

CBCS Summer School on Cardiovascular Sciences
“From Basic Mechanisms to Clinical Application“- 15th June 2011

Cardiac Stem Cells

Joost Sluiter



University Medical Center
Utrecht

Utrecht, the Netherlands



Cardiac regeneration

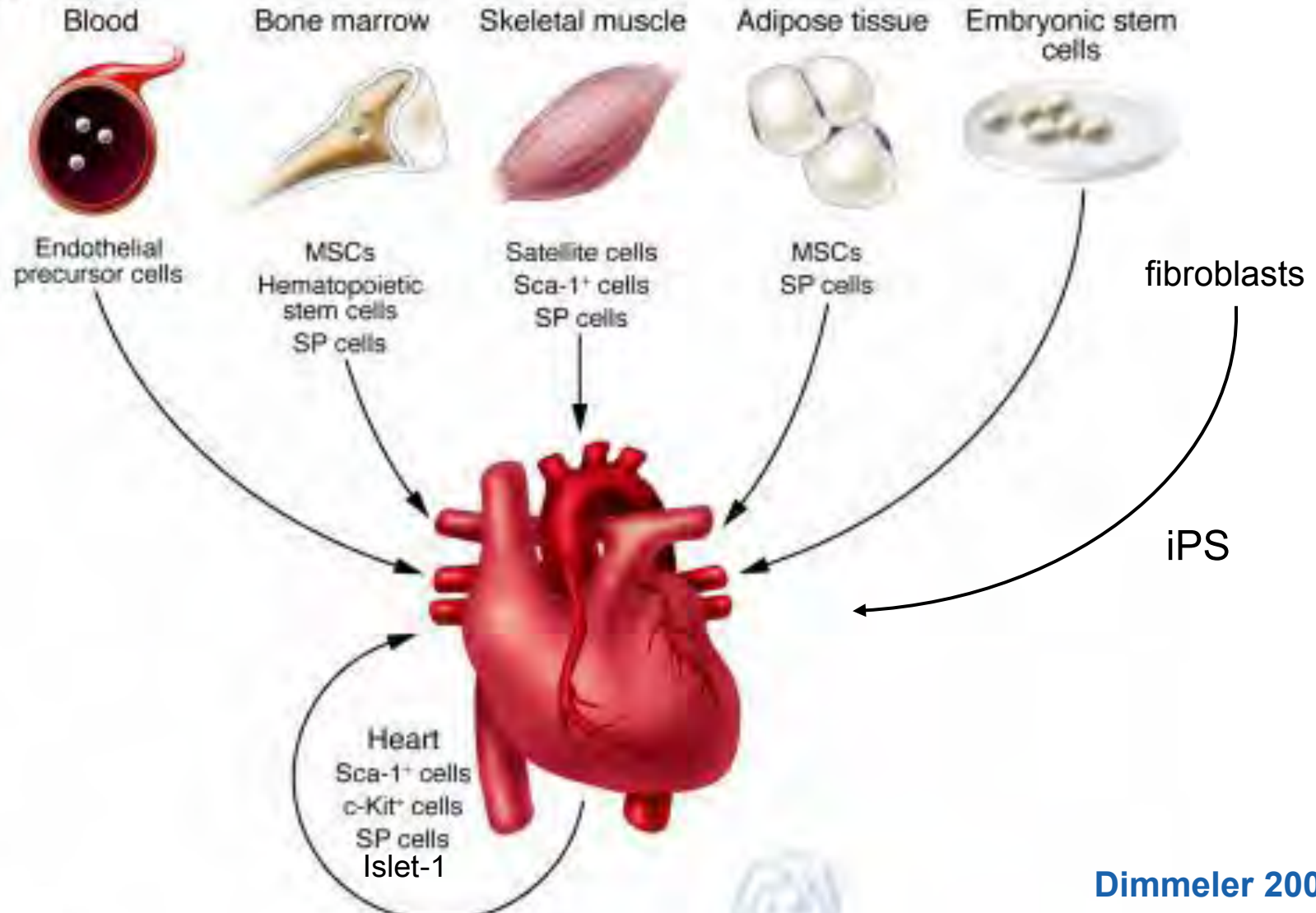


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<http://mediworldrkr.blogspot.com/2010/08/regenerating-heart-tissue.html>

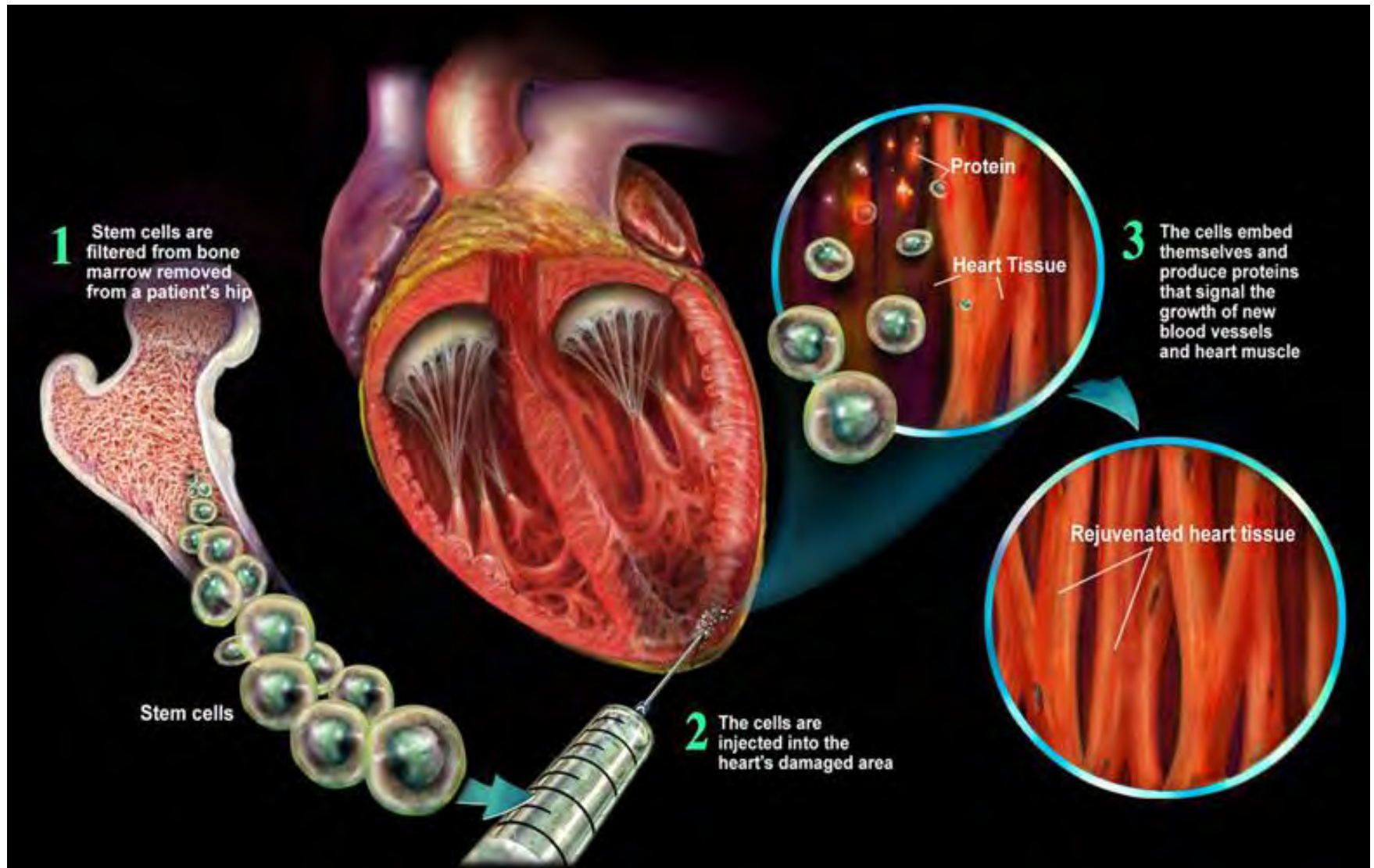
Stem cells for cardiac repair



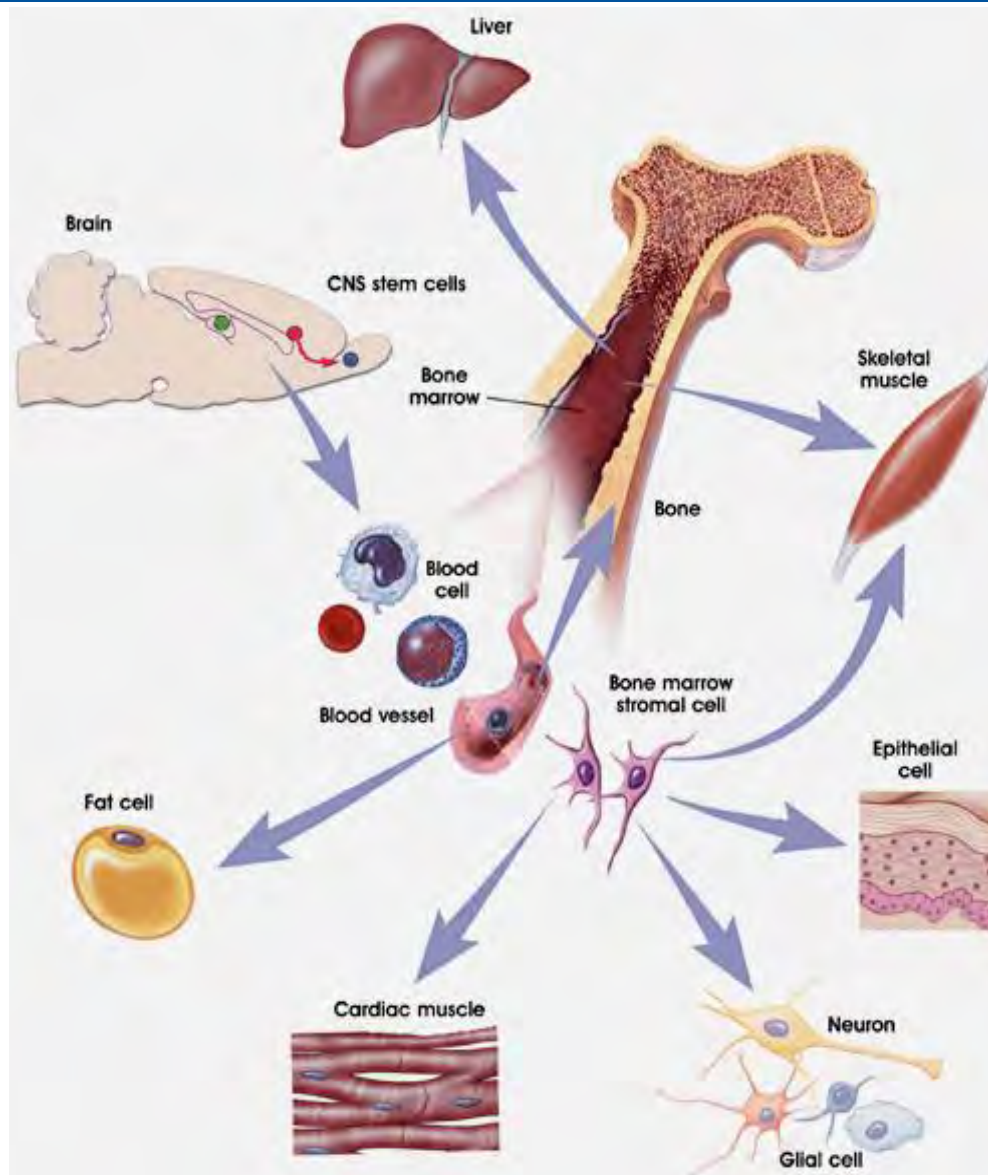
Cardiac cell therapy

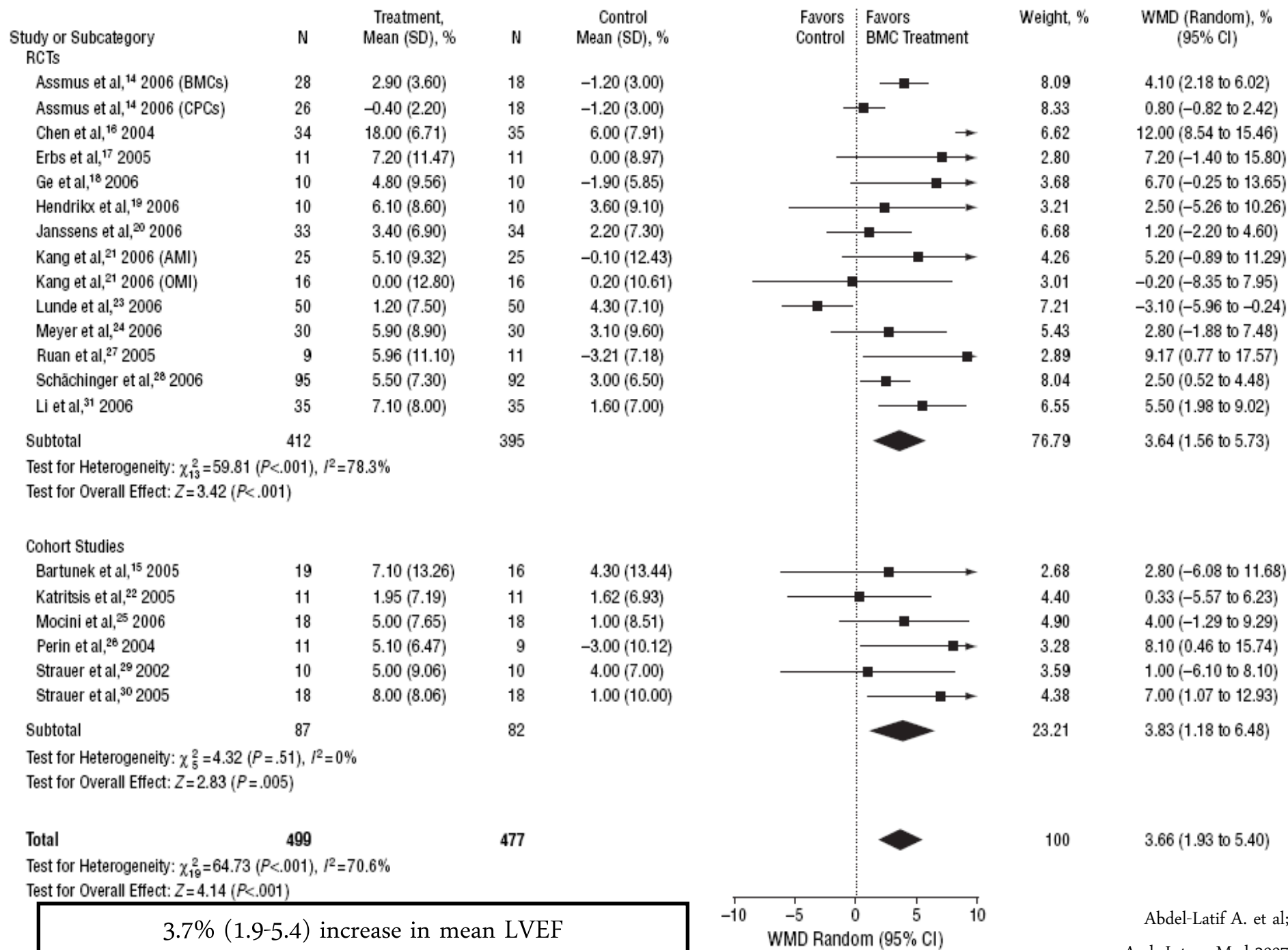


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Bone marrow: source of somatic stem cells

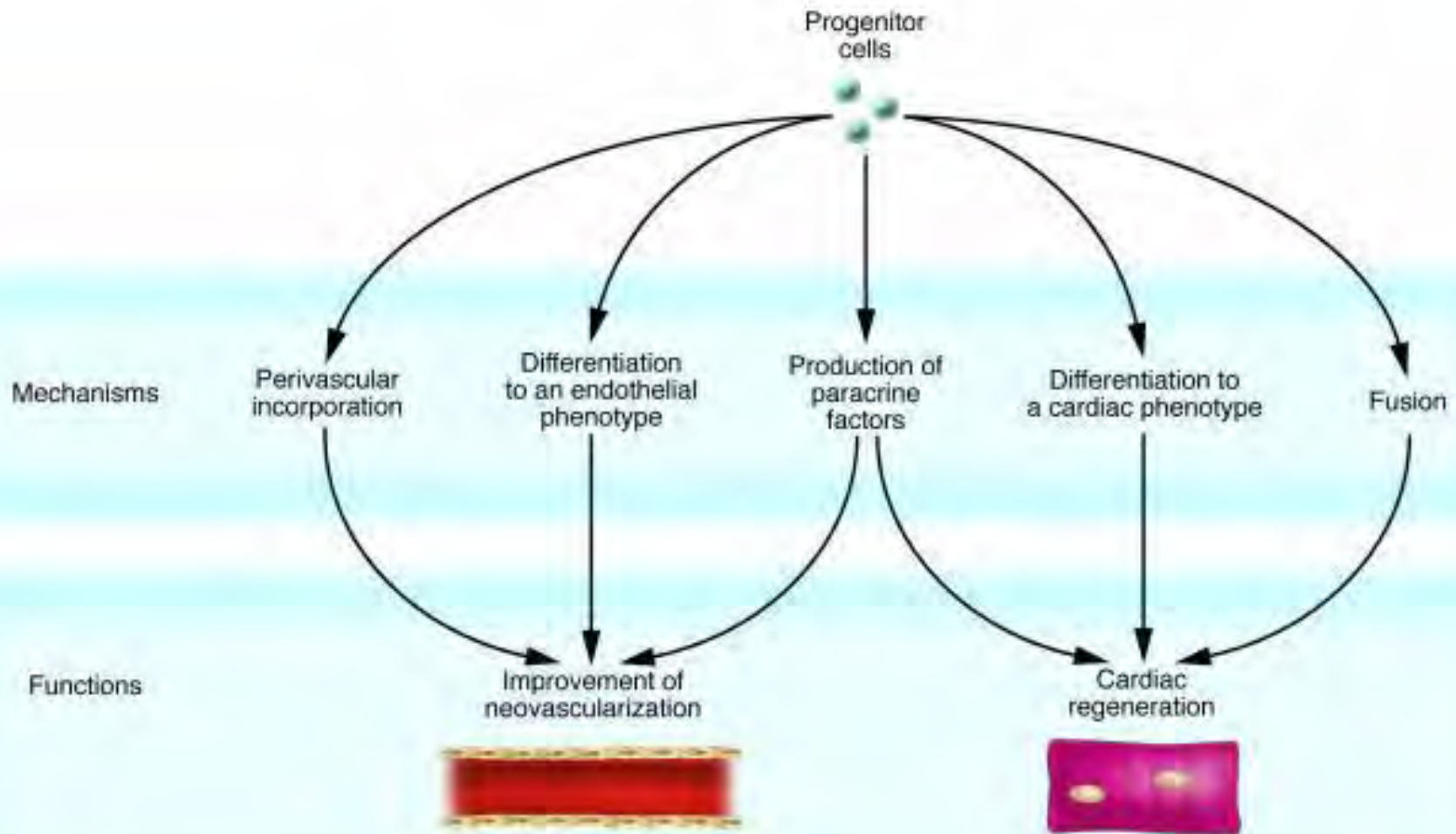




3.7% (1.9-5.4) increase in mean LVEF

-10 -5 0 5 10
WMD Random (95% CI)

Mechanisms of actions to repair the heart

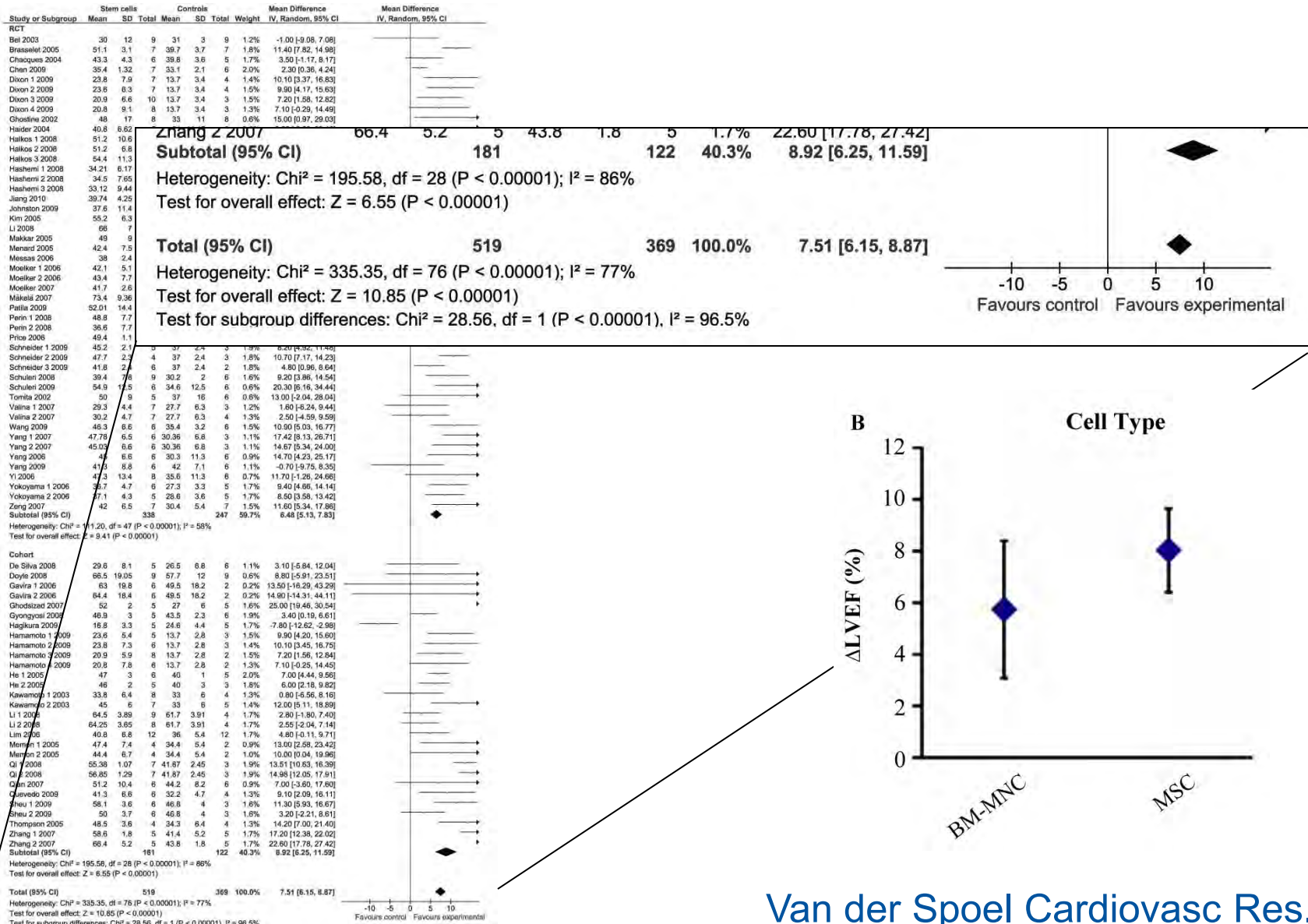




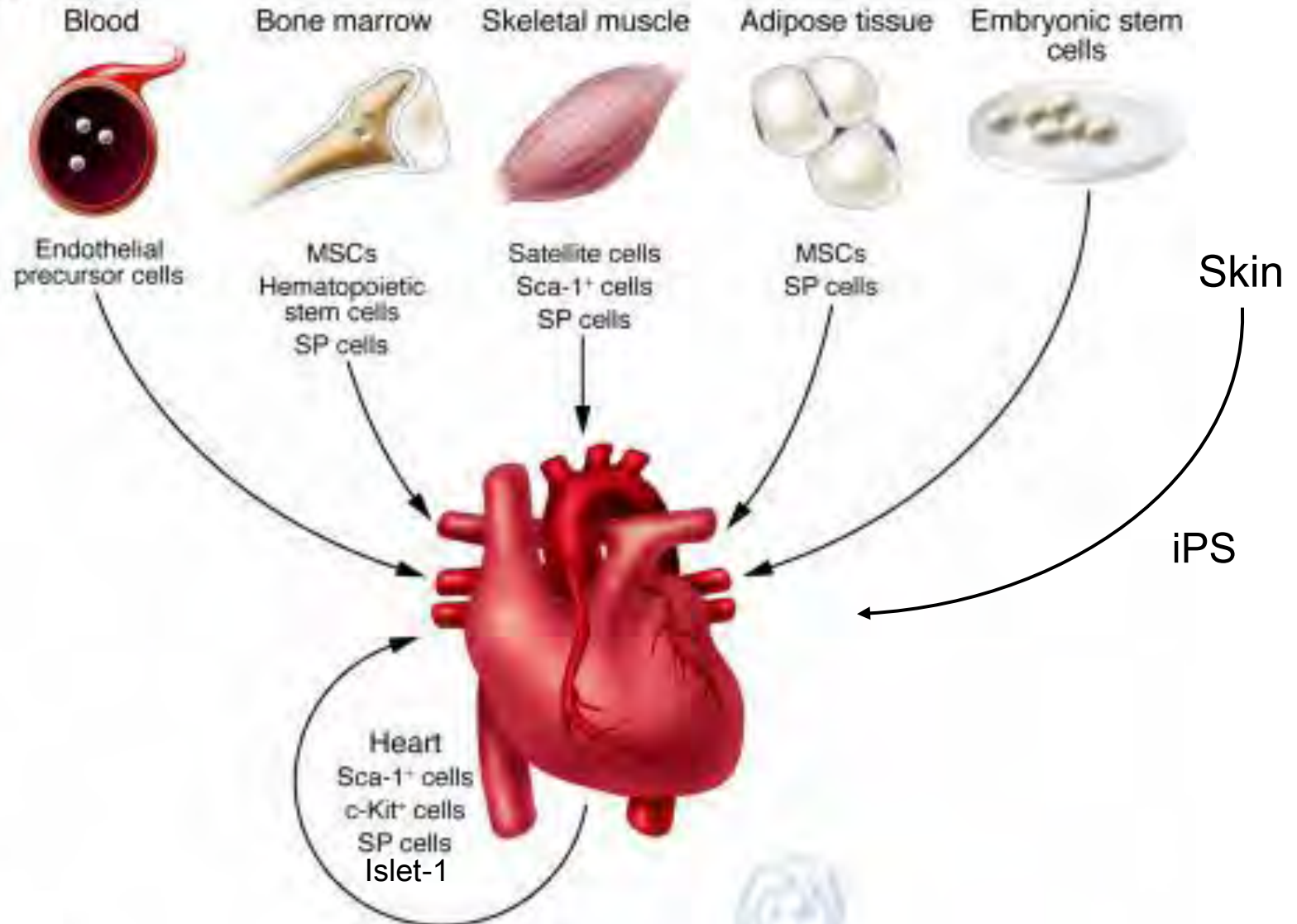
Improvements cell therapy

- Cell type
- Delivery method
- Number of cells
- Timing of delivery
- Survival / differentiation

meta-analysis of large animal models of ischemic heart disease

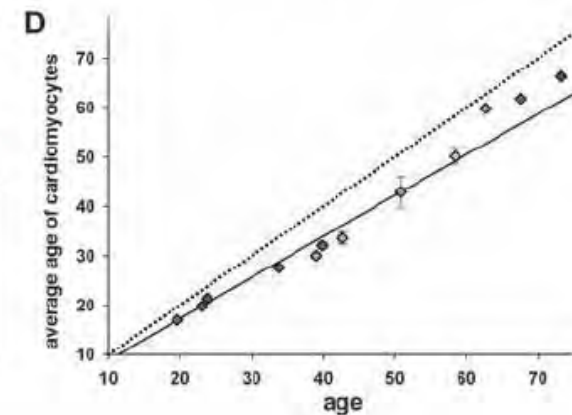
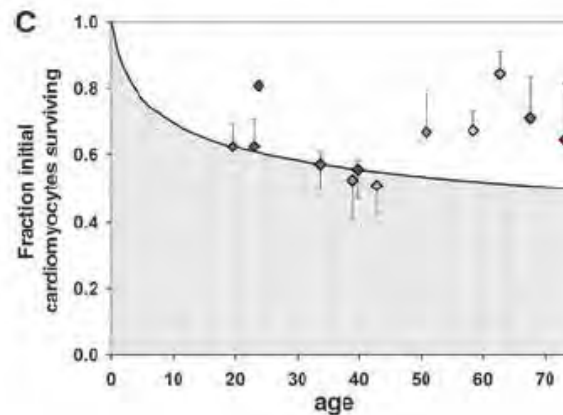
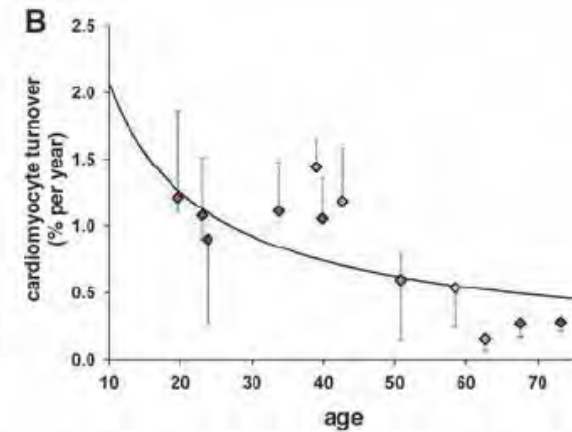
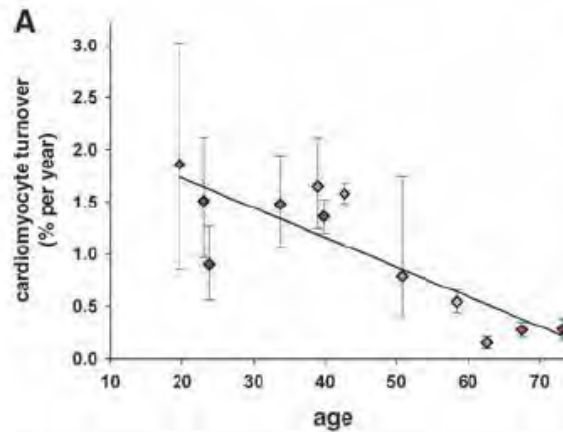


Cell type



Cardiac turnover (cardiomyocytes)

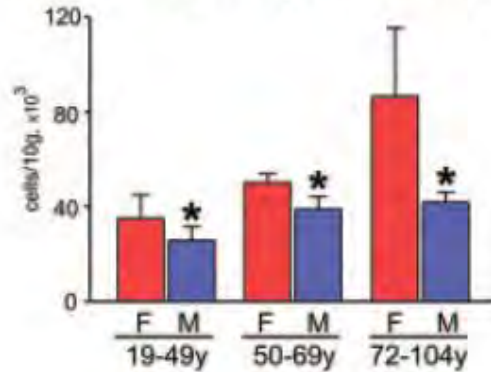
- Integration of carbon-14, (nuclear bomb tests, Cold War)
- Annually turnover;
 - Age 25: 1%
 - Age 75: 0.45%
 - non-CM: 18%
- Fewer than 50% of cardiomyocytes are exchanged during a normal life span.



Cardiac turnover



Functionally Competent hCSCs

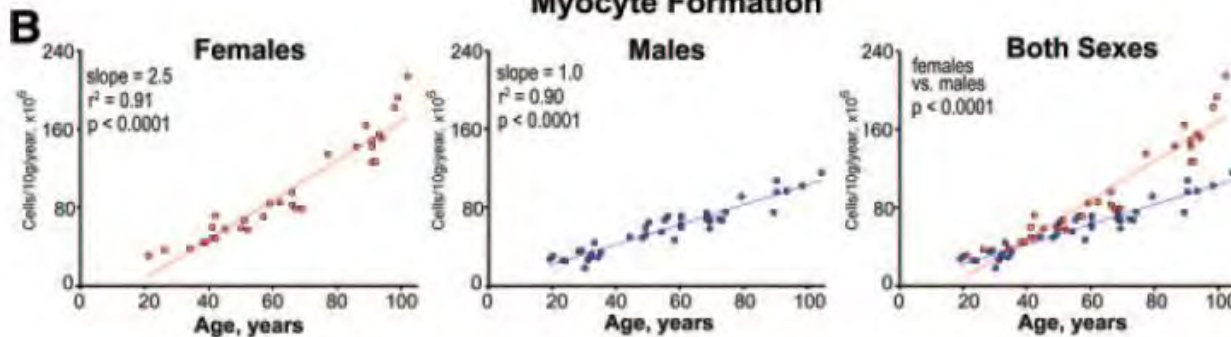


Annual myocyte turnover
2- 60 -100 years old:

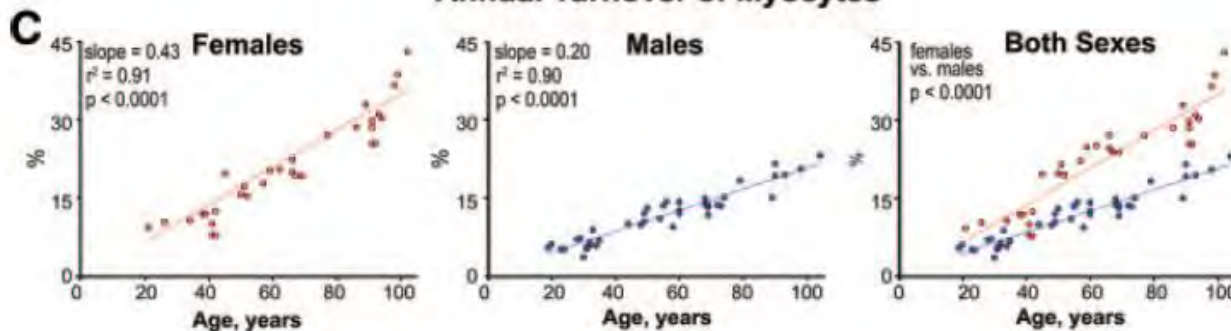
F: 10 -14 – 40 %

M: 7 – 12 – 32%

Myocyte Formation



Annual Turnover of Myocytes



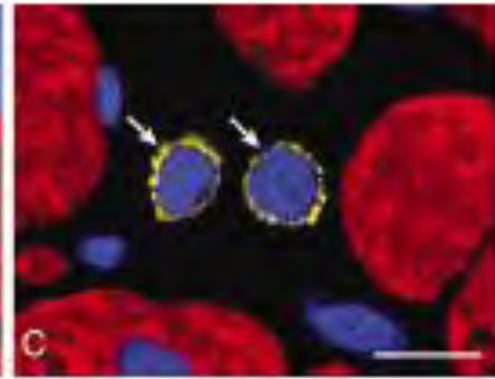
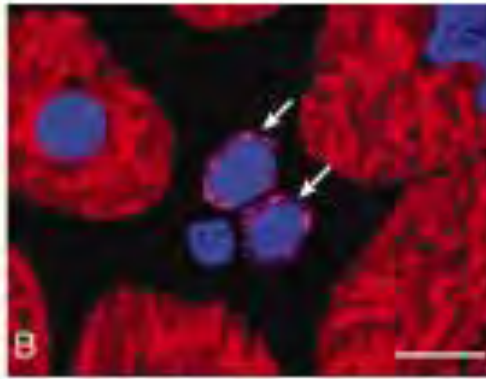
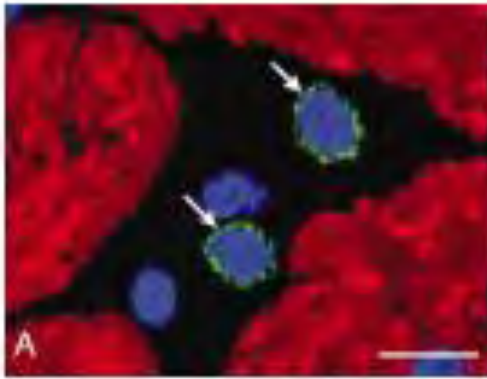
Stem cells in the adult heart



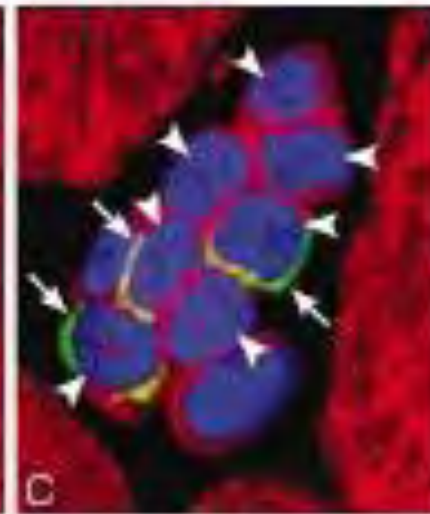
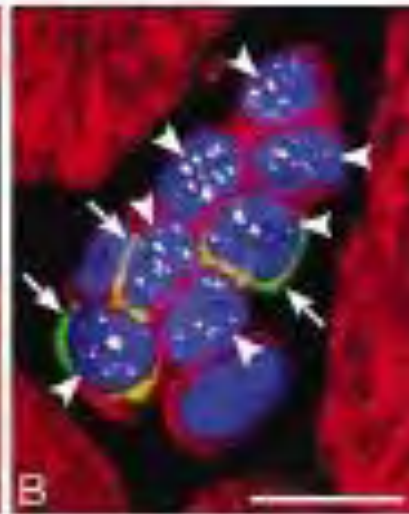
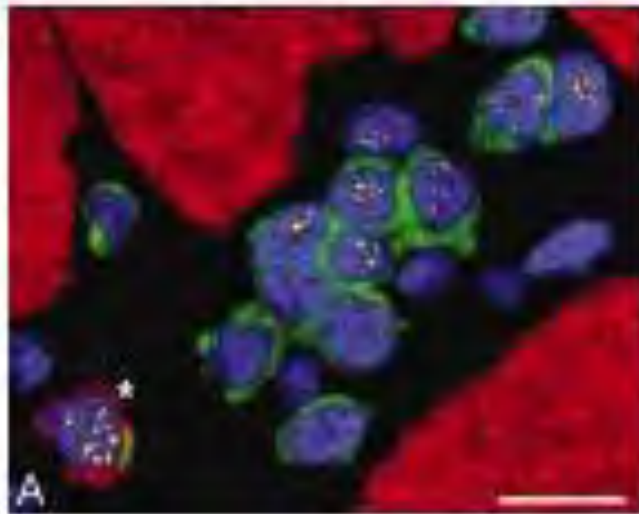
c-kit

MDR-1

Sca-1 like



Urbanek, 2003

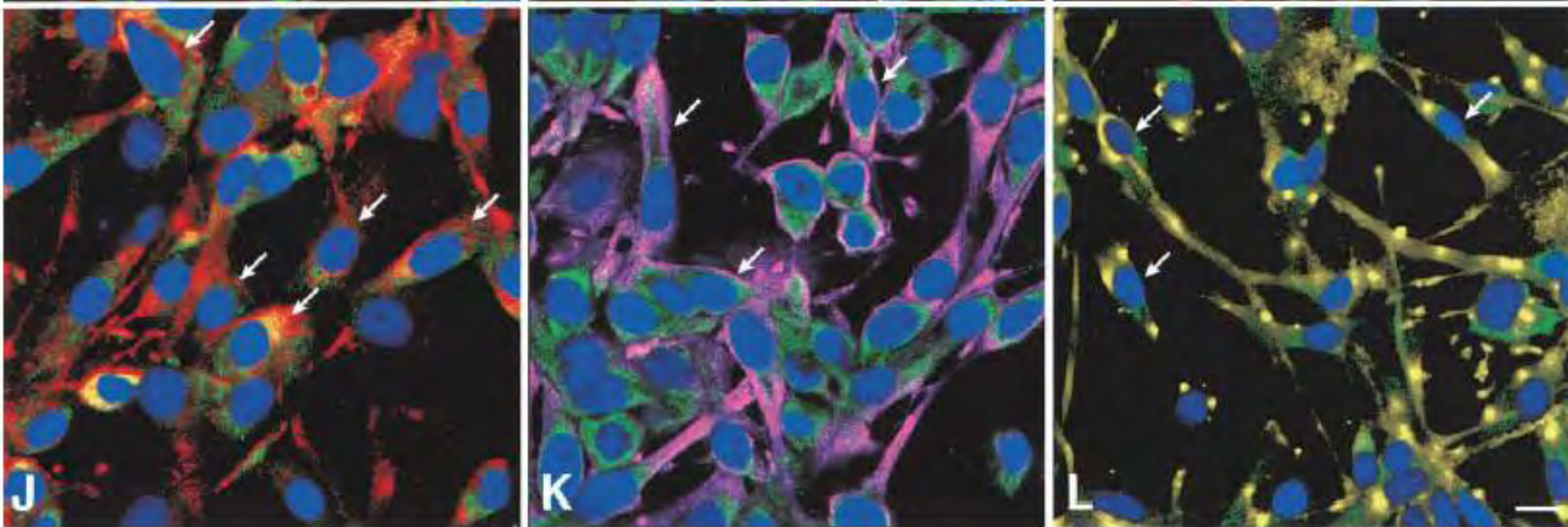


c-kit/GATA-4

c-kit/Tel

c-kit/MCM5

CSC can differentiate into cardiomyocytes, SMC and EC



Red: Cardiac myosin

Magenta: α -SM actin

Yellow: Von Willibrand factor

Blue: nuclei

Green: EGFP labeled CSC

Beltrami et al, Cell 2003

Dawn et al, PNAS 2005

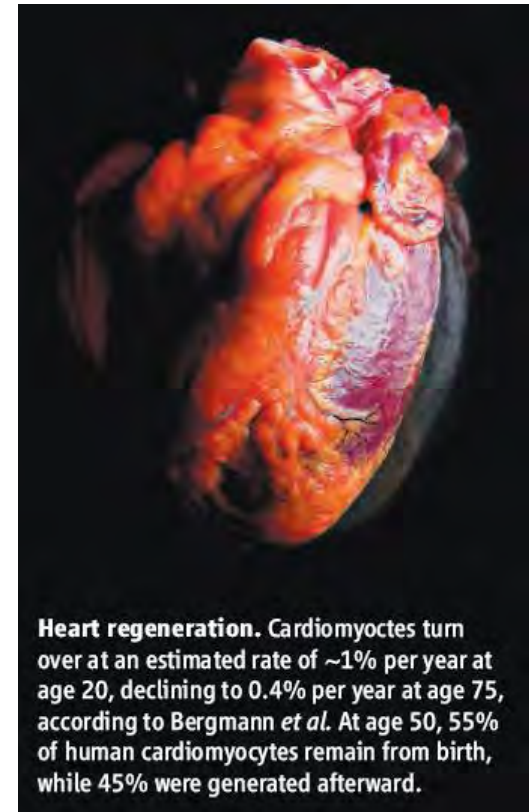
Bearzi et al, PNAS 2007

Cardiac-derived stem cells?



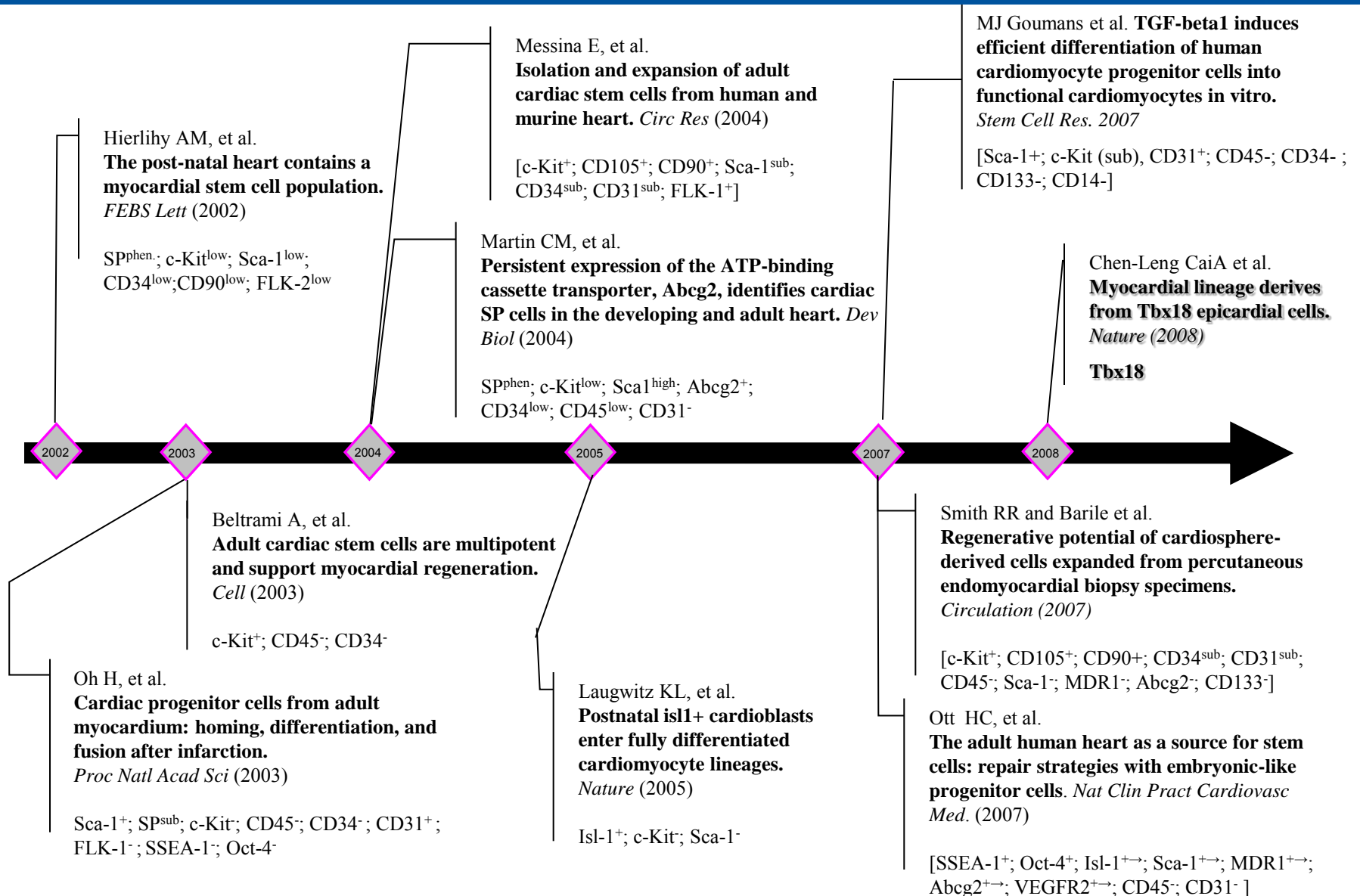
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- Cells reside in the heart
- Have the potential to differentiate
- Inherently programmed to reconstitute cardiac tissue
- Heightened predisposition to adopt cardiac muscle fate



Heart regeneration. Cardiomyocytes turn over at an estimated rate of ~1% per year at age 20, declining to 0.4% per year at age 75, according to Bergmann *et al.* At age 50, 55% of human cardiomyocytes remain from birth, while 45% were generated afterward.

Major discoveries in 2002-2008



Cardiac derived progenitor / stem cells



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- Cardiac stem cell (CSC)
- Cardiac progenitor cell (CPC)
- Cardiomyocyte progenitor cell (CMPC)
- Cardiac side population cells (SP)
- Cardiospheres
- Etc.....



CSC type	Ref	Tissue	Stem cell markers		Cardiac specific TF (at isolation)	Cardiac myocyte gene* (<i>in vitro</i> diff.)	<i>In vivo</i> myocytic diff.	<i>In vivo</i> functional studies
			Positive	Negative				
<i>c-kit</i>	2, 45, 47	Rat Pig Human	c-kit	CD34 CD45 Lineage	GATA4 GATA5 MEF2C NKX2.5	α -sarcomeric actin, cardiac myosin, desmin, connexin 43	Yes (also into SMC, EC, fibroblast)	Yes
<i>Sca-1</i>	33, 35, 39, 44	Mouse Human	Sca-1 CD31	c-kit (+/-) CD34 CD45 Lineage	GATA4 GATA5 MEF2C NKX2.5	Troponin I, α - sarcomeric actin, cardiac myosin, MHC (α and β), desmin, connexin 43	Yes	Yes
<i>Side population</i>	31, 41, 42	Mouse	Abcg2 Sca-1	CD31 c-kit (low) CD34 (low) CD45(low)	GATA4 MEF2C NKX2.5	Troponin, α -cardiac actinin	ND	ND
<i>Cardiospheres</i>	38, 49	Mouse Human	c-kit, Sca-1 MDR	CD34 Lineage	GATA4	Troponin, α -MHC	ND	Yes
<i>Islet-1</i>	43	Neonatal mouse	Ils-1	Sca-1 c-kit CD31	NKX2.5 GATA4	Troponin T, α - cardiac actinin	ND	ND

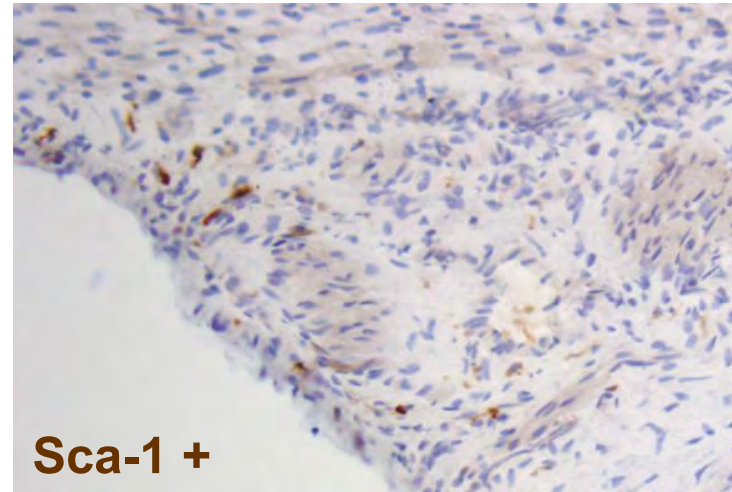
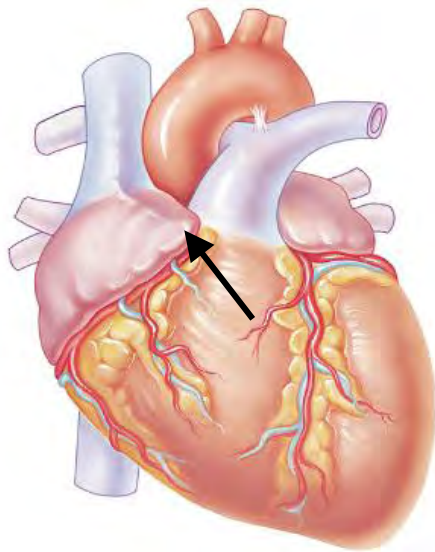
* Cardiac myocytic markers were *negative* at isolation.

CSC, cardiac stem cell; TF, transcription factors, ND, not determined; diff, differentiation; MHC, myosin heavy chain

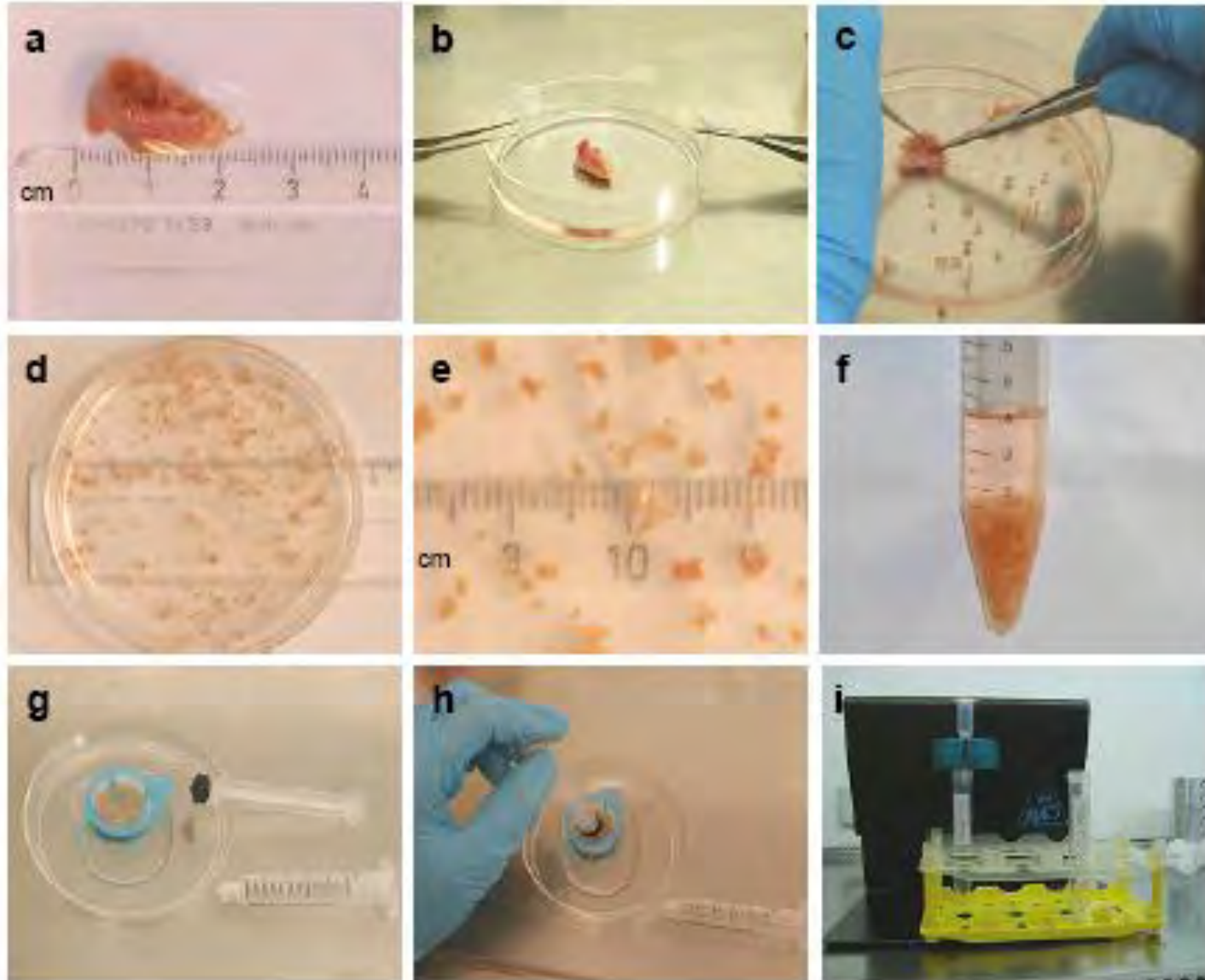
Cardiomyocyte progenitor cells (CMPCs)



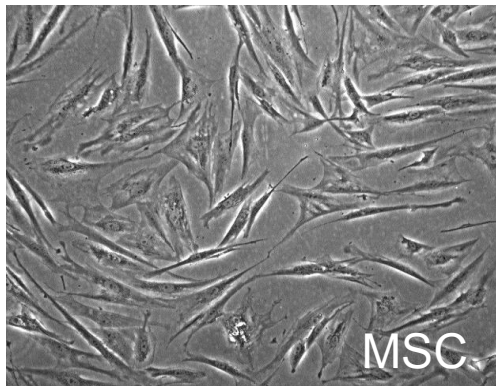
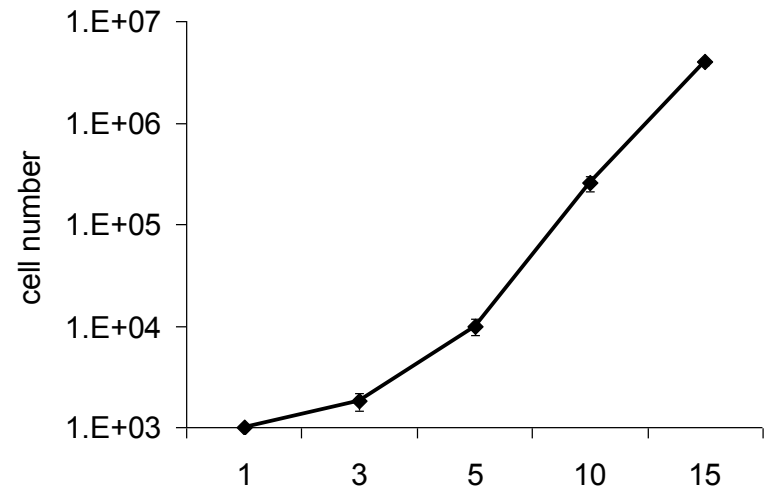
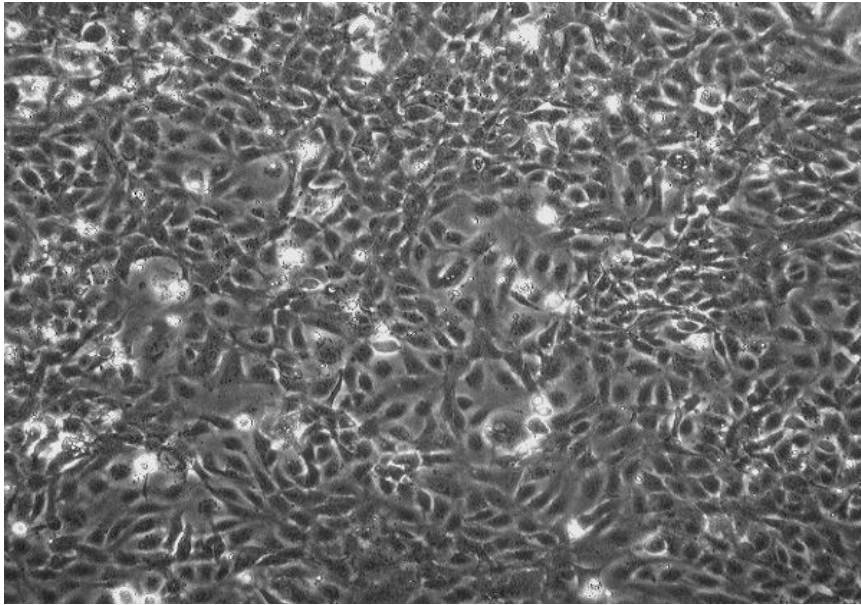
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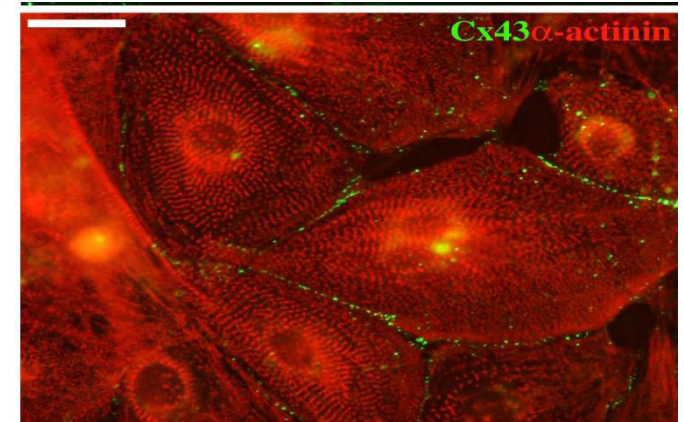
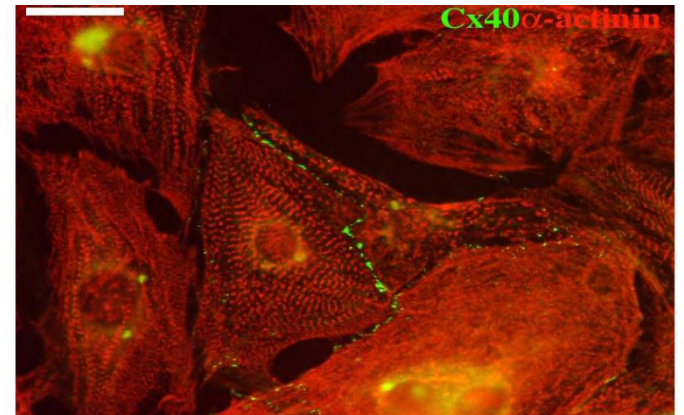
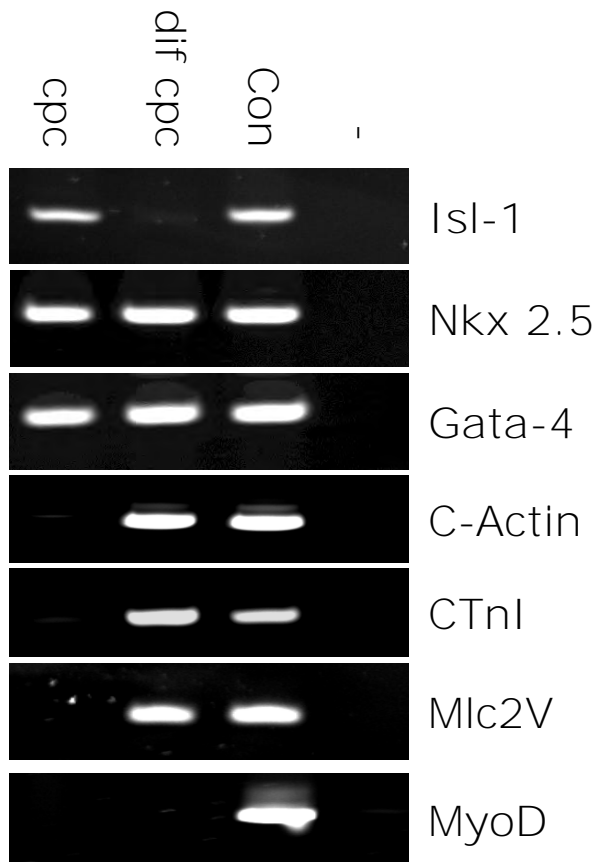
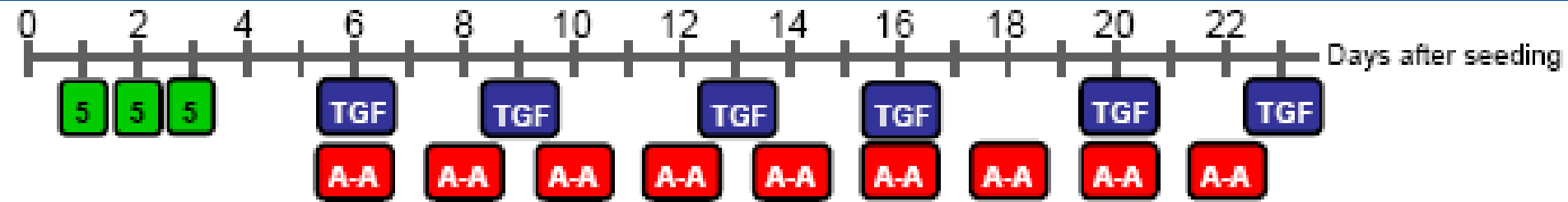
CMPCs



CMPC proliferation



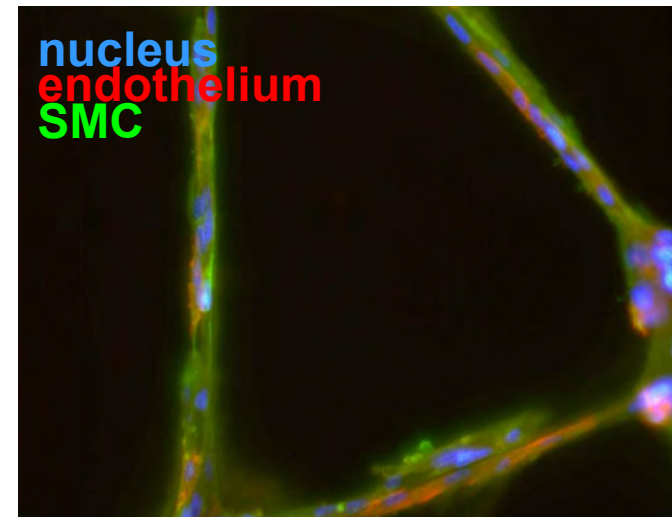
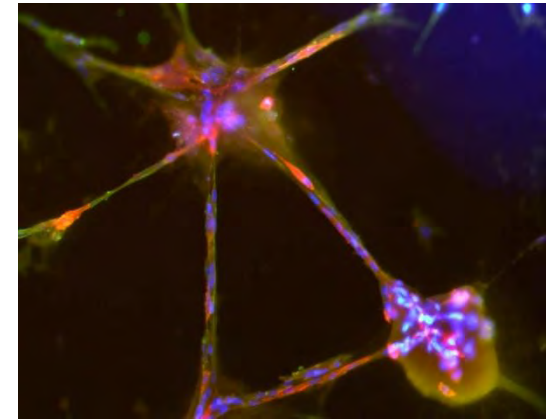
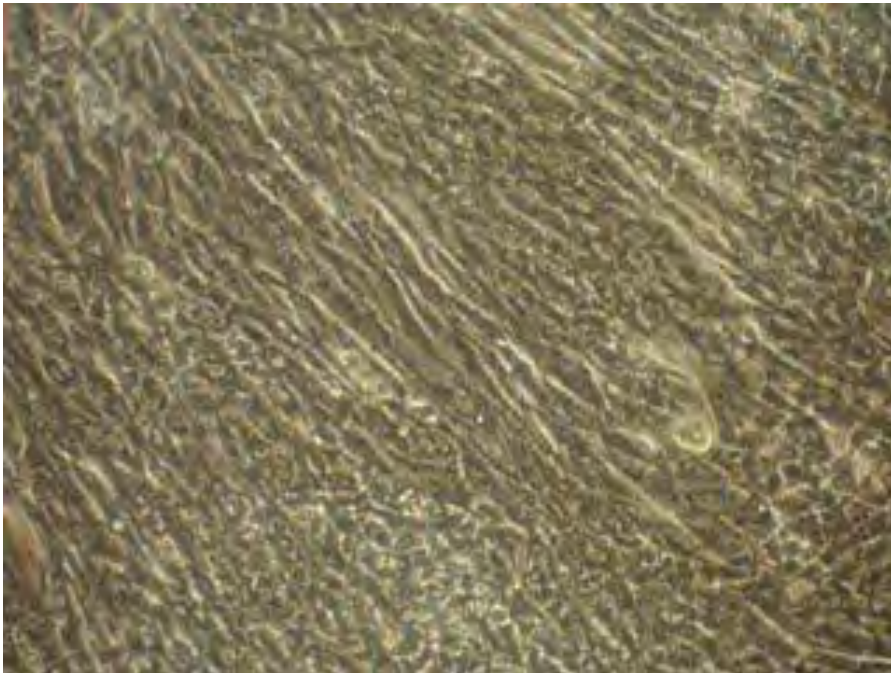
CMPC Differentiation - Cardiomyocytes



CMPC Differentiation



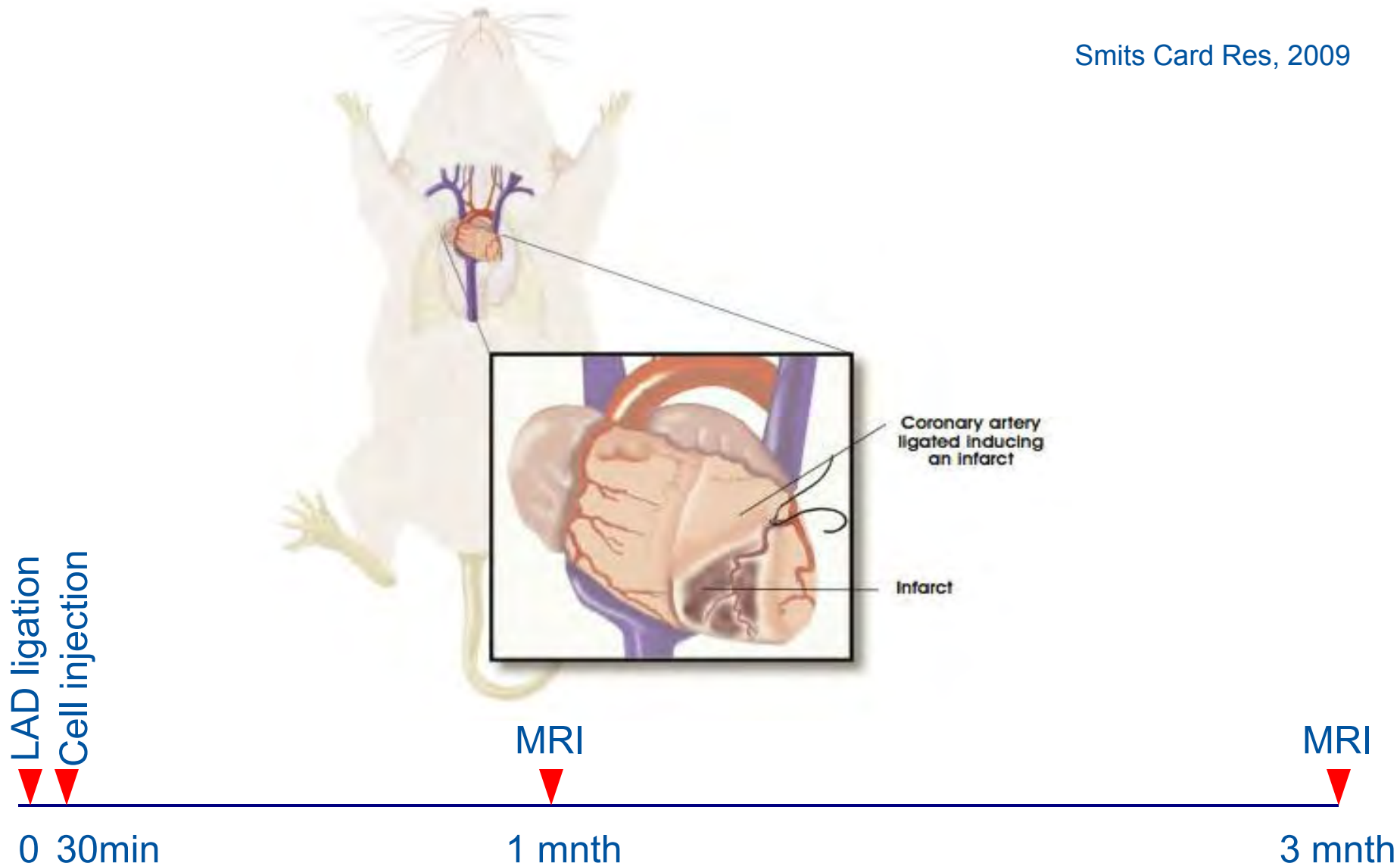
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Myocardial infarction - mouse



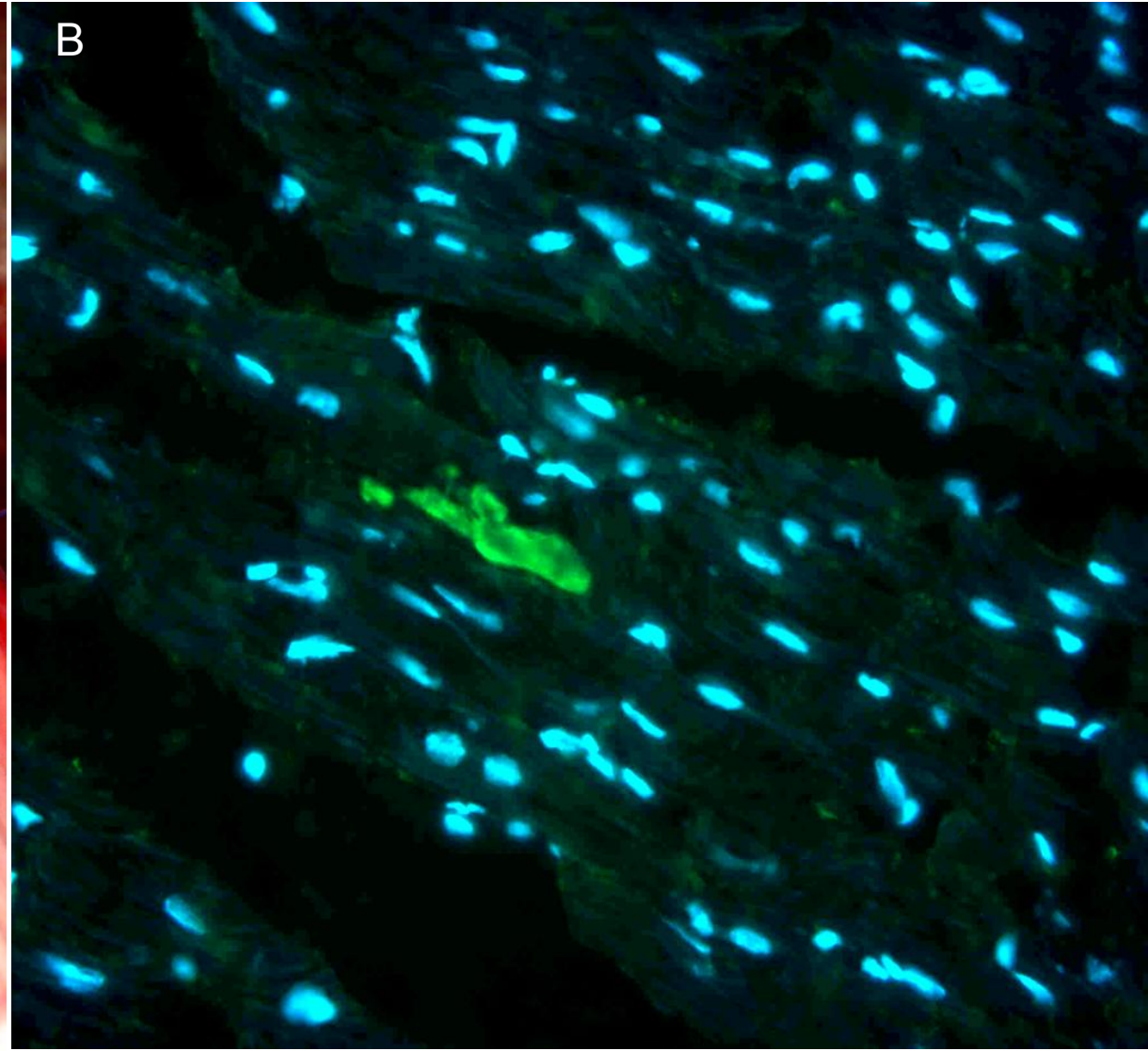
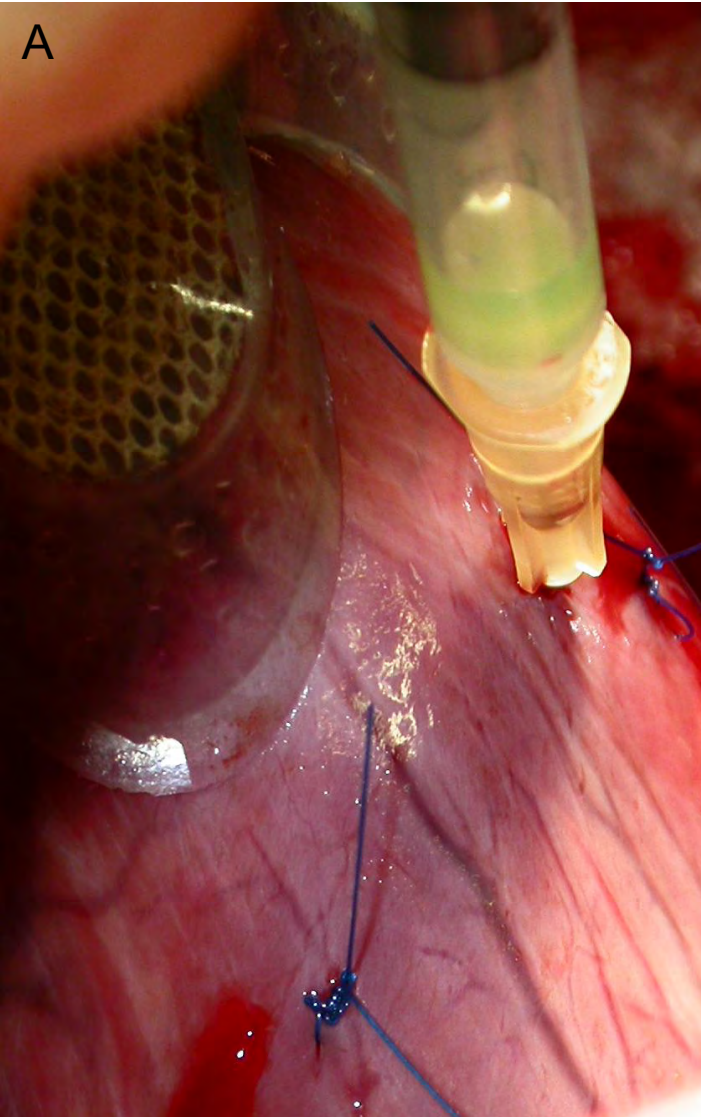
Smits Card Res, 2009



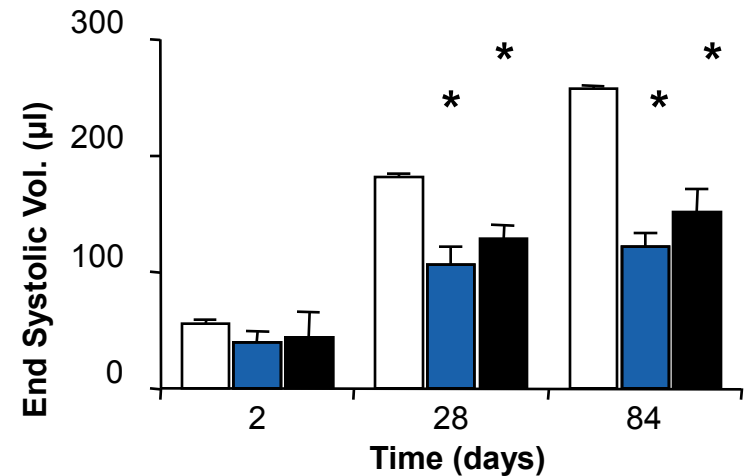
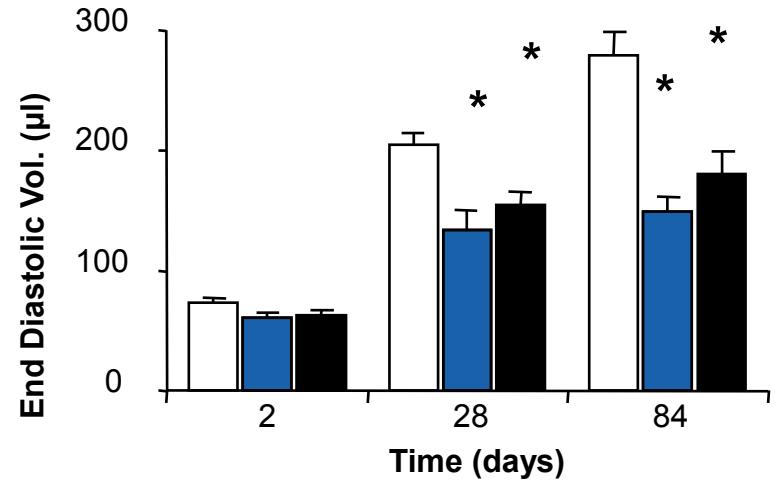
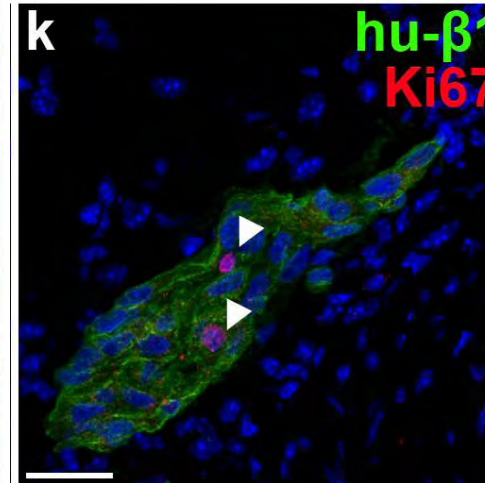
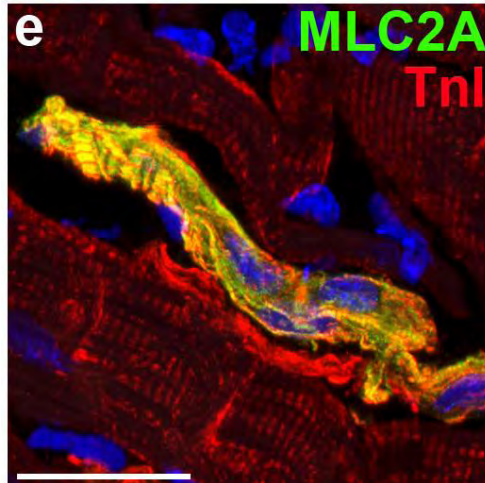
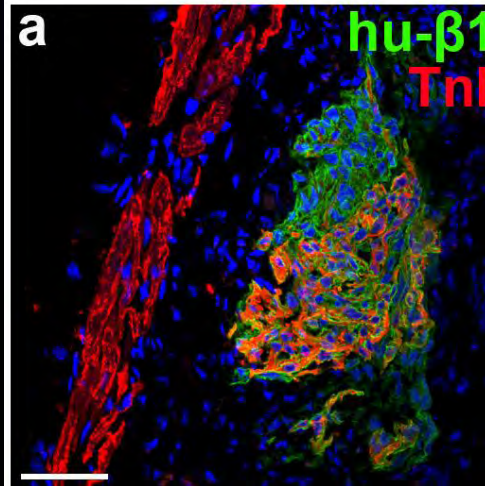
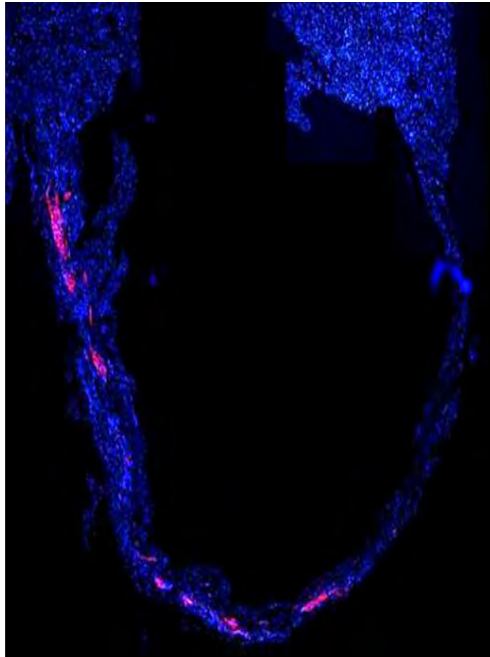
CMPC injection into porcine heart 2 weeks post MI



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CMPC Cell therapy – SCID mice



Optimizations

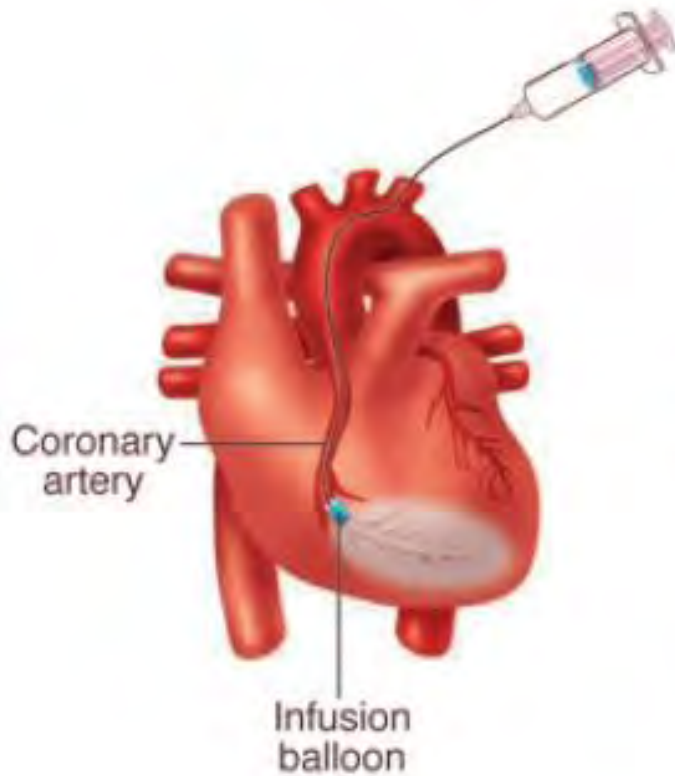


- Cell type
- Delivery method
- Number of cells
- Timing of delivery
- Survival / differentiation

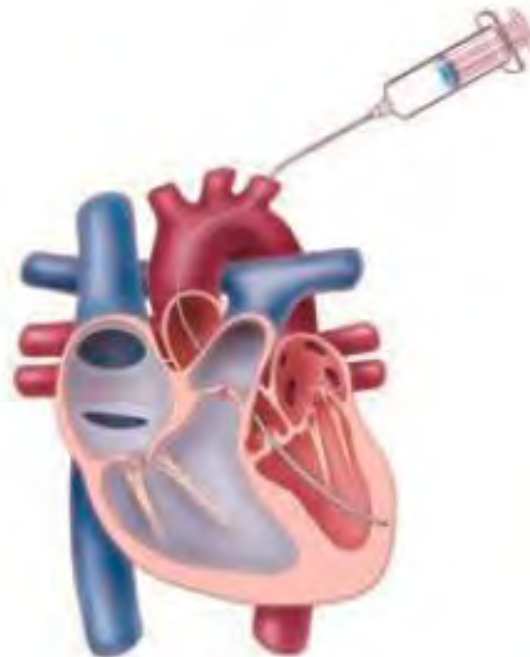
Delivery



Intracoronary infusion



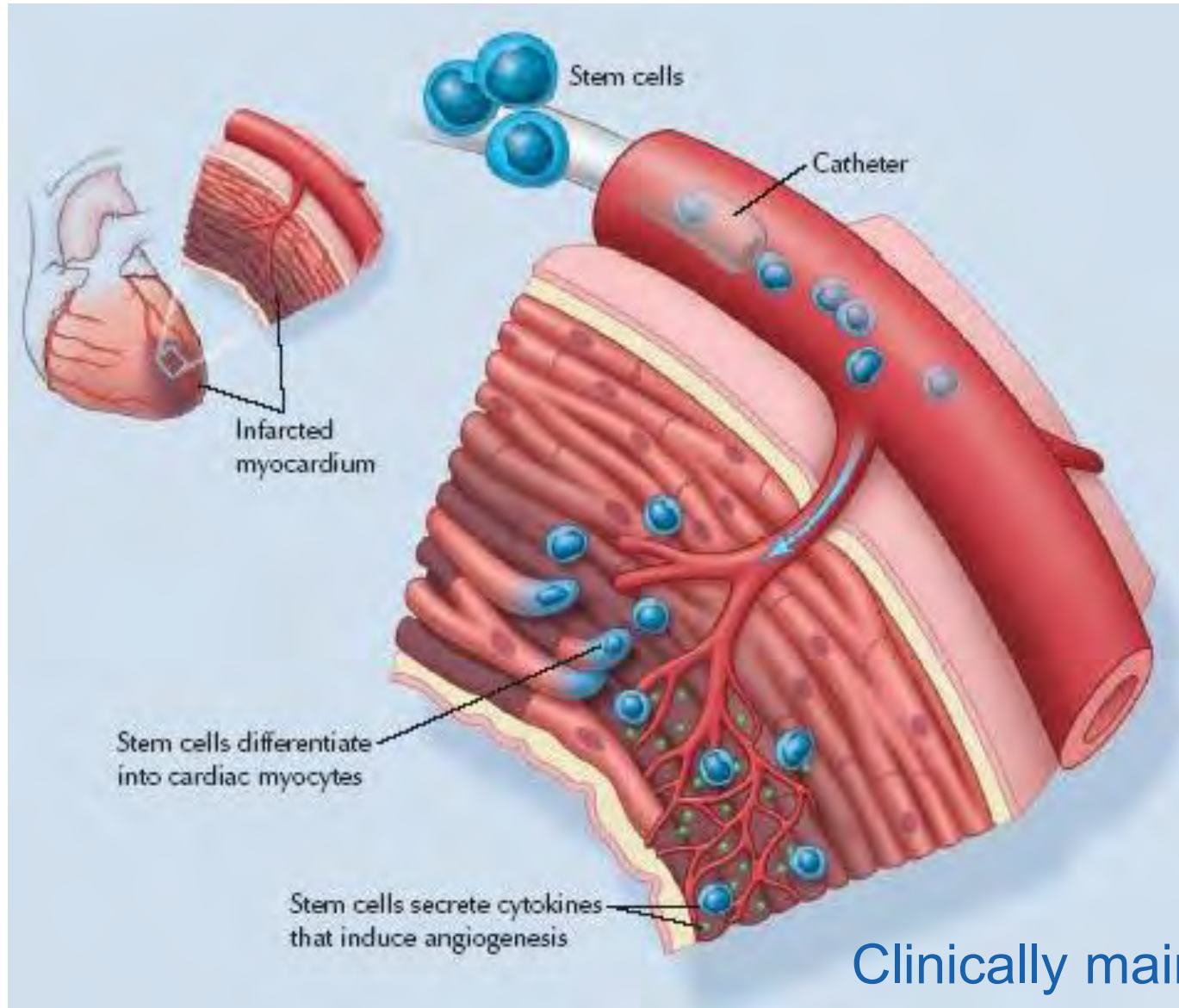
Catheter-based intramyocardial needle injection



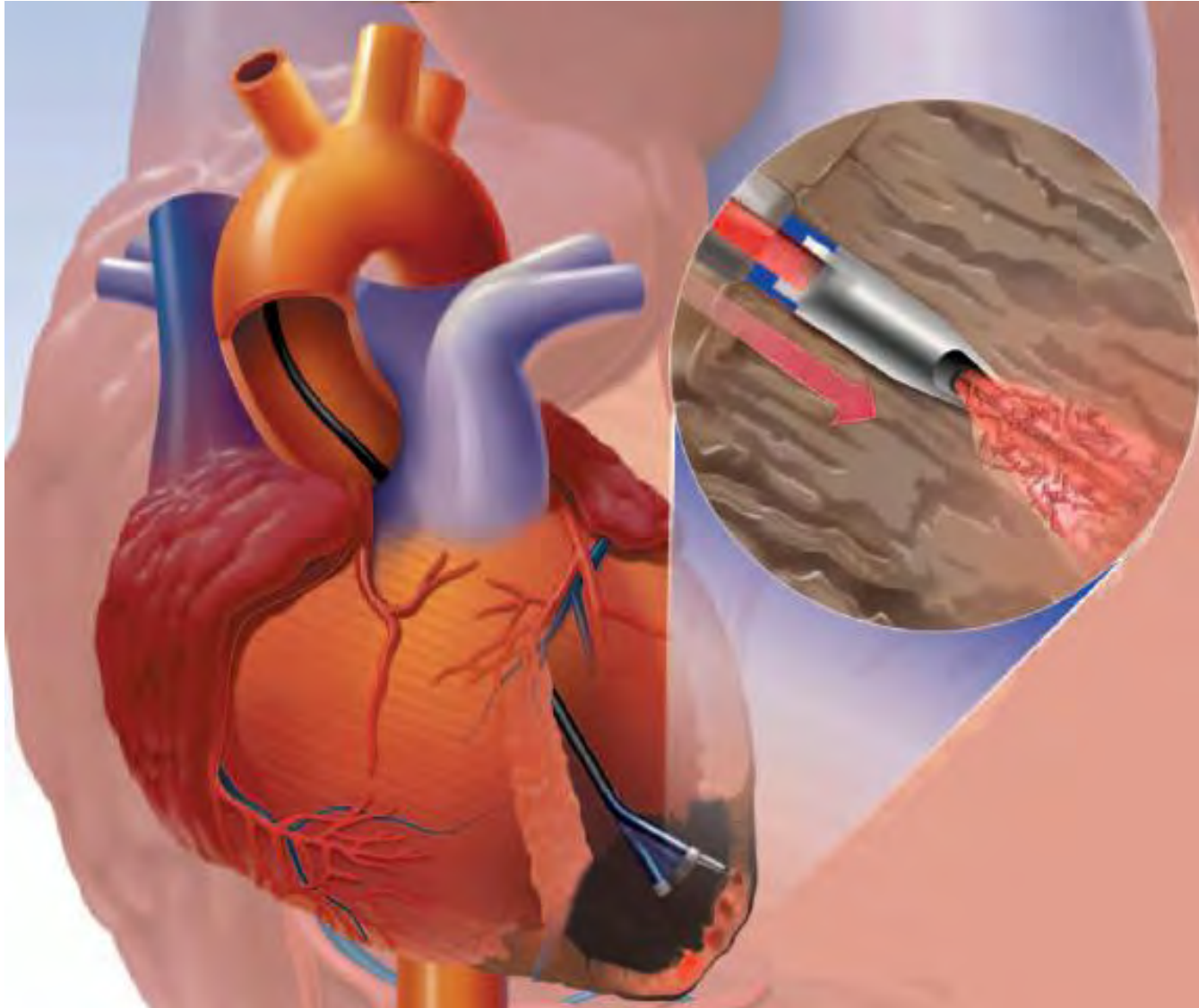
Direct intramyocardial injection during surgery



Infusion



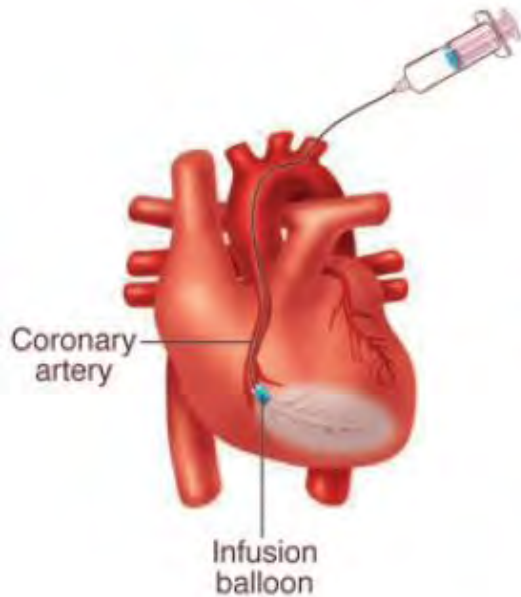
Catheter based intra-myocardial injection



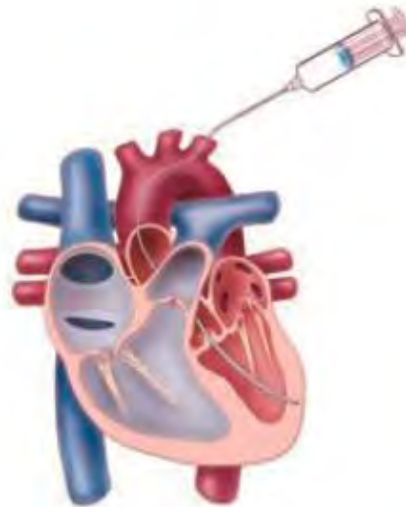
Cell delivery



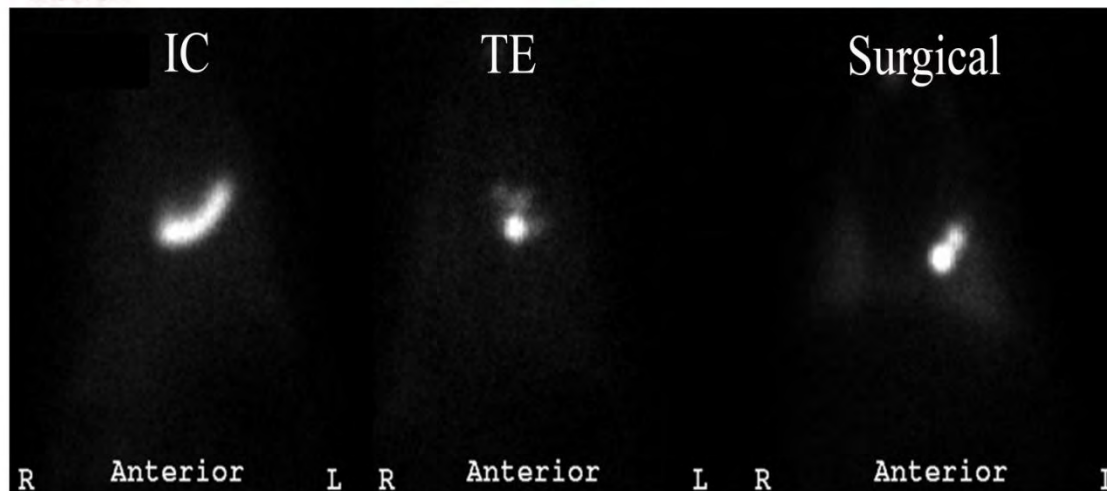
Intracoronary infusion



Catheter-based intramyocardial needle injection



Direct intramyocardial injection during surgery

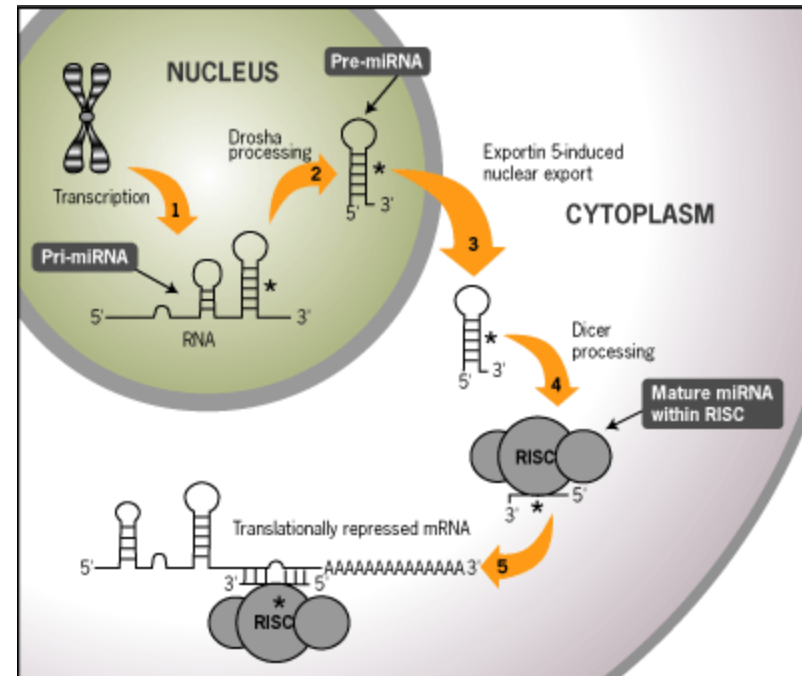
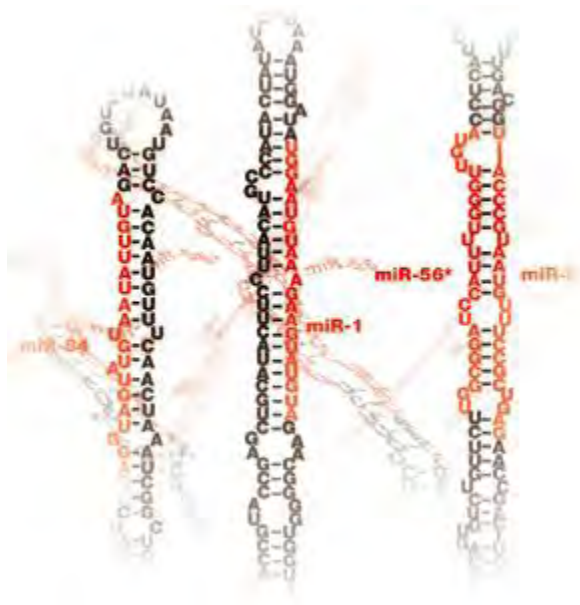


Optimizations

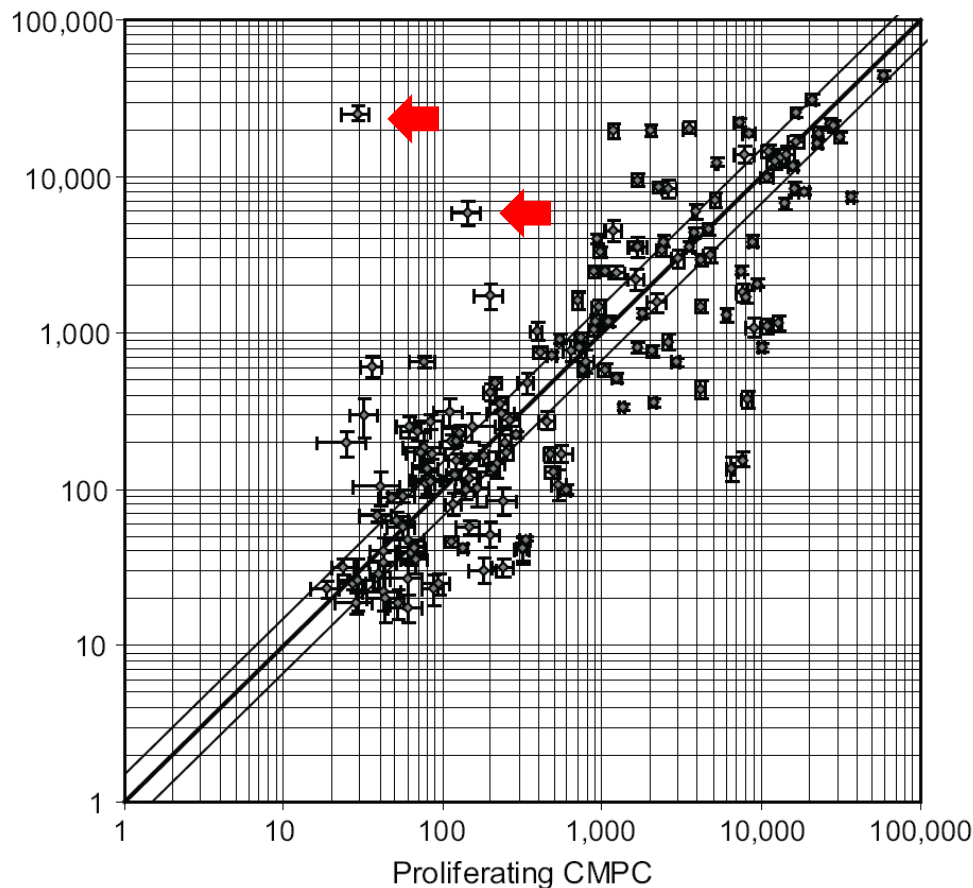


- Cell type
- Delivery method
- Number of cells
- Timing of delivery
- Survival / differentiation

MicroRNA biogenesis



Differential miRNA expression in proliferating / differentiated CMPCs

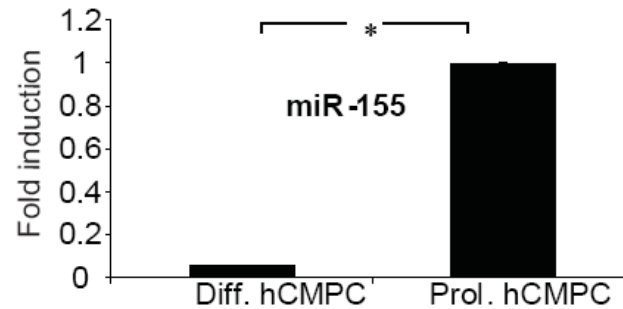
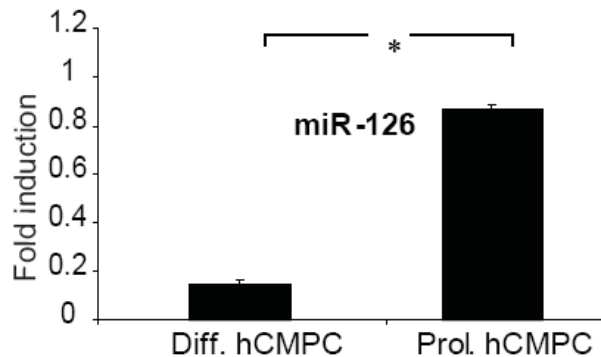
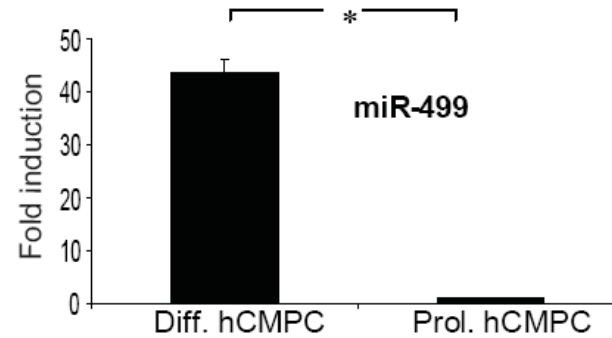
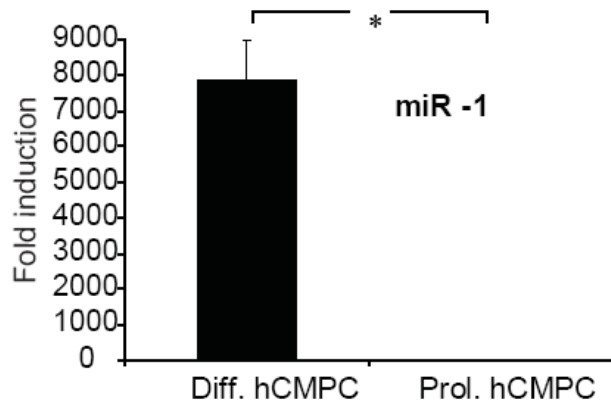


	miRNA ID	Proliferating CMPC	Differentiated CMPC	log2	fold induction
1	hsa-miR-1	26.1	24058.8	9.91	962.3
2	hsa-miR-499	10.9	1372.2	7.07	133.9
3	hsa-miR-133a	9.7	715.5	6.28	77.6
4	hsa-miR-133b	15.6	727.8	5.72	52.9
5	hsa-miR-335	128.9	5829.0	5.42	42.8
6	hsa-miR-450	32.2	643.7	4.40	21.2
7	hsa-miR-542-3p	12.5	245.1	4.21	18.5
8	hsa-miR-143	1143.5	19731.0	4.04	16.5
9	hsa-miR-217	8.8	122.1	3.79	13.8
10	hsa-miR-204	27.9	330.7	3.68	12.8
11	hsa-miR-203	20.0	181.2	3.54	11.6
12	hsa-miR-210	31.3	295.4	3.35	10.2
13	hsa-miR-145	2015.3	19156.3	3.34	10.2
14	hsa-miR-422b	68.4	630.5	3.21	9.3
15	hsa-miR-218	221.0	1798.7	3.10	8.6
16	hsa-miR-214	3645.1	20597.9	2.55	5.9
17	hsa-miR-152	1709.7	10020.7	2.51	5.7
18	hsa-miR-30d	918.3	4009.8	2.07	4.2
19	hsa-miR-374	59.6	249.2	2.06	4.2

	miRNA ID	Proliferating CMPC	Differentiated CMPC	log2	fold repression
1	hsa-miR-146a	7683.9	155.1	-5.59	48.1
2	hsa-miR-155	6457.5	127.6	-5.53	46.2
3	hsa-miR-126	8375.6	380.6	-4.48	22.3
4	hsa-miR-19a	192.5	12.1	-3.99	15.9
5	hsa-miR-31	10019.8	781.9	-3.66	12.7
6	hsa-miR-222	13140.2	1124.8	-3.48	11.2
7	hsa-miR-221	11128.8	1057.9	-3.32	10.0
8	hsa-miR-20b	4227.6	439.9	-3.27	9.6
9	hsa-miR-542-5p	181.9	20.2	-3.13	8.7
10	hsa-miR-625	121.3	13.3	-3.08	8.4
11	hsa-miR-663	9058.1	1057.9	-3.04	8.2
12	hsa-miR-602	303.5	42.3	-2.95	7.7
13	hsa-miR-612	226.6	29.3	-2.93	7.6
14	hsa-miR-323	309.9	44.1	-2.93	7.6
15	hsa-miR-29b	121.7	17.9	-2.84	7.2
16	hsa-miR-299-5p	331.7	47.2	-2.81	7.0
17	hsa-miR-494	2099.8	353.4	-2.60	6.1
18	hsa-miR-212	190.2	33.6	-2.57	5.9
19	hsa-miR-18a	591.4	99.8	-2.57	5.9
20	hsa-miR-638	37354.6	7219.1	-2.33	5.0
21	hsa-miR-329	540.3	110.4	-2.32	5.0
22	hsa-miR-106a	5998.6	1252.7	-2.27	4.8
23	hsa-miR-17-5p	8154.2	1645.0	-2.22	4.6
24	hsa-miR-20a	9392.7	2119.2	-2.21	4.6
25	hsa-miR-19b	2901.6	660.8	-2.14	4.4
26	hsa-miR-505	202.7	50.0	-2.09	4.3
27	hsa-miR-15b	7703.7	1734.1	-2.03	4.1

µParaFlo™ microfluidic chip technology (LCSciences Houston)

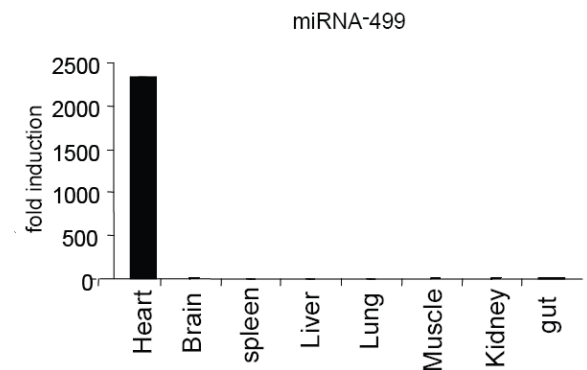
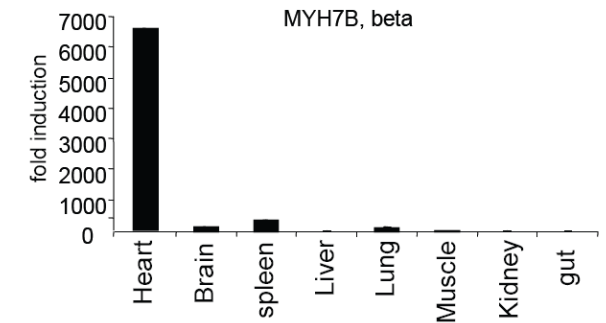
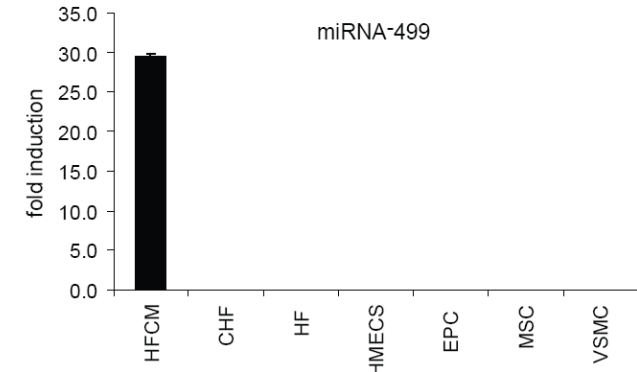
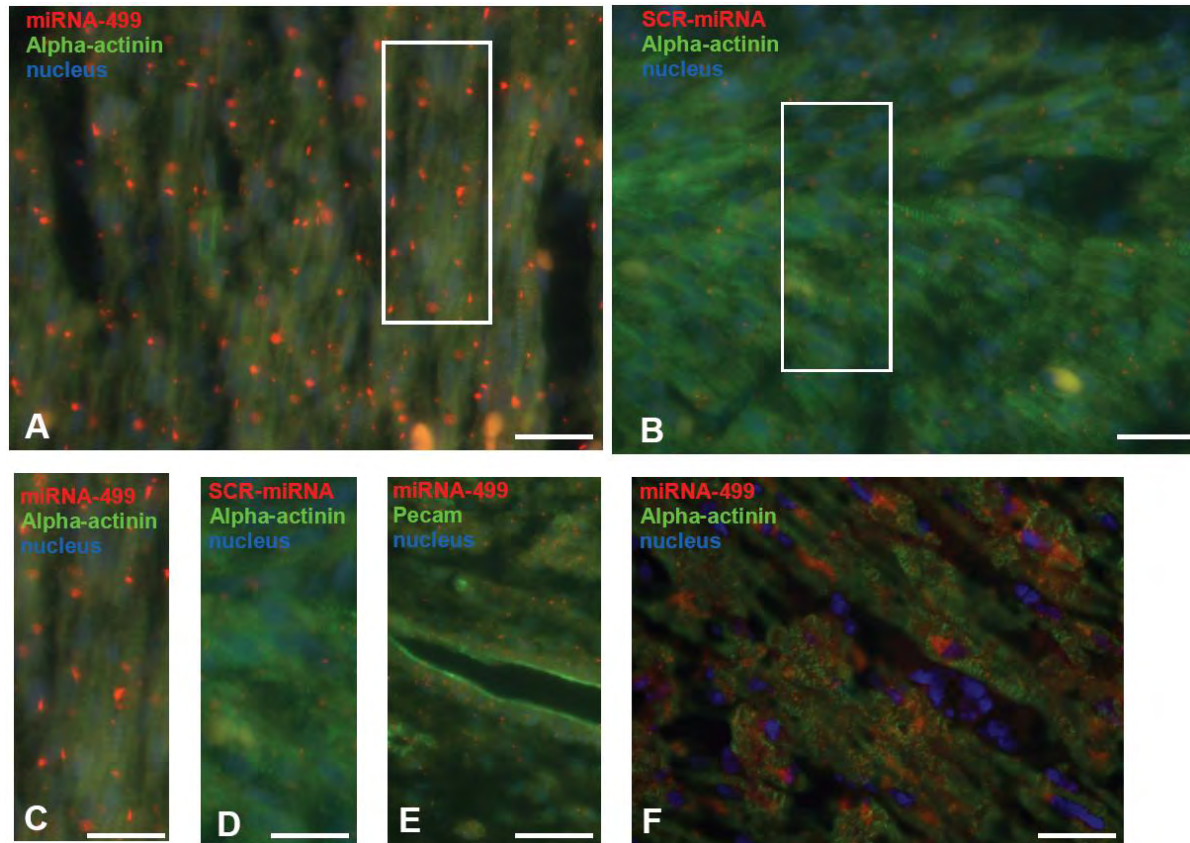
Validation by qRT-PCR



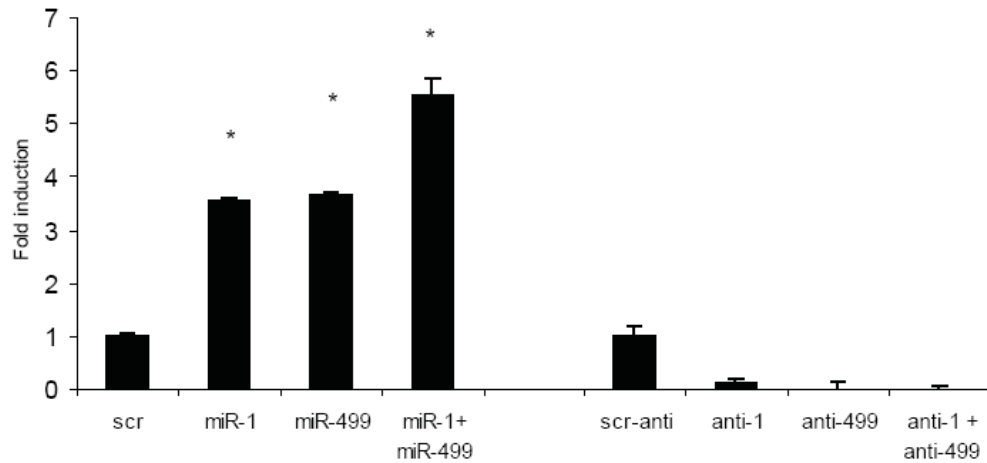
miRNAs may inhibit
or enhance CMPC:

- Proliferation**
- Differentiation**
- Survival**
- Migration**
- Integration**

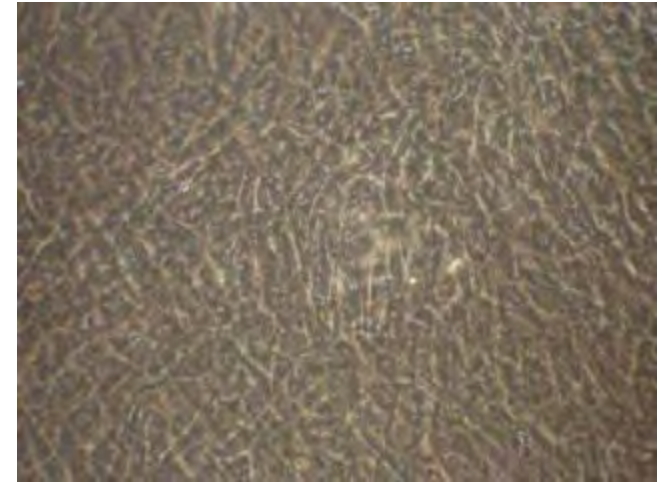
miR-499 is localized in cardiomyocytes



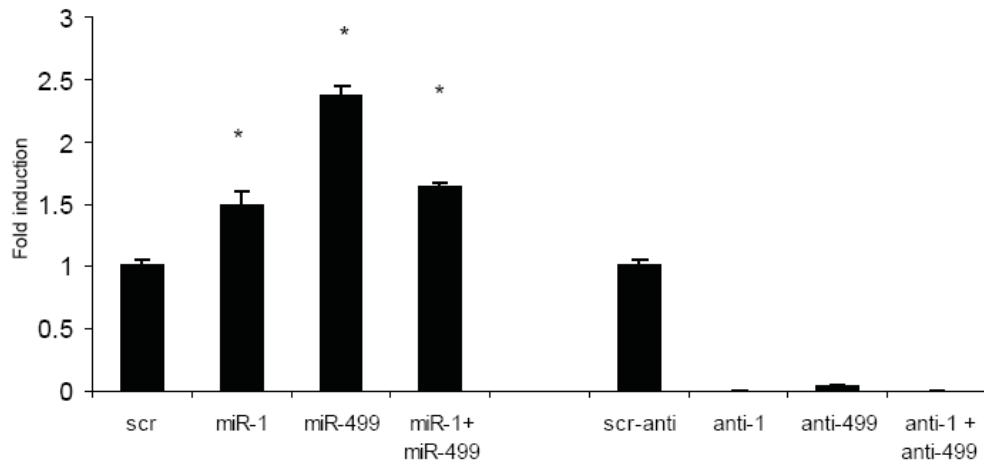
CMPC differentiation – miR-1 and miR-499



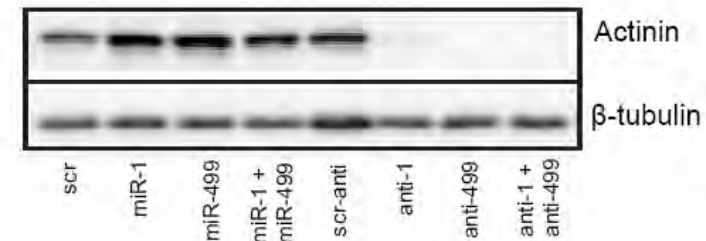
Trop T



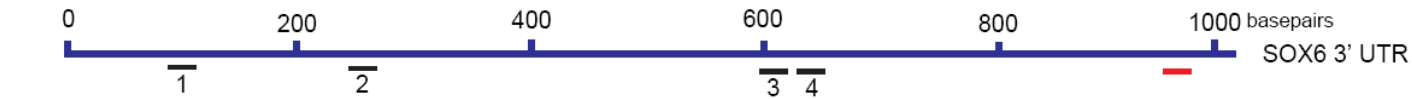
6 days differentiation



Mlc2v

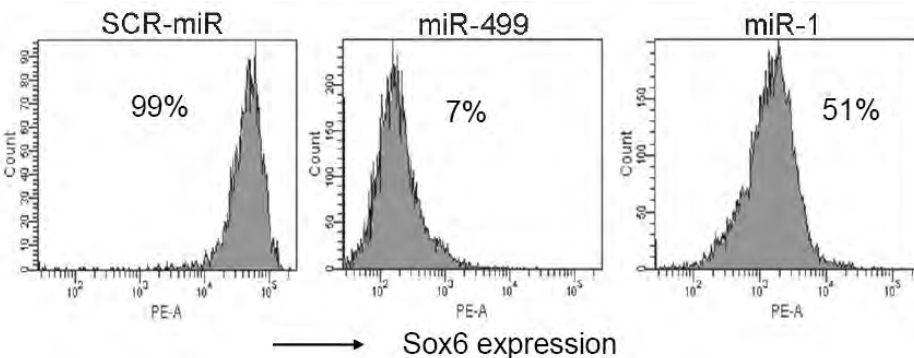
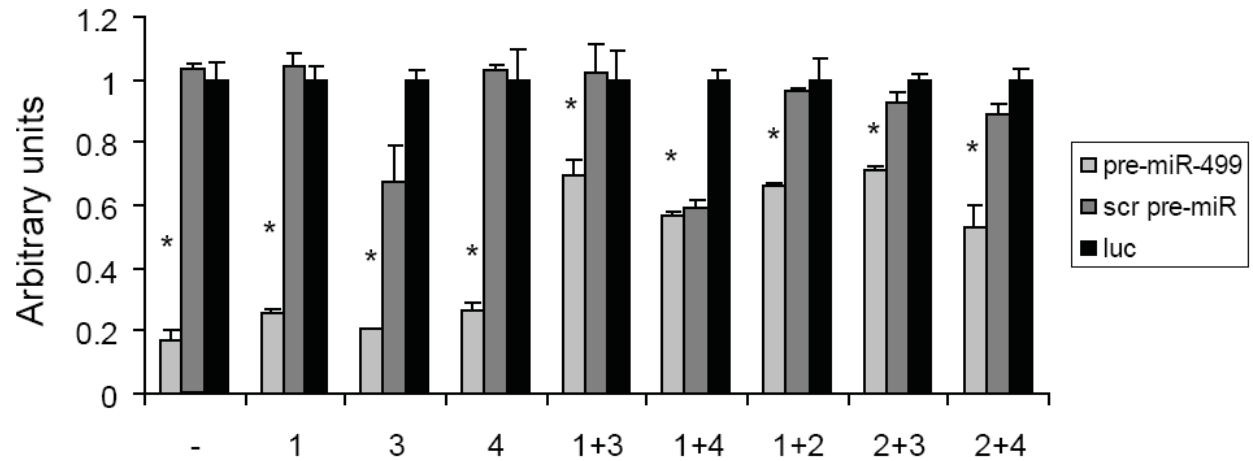
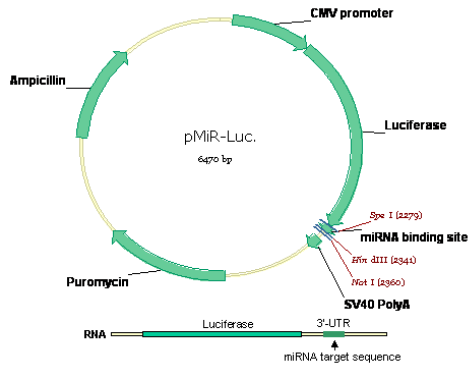


Predicted miR-499 target – sox6



— miR-499
— miR-1

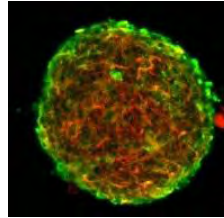
1 = 123-125, 7mer-1A
2 = 287-289, 7mer-m8
3 = 600-602, 8mer
4 = 623-625, 7mer-m8



Regulation of muscle differentiation



CMPC

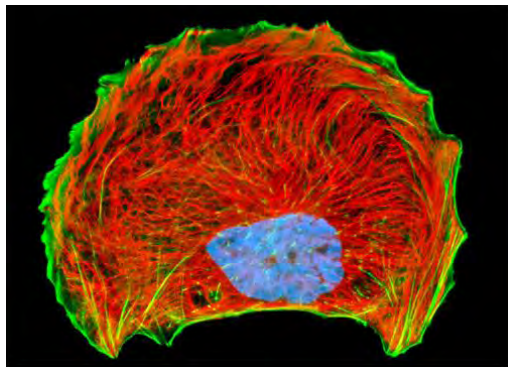


HDAC4

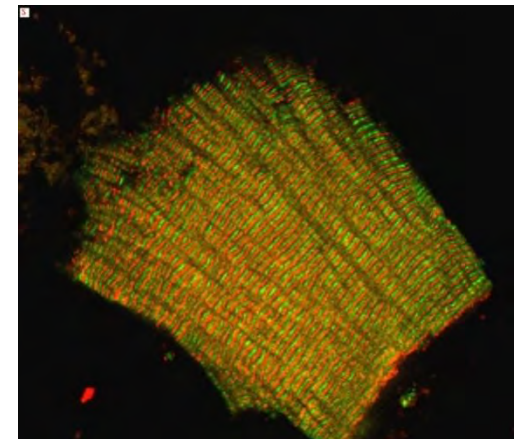
SOX6

miR-499

miR-1



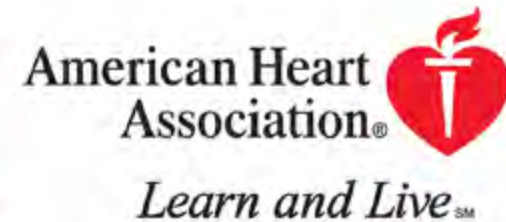
Smooth muscle cells



Cardiomyocytes

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Human Cardiac Stem Cell Differentiation Is Regulated by a Mircrine Mechanism

Toru Hosoda, Hanqiao Zheng, Mauricio Cabral-da-Silva, Fumihiko Sanada, Noriko Ide-Iwata, Barbara Ogórek, João Ferreira-Martins, Christian Arranto, Domenico D'Amario, Federica del Monte, Konrad Urbanek, David A. D'Alessandro, Robert E. Michler, Piero Anversa, Marcello Rota, Jan Kajstura and Annarosa Leri

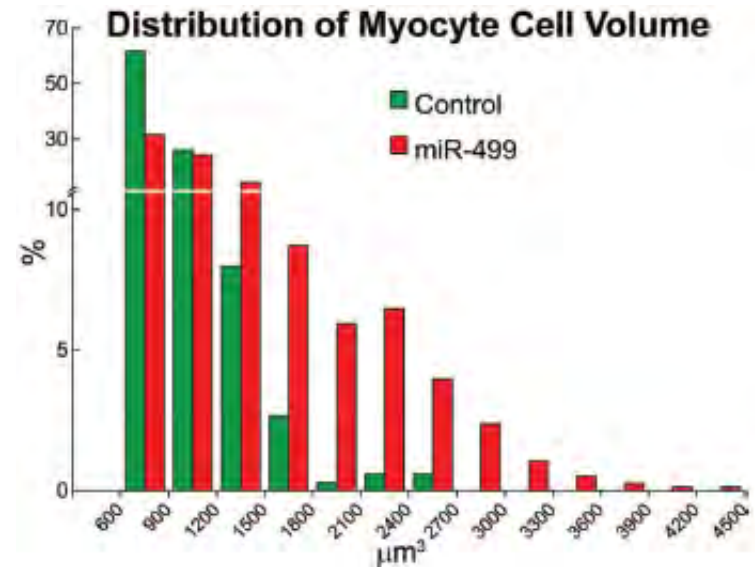
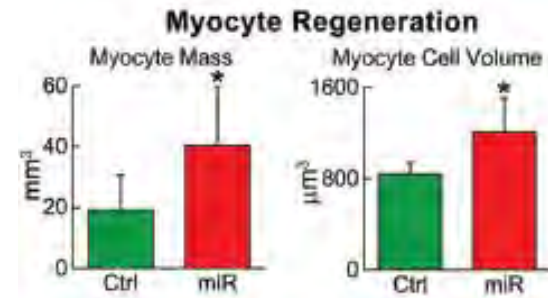
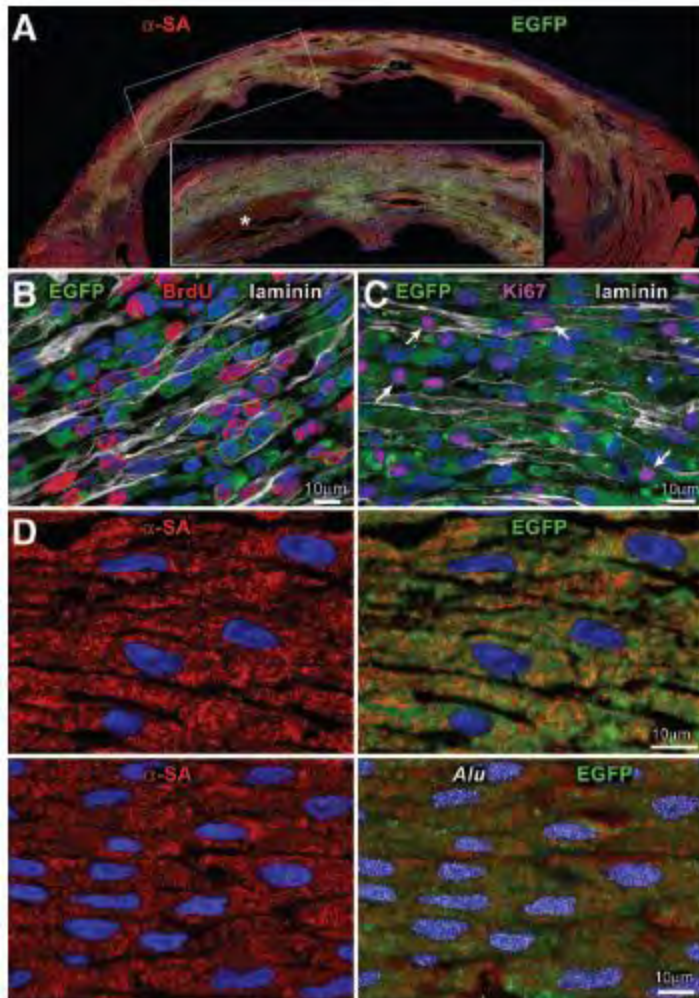
Circulation 2011;123:1287-1296; originally published online Mar 14, 2011;

DOI: 10.1161/CIRCULATIONAHA.110.982918

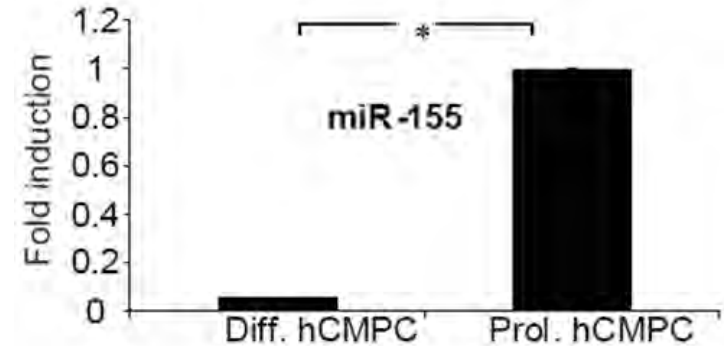
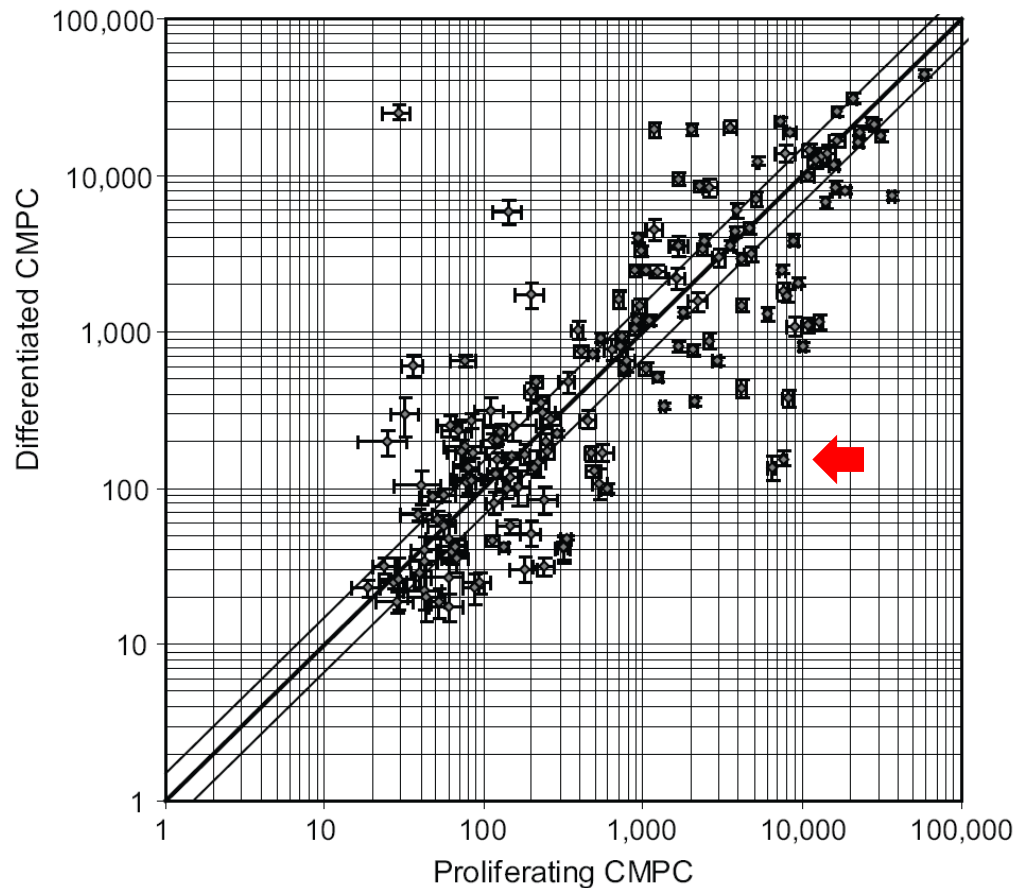
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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miR-499 induced more mature myocytes

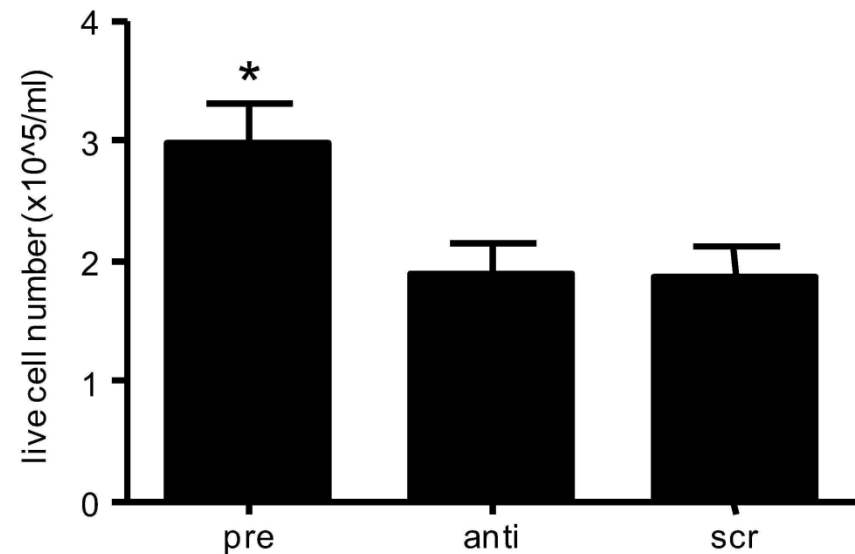
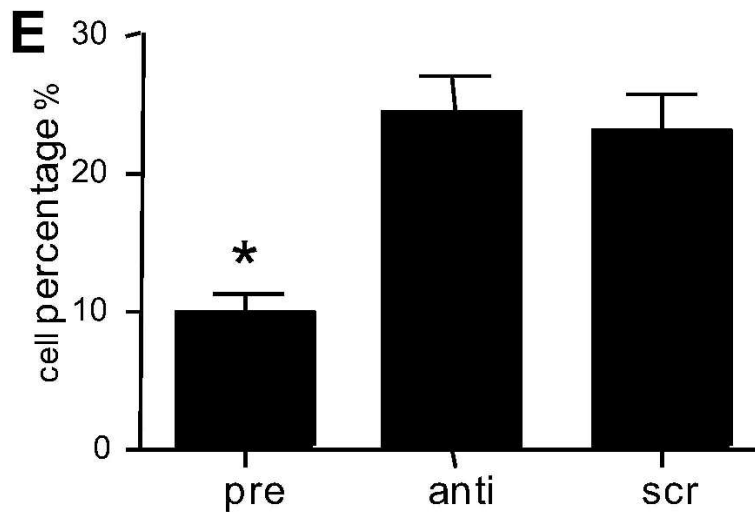
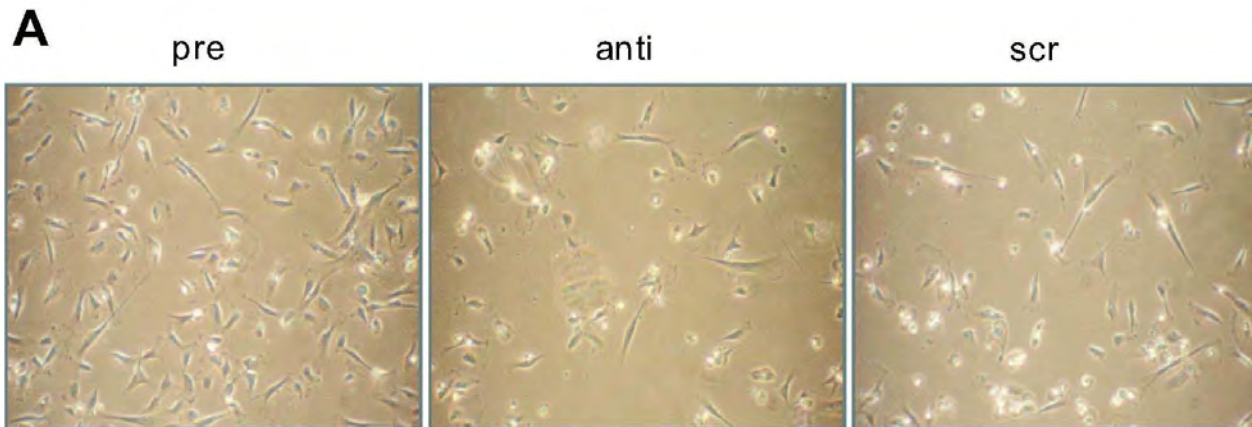


CMPC survival – miR-155

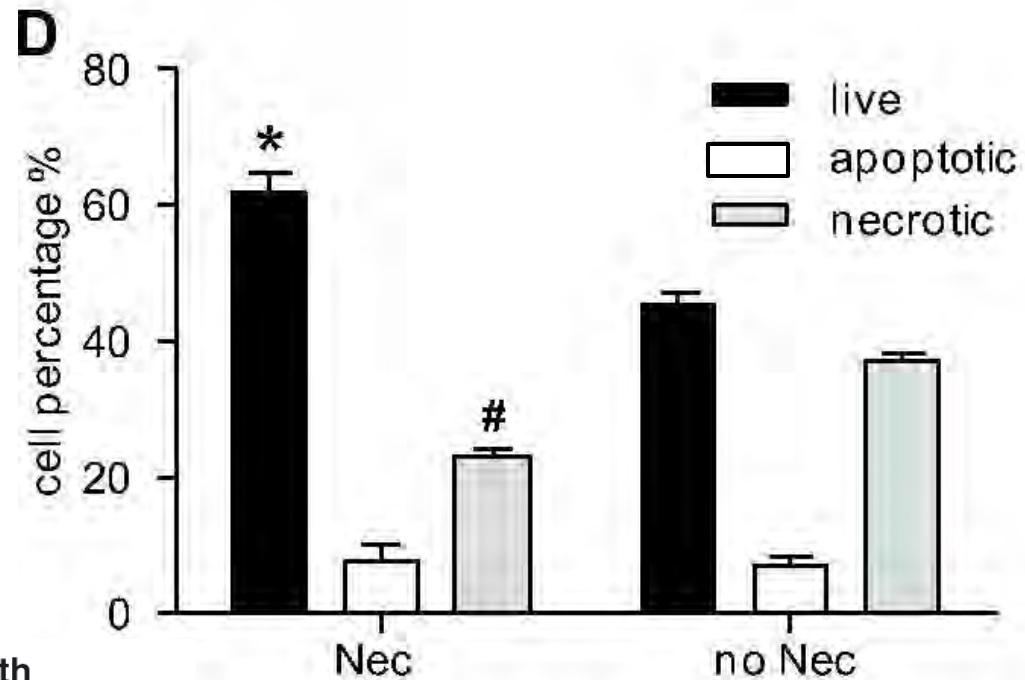
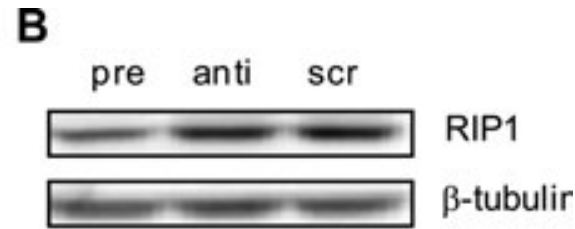
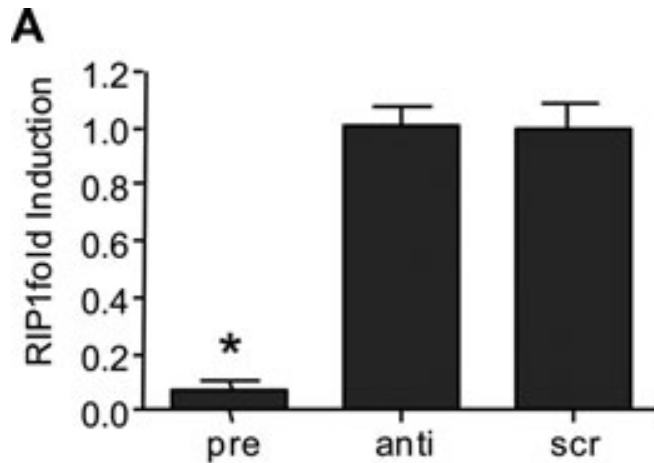


	miRNA ID	Proliferating CMPC	Differentiated CMPC	log2	fold repression
1	hsa-miR-146a	7683.9	155.1	-5.59	48.1
2	hsa-miR-155	6457.5	127.6	-5.53	46.2
3	hsa-miR-126	8375.6	380.6	-4.48	22.3
4	hsa-miR-19a	192.5	12.1	-3.99	15.9
5	hsa-miR-31	10019.8	781.9	-3.66	12.7
6	hsa-miR-222	13140.2	1124.8	-3.48	11.2
7	hsa-miR-221	11128.8	1057.9	-3.32	10.0
8	hsa-miR-20b	4227.6	439.9	-3.27	9.6
9	hsa-miR-542-5p	181.9	20.2	-3.13	8.7
10	hsa-miR-625	121.3	13.3	-3.08	8.4
11	hsa-miR-663	9058.1	1057.9	-3.04	8.2
12	hsa-miR-602	303.5	42.3	-2.95	7.7
13	hsa-miR-612	226.6	29.3	-2.93	7.6
14	hsa-miR-323	309.9	44.1	-2.93	7.6
15	hsa-miR-29b	121.7	17.9	-2.84	7.2
16	hsa-miR-299-5p	331.7	47.2	-2.81	7.0
17	hsa-miR-494	2099.8	353.4	-2.60	6.1
18	hsa-miR-212	190.2	33.6	-2.57	5.9
19	hsa-miR-18a	591.4	99.8	-2.57	5.9
20	hsa-miR-638	37354.6	7219.1	-2.33	5.0
21	hsa-miR-329	540.3	110.4	-2.32	5.0
22	hsa-miR-106a	5998.6	1252.7	-2.27	4.8
23	hsa-miR-17-5p	8154.2	1645.0	-2.22	4.6
24	hsa-miR-20a	9392.7	2119.2	-2.21	4.6
25	hsa-miR-19b	2901.6	660.8	-2.14	4.4
26	hsa-miR-505	202.7	50.0	-2.09	4.3
27	hsa-miR-15b	7703.7	1734.1	-2.03	4.1

Pre-miR-155 increased cell survival and reduced cell death

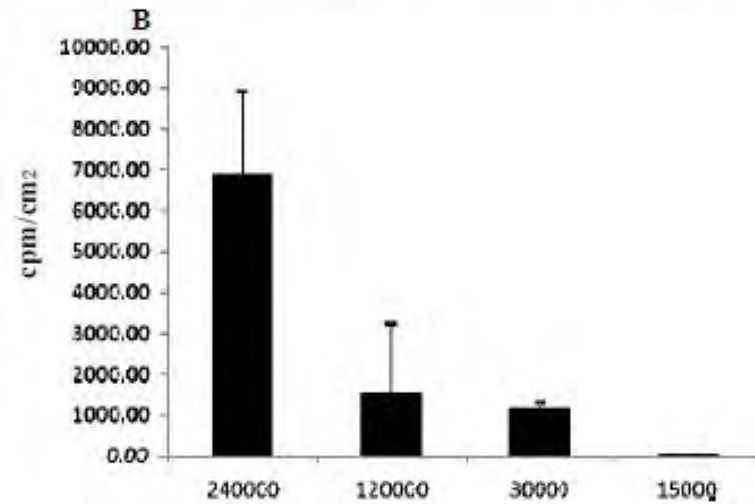
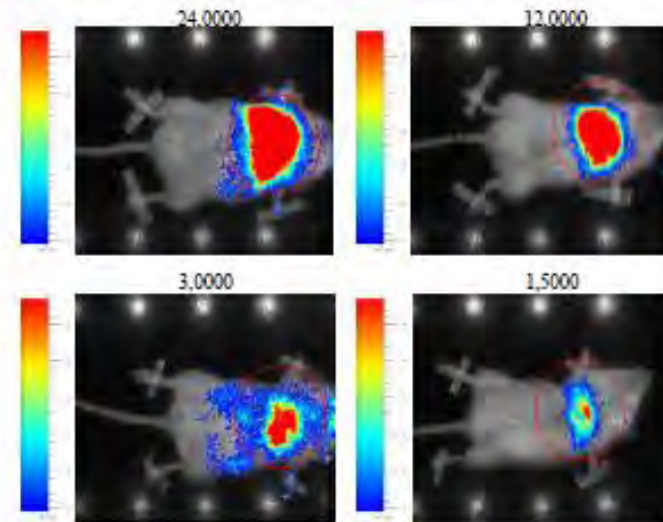


miRNA-155 – RIP1

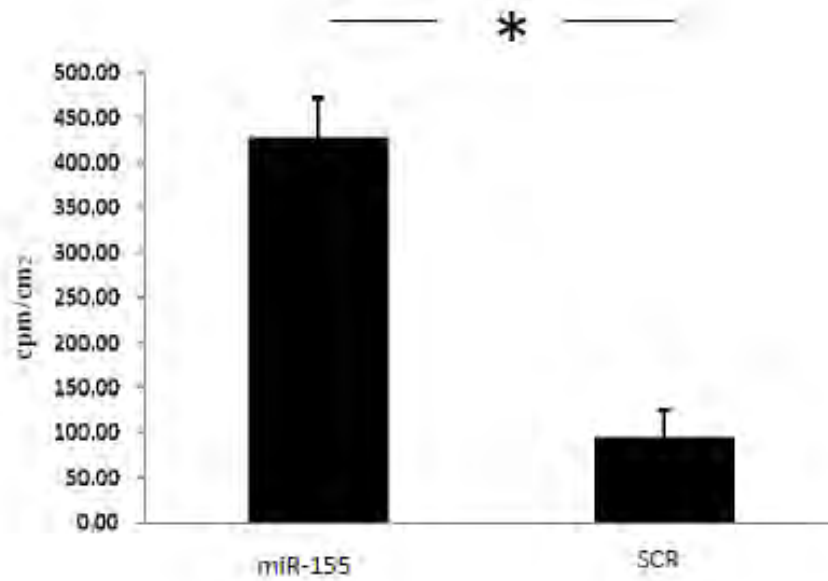


MicroRNA-155 prevents necrotic cell death
in human cardiomyocyte progenitor cells *via* targeting RIP1

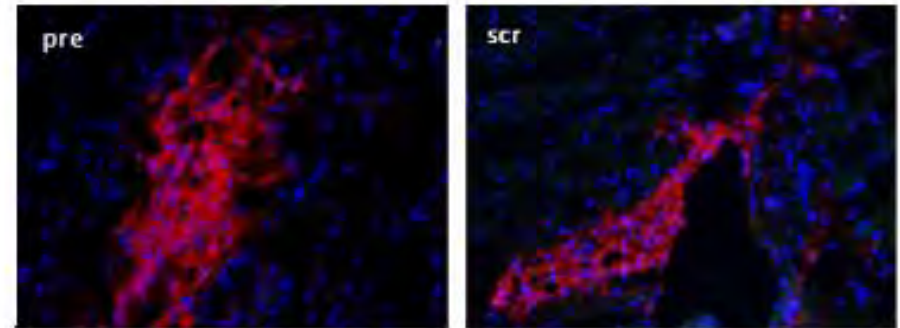
BLI imaging to monitor cell survival



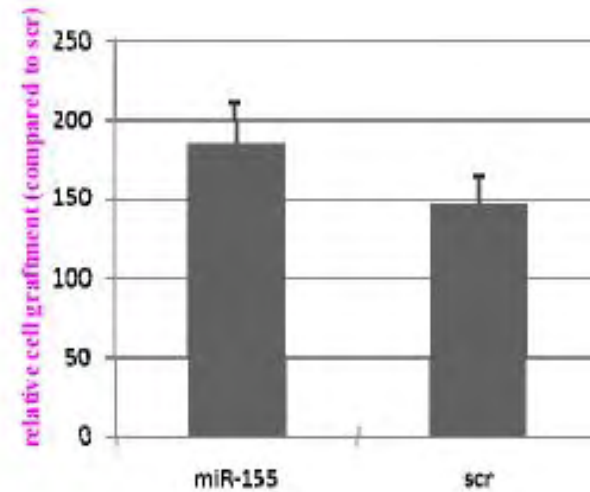
miR-155 improves CMPC survival



A



B



Possible Mechanisms

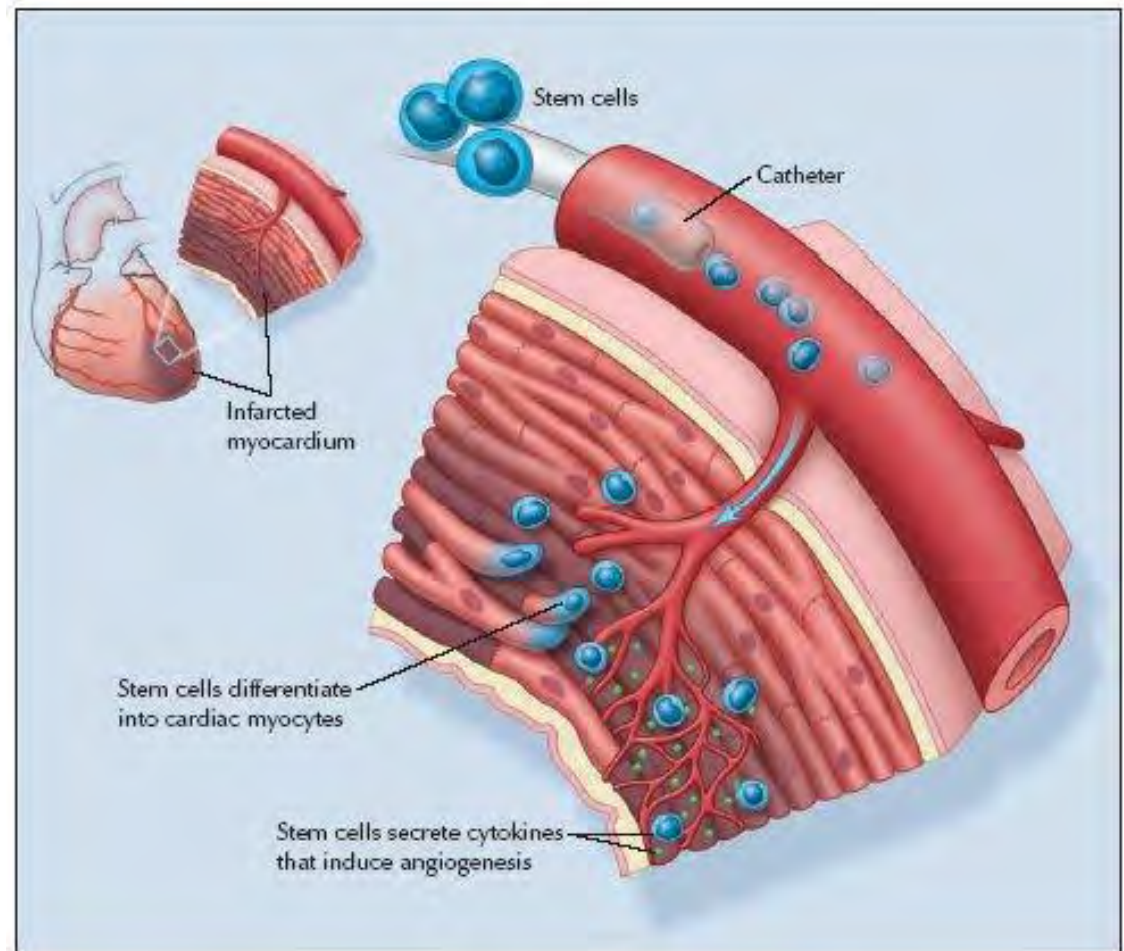


Myocyte regeneration

Neovascularization

Decreased apoptosis

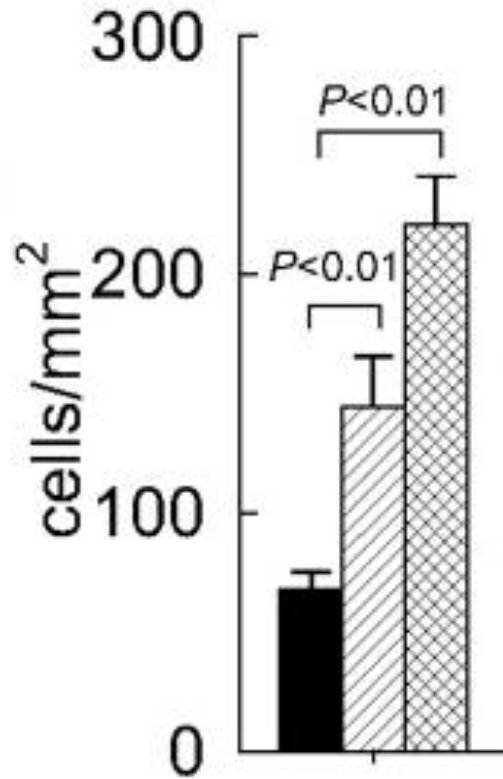
Decreased fibrosis



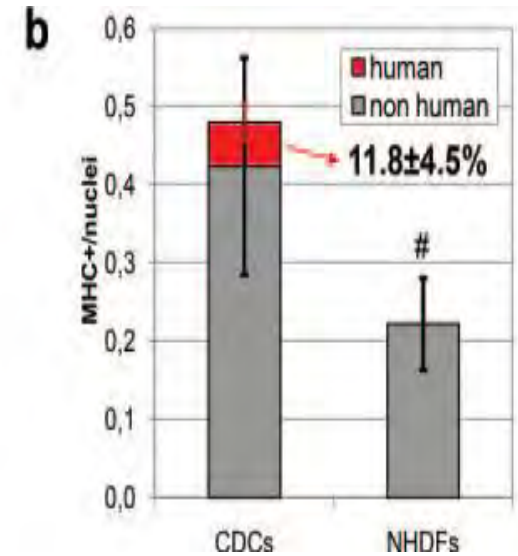
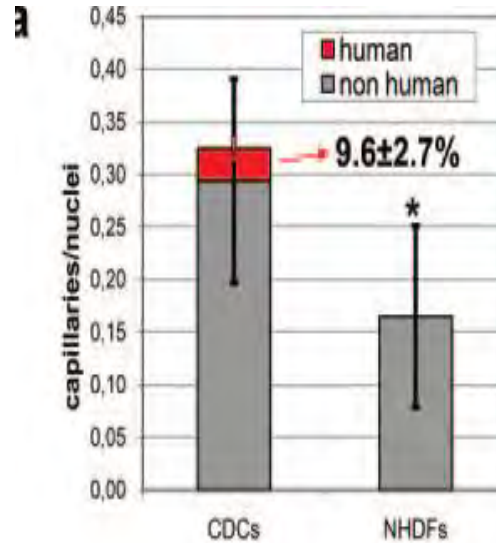
Endogenous activation by paracrine effects



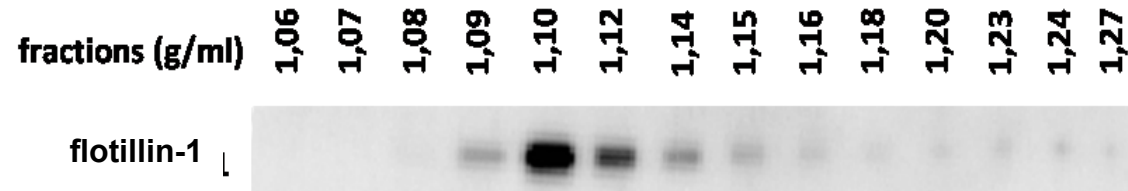
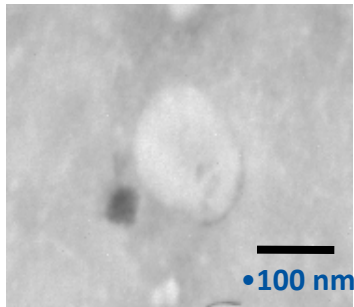
endogenous cardiac stem cells



- No cells injected
- ▨ Cells injected, no cells detected
- ▩ Cells injected, cells detected

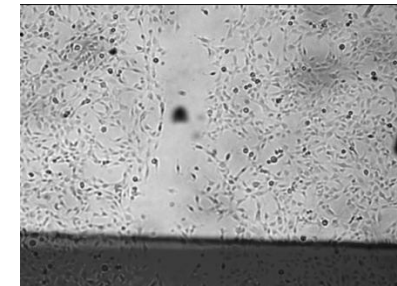
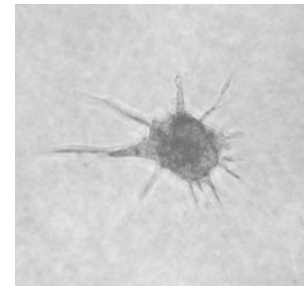
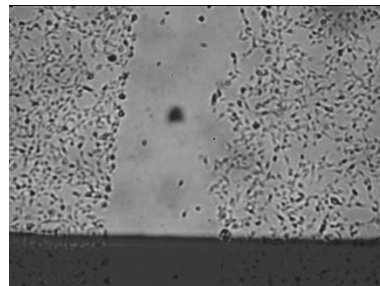
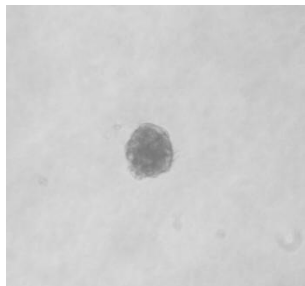


Paracrine effects - exosomal activation



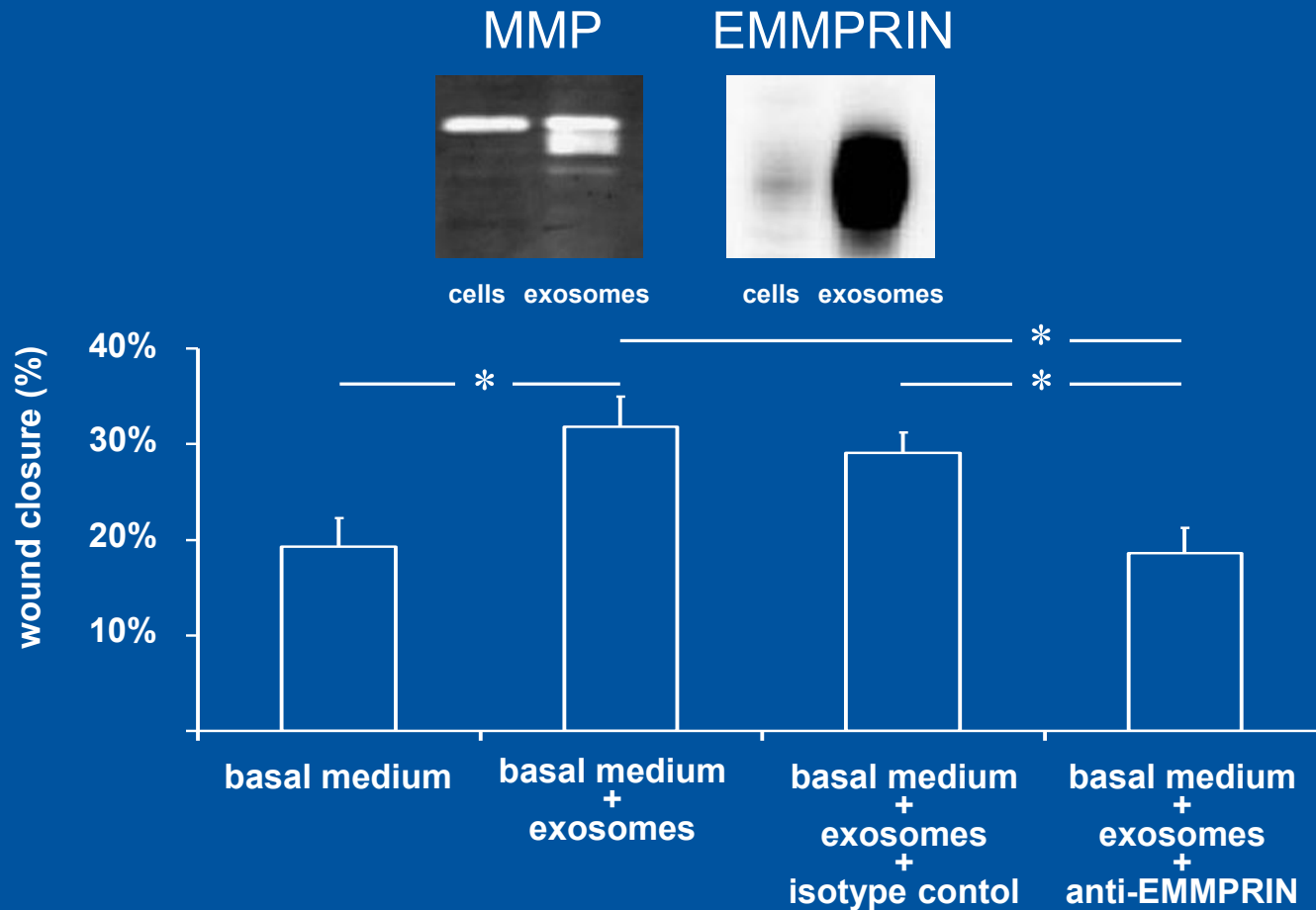
NO EXOSOMES

WITH EXOSOMES



Exosomal signaling - EMMPRIN

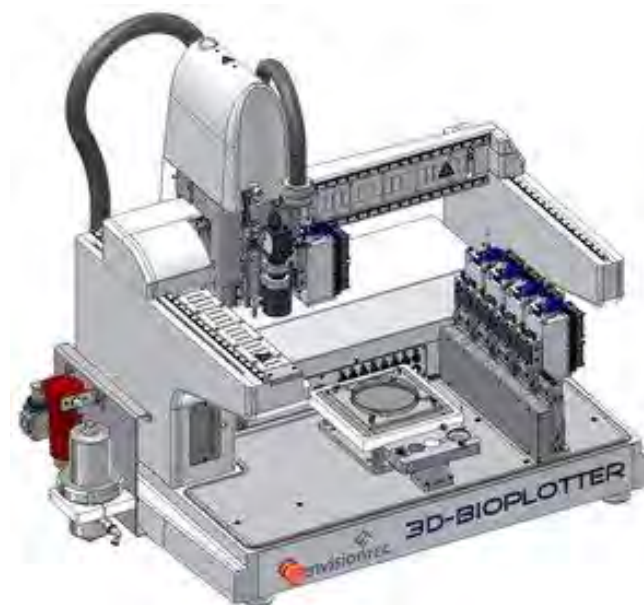
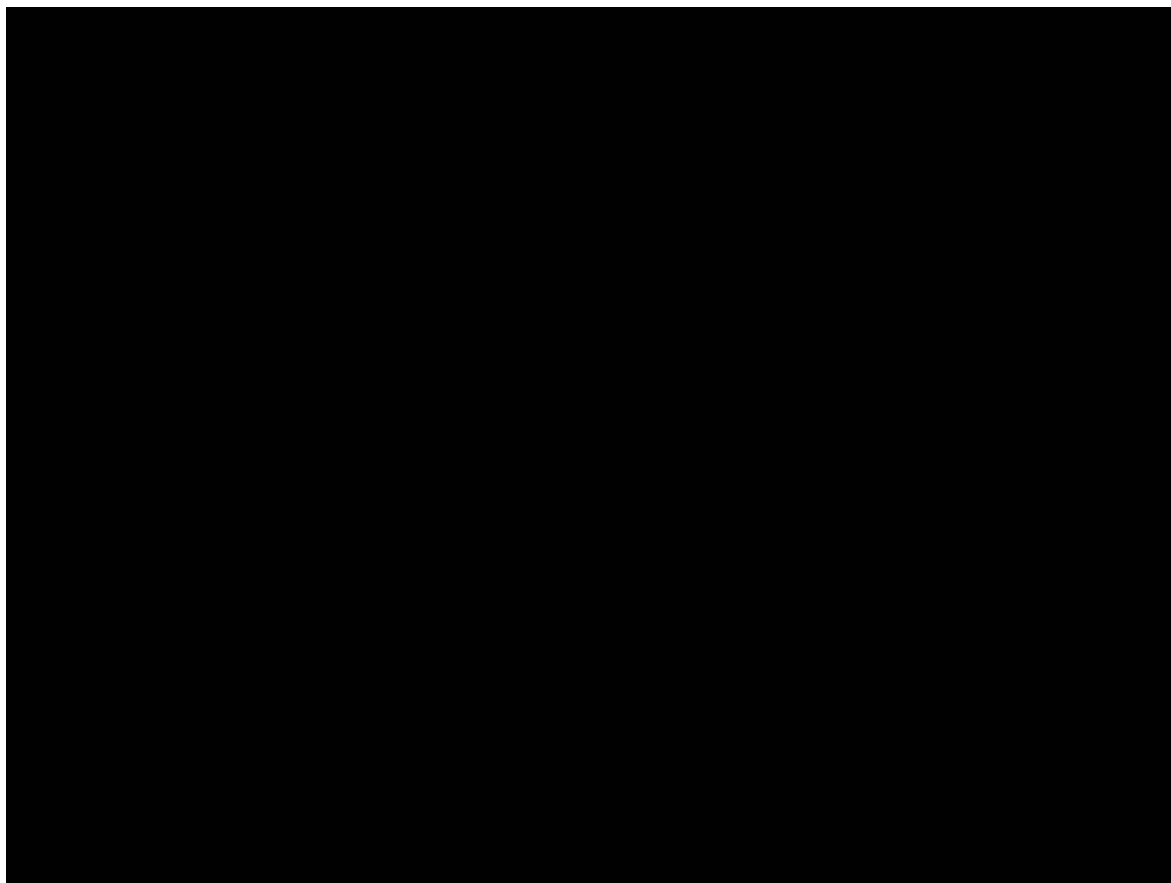
**CMPC& MSC exosomes
are enriched for MMP & EMMPRIN**



3D BioPlotter



University Medical Center
Utrecht



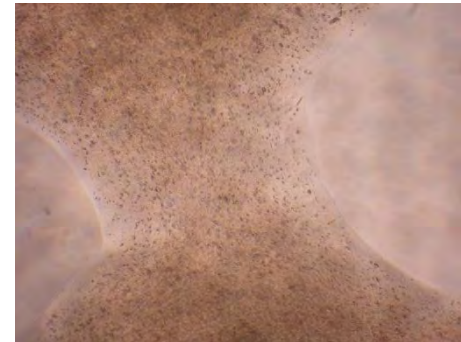
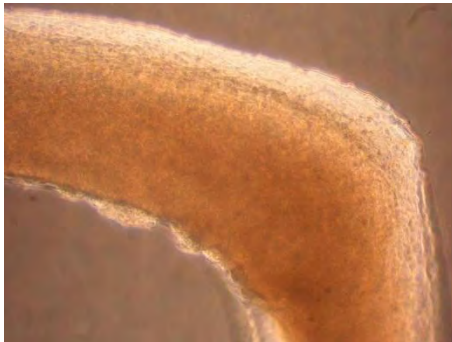
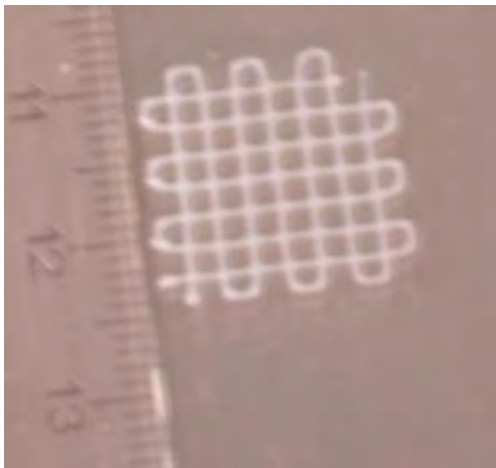
Gaetani submitted

Printed Alginate Matrix and cells

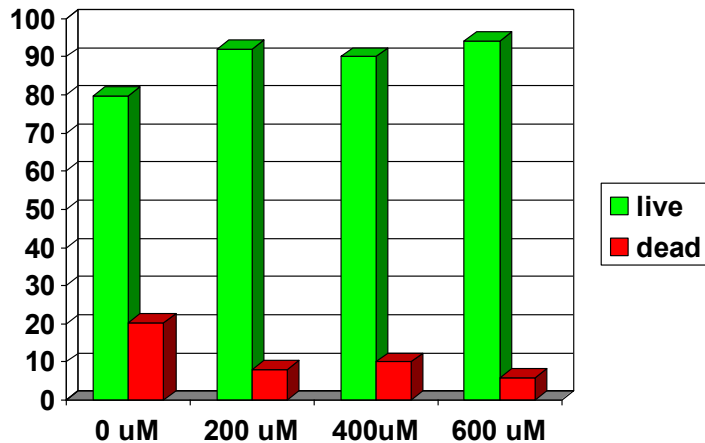
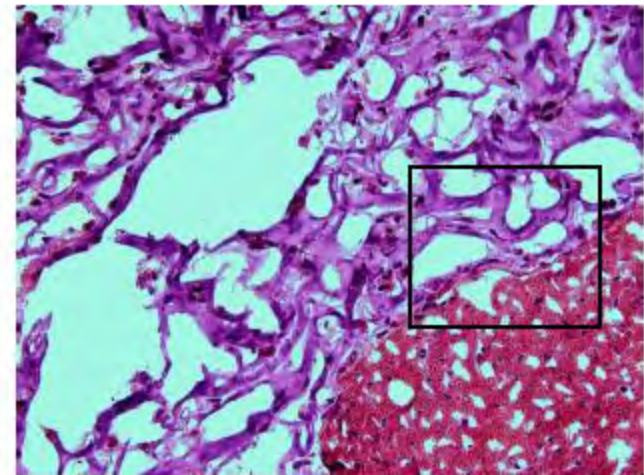
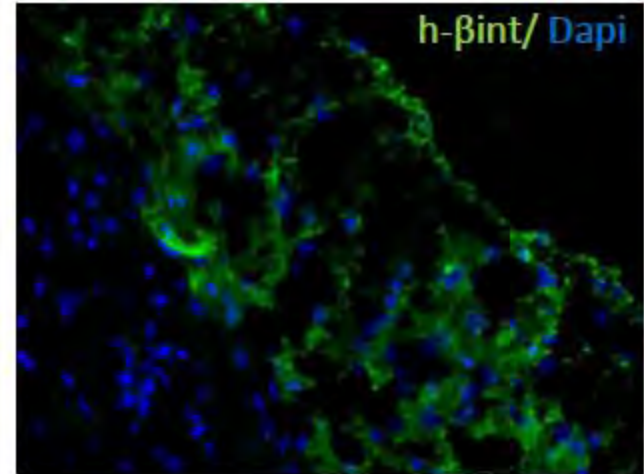
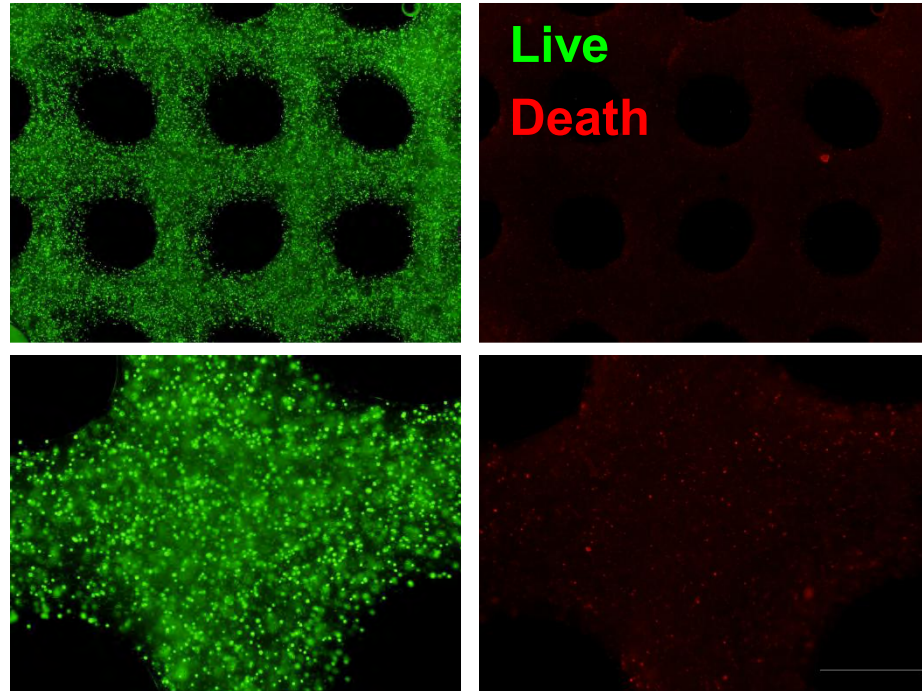


University Medical Center
Utrecht

15×10^6 cells/ml gel printed in 10% (w/v) alginate in medium



Viability



- Several cell types tested, but ESC and CSC most promising for cardiac regeneration
- Intra-myocardial injection most optimal delivery, but still very inefficient
- Tissue engineering: feasible but time consuming and expensive approach
- Cell number (2×10^9 loss, injected 1×10^7 - 10^8)
- Timing of injection, immediately/later

Several items need optimization for future steps into clinic.....



Acknowledgements



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