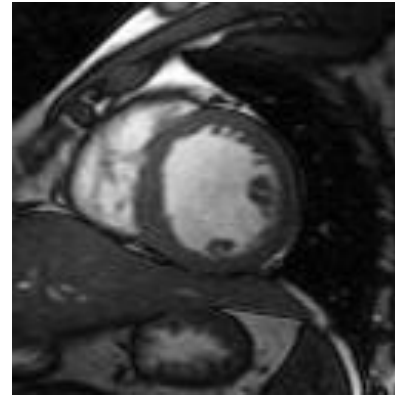
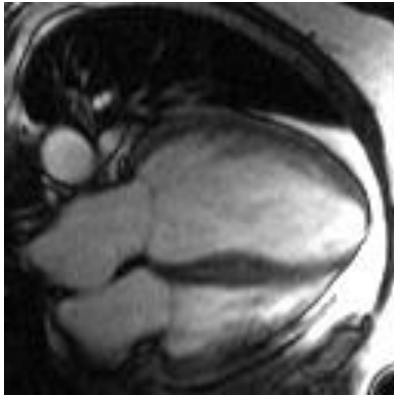
Pat # 6 RGS

Cine

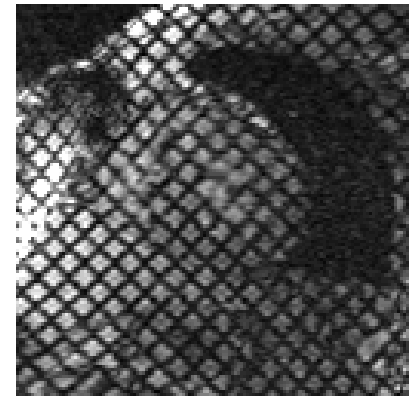
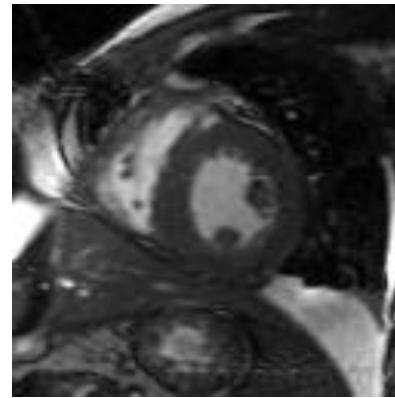
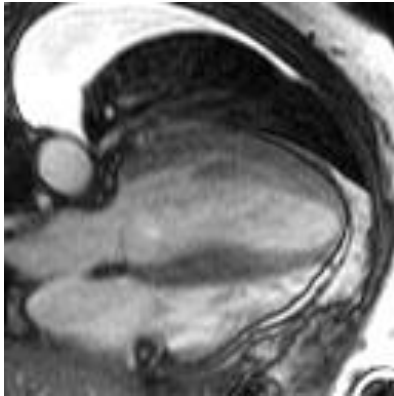
Cine

Tagging

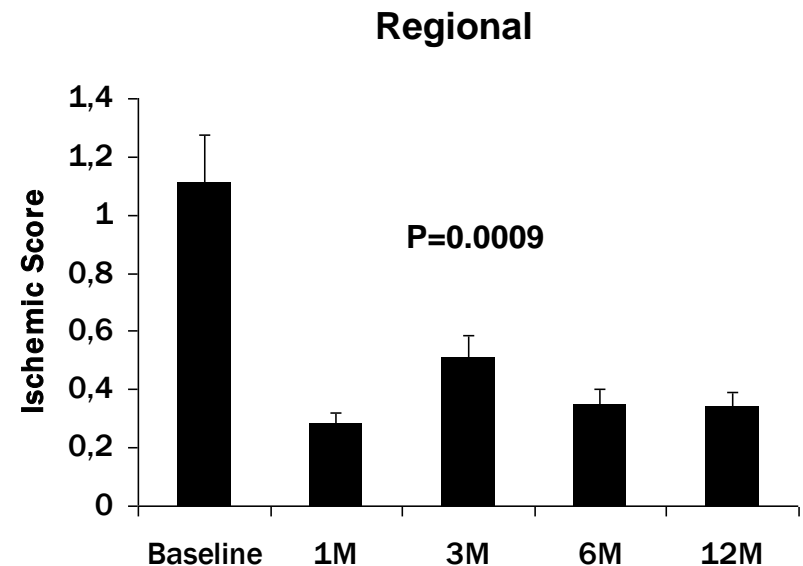
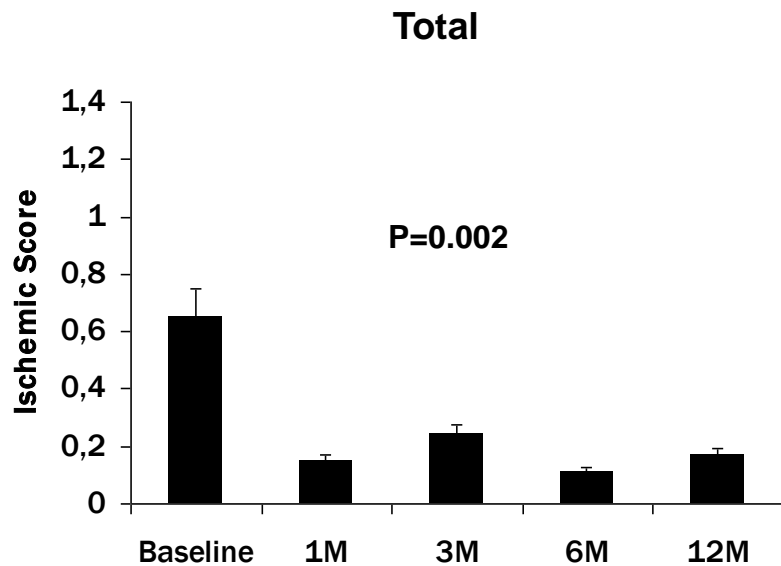
Pre- op



1 month
Post - op



Total and Regional LV Ischemic Score Assessed by MRI

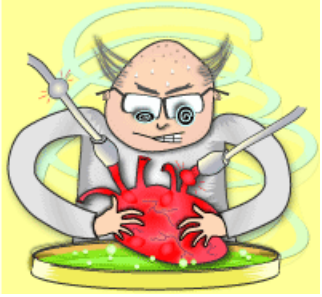


Efficacy – Controlled Double Blind Randomized Trials:

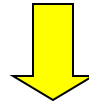
1. BMSC in Chronic CAD (Incomplete CABG) (N=140, 1:1)
2. BMSC in Chronic CAD (TMLR) (N=50, 1:1)

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Biological Cardiac Repair



Cell replacement - “biological cardiomyoplasty”
Angiogenesis

1. The Naïve Approach



2. The “Awe” Approach (surprise)



3. The Rational Approach



Evidence for Structural
&
Functional Benefit
(Mechanism of Action?)



Controlled
&
Rational Use

Special Challenges Remain

Encouraging data obtained in rodents must be rapidly tested in more suitable models (e.g. swine)

Description of detailed mechanism of action (may allow the use of small molecules)

“Efficient” source of cardiomyocytes

To obtain cell/tissue integration to perform as a functional sincition

Acknowledgements



**Lab Genetics & Molecular Cardiology
Heart Institute (InCor)/Univ São Paulo Med Sch**

**Bryan Strauss
Ayumi A. Miyakawa
Maria de Lourdes Junqueira
Renata Carmona
Mariliza Velho
Monica N. Bezerra**

**Vinicius Bassaneze
Juliana Nakamuta
Chester Bittencourt
Luciene Campos
Leonardo Santos
Thais Girao Silva
Giovana Goncalves
Maria Elena Danoviz
Gabriela Venturini
Rafael Dariolli
Leonardo Jensen
Camila Zogbi**

**Cell Therapy Clinical Coordinator
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**Hematologist
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**CV Surgery InCor
Noedir Stolf
Sergio A Oliveira
L F Dalan**

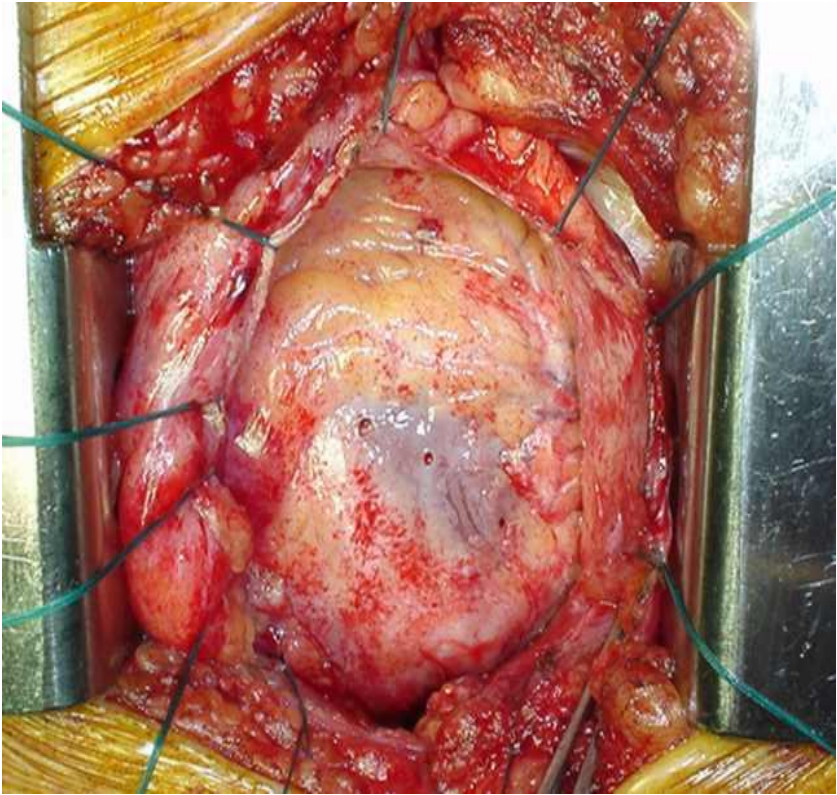
**Tucci's Group – EPM-UNIFESP
Paulo J. F. Tucci**

All InCor Teams

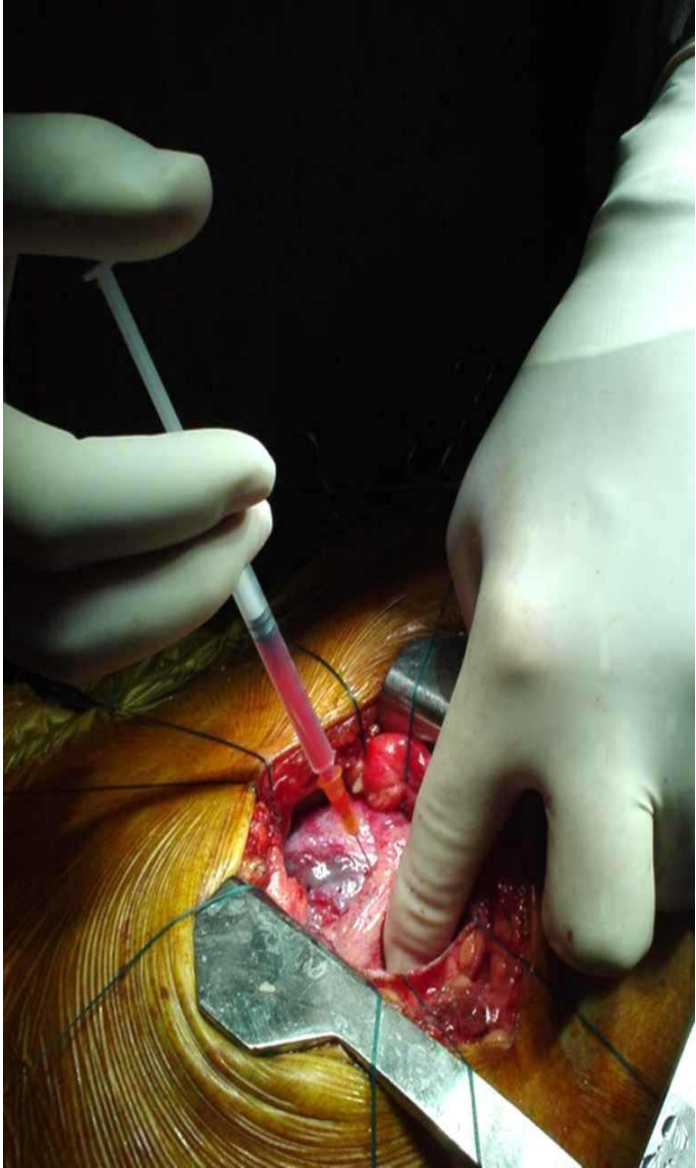
**Funding: FAPESP, CNPq, F Zerbini,
MCT-FINEP, MS-DECIT**

Combined Strategy: TMLR & Cell Transplantation

Combined Strategy



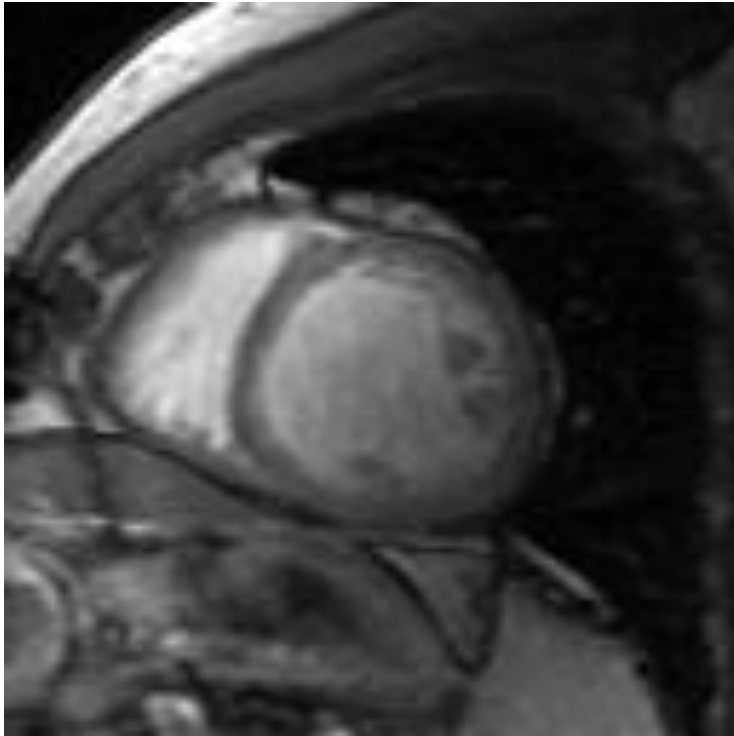
+



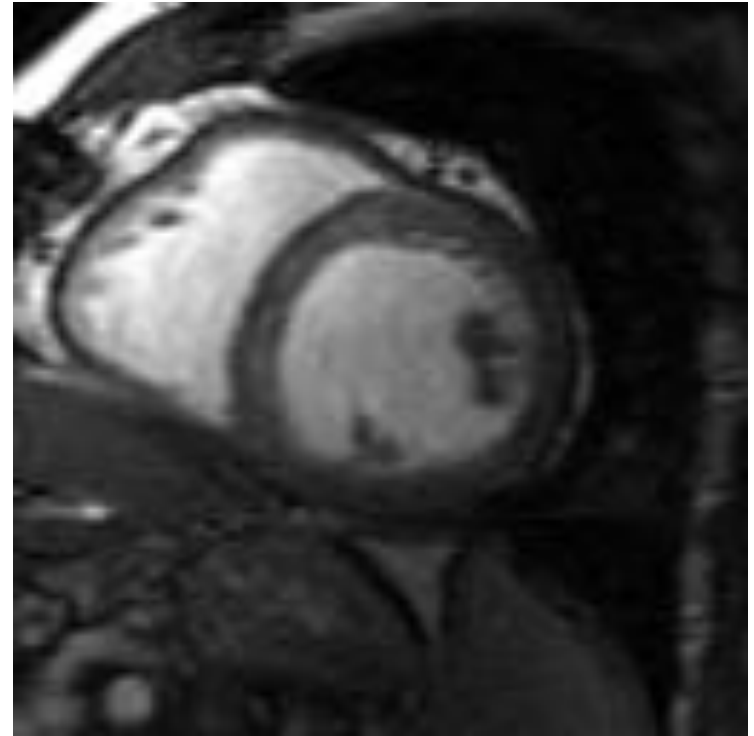
Cardiac MRI

Injected Area: Anterior Wall

Pre-

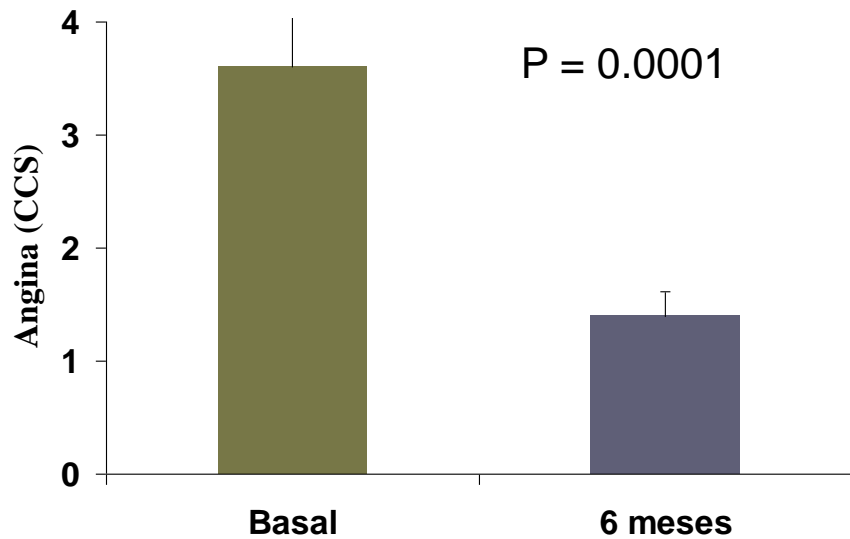


6 mos Post-

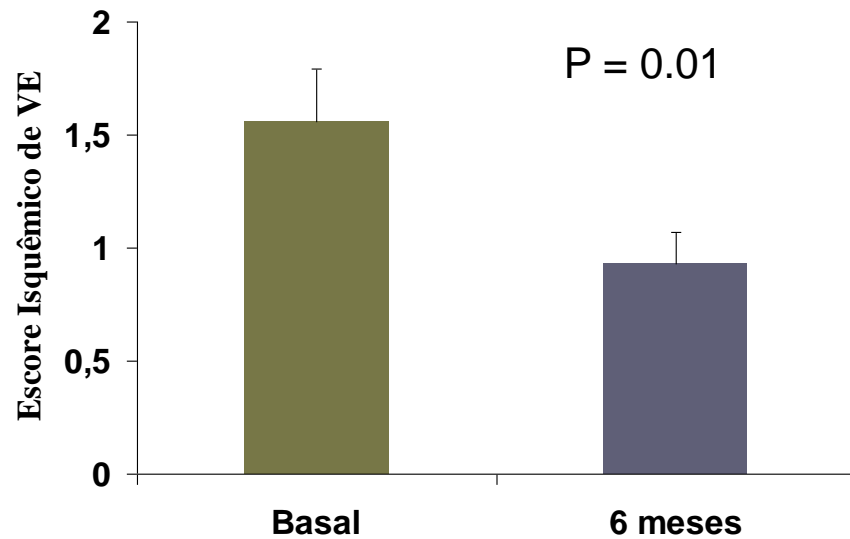


Combined Strategy: Functional Assessment

Angina – Functional Class



LV Ischemic Score



N = 9