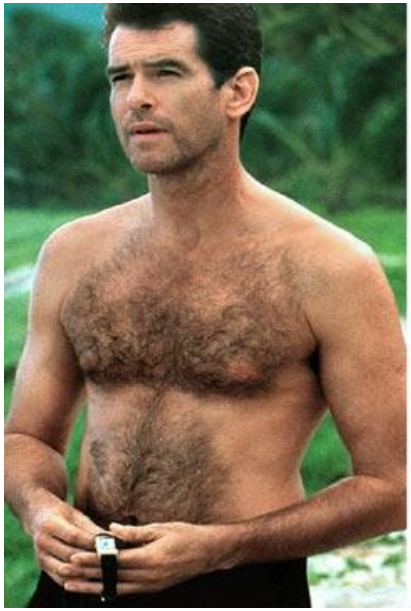
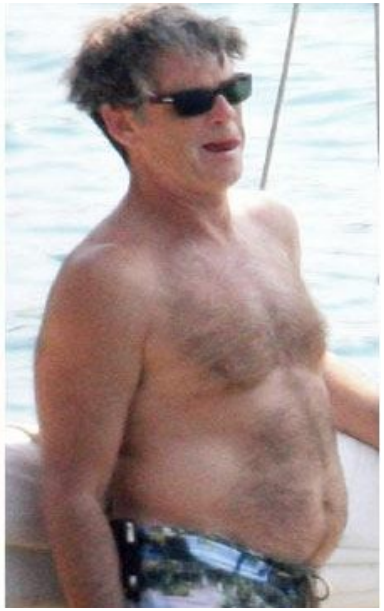
7. REGENERATIVE MEDICINE[1] AND CELL REPLACEMENT THERAPY[2]

[1] Therapy that enables the body to repair, replace, restore and regenerate damaged or diseased cells, tissues and organs.

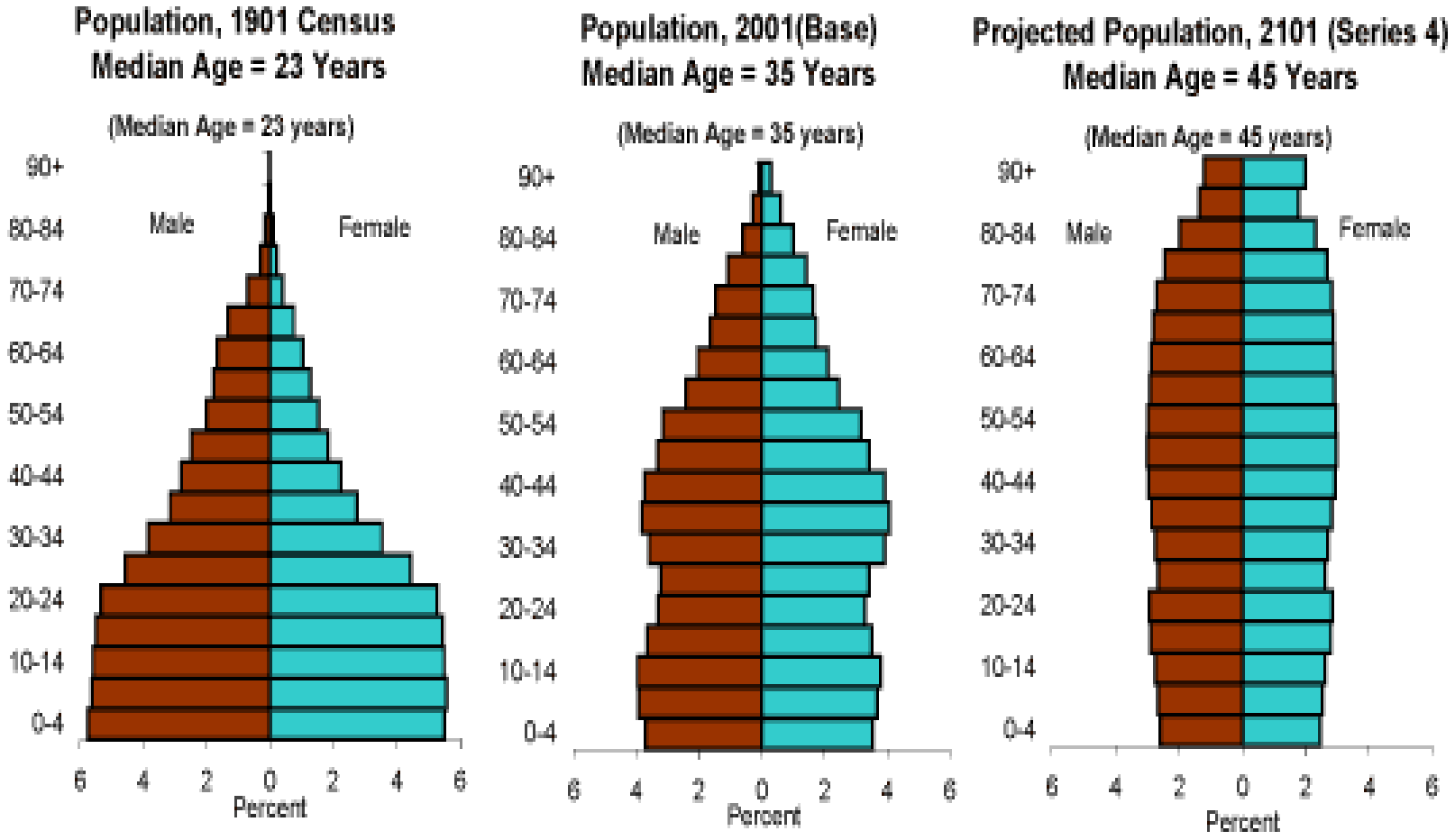
[2] The prevention, treatment, cure or mitigation of disease or injuries in humans by the administration of autologous, allogeneic or xenogeneic cells that have been manipulated or altered ex vivo.

Why do we need it?

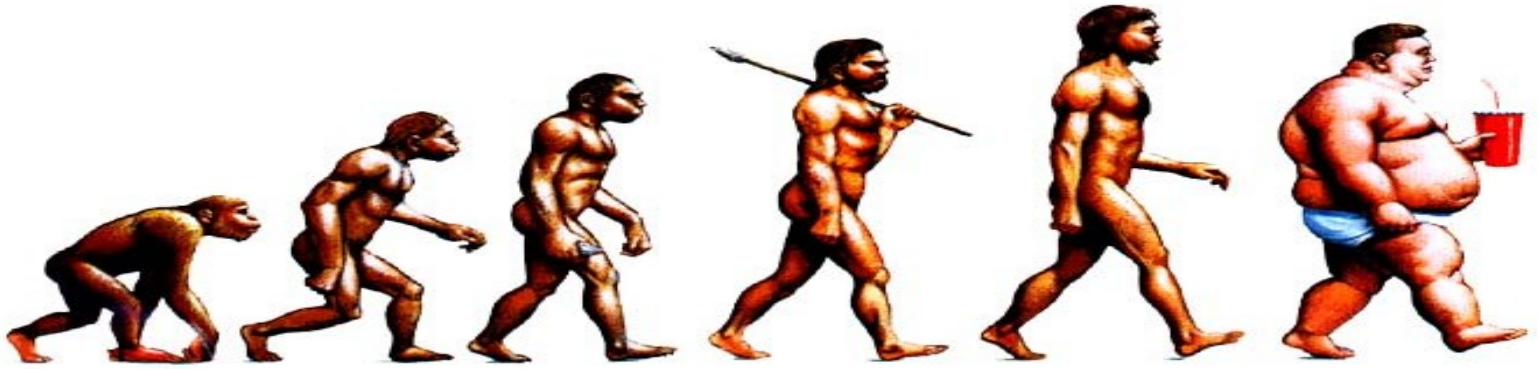


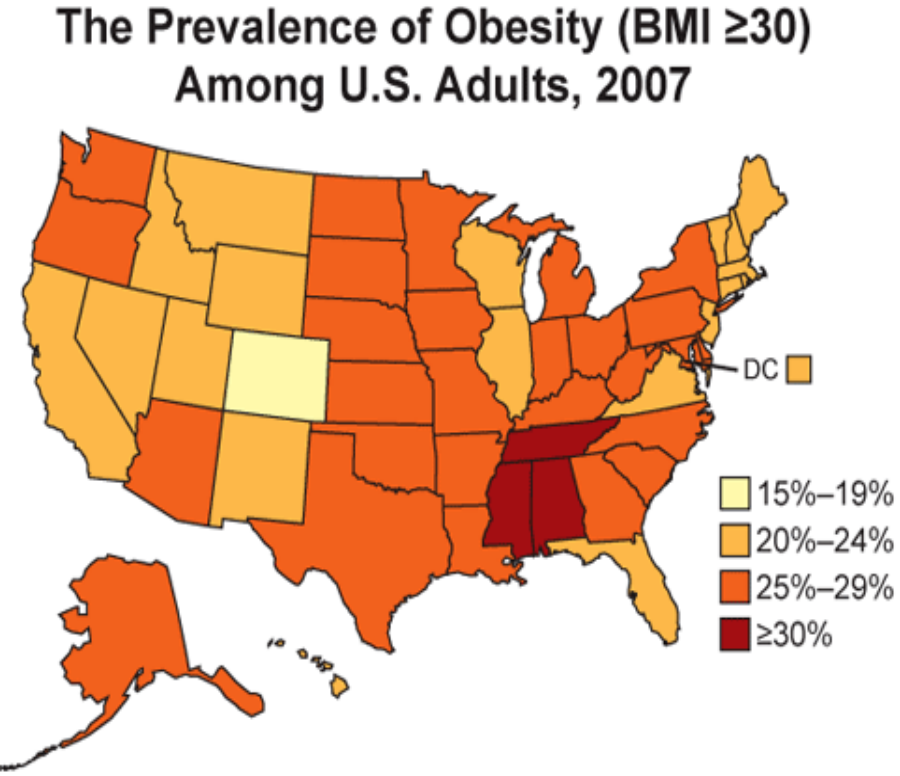


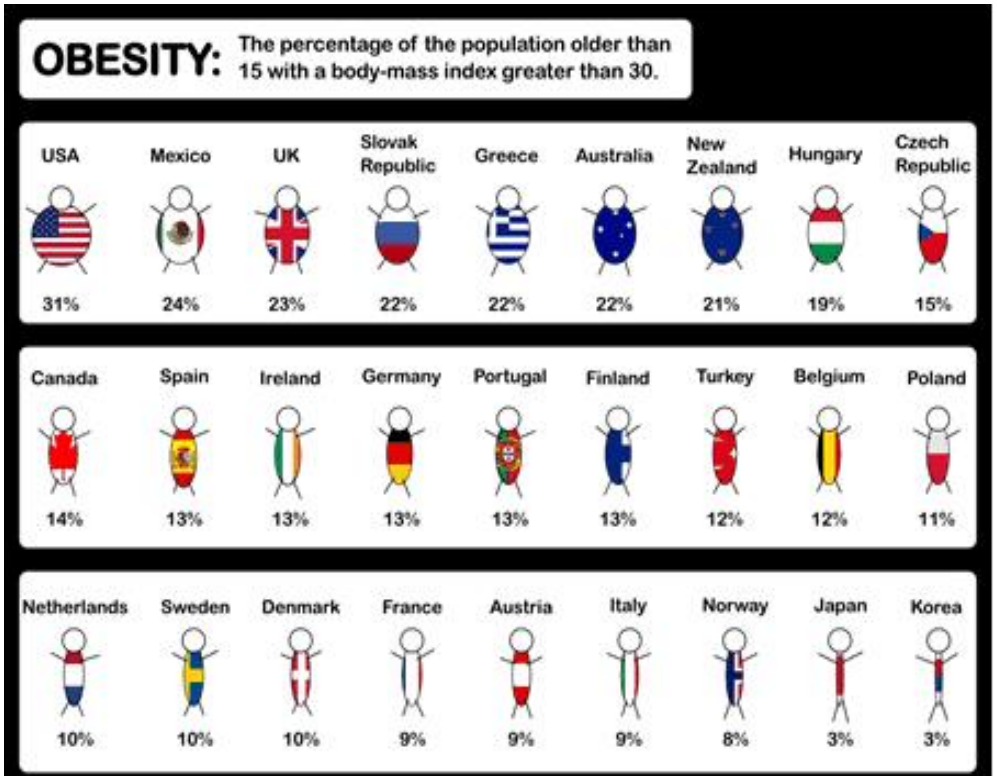
Aging population



Obesity




The Prevalence of Obesity (BMI ≥30)
Among U.S. Adults, 2007
DC
15%–19%
20%–24%
25%–29%
≥30%


OBESITY: The percentage of the population older than 15 with a body-mass index greater than 30.
USA 31%
Mexico 24%
UK 23%
Slovak Republic 22%
Greece 22%
Australia 22%
New Zealand 21%
Hungary 19%
Czech Republic 15%
Canada 14%
Spain 13%
Ireland 13%
Germany 13%
Portugal 13%
Finland 13%
Turkey 12%
Belgium 12%
Poland 11%
Netherlands 10%
Sweden 10%
Denmark 10%
France 9%
Austria 9%
Italy 9%
Norway 8%
Japan 3%
Korea 3%

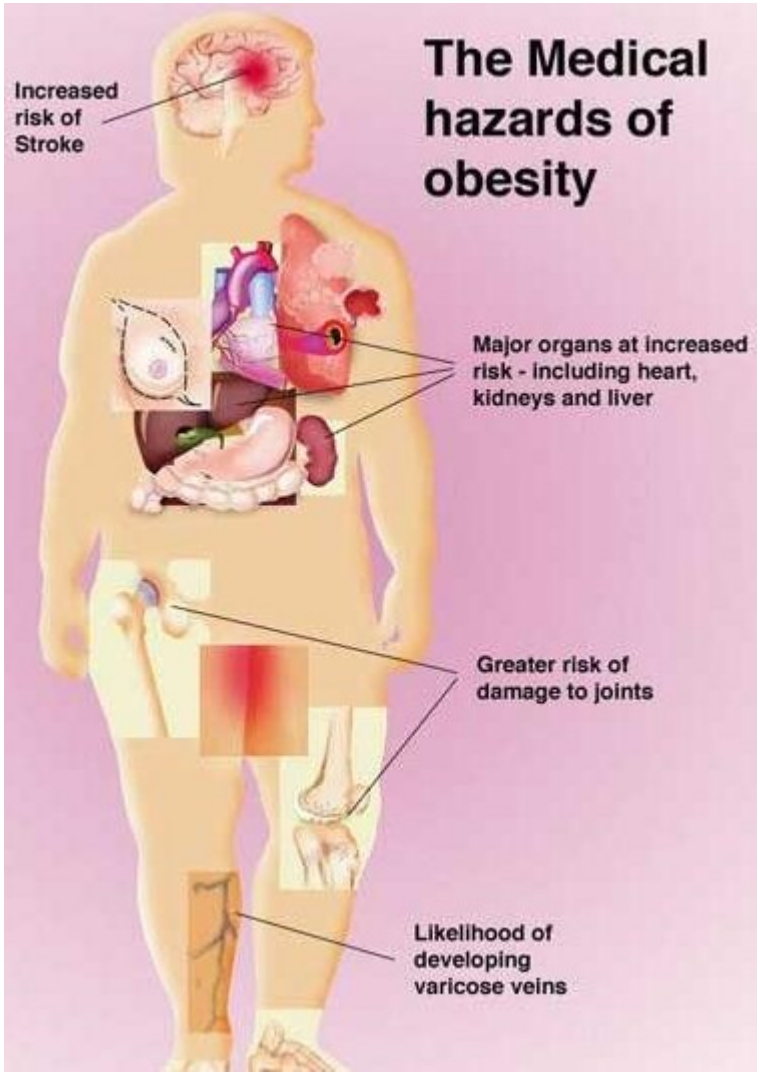
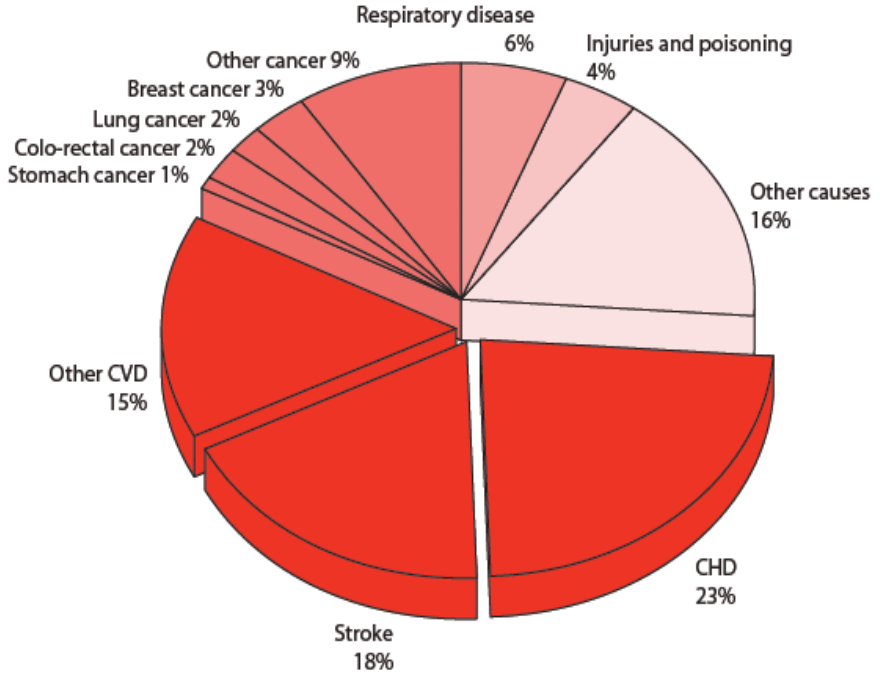


Figure 1.1b Deaths by cause, women, latest available year, Europe



The source#1: EMBRYONIC STEM CELLS

REPORTS

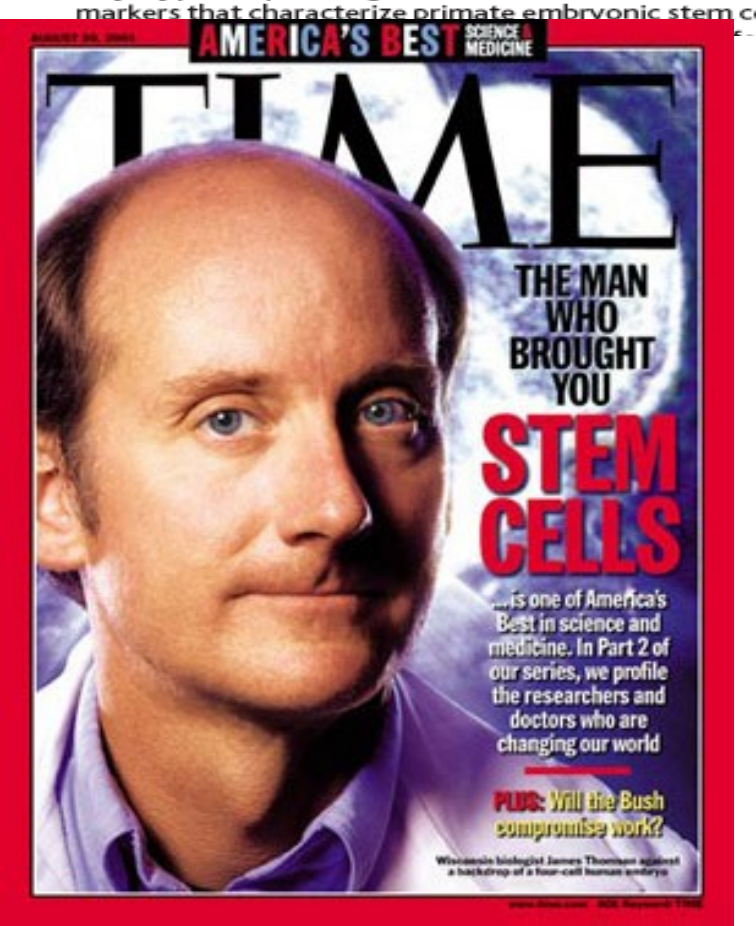
Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

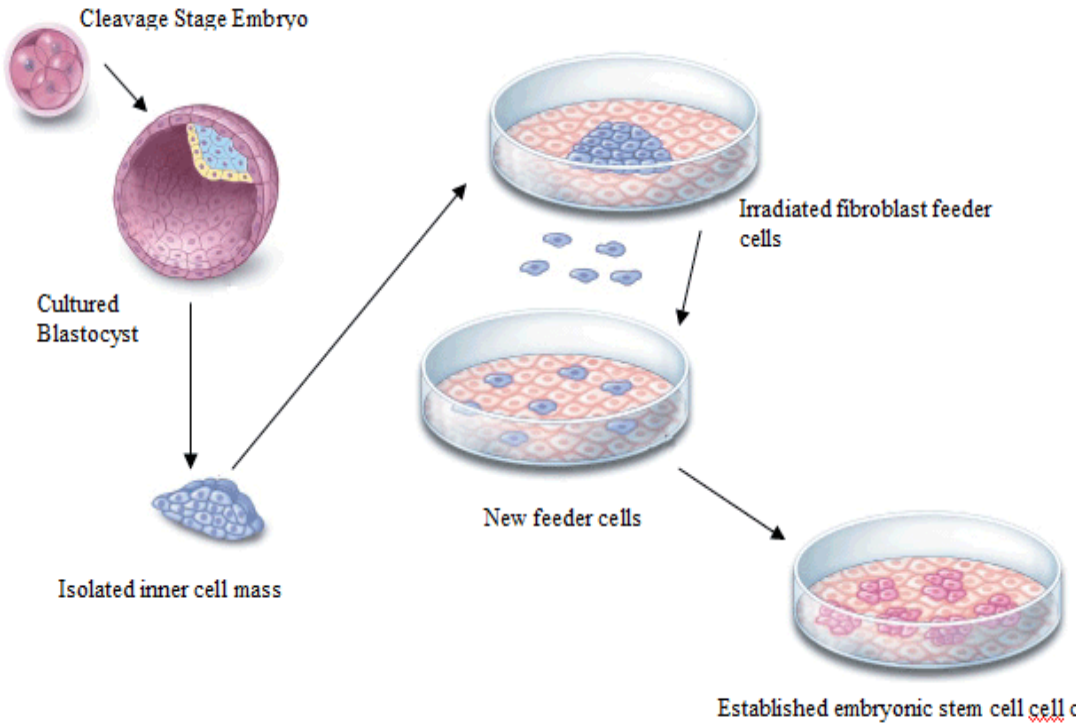
Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize

XX karyotype after 6 months of culture and has now been passaged continuously for more than 8 months (32 passages). A period of replicative crisis was not observed for any of the cell lines.

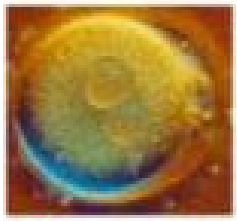
The human ES cell lines expressed high levels of telomerase activity (Fig. 2). Telomerase is a ribonucleoprotein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative life-span (*7*, *8*). Telomerase expression is highly correlated with immortality in human cell lines, and reintroduction of telomerase activity into some diploid human somatic cell



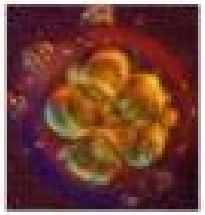
Science. 1998 Nov 6;282(5391):1145-7



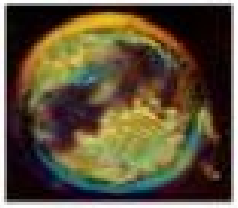
Human Developmental Continuum →



Single-cell Embryo



3-day Embryo

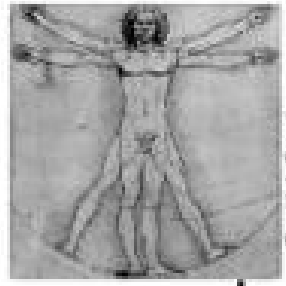


5-7 day Embryo

4-week Embryo

6-week Embryo

Infant



Adult

Embryonic Stem (ES) cells
Totipotent

Embryonic Germ (EG) cells
(primordial germ cells)
Pluripotent

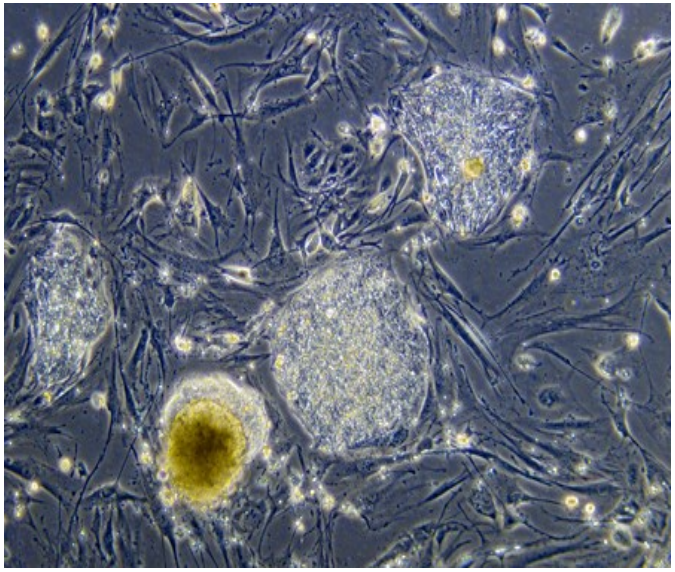
Fetal Tissue Stem cells
Pluripotent or Multipotent

Cord Blood Stem cells
Placental Stem cells
Pluripotent or Multipotent

"Adult" Stem cells
Pluripotent or Multipotent

Teratocarcinoma (germ cell tumor)

Embryonal Carcinoma (EC) cells
Pluripotent



The source#2: ADULT STEM CELLS

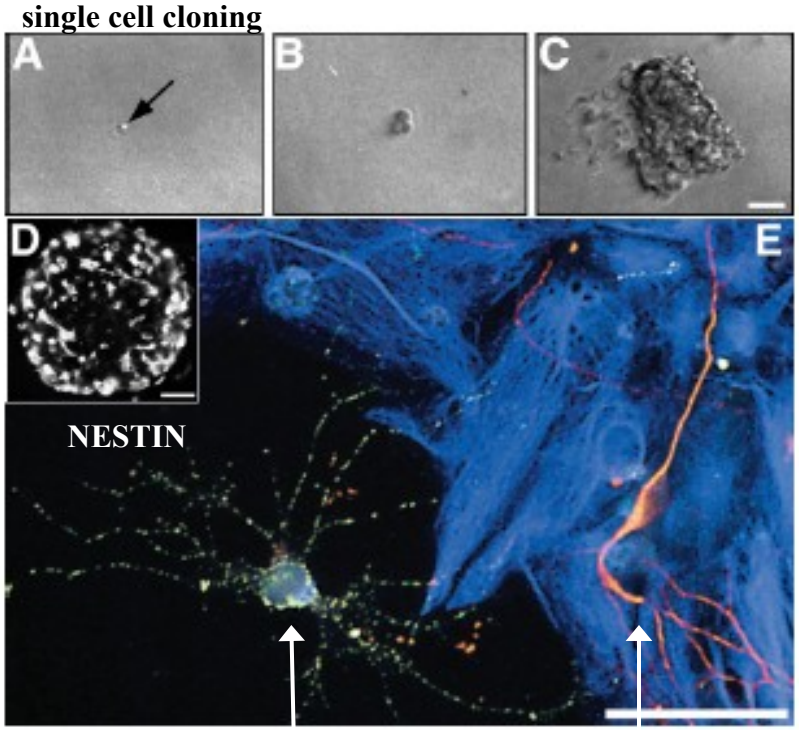
Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells in Vivo

Christopher R. R. Bjornson,*†‡ Rodney L. Rietze,*§
Brent A. Reynolds, M. Cristina Magli, Angelo L. Vescovi‡

Stem cells are found in various organs where they participate in tissue homeostasis by replacing differentiated cells lost to physiological turnover or injury. An investigation was performed to determine whether stem cells are restricted to produce specific cell types, namely, those from the tissue in which they reside. After transplantation into irradiated hosts, genetically labeled neural stem cells were found to produce a variety of blood cell types including myeloid and lymphoid cells as well as early hematopoietic cells. Thus, neural stem cells appear to have a wider differentiation potential than previously thought.

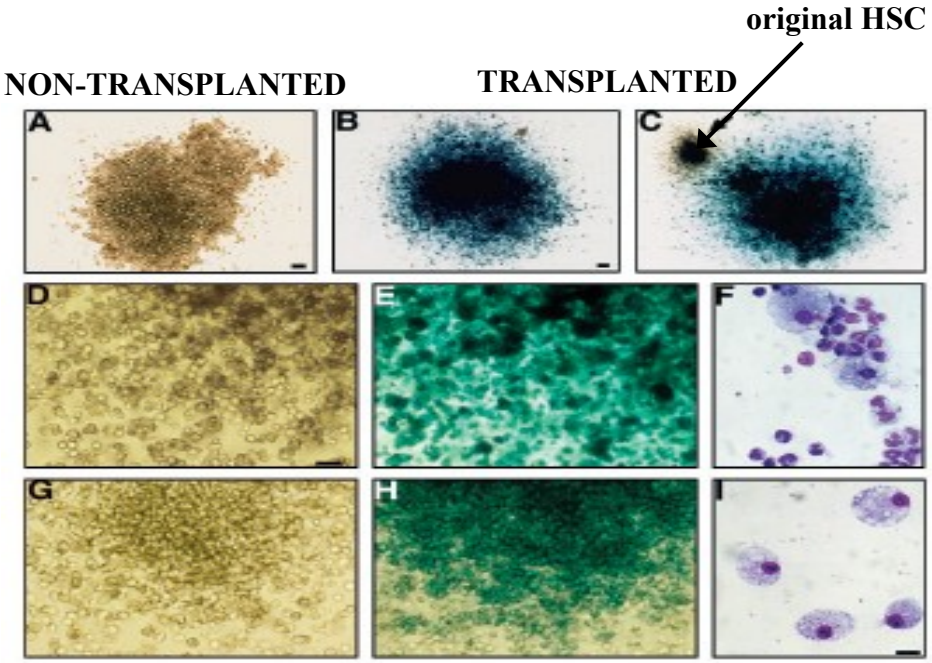
22 JANUARY 1999 VOL 283 SCIENCE www.sciencemag.org

Neural stem cell validation



differentiation

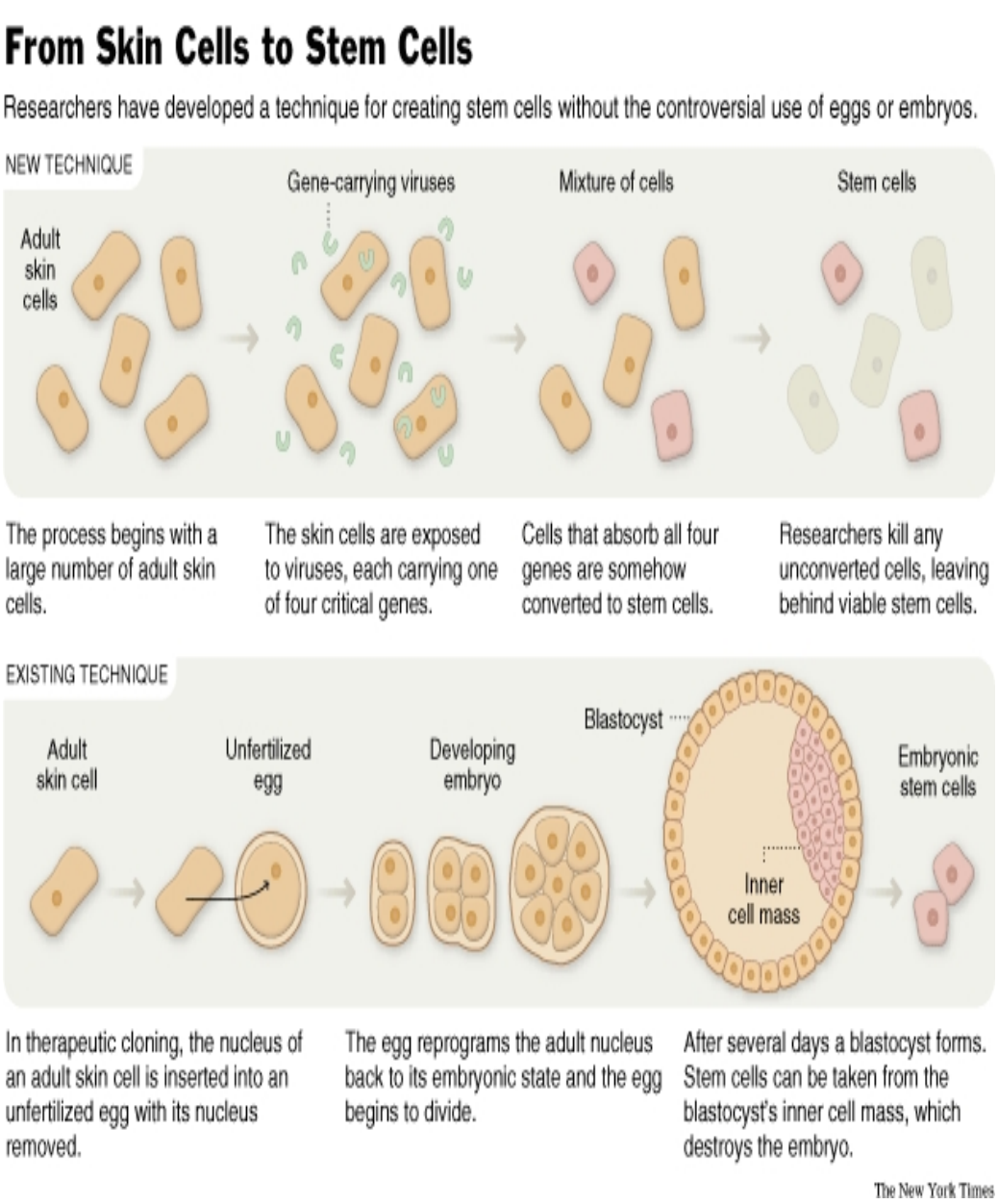
Hematopoietic stem cell validation in irradiated mice trasnplanted with β-gal-labeled NSC (in vitro BM clonogenic assay)

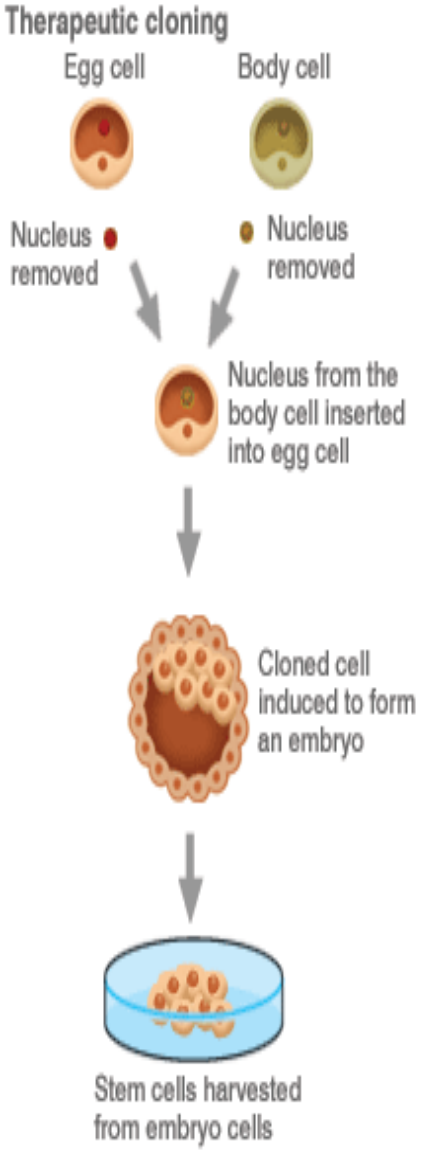


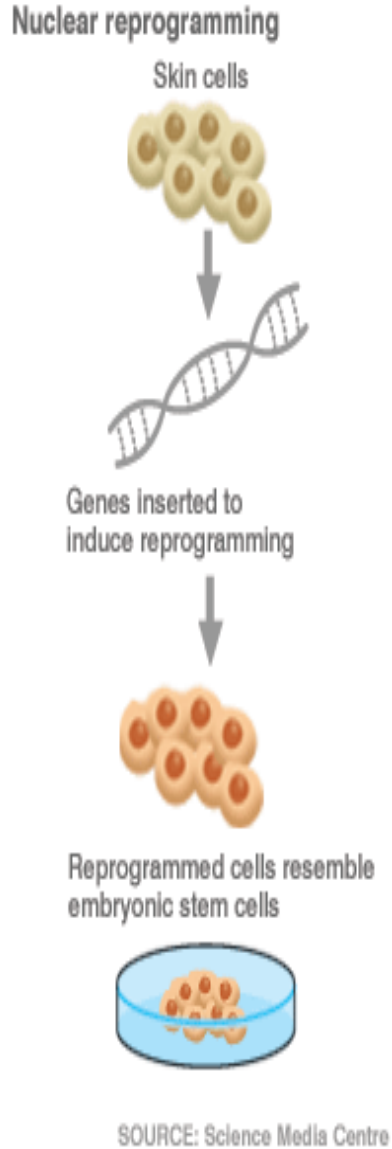
granulocyte/macrophage

macrophage

The source#3: INDUCIBLE PLURIPOTENT CELLS (iPS)







2006- INDUCIBLE PLURIPOTENT CELLS (iPS)

Cell

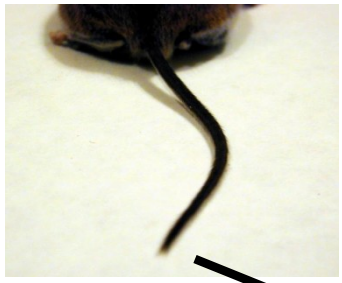
Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

SUMMARY

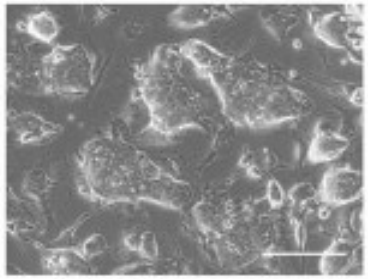
Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors

or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.



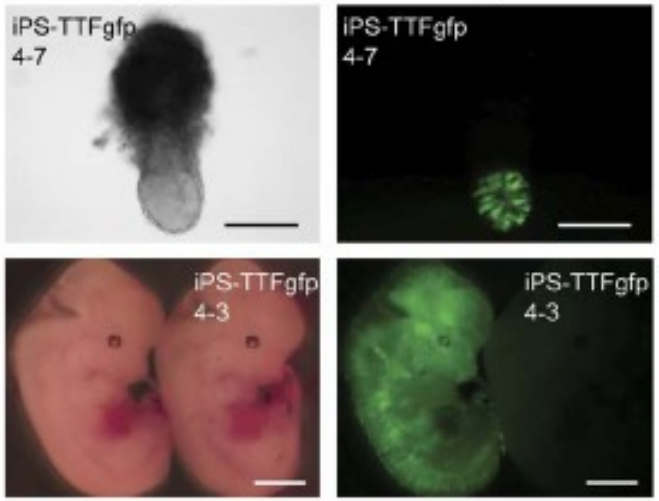
Oct3/4
Sox2
c-Myc
Klf4

A

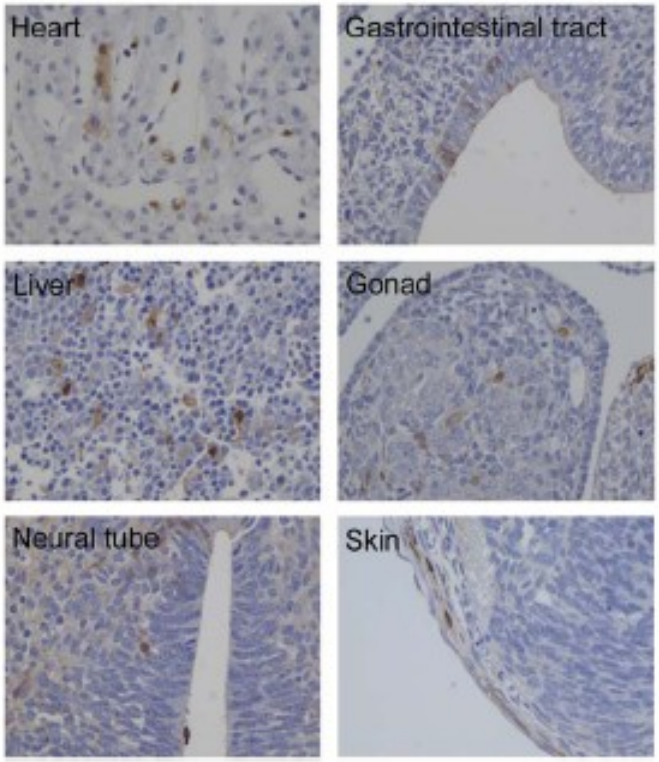


B

C



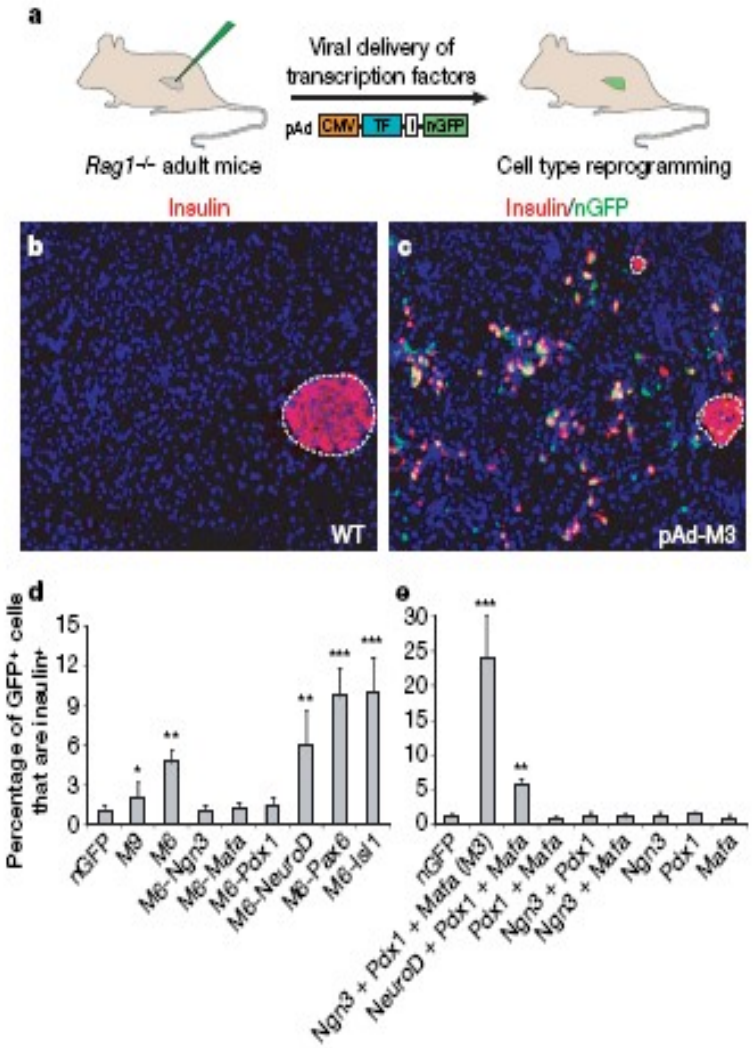
D



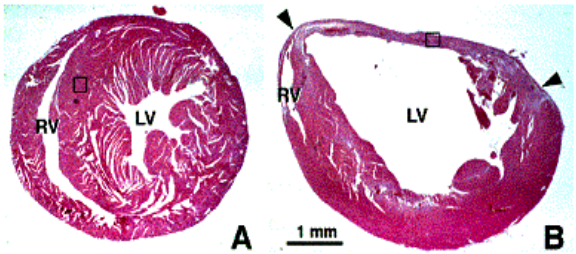
ARTICLES

In vivo reprogramming of adult pancreatic exocrine cells to β-cells

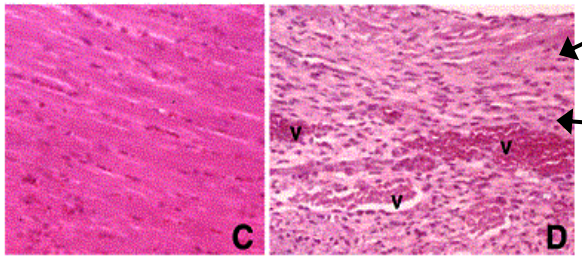
Qiao Zhou[1], Juliana Brown[2], Andrew Kanarek[1], Jayaraj Rajagopal[1] & Douglas A. Melton[1]



HOW TO REPAIR BROKEN HEART?



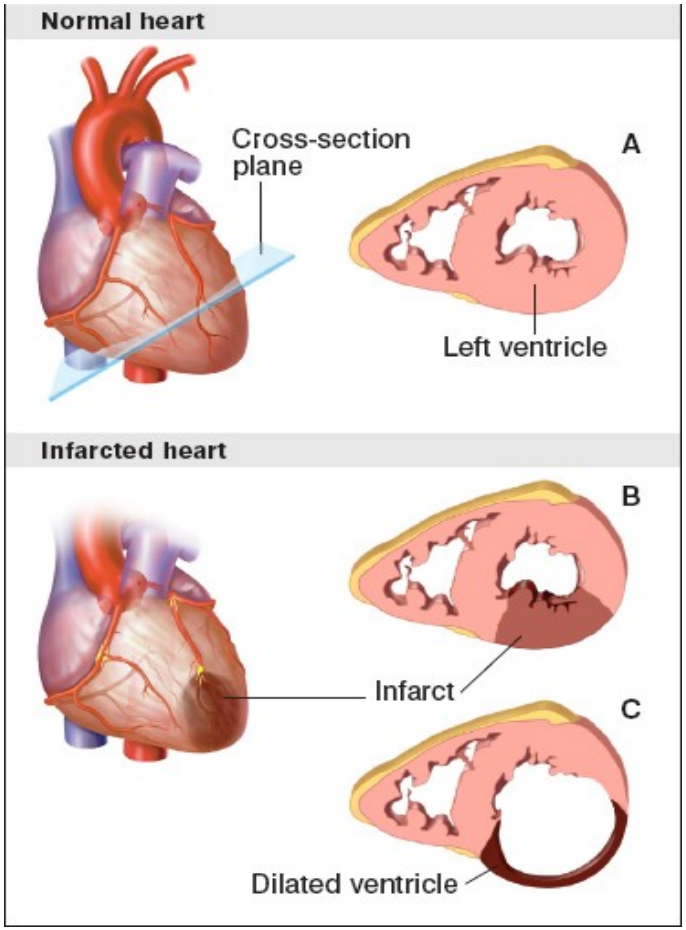
→ infarcted area

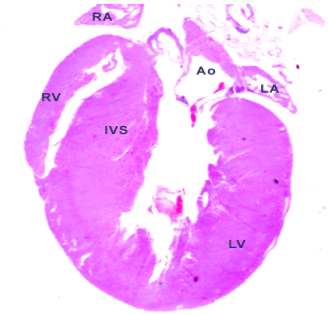


necrotic muscle (red)

extensive fibrosis (pink)

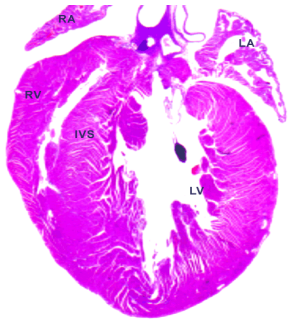
v - blood vessel





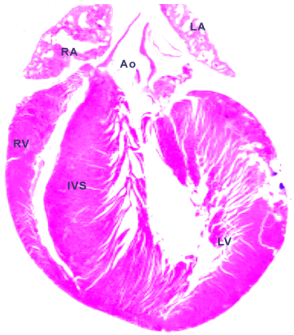
Sham Heart

Ao, Aortic artery; IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle



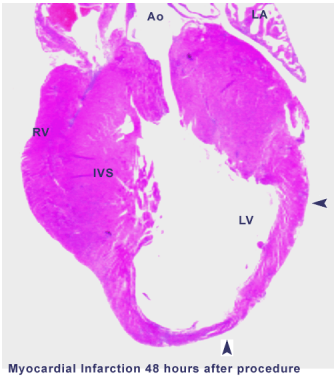
Myocardial Infarction 4 hours after procedure

IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle



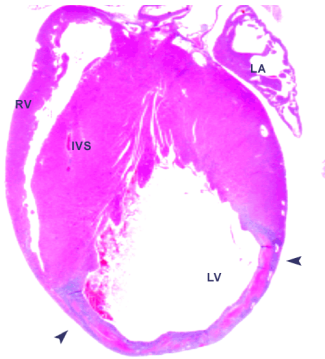
Myocardial Infarction 24 hours after procedure

Ao, Aortic artery; IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle



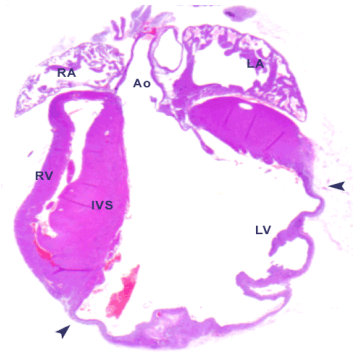
Myocardial Infarction 48 hours after procedure

Ao, Aortic artery; IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle



Myocardial Infarction 1 week after procedure

IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle

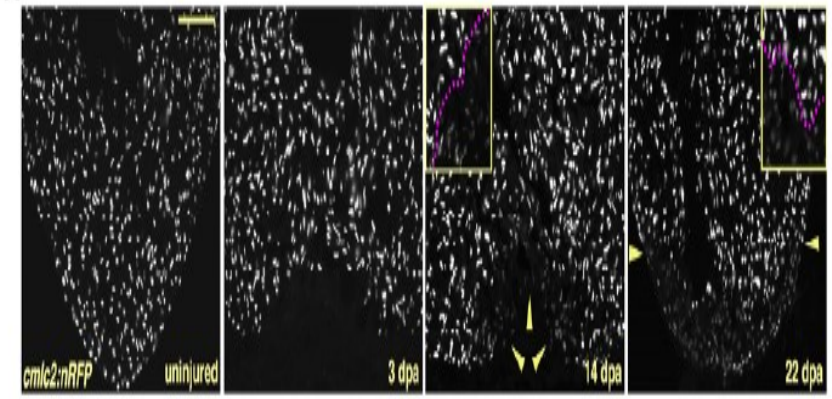


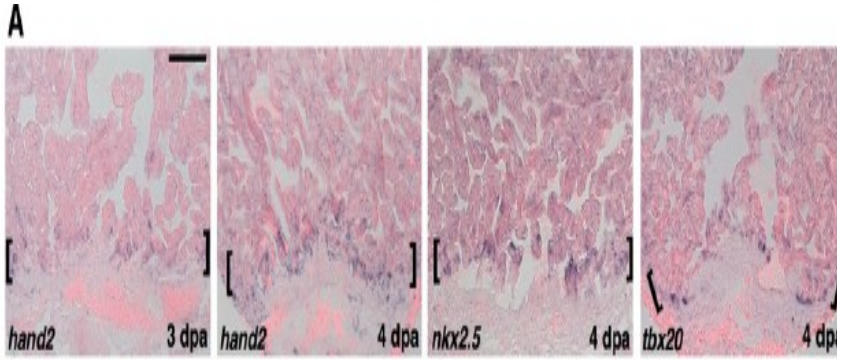
Myocardial Infarction 8 weeks after procedure

Ao, Aortic artery; IVS, Interventricular septum;LA, Left atrium; LR Left ventricle; RA, Right atrium; RL, Right ventricle

Fish heart regenerates from undifferentiated (de-differentiated?) progenitor cells

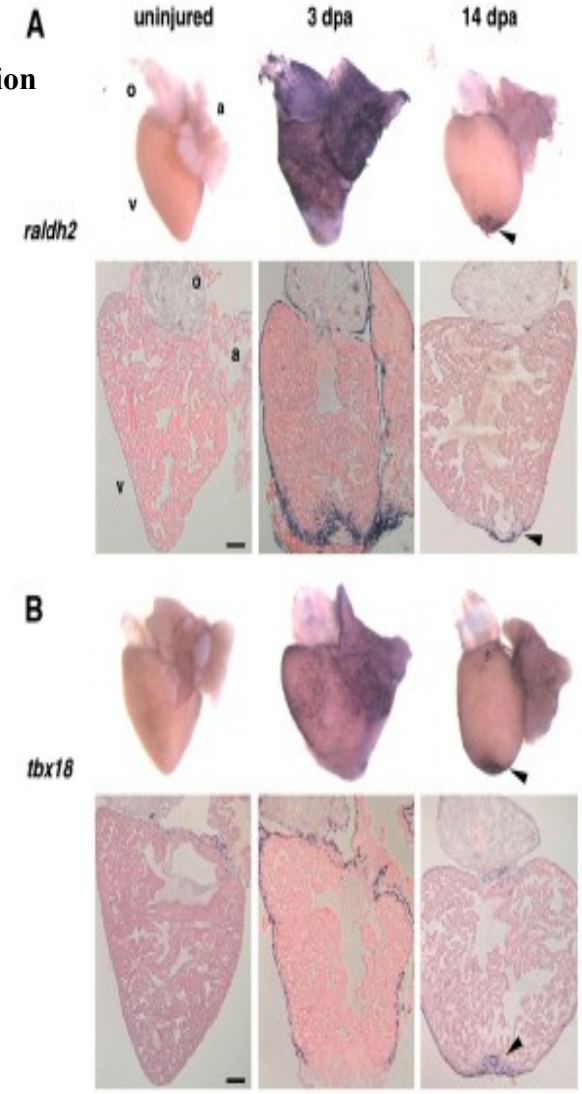
nuclear-dsRed reporter – differentiated high expression, differentiating – low expression. dpa - days after amputation

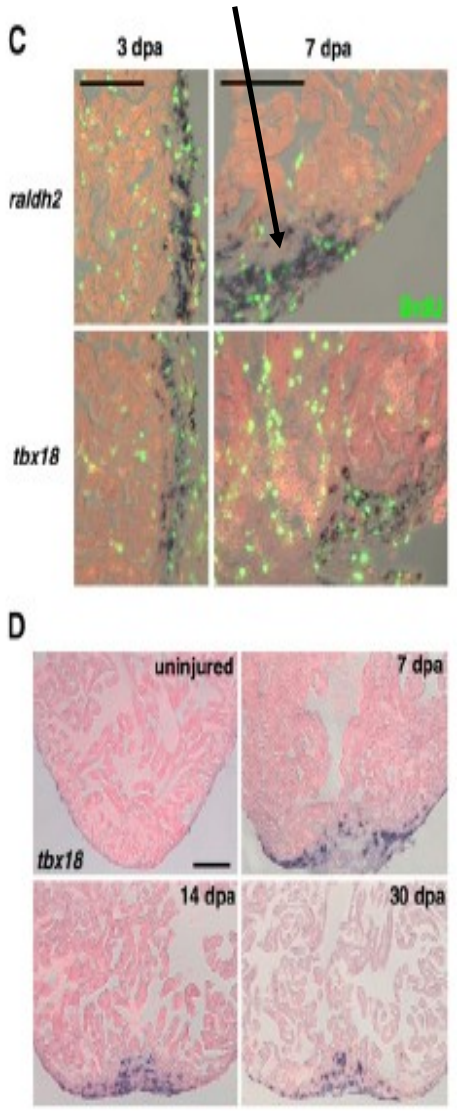




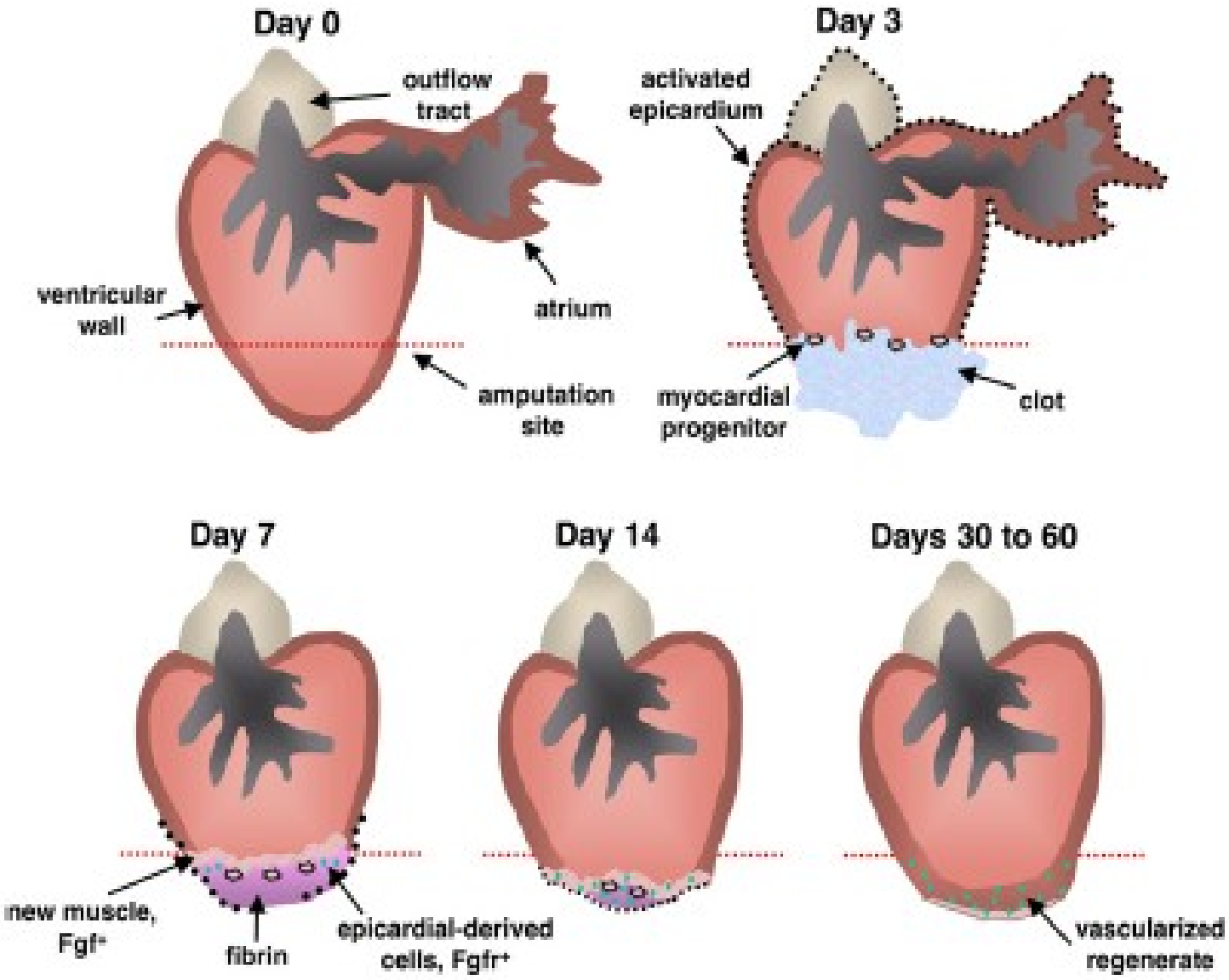
pre-cardiac markers

epicardial invasion via EMT

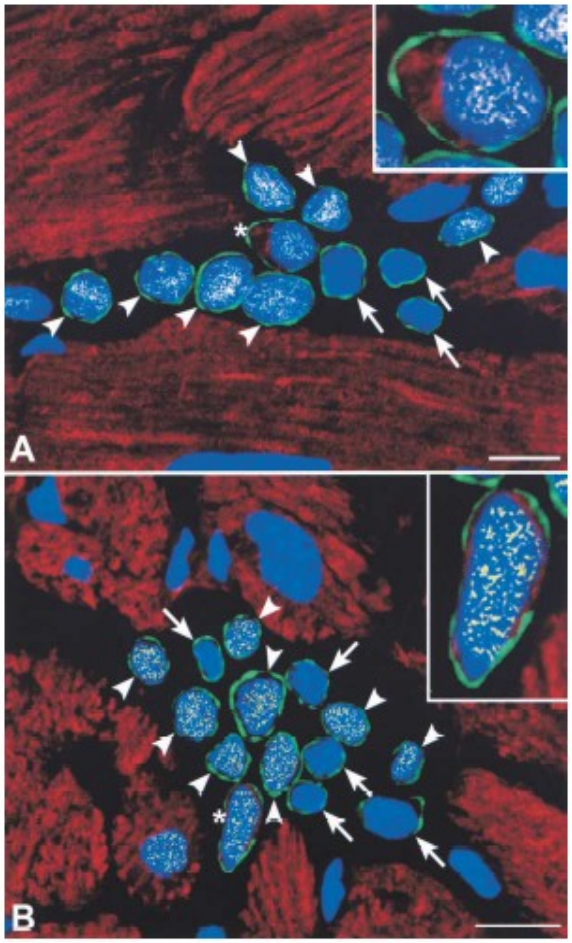


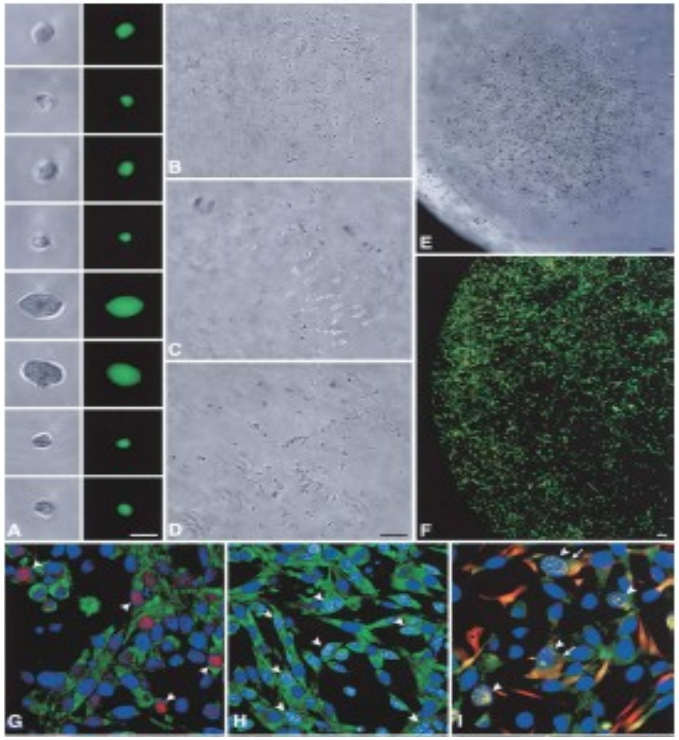


epicardial tissue (*tbx18* and *raldh2* – markers of embryonic epicardium)


Day 0
outflow tract
ventricular wall
atrium
amputation site
Day 3
activated epicardium
myocardial progenitor
clot
Day 7
new muscle, Fgf+
fibrin
epicardial-derived cells, Fgfr+
Day 14
Days 30 to 60
vascularized regenerate

Adult rat heart contains resident cardiac stem cells that can be isolated and expanded *in vitro*……

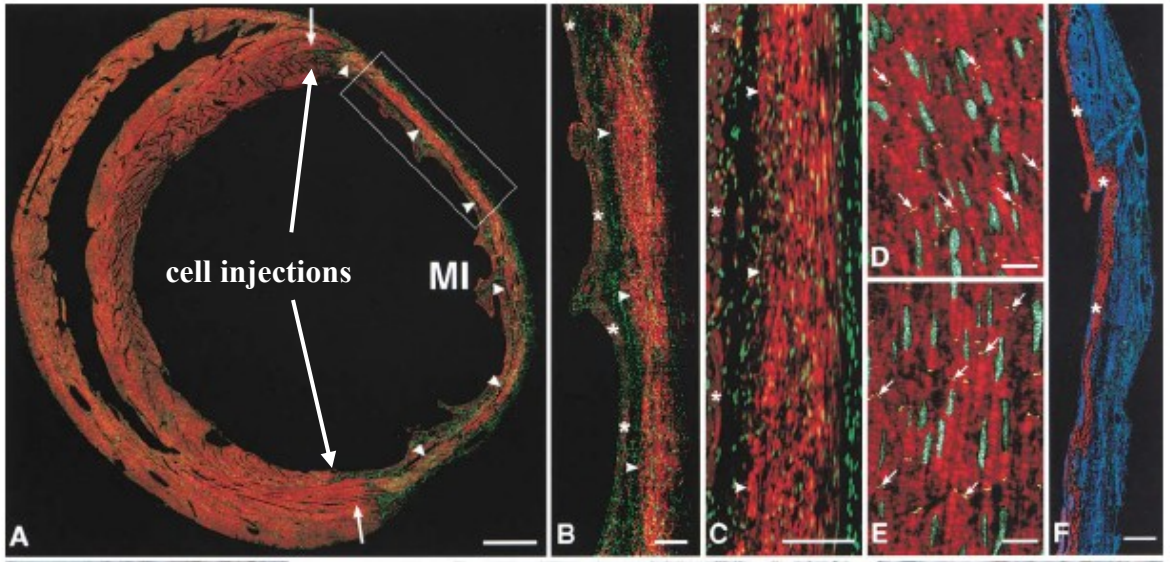




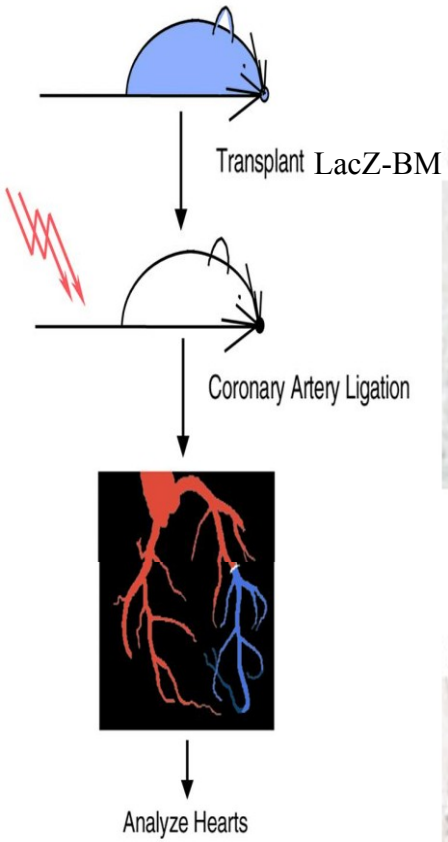
Lin (none) – blood lineage
c-kit+ (green) - stem cells
Nkx2.5 (white) – early cardiac
sarcomeric actin (red)- cardiac
MEF2C (yellow dots)- early cardiac
GATA4 (magenta) – early cardiac
cardiac myosin (orange)

…and used to repair infarcted heart

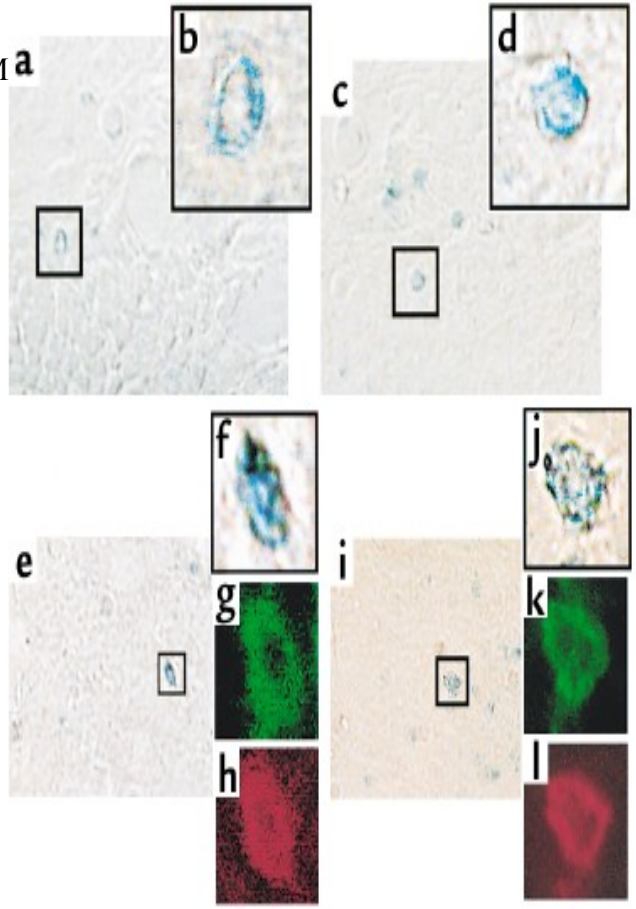
red – cardiac myosin
green -PI
yellow (D) – connexin 43
yellow (E)- N-cadherin
(F) - non-treated tissue (blue – collagen)

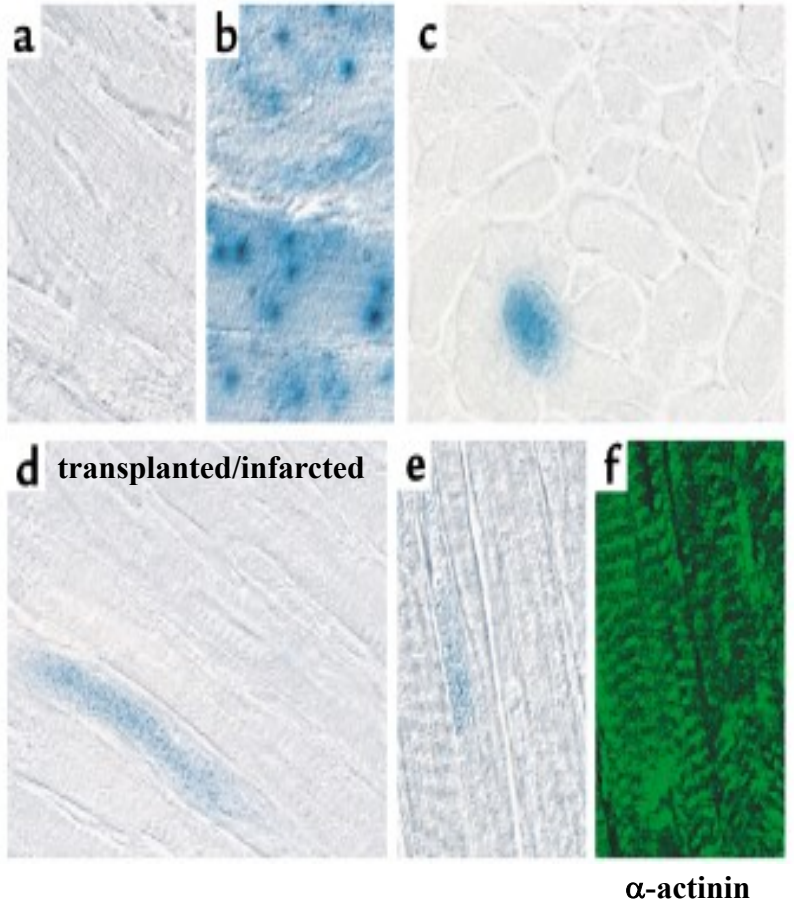


Hematopoietic stem cells can regenerate endothelium and cardiac muscle in ischemic heart



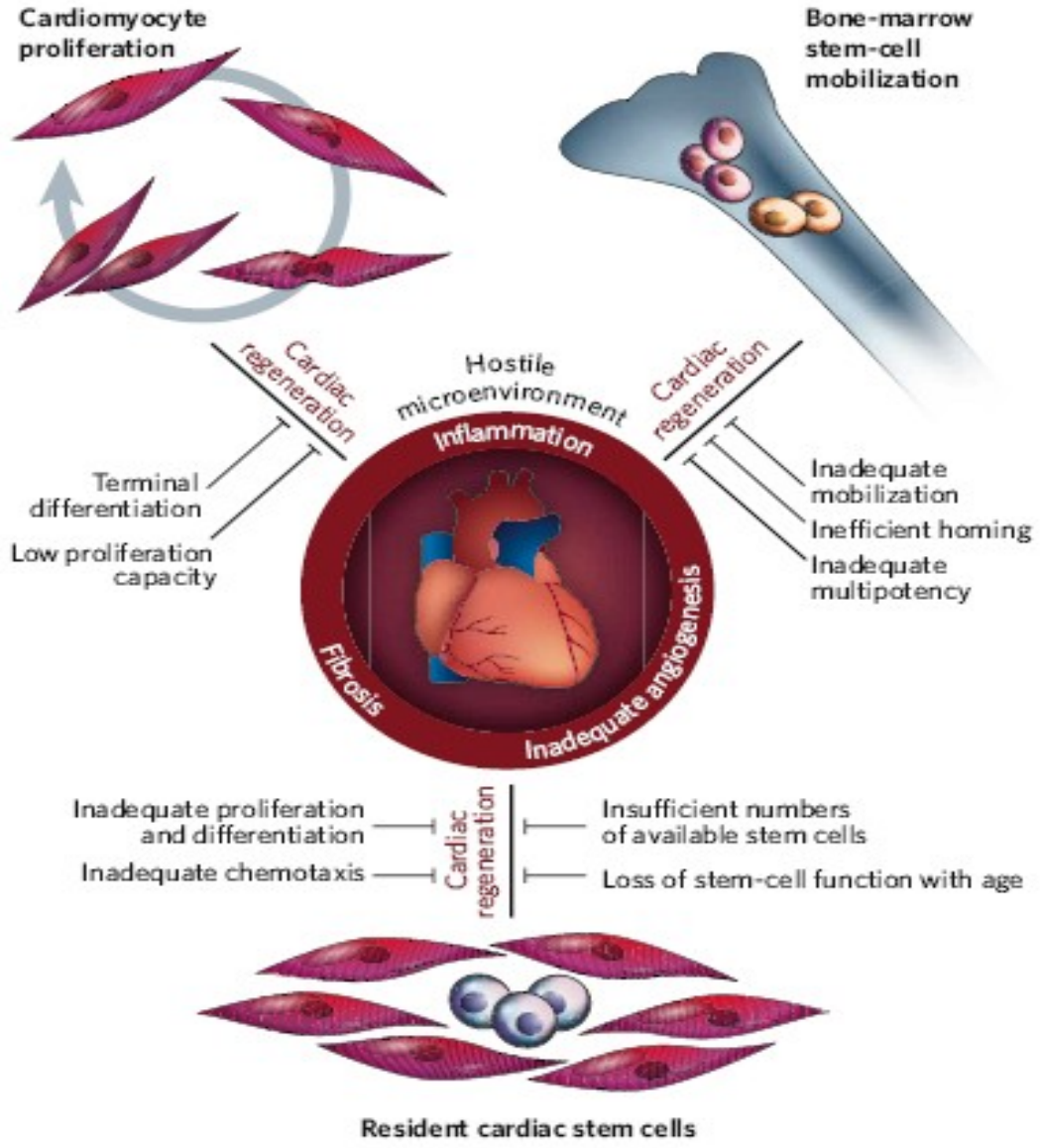
Flt-1 (red)
ICAM-1 (green)
LacZ (blue)





Mammalian heart can regenerate! (at least during its physiological renewal)

But does it happen in disease?



SKELETAL MYOBLASTS

- remain committed to skeletal muscle fate
- do not form gap junctions to couple with host myocardium, do not beat in synchrony with the rest of the heart

ADULT HEMATOPOIETIC STEM CELLS

+ differentiate well into endothelial and smooth muscle compartments of the heart veins
- differentiate poor into the myocardium
- fuse with myocardial cells

ENDOTHELIAL PROGENITORS

+ excellent in infarct revascularisation
- poor contribution to myocardium

MESENCHYMAL STEM CELLS (BM-derived)

- do not fully transdifferentiate to myocardium
- do not form connections or contract

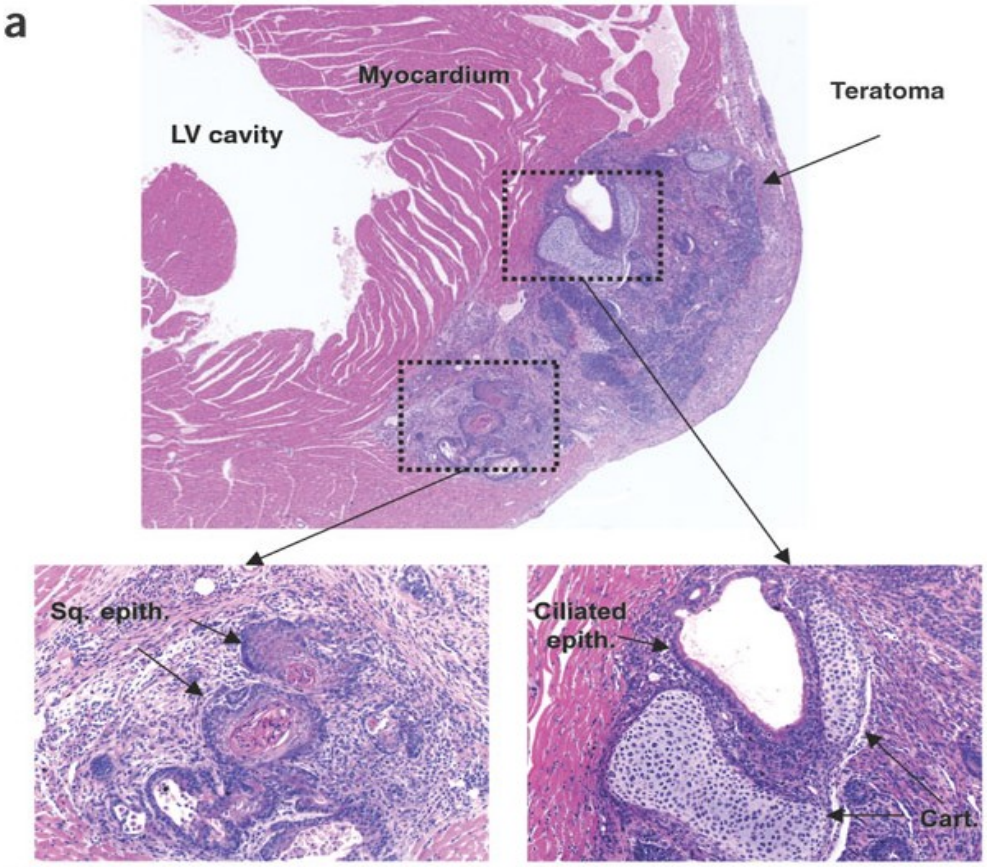
RESIDENT MYOCARDIAL PROGENITORS

+ differentiate into cardiomyocytes (partially), smooth muscle cells and endothelia
- fuse with cardiomyocytes

MOVIE – hESC-derived cardiomyocytes in gelatin cell culture (Histone 2BeGFP)

HUMAN EMBRYONIC STEM CELLS

+ excellent cardiac potential, full functional differentiation into cardiomyocytes
+ specific differentiation into ventricular, atrial and nodal/pacemaker cells possible
- inefficient cardiogenesis
- stem cells often carried-over in transplant



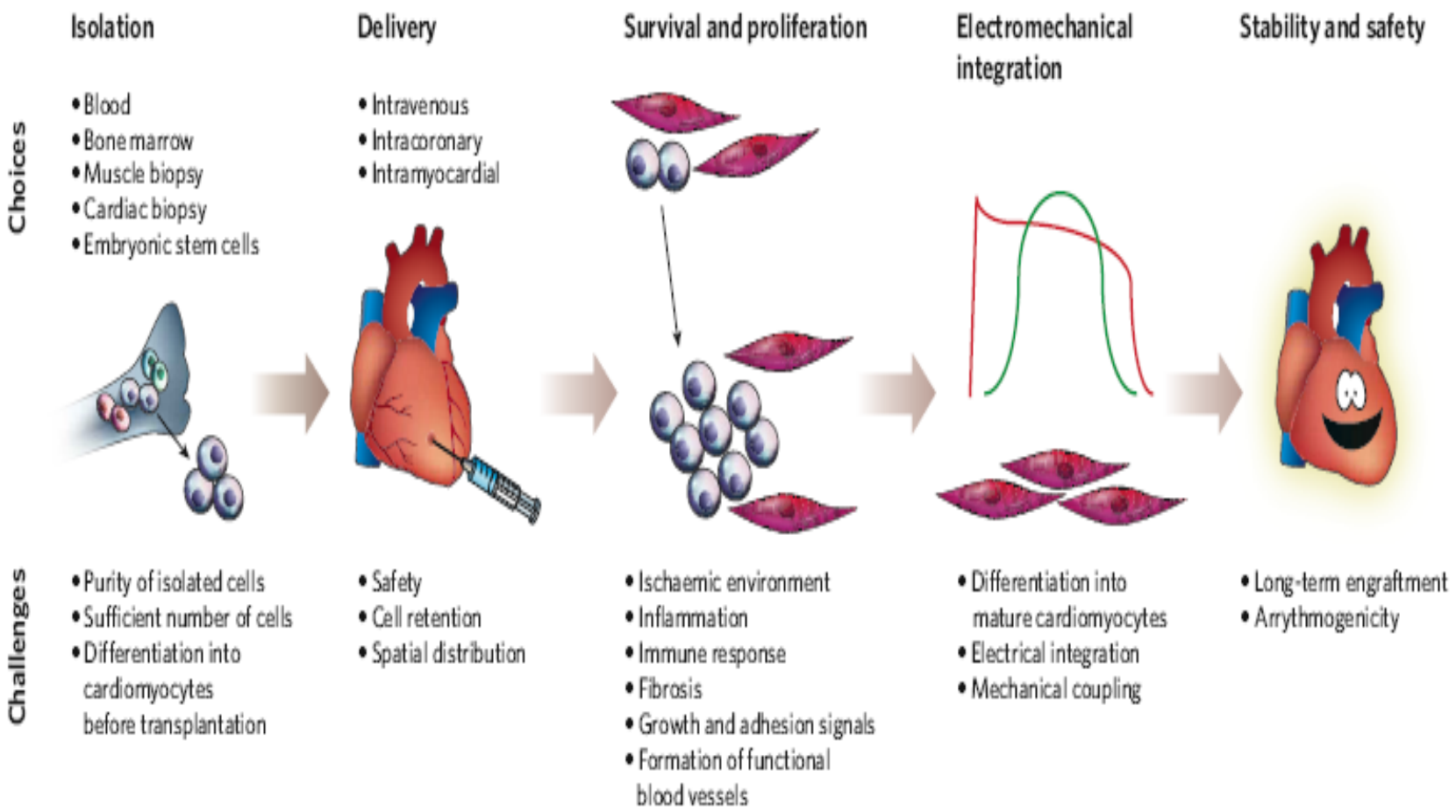

Isolation
Delivery
Survival and proliferation
Electromechanical integration
Stability and safety
Choices
• Blood
• Bone marrow
• Muscle biopsy
• Cardiac biopsy
• Embryonic stem cells
• Intravenous
• Intracoronary
• Intramyocardial
Challenges
• Purity of isolated cells
• Sufficient number of cells
• Differentiation into cardiomyocytes before transplantation
• Safety
• Cell retention
• Spatial distribution
• Ischaemic environment
• Inflammation
• Immune response
• Fibrosis
• Growth and adhesion signals
• Formation of functional blood vessels
• Differentiation into mature cardiomyocytes
• Electrical integration
• Mechanical coupling
• Long-term engraftment
• Arrythmogenicity

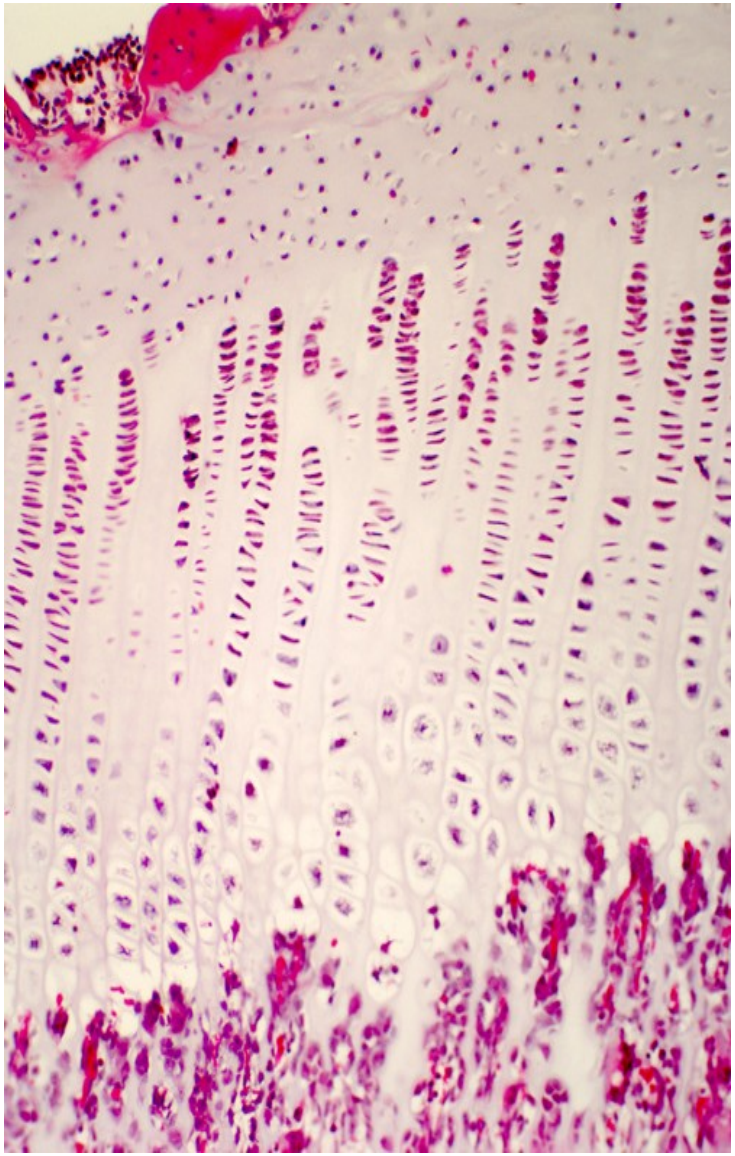
Table 1 | Overview of clinical trials of stem-cell or progenitor-cell delivery to the heart

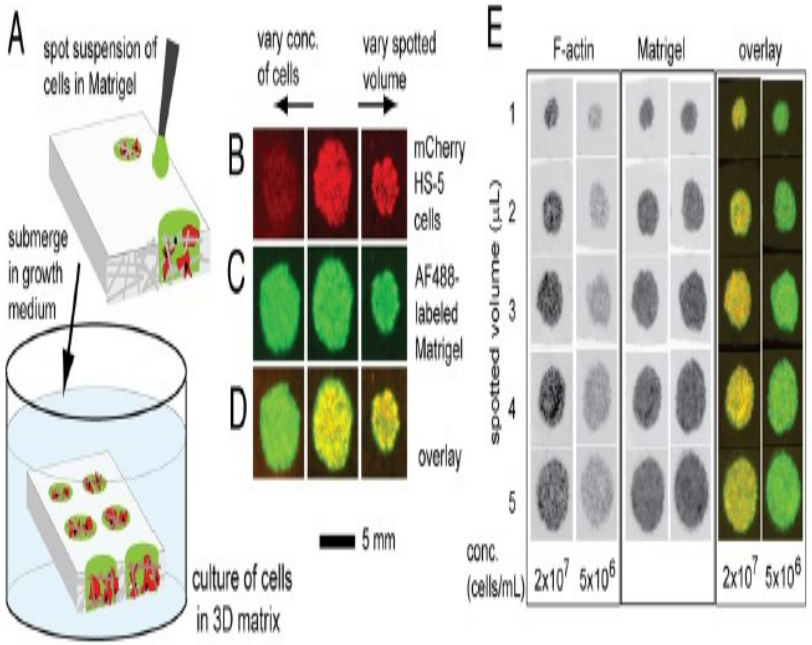
Cell type	Study design	Number of patients*	Mean follow-up duration (months)	Number of cells injected	Route of injection	Ejection fraction versus control (%)†	Source‡
BMMNC	R-SB	60	12	10^8	Intracoronary	+7.0 ($P=0.03$)	Meluzin *et al.*[67] (2007)
	R-SB	51	3	2×10^8	Intracoronary	+4.1 ($P=0.001$)	Assmus *et al.*[32] (2006)
	R-SB	66	3	10^8	Intracoronary	+3 ($P=0.04$)	Meluzin *et al.*[68] (2006)
	R-SB	204	12	2.4×10^8	Intracoronary	Decreased mortality	Schächinger *et al.*[69] (2006)
	R-SB	20	6	4×10^7	Intracoronary	+6.7 (NS)	Ge *et al.*[32] (2006)
	R-SB	20	4	6×10^7	TEIM	+2.5 (NS)	Hendrikx *et al.*[32] (2006)
	R-DB	67	4	1.7×10^8	Intracoronary	+1.2 (NS)	Janssens *et al.*[32] (2006)
	R-SB	100	6	8.7×10^7	Intracoronary	-3.0 ($P=0.05$)	Lunde *et al.*[32] (2006)
	R-SB	60	18	2.5×10^9	Intracoronary	+2.8 (NS)	Meyer *et al.*[32] (2006)
	Cohort§	36	3	3×10^8	TEIM	+4.0 (NS)	Mocini *et al.*[32] (2006)
	R-SB	204	4	2.4×10^8	Intracoronary	+2.5 ($P=0.01$)	Schächinger *et al.*[32] (2006)
	Cohort§	36	3	9×10^7	Intracoronary	+7.0 ($P=0.02$)	Strauer *et al.*[32] (2005)
	Cohort§	20	12	2.6×10^7	TEIM	+8.1 (NS)	Perin *et al.*[32] (2004)
	Cohort§	20	3	2.8×10^7	Intracoronary	+1.0 (NS)	Strauer *et al.*[32] (2002)
CPC	Cohort§	54	6	5×10^9	Intracoronary	+6.0 ($P=0.04$)	Tatsumi *et al.*[70] (2007)
	Cohort§	73	6	2×10^9	Intracoronary	+2.8 (NS)	Choi *et al.*[71] (2007)
	R-SB	47	3	2×10^7	Intracoronary	+0.8 (NS)	Assmus *et al.*[32] (2006)
	R	82	6	1.4×10^9	Intracoronary	-0.2 (NS)	Kang *et al.*[32] (2006)
	Cohort§	70	6	7.3×10^7	Intracoronary	+5.5 ($P=0.04$)	Li *et al.*[32] (2006)
	SB	26	3	7×10^7	Intracoronary	+7.2 (NS)	Erbs *et al.*[32] (2005)
CD133+	Cohort§	27	6	NA	Intramyocardial ‖	NA	Ahmadi *et al.*[72] (2007)
	Cohort§	55	6	6×10^6	Intramyocardial ‖	+6.3 ($P=0.02$)	Stamm *et al.*[73] (2007)
	Cohort§	35	4	1.3×10^7	Intracoronary	+2.8 (NS)	Bartunek *et al.*[32] (2005)
CD34+	R-DB	24	6	3.5×10^7	TEIM	NA	Losordo *et al.*[74] (2007)
SMB	R-DB	97	6	NA	Intramyocardial ‖	+3 ($P<0.04$)	MAGIC[22] (2007)
	Cohort§	26	12	2.5×10^8	Intramyocardial ‖	+14.5 ($P<0.01$)	Gavira *et al.*[75] (2006)
	Cohort§	12	12	2.1×10^8	TEIM	+11.6 ($P<0.05$)	Ince *et al.*[76] (2004)
MSC	R	48	12	5×10^6	Intracoronary	-3 (NS)	Chen *et al.*[77] (2006)
	R-SB	69	6	6×10^{10}	Intracoronary	+12.0 ($P=0.01$)	Chen *et al.*[32] (2004)
MSC + EPC	Cohort§	22	4	3×10^6	Intracoronary	+0.3 (NS)	Katritsis *et al.*[32] (2005)
BMC	R-DB	20	6	NA	Intracoronary	+9.2 ($P<0.05$)	Ruan *et al.*[32] (2005)

BMC, bone-marrow-derived cells (unspecified); BMMNC, bone-marrow mononuclear cell; CPC, circulating progenitor cell; DB, double blinded; EPC, endothelial progenitor cell; MSC, mesenchymal stem cell; NA, not available; NS, not significant; R, randomized; SB, single blinded; SMB, skeletal myoblast; TEIM, transendocardial intramyocardial injection. *The number of patients is the sum of individuals in the control and treatment groups; almost all studies have equal numbers in each group. †Ejection fraction is the proportion of blood in the left ventricle that is ejected into the aorta during each heartbeat; this is a measure of cardiac function. ‡The author names refer to the original report, and the reference number cited indicates either the original report or a meta-analysis (or review) in which the original report is discussed. §Cohort denotes a non-randomized and non-blinded study. ‖Intramyocardial indicates injection through the epicardial side of the heart.

Paper-supported 3D cell culture for tissue-based bioassays

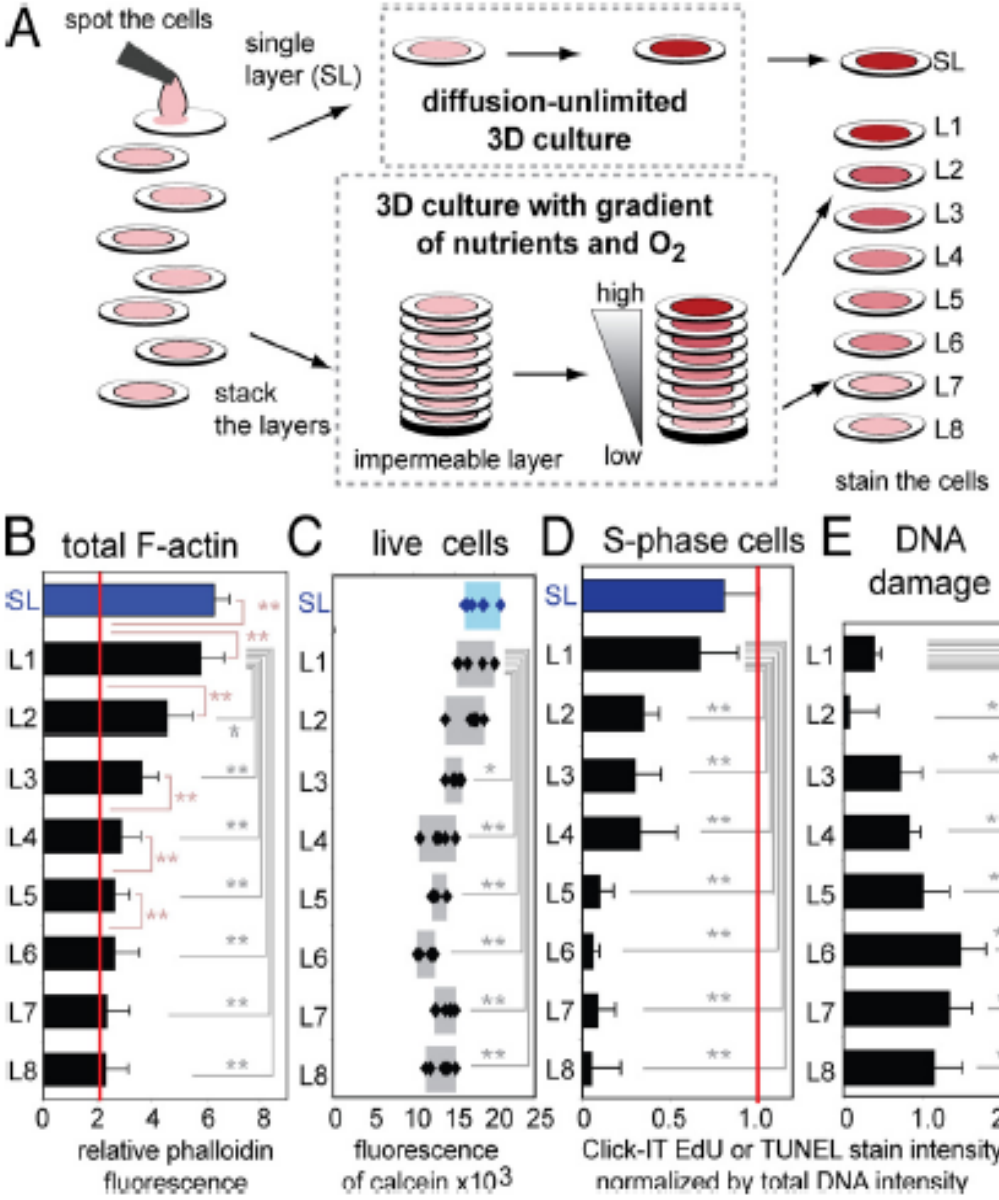
PNAS | November 3, 2009 | vol. 106 | no. 44 | 18457–18462

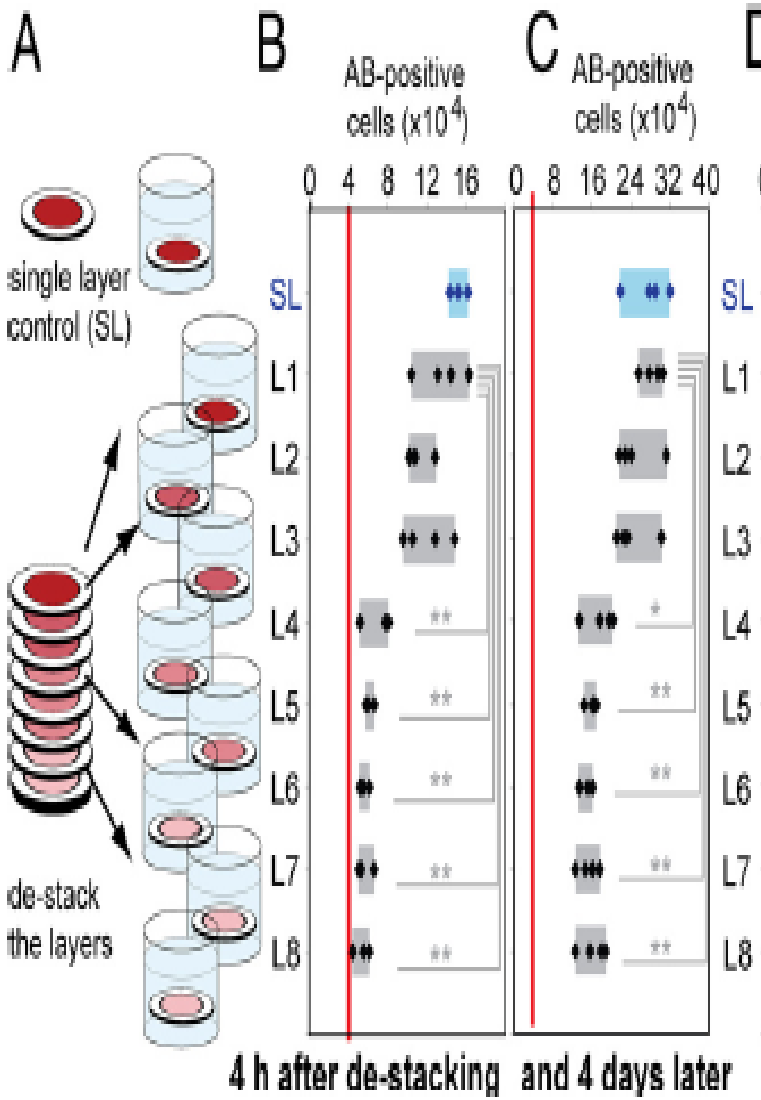


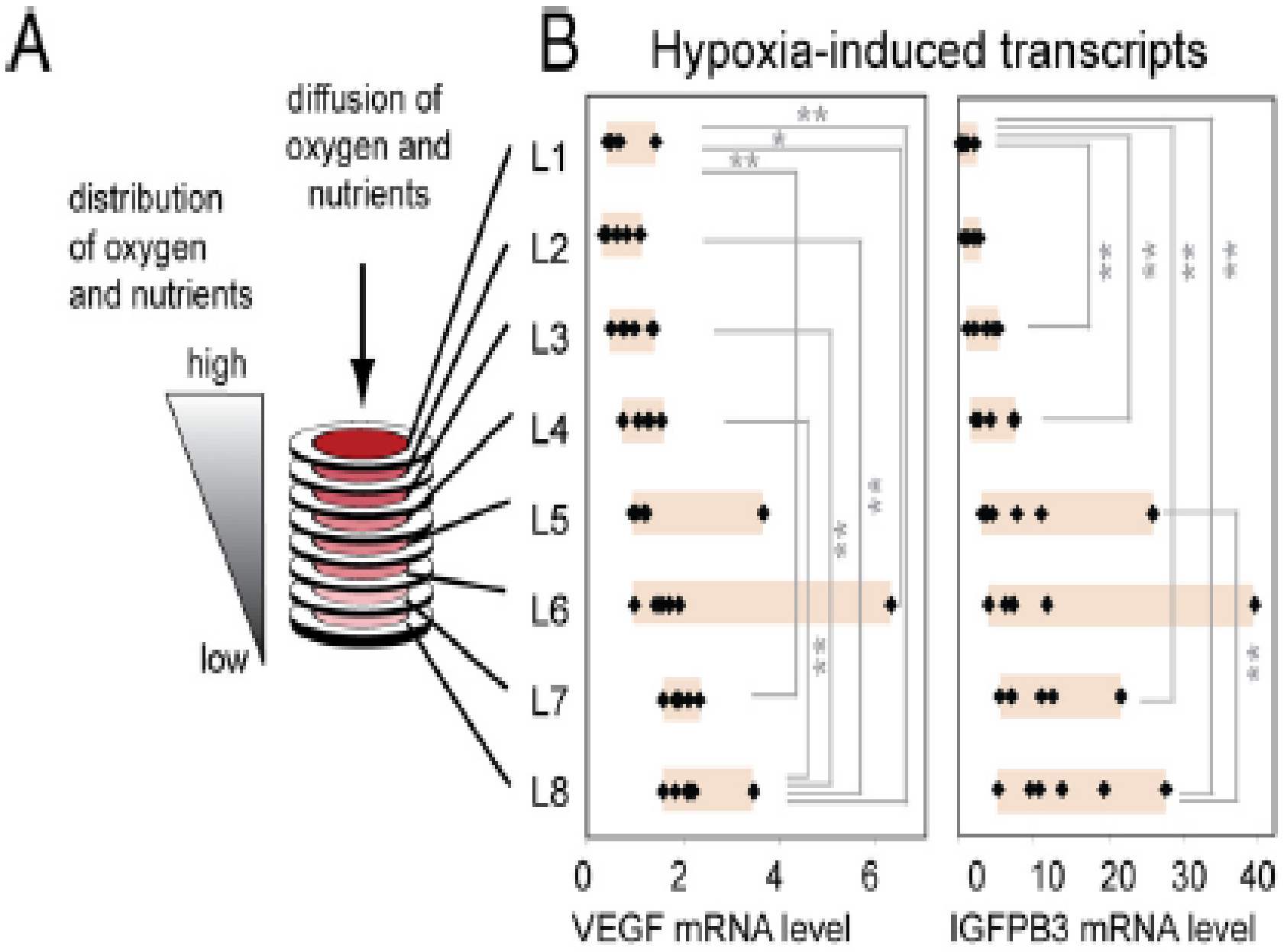


MDA-MB-231, 9 days of growth

Alamar blue conversion for cell proliferation (red line-seeded cells)

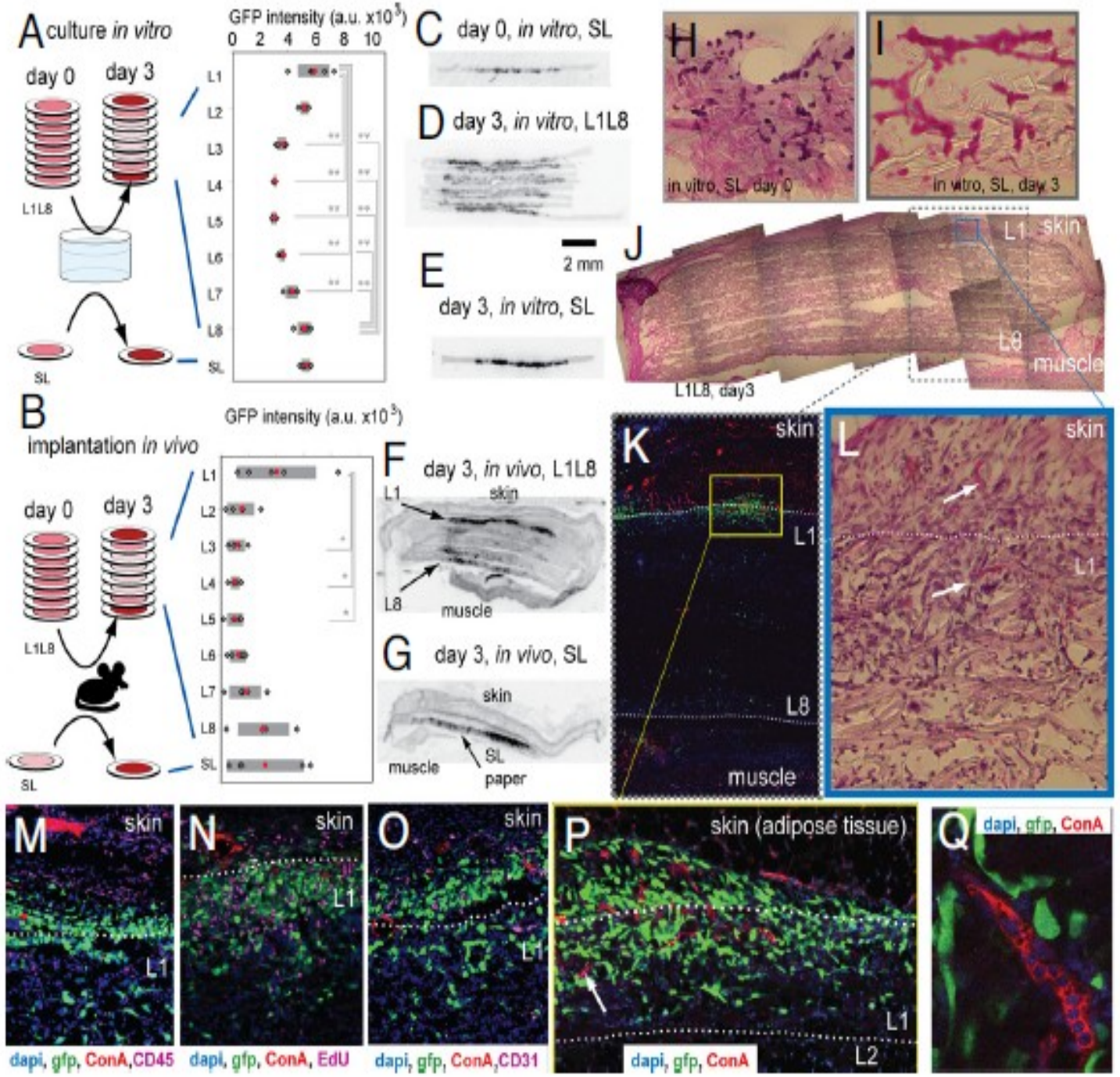





A
diffusion of oxygen and nutrients
distribution of oxygen and nutrients
high
low
B
Hypoxia-induced transcripts
L1
L2
L3
L4
L5
L6
L7
L8
0
2
4
6
VEGF mRNA level
0
10
20
30
40
IGFPB3 mRNA level

LLC – Lewis lung carcinoma cells

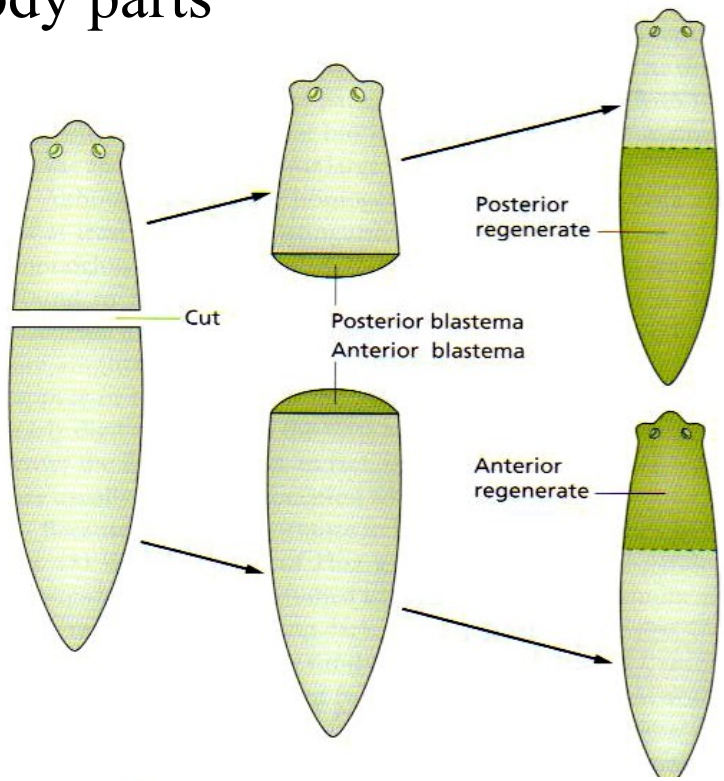
CD45 – hematopoietic
CD31- endothelial
ConA-rhodamine injection - capillary

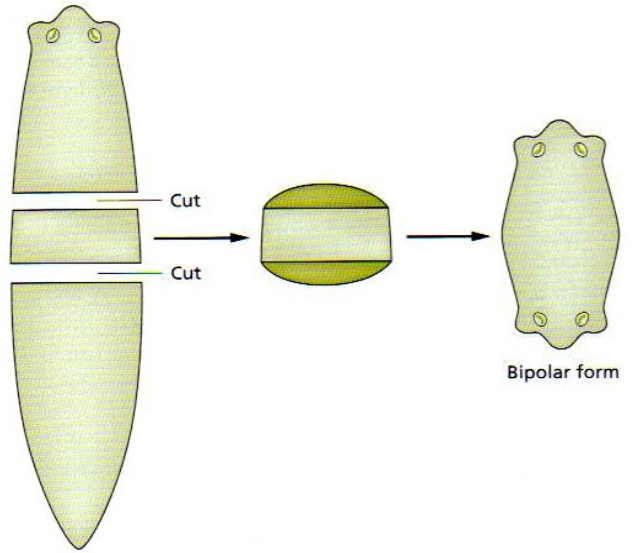


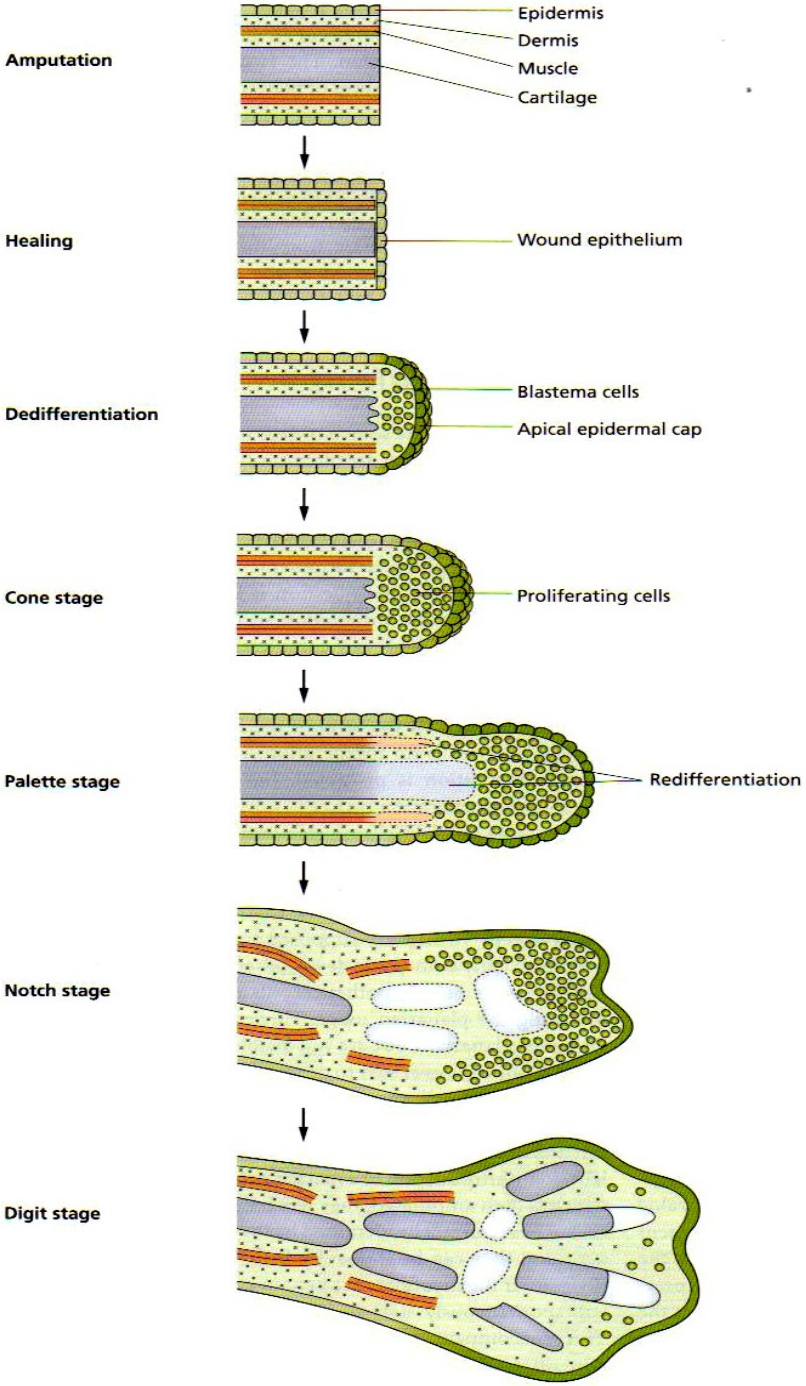
Regeneration of body parts

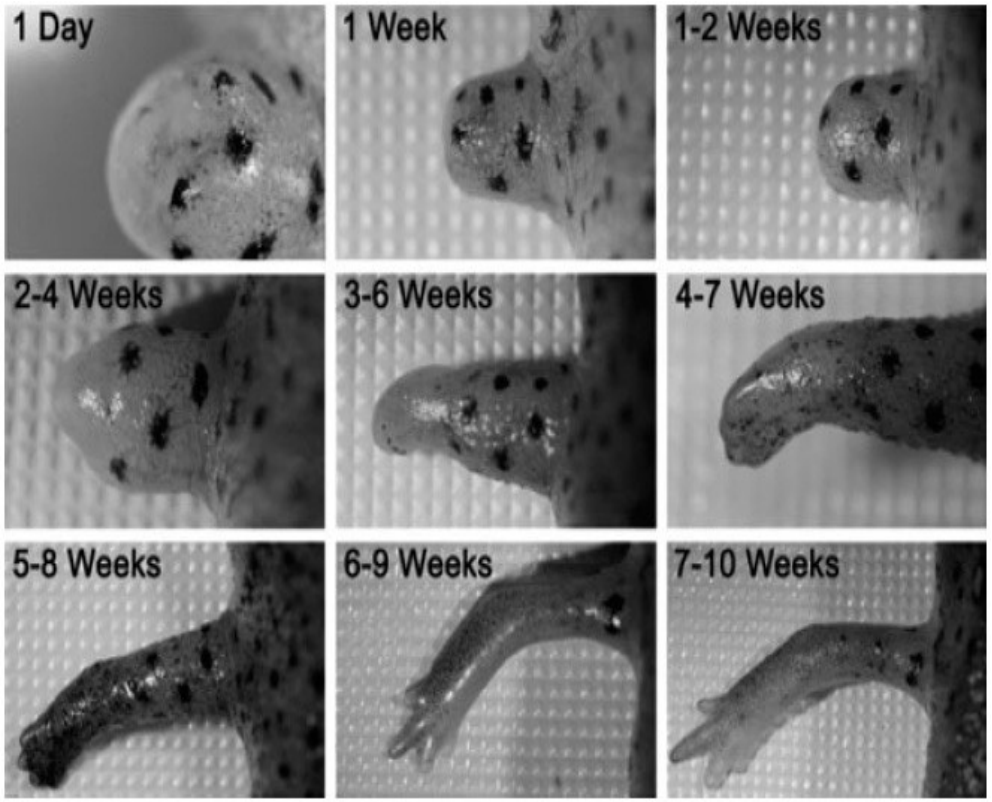
<u>REGENERATION IN PLANARIANS:</u> Being the simplest animals with bilateral symmetry, planarians are in a constant cell turnover. Their bodies contain up to 20% of so called neoblasts, characterized by the expression of ATP-dependent RNA helicase similar to Drosophila vasa protein. Neoblasts divide and contain the population of totipotent cells that can form all 15 cell types of the planarian tissues.

Following transection, there is a muscular contraction limiting the area of the cut followed by the formation of the wound epithelia that makes up regeneration blastema. The blastema enlarges and redifferentiates to form missing structures. The mechanism of a polarity decision, whether to be a head or tail, is poorly understood and does not likely involve the Hox genes.









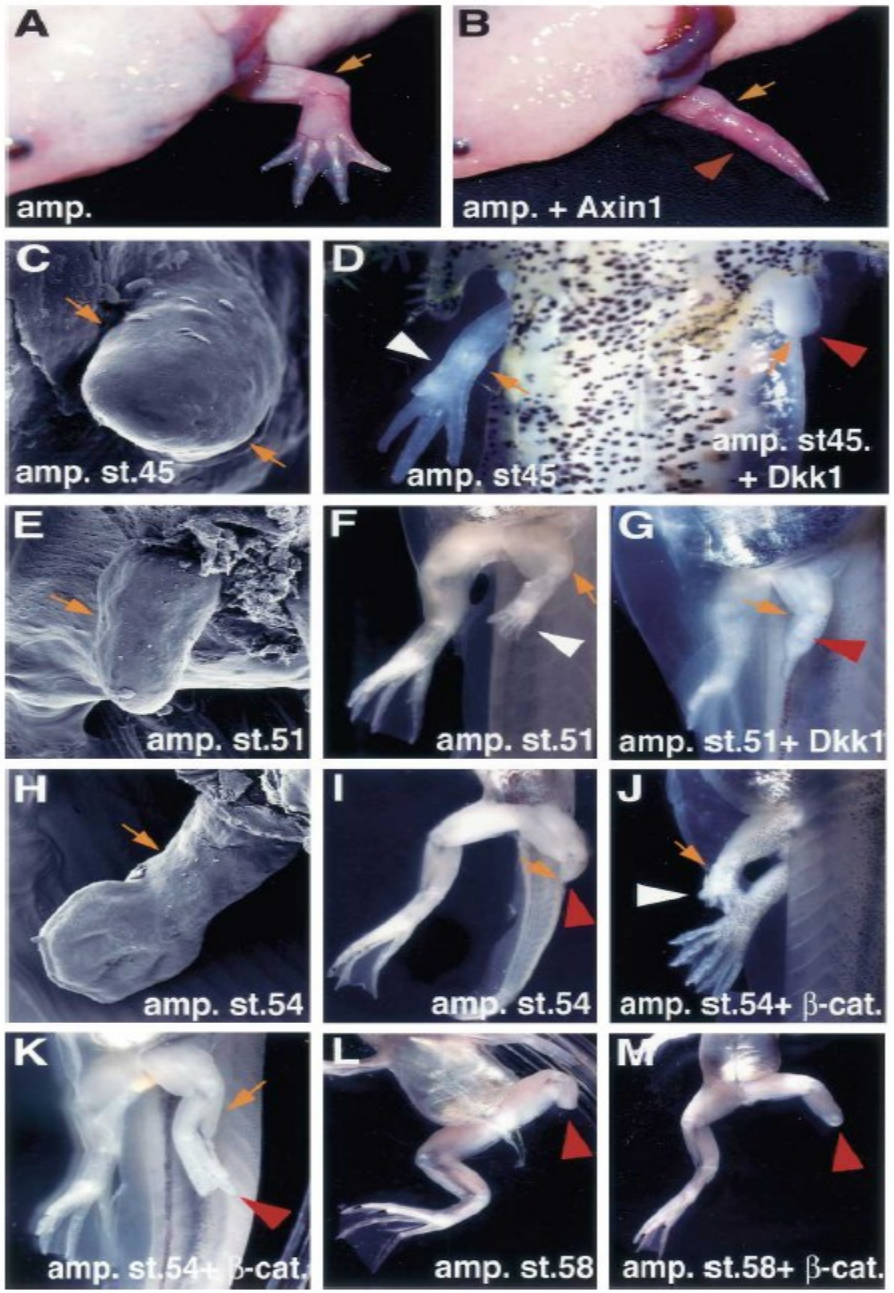
VERTEBRATE LIMB REGENERATION:

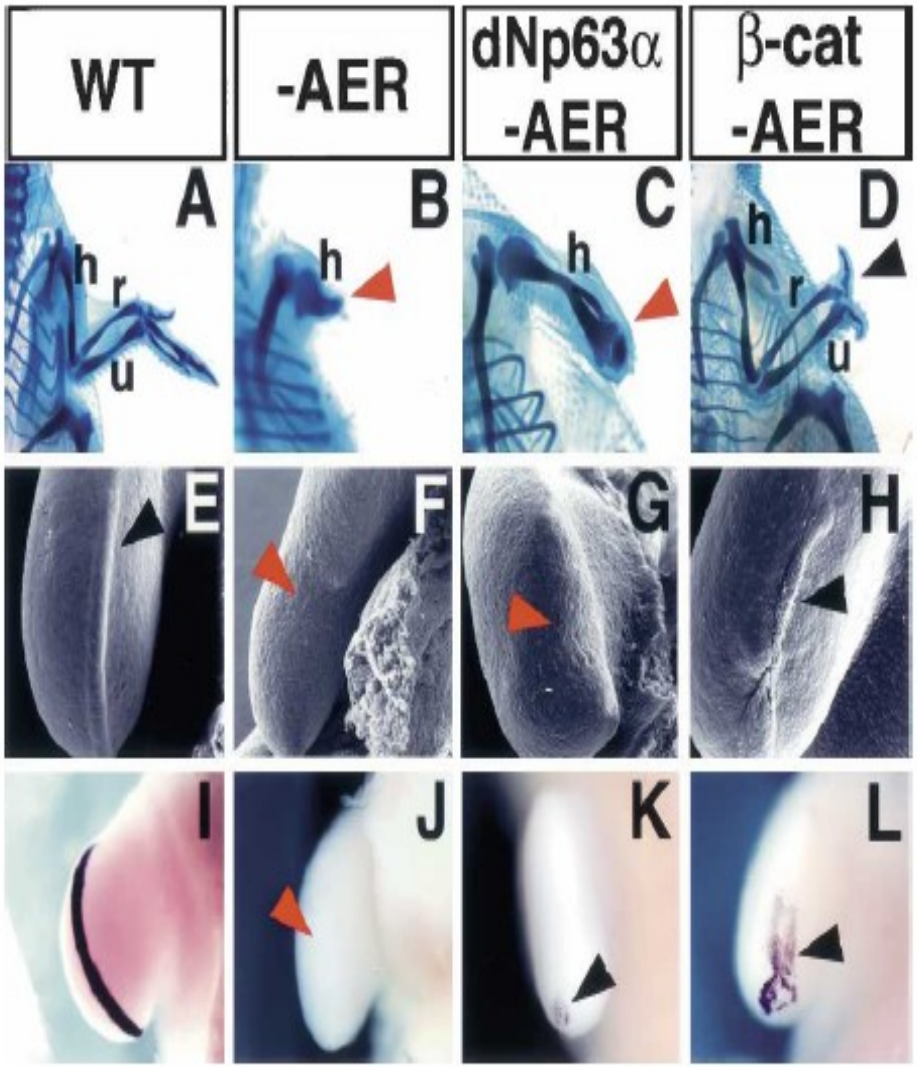
Among the vertebrates only certain amphibians can regenerate limbs after surgical removal. These include anuran tadpoles that can regenerate limbs before they reach the metamorphosis as well as many urodele species that regenerate limbs during both larval and adult life.

After limb amputation, a wound epithelia forms via migration of epidermal cells over the cut surface followed by dedifferentiation of an underlying tissue. The blastema consists of loose-packed mesenchymal cells surrounded by thick epidermal jacket. The blastema proliferates and then the limb structures redifferentiate in the proximal-distal sequence.

Wnt/β-catenin signaling regulates vertebrate limb regeneration

Genes Dev. 2006 20: 3232-3237





Can mammals regenerate body parts?

A New Murine Model for Mammalian Wound Repair and Regeneration

CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY
Vol. 88, No. 1, July, pp. 35–45, 1998

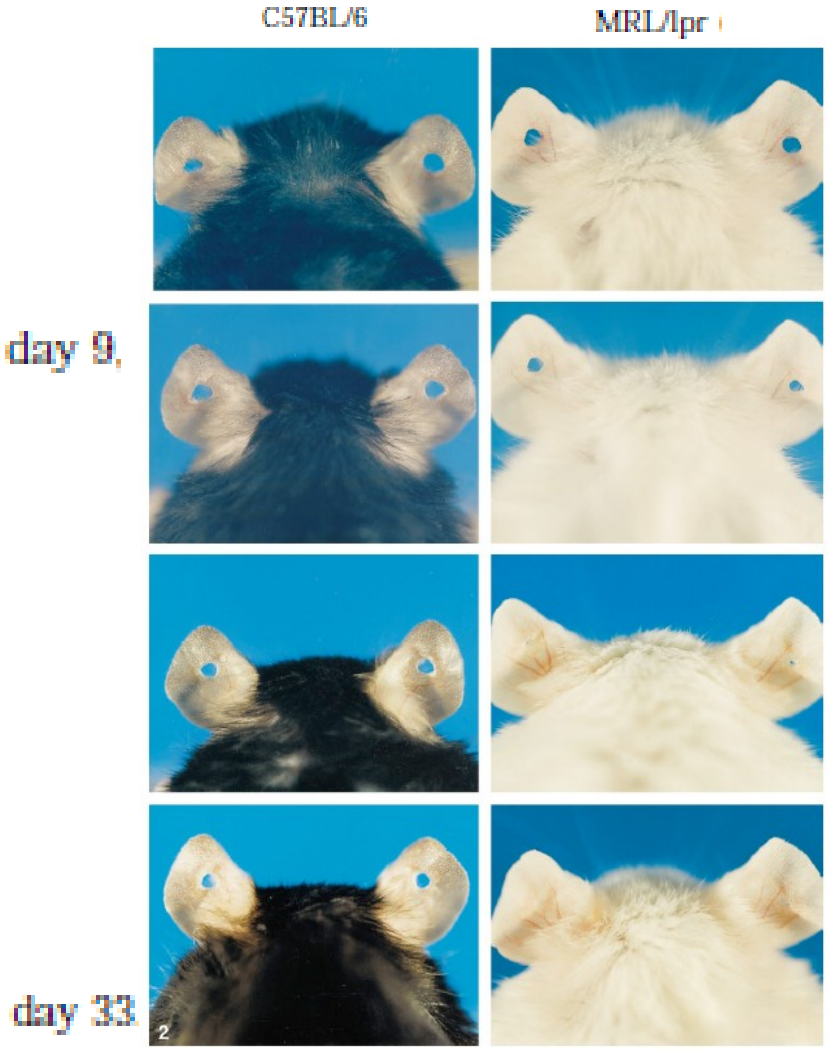


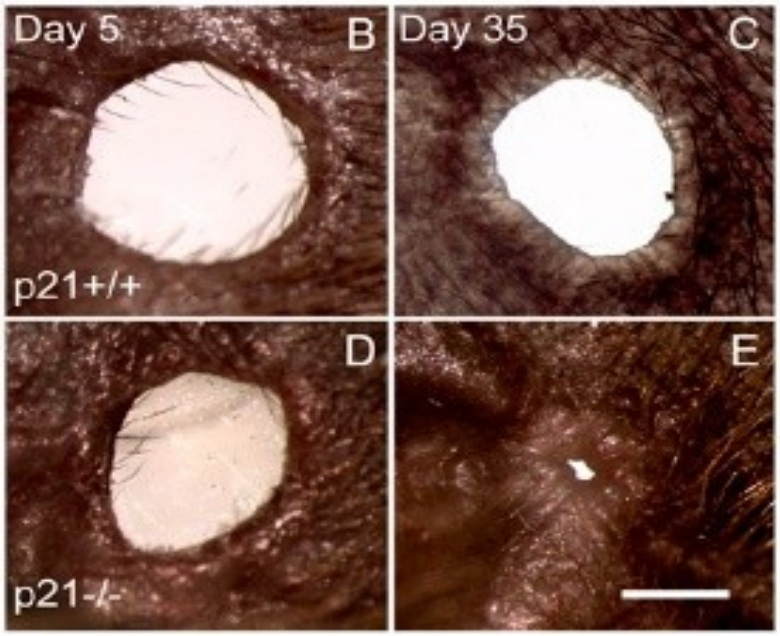
Table 4. Candidate genes in genomic intervals containing QTLs

OTL	Mouse Genome Database, centi-morgans	Candidate genes in interval
heal1	33	Comp, cartilage oligomeric matrix protein
	39	pdw, proportional dwarf
	42	Os, oligosyndactylism
	46	Gna0
	51.5 to 67	Cadherin family
heal2	7	Nid, nidogen
	8	Gli3, GLI-Kruppel family member GL13
	10	Amph, amphiphysin
	10	Inhba, inhibin beta-A
	10	Rasl1, Ras-like, family 1
heal3	32	Msx2, hox8
	32.5	Fgfr4, fibroblast growth factor receptor
	33	mes, mesenchymal dysplasia
	36	Tgfbi, transforming growth factor induced
	44	Cspg2, chondroitin sulfate proteoglycan
	45	Rasa, ras p21 GTPase activating protein
	56	Gpcrl8, G-protein coupled receptor 18
	62	Itga 1,2, integrin alpha 2 (Cd49b)
heal4	51.6	Pdgfec, platelet derived growth factor
	56.8	Col2a1, procollagen, type 11, alpha 1
	56.8	Ela1, elastase 1
	57	Emb, embigin
	57.1	Hoxc, homeo box C cluster
	57.1	Rarg, retinoic acid receptor, gamma
	57.5	Dhh, desert hedgehog homolog
	58.7	Krt2, keratin gene complex 2
	60	Itga5, integrin alpha 5
	61.1	Itgb7, integrin beta 7
	63	Glycam 1 adhesion molecule
heal5	40	Fos, FBJ osteosarcoma oncogene
	41	Tgfb3, transforming growth factor, beta
	44.6	Chx10, C elegans ceh-10 homeo domain con
	45	Pgf, placental growth factor

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11792–11797, September 1998
Genetics

Lack of p21 expression links cell cycle control and appendage regeneration in mice

PNAS | March 30, 2010 | vol. 107 | no. 13 | 5845–5850



INJURY → Replication Stress → p53 ↑ → p21 ↑ G1 Arrest SCAR FORMATION

p21 ↓ G2M Pause REGENERATION

Heart regeneration in adult MRL mice

John M. Leferovich*, Khamilia Bedelbaeva*, Stefan Samulewicz*, Xiang-Ming Zhang*, Donna Zwas†, Edward B. Lankford†, and Ellen Heber-Katz*§

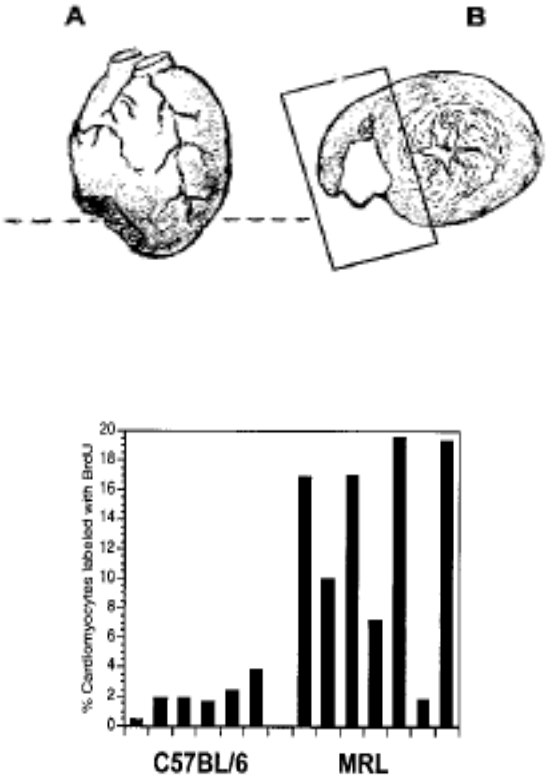
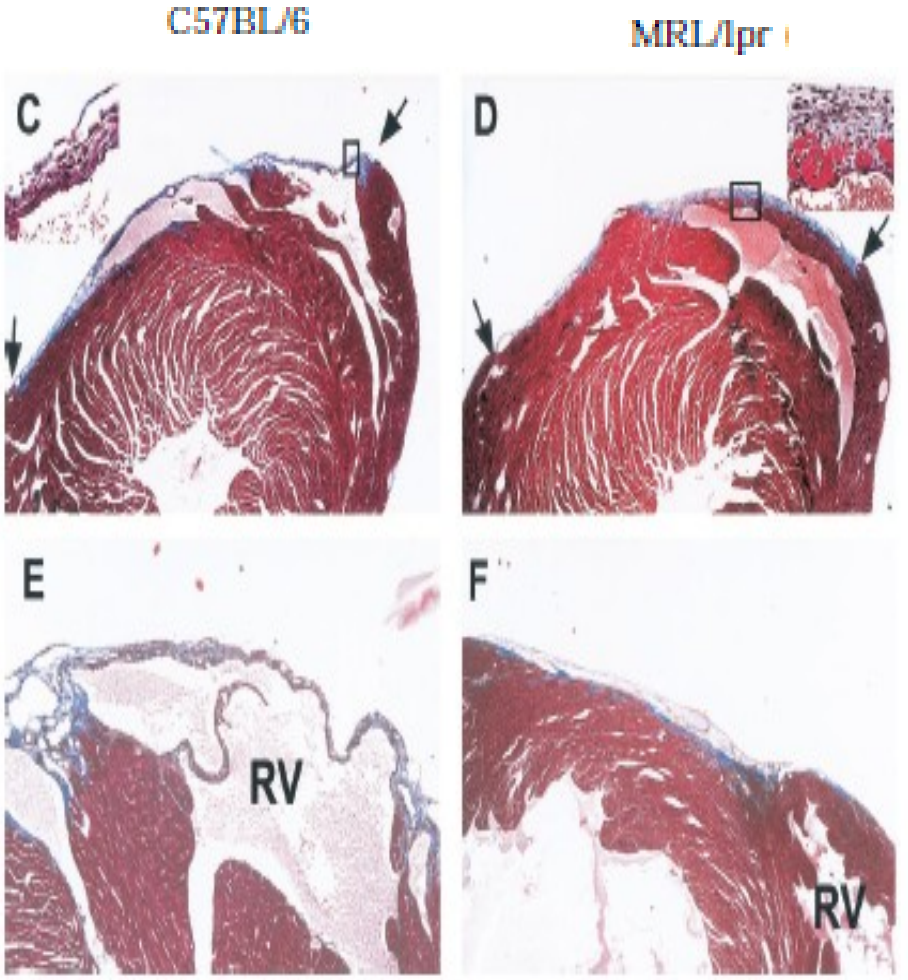


Fig. 4. Labeling and indices of BrdUrd-positive myonuclei in individual hearts of C57BL/6 mice, $n = 6$ (*Left*) and MRL mice, $n = 7$ (*Right*), 60 days after cryoinjury. Labeling indices were obtained from double-labeled tissue in which nuclei were first stained with anti-BrdUrd antibody and then costained with DAPI. A total of 1,000 DAPI-positive nuclei were counted from each heart sample, and the mitotic index was calculated from the number of BrdUrd-labeled nuclei divided by the number of DAPI-positive nuclei × 100. Histograms were assembled from counts made from the area of the initial cryoinjury.



THE ROYAL SOCIETY

Published online 8 April 2004

The scarless heart and the MRL mouse

Ellen Heber-Katz*, John Leferovich, Khamilia Bedelbaeva, Dmitri Gourevitch and Lise Clark

ELSEVIER

Cardiovascular Pathology 17 (2008) 6–13

Original Article

Absence of regeneration in the MRL/MpJ mouse heart following infarction or cryoinjury

Thomas E. Robey, Charles E. Murry*

ELSEVIER

Cardiovascular Pathology 17 (2008) 32–39

Original Article

The MRL mouse heart does not recover ventricular function after a myocardial infarction

Massimo Cimini, Shafie Fazel, Hiroko Fujii, Sun Zhou, Gilbert Tang, Richard D. Weisel, Ren-Ke Li*

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Received 10 August 2006; received in revised form 20 April 2007; accepted 28 June 2007

J. Anat. (2005) **207**, pp603–618

REVIEW

Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals?

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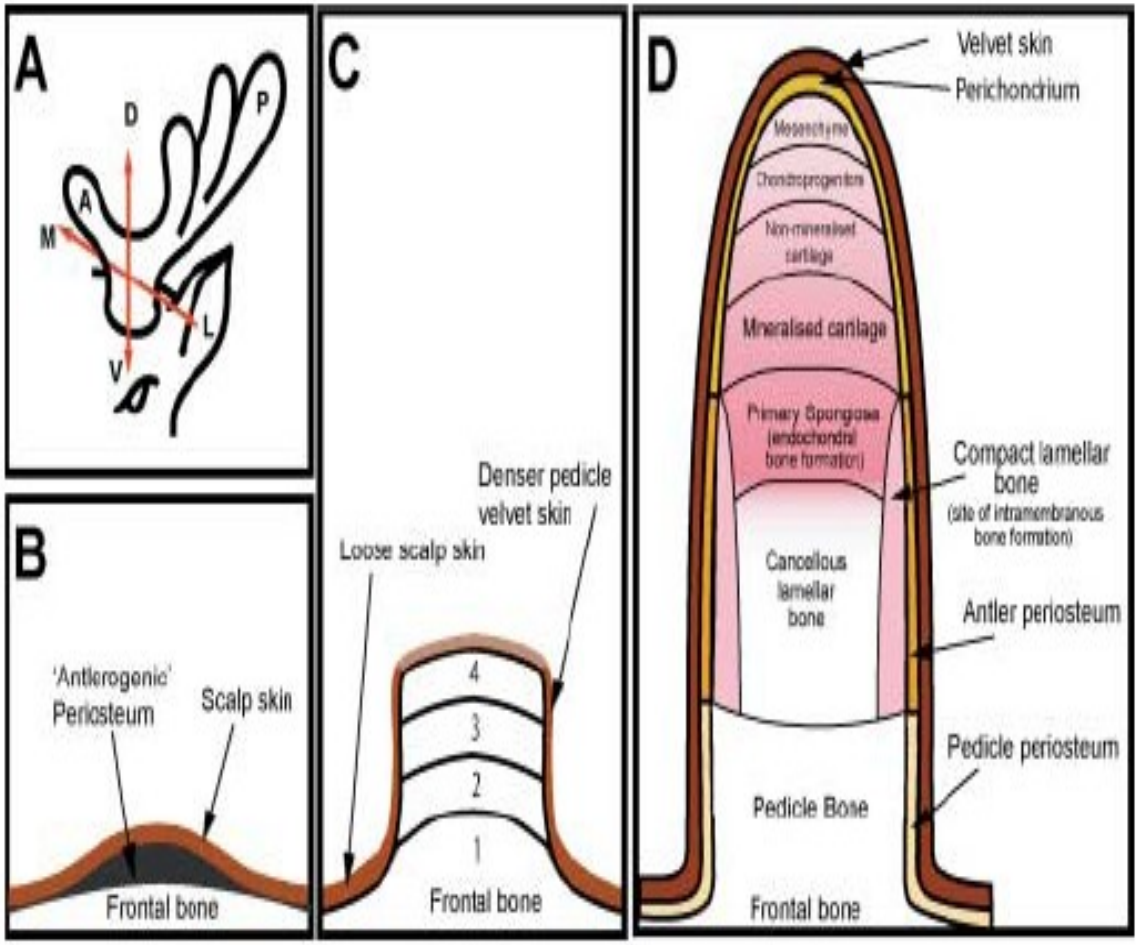
Department of Basic Sciences, The Royal Veterinary College, London, UK

Abstract

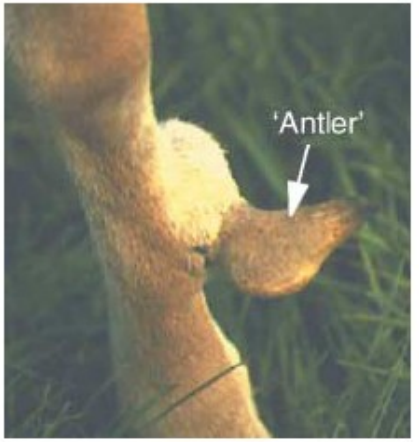
Many organisms are able to regenerate lost or damaged body parts that are structural and functional replicates of the original. Eventually these become fully integrated into pre-existing tissues. However, with the exception of deer, mammals have lost this ability. Each spring deer shed antlers that were used for fighting and display during

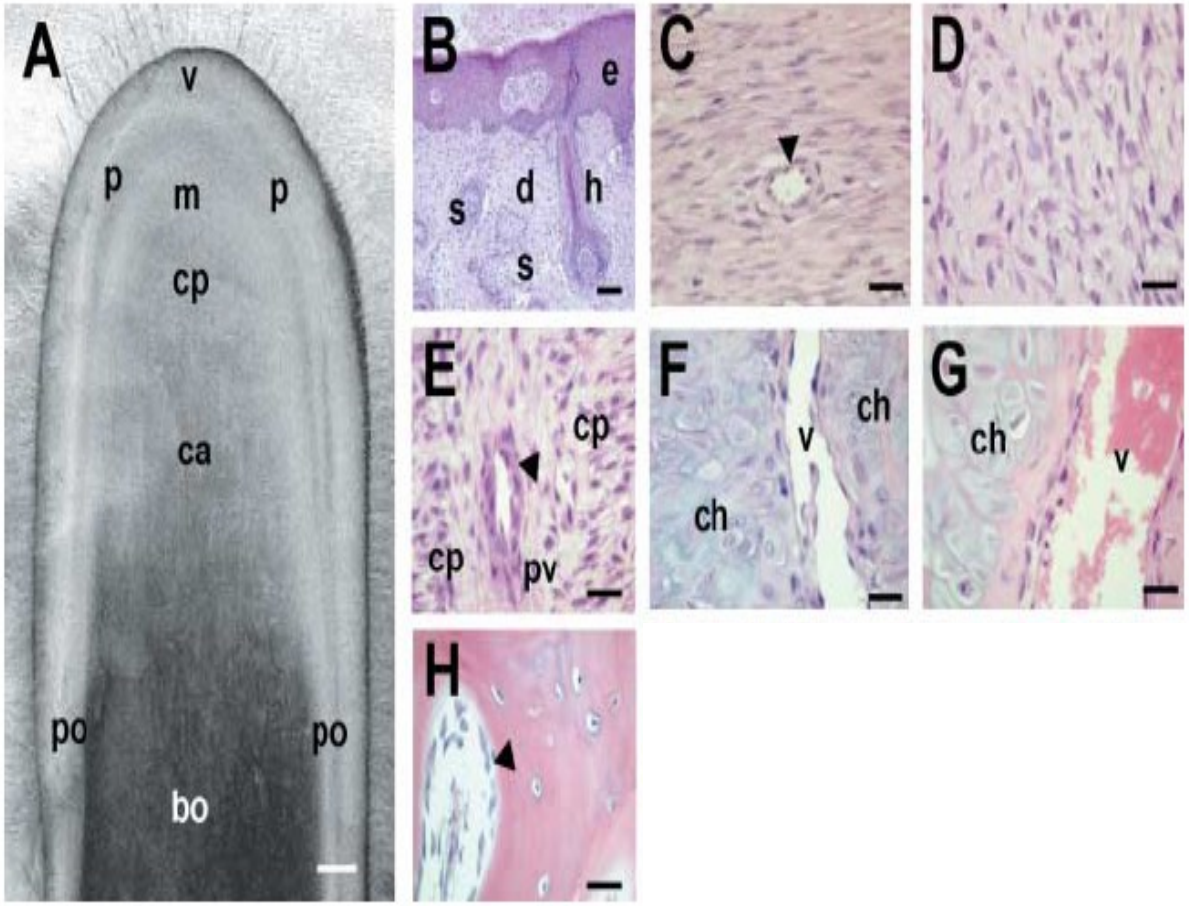


(C) Stages of pedicle development

1 - intramembranous ossification
2 - transitional ossification
3 - endochondral ossification
4 - endochondral ossification and skin formation

Antler growth from transplanted perichondrium into the metacarpal bone





(B) Velvet skin
e - epidermis
d - dermis
h - hair follicle
s – gland

(C) Fibrous perichondrium
arrow – blood vessel

(D) Mesenchymal growth zone

(E) Chondroprogenitor region

(F) non-mineralized cartilage
v - blood vessel

(G) mineralized cartilage

(H) spongy bone

v – velvet skin
p –perichondrium
m- mesenchyme
cp – chondroprogenitor region
c- cartilage
bo – bone
p – periosteum