Stem cells and heart function – how to repair a broken heart?

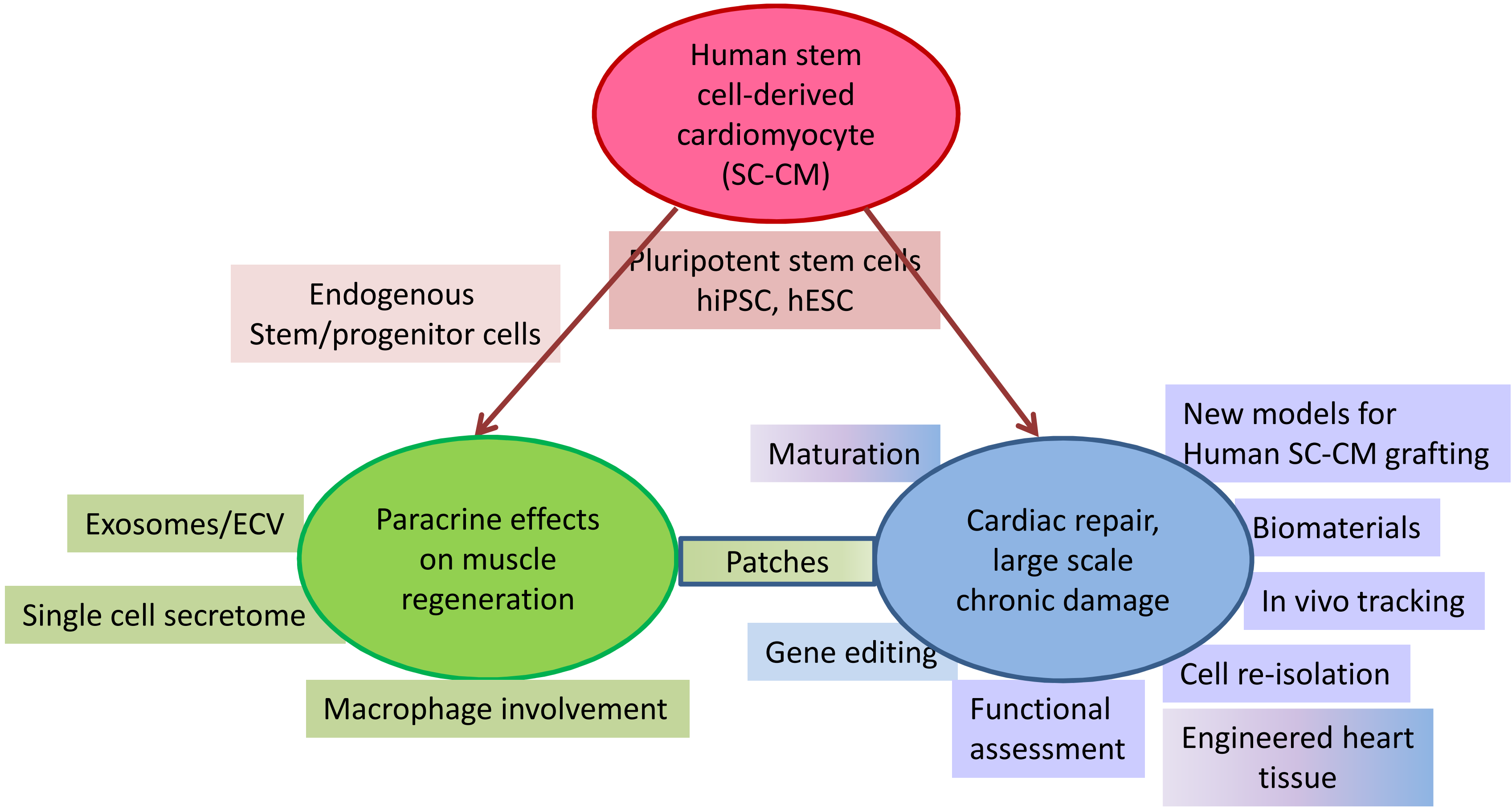
Professor Sian E. Harding

Director, Imperial BHF Centre for Cardiac Regeneration

Imperial BHF Centre for Cardiac Regeneration 2017-2021

Human stem cell-derived cardiomyocytes (SC-CM)

in vivo cardiac muscle regeneration and **paracrine-mediated repair**



Natural history of heart failure

Heart attack
Valve disease
Genetic defect
Alcohol/drugs
Infection/sepsis

Damage ← Repair

↓

Heart thickening
Dilation
Volume loading
Sympathetic stimulation

Apparent recovery/ compensation

Gene therapy
Revascularisation

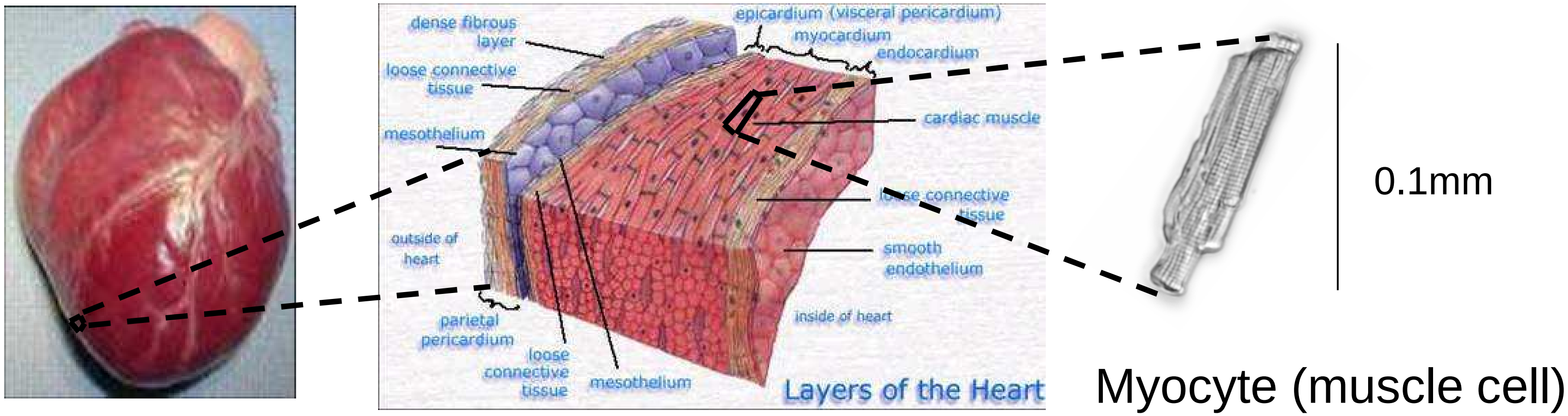
↓ ← Drugs

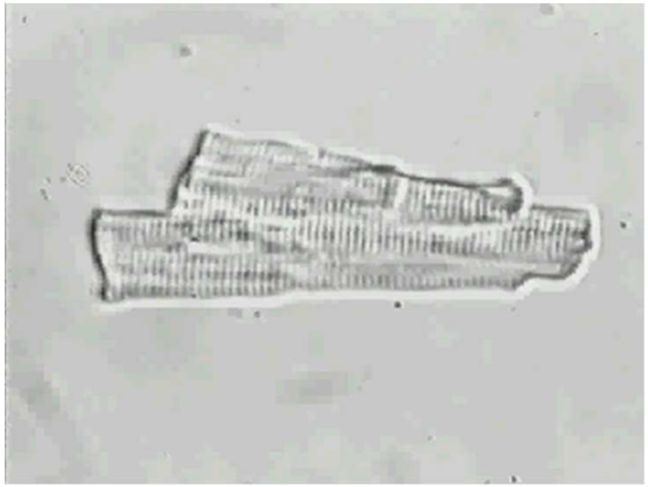
Decompensation/ Heart failure

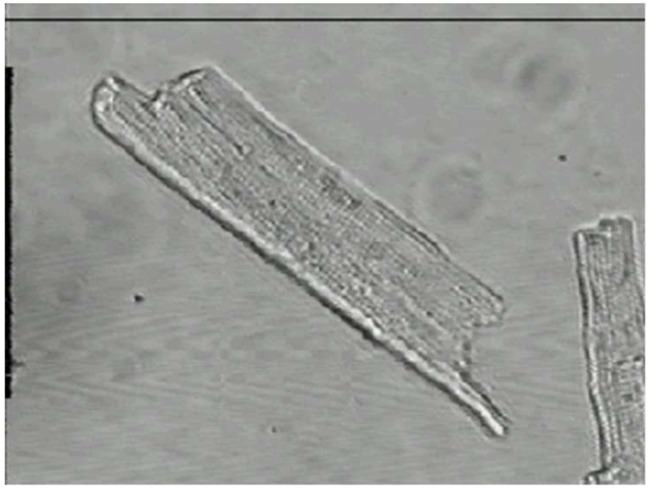
↓ ← Devices
Transplantation

Death

Structure of the contracting myocardium

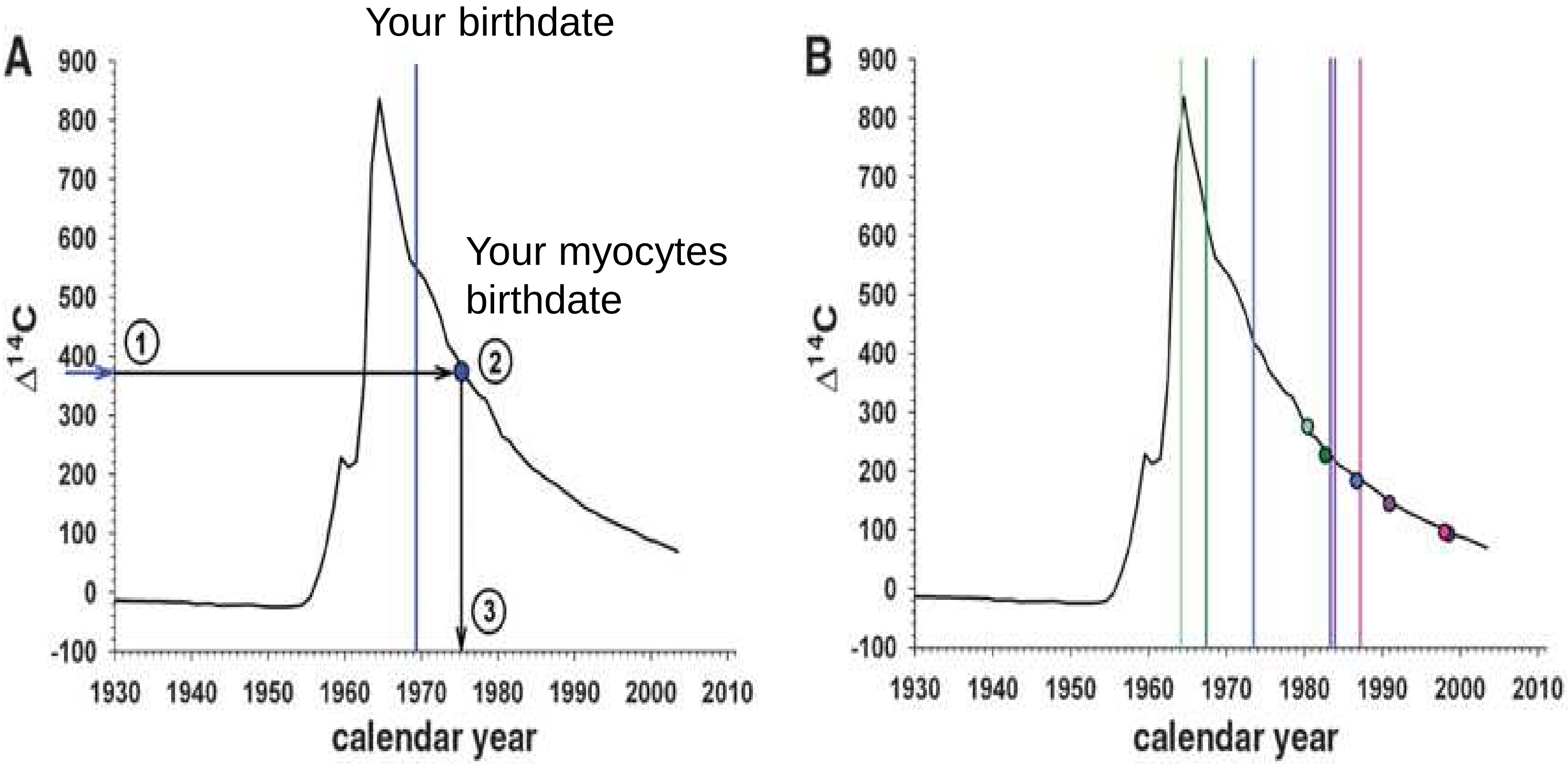






Regeneration of cardiomyocytes? Carbon dating the heart

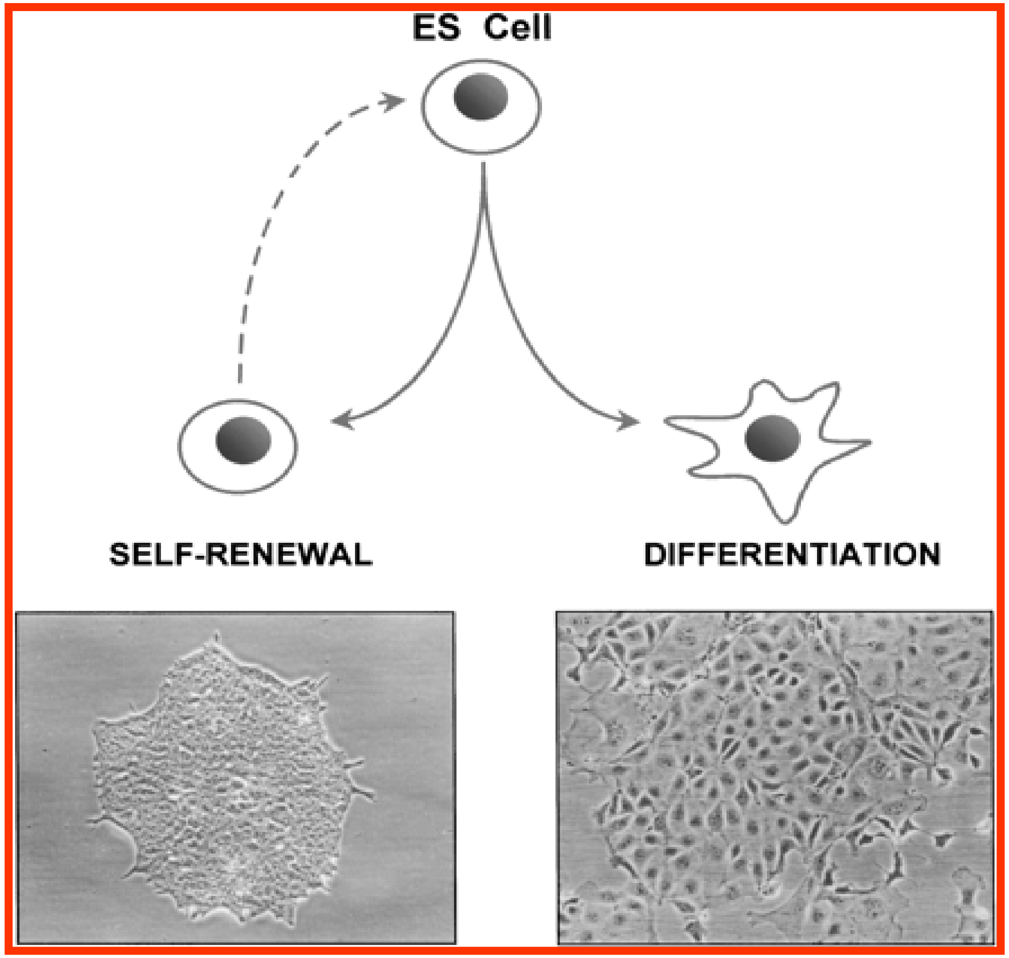
14C levels in
the atmosphere



50% of cardiomyocytes are present at birth. Turnover rate is ~1% per year at age 25, and 0.45% per year at age 75

Bergmann, Science 2009

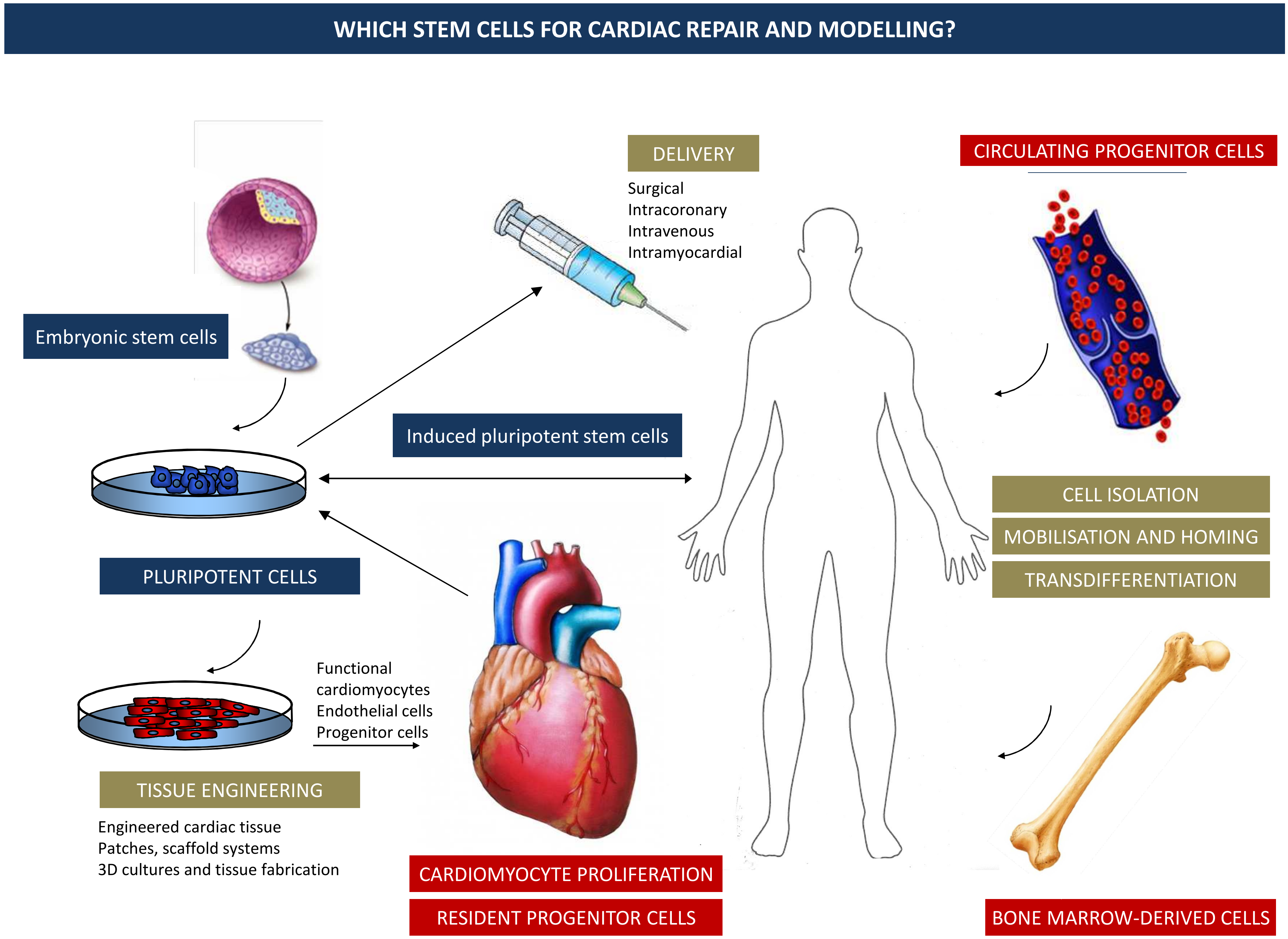
Can stem cells repair the heart? What is a stem cell?



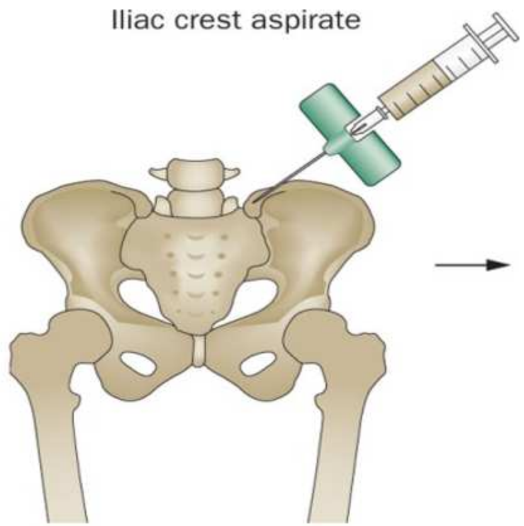
Adapted from A.Smith Annu.Cell Dev.Biol (2001) 17:435-62

- Undifferentiated cells with capacity for prolonged or unlimited self renewal
- Asymmetric cell division
- Pluripotent (any cells except placenta)
- Stem/progenitors - in adult tissues differentiate to more limited range to maintain normal tissue (multipotent)

Differentiation : is the process by which a less specialized cell becomes a more specialized cell type


WHICH STEM CELLS FOR CARDIAC REPAIR AND MODELLING?
Embryonic stem cells
DELIVERY
Surgical
Intracoronary
Intravenous
Intramyocardial
CIRCULATING PROGENITOR CELLS
Induced pluripotent stem cells
PLURIPOTENT CELLS
CELL ISOLATION
MOBILISATION AND HOMING
TRANSDIFFERENTIATION
Functional
cardiomyocytes
Endothelial cells
Progenitor cells
TISSUE ENGINEERING
Engineered cardiac tissue
Patches, scaffold systems
3D cultures and tissue fabrication
CARDIOMYOCYTE PROLIFERATION
RESIDENT PROGENITOR CELLS
BONE MARROW-DERIVED CELLS

Bone Marrow Stem Cells (BMC)

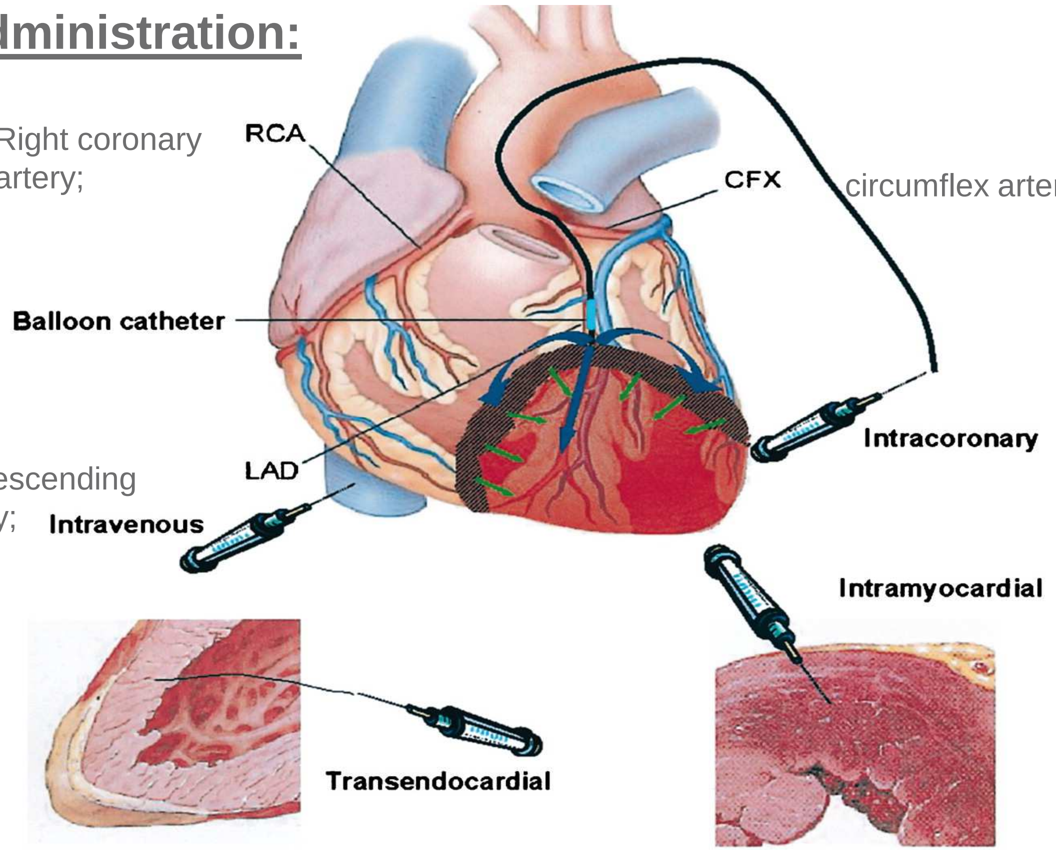


Route of administration:

Right coronary artery;

circumflex artery.

Left anterior descending coronary artery;



Results of bone marrow stem cell implantation for heart disease

- Started around10 years ago with small safety trials

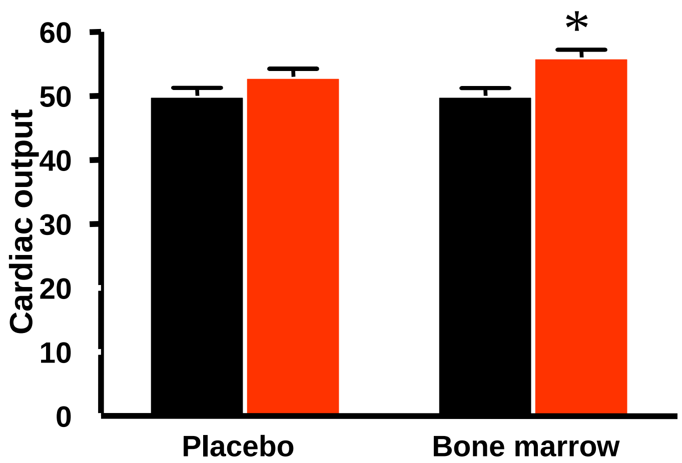
• Now more than 500 treated and 500 control patients in double-blind randomised placebo-controlled trials

- Procedure is safe in the short and medium term

- Some benefit, but not very large

- But, not producing many new myocytes
  - new blood vessels?
  - secreted protective factors?

Cardiac output (ejection fraction)



Human embryonic stem cells discovered in 1998

In vitro fertilization day 1

Embryos frozen at 1-7 days
(at this point, ~80% embryos do not implant either naturally or after IVF)

Unused embryos must be destroyed

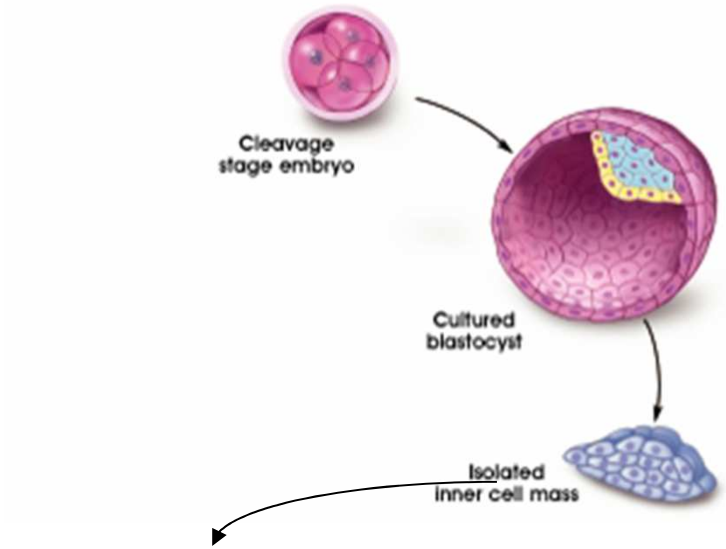
Permission requested at that point to use for research

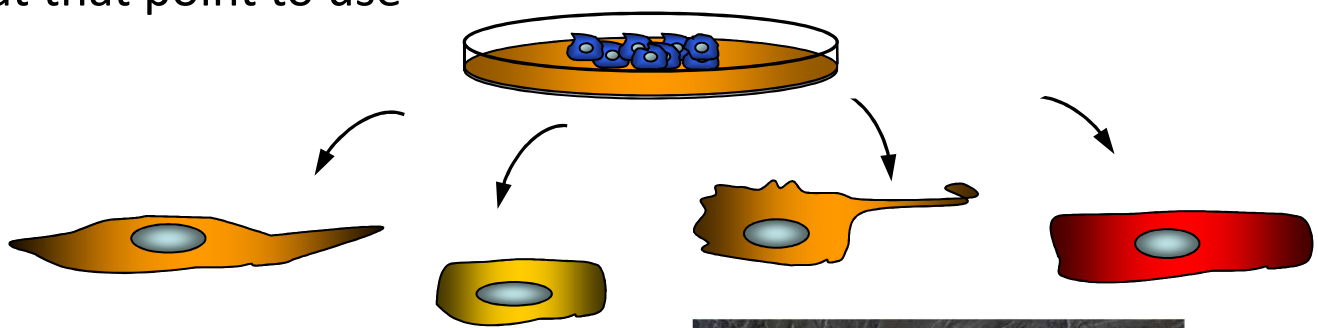
Cell line made

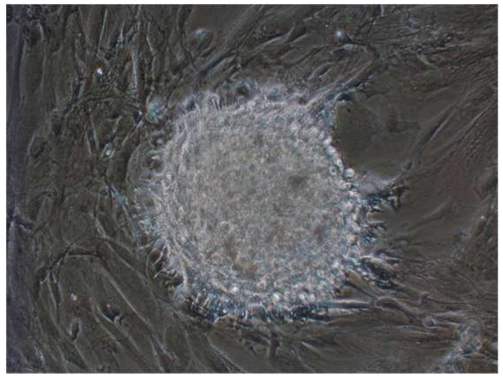
Held in Stem Cell Bank

Distributed free to researchers

Can become any cell type in the body





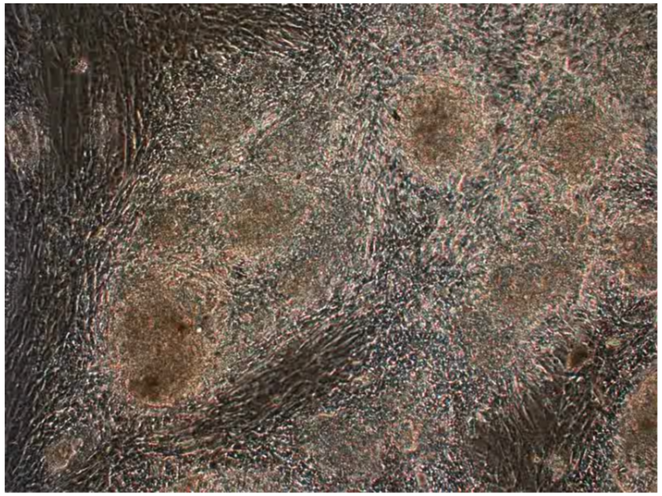


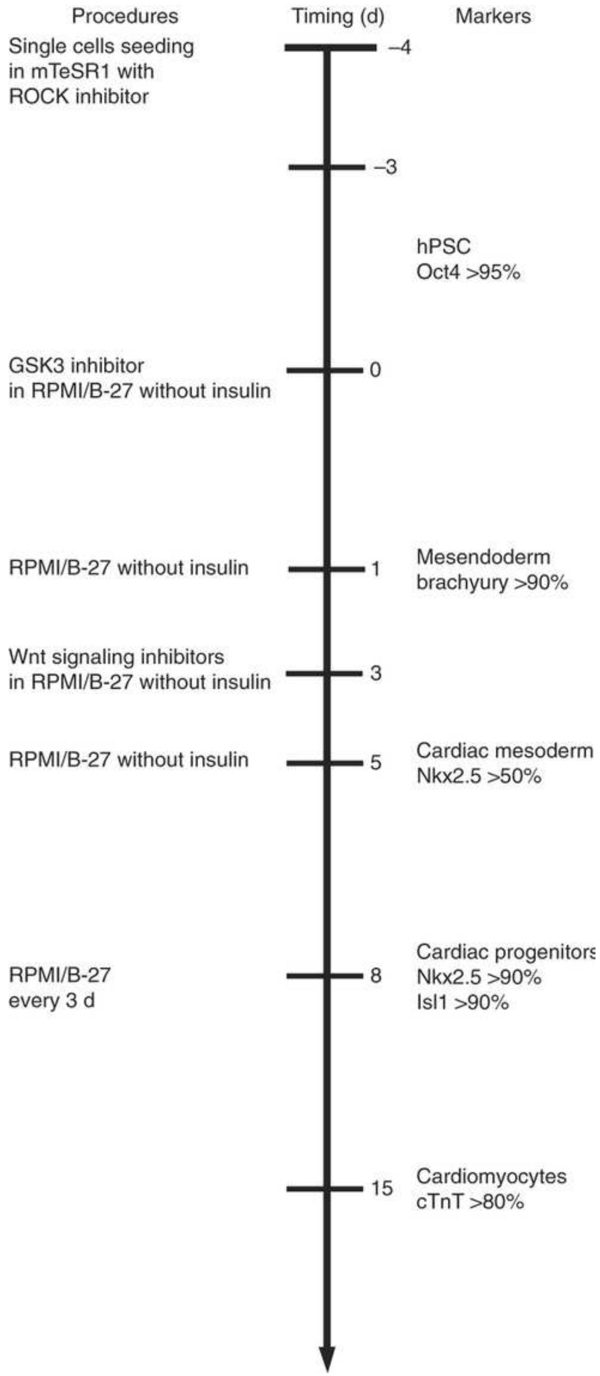
nature protocols

NATURE PROTOCOLS | PROTOCOL

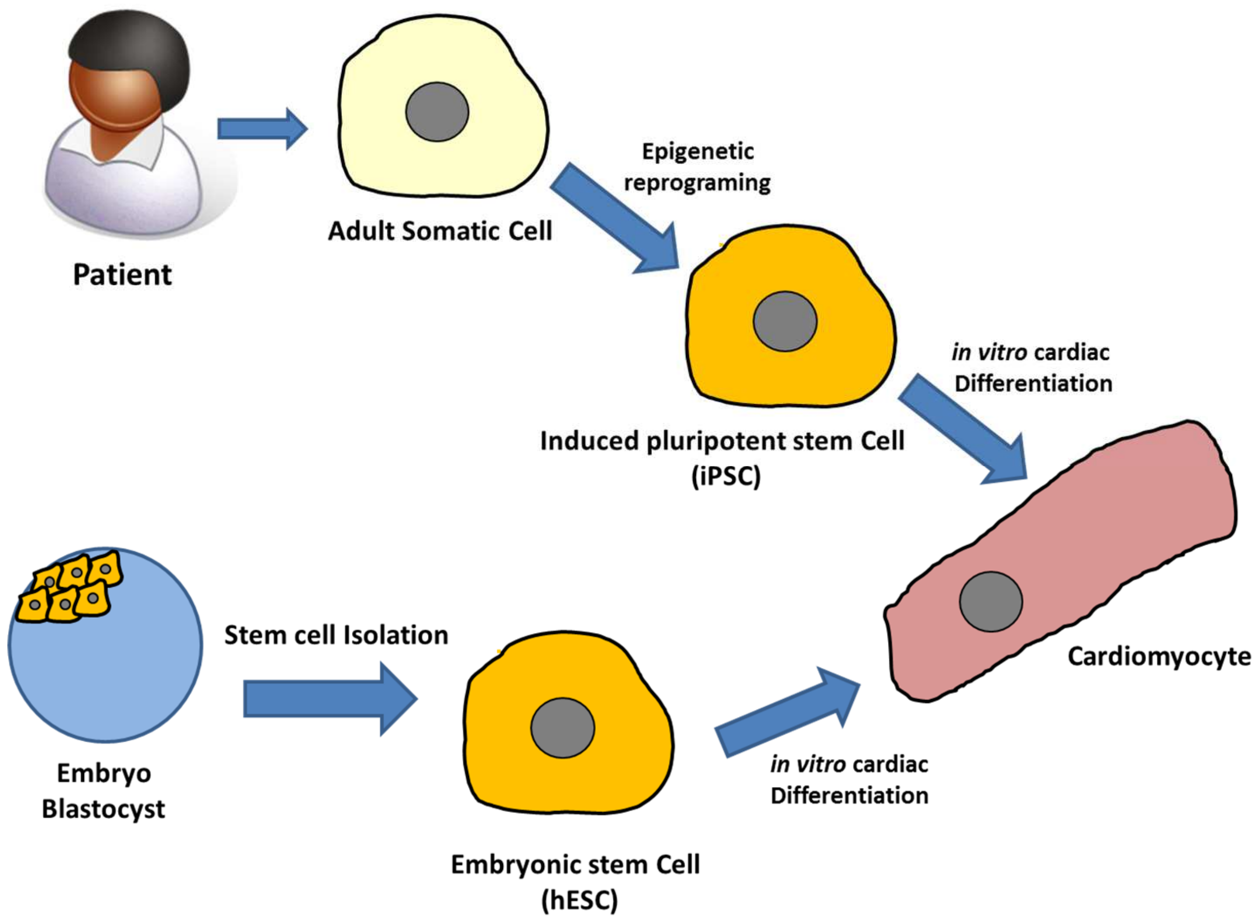
Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions

Xiaojun Lian, Jianhua Zhang, Samira M Azarin, Kexian Zhu, Laurie B Hazeltine, Xiaoping Bao, Cheston Hsiao, Timothy J Kamp & Sean P Palecek





Pluripotent stem Cell-Derived Cardiomyocytes

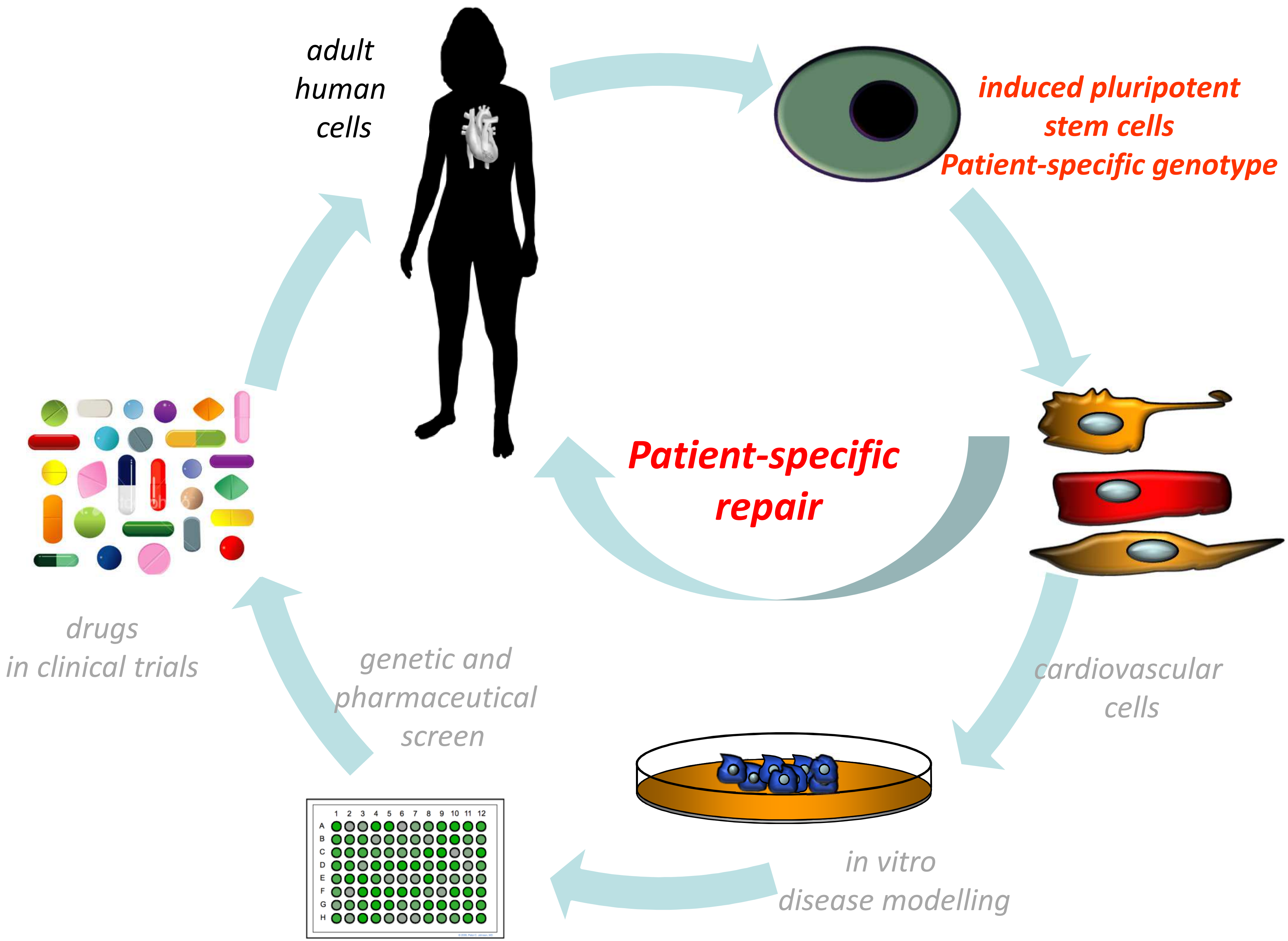


Human induced pluripotent stem cells -2008
Nobel Prize 2012

- Skin fibroblasts are treated with retroviruses carrying “stemness” factors discovered in embryonic stem cells

- They form embryonic-like stem cells which differentiate into many cell types (including cardiomyocytes):-

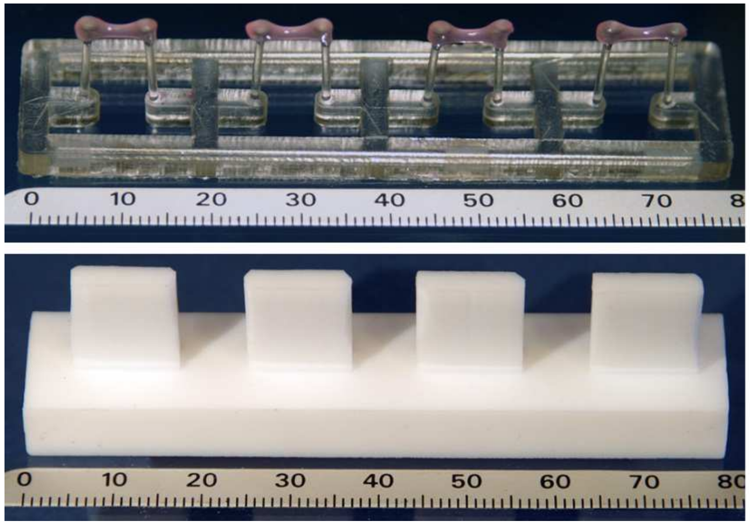
- This produces person-specific stem cells with potential for immune matching

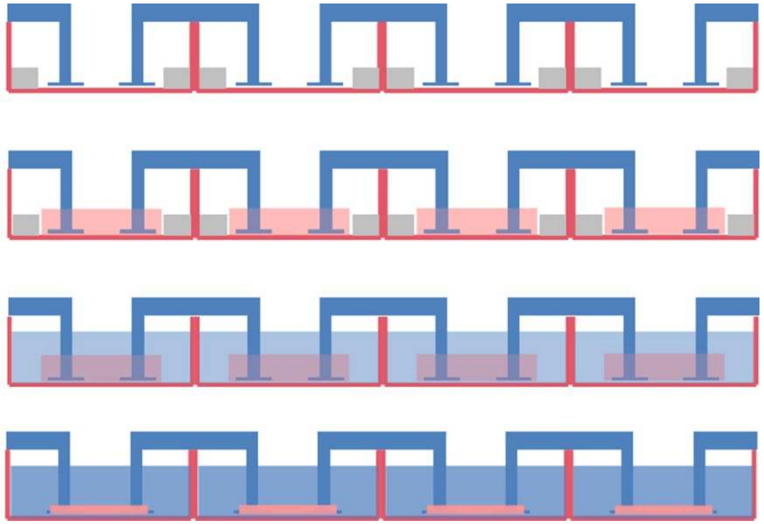

adult
human
cells
induced pluripotent
stem cells
Patient-specific genotype
Patient-specific
repair
cardiovascular
cells
in vitro
disease modelling
genetic and
pharmaceutical
screen
drugs
in clinical trials

3D constructs for implantation and modelling

Fibrin-based mini engineered heart tissue (FBME)

Engineered heart tissue: three-dimensional, force-generating,
reconstituted heart tissue



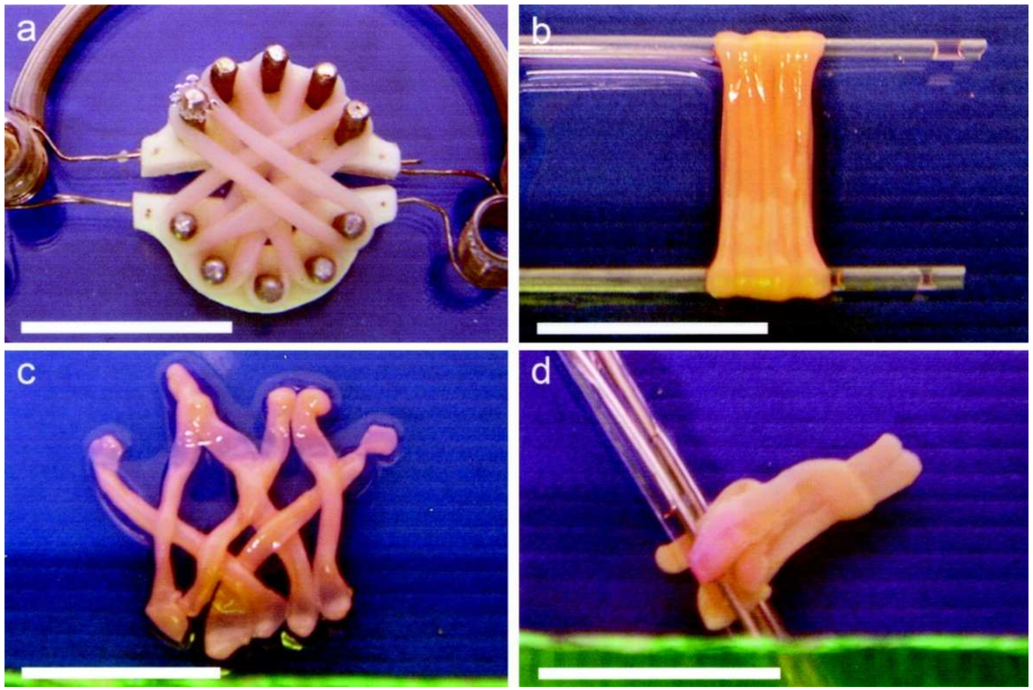


- Generation under standardized conditions

Thomas Eschenhagen, Hamburg

Engineered heart tissue

Human stem cell-derived cardiomyocytes in fibrin



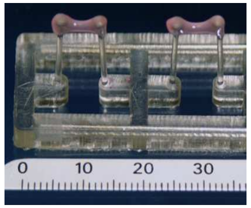
Imperial College London

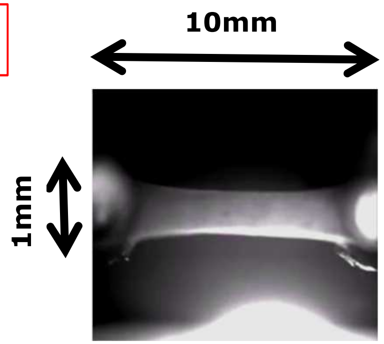
Upscaled engineered heart tissue

- Second generation EHTs significant up-scaling:
  - 1 EHT per well in 6 well plate
  - 1.7ml of master mix compared to 100ul
  - 15-20 vs 0.5 million cells per EHT

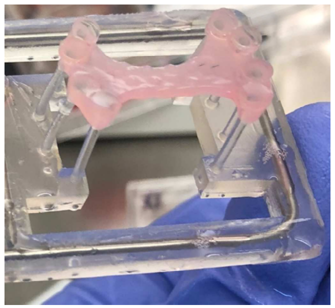
- Master mix and manufacturing similar

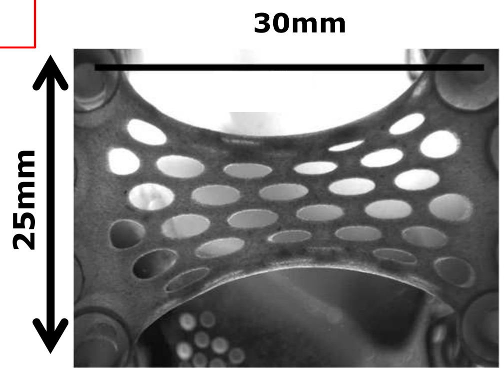
First Generation

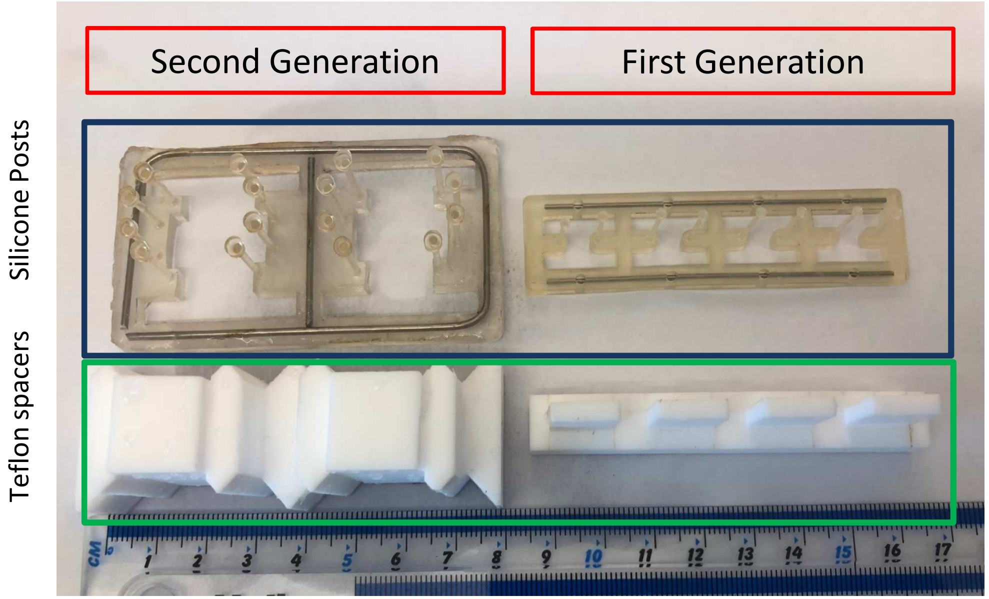




Second Generation





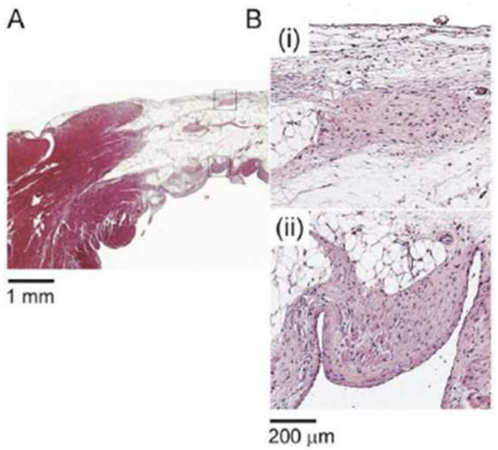


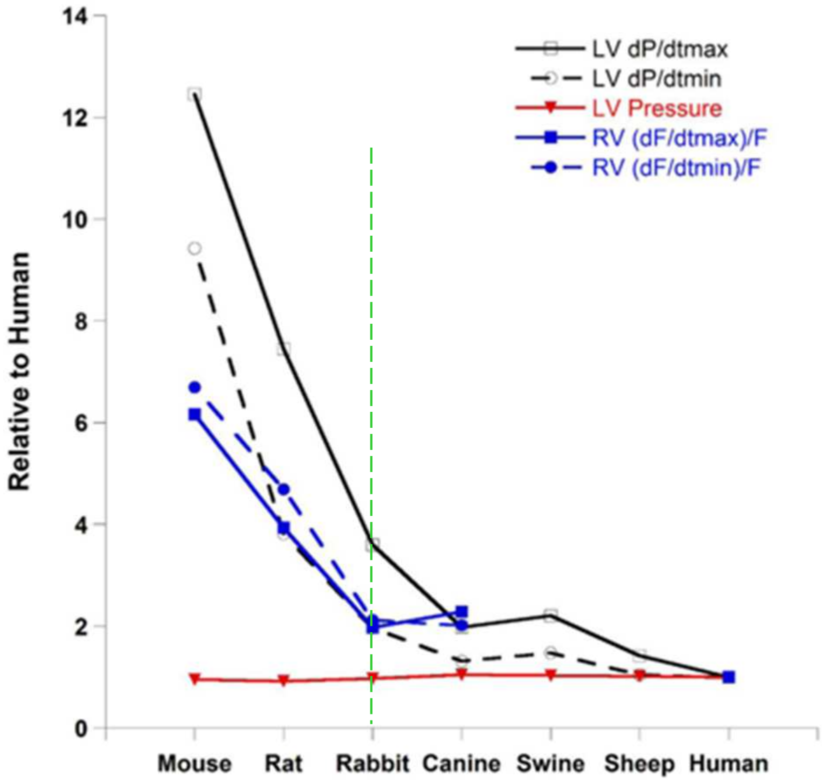
- Upscaling achieved predominantly by development of silicone posts and teflon spacers

Prof Eschenhagen, Dr F Weinberger, Dr T Owen

In vivo rabbit model of myocardial infarction- a bridge/replacement for large animal studies

- Rabbit myocytes have similar mechanisms of repolarization / action potential morphology
- Scar morphology is similar
- Heart failure/post myocardial infarction syndrome has human similarities
- The lack of collateral circulation enables a consistent infarct size
- Relatively tolerant of immunosuppression



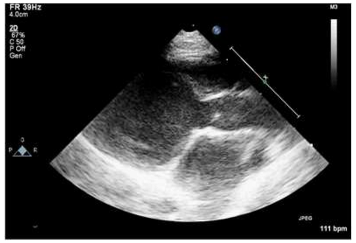


Milani-Nejad et al. Pharmacol Ther. 2014 Mar; 141(3): 235–249.

Rabbit in-vivo grafting protocol

Thanks to Hannah Jones, Phil Rawson, Alasdair Gallie, Lindsay Benson

Functional changes

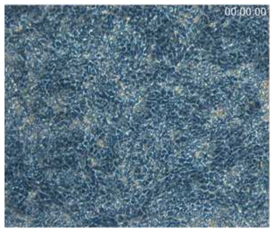


Arrhythmia

Telemetry

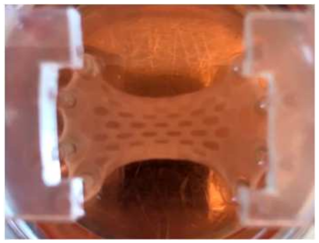
- 56 days

iPSC-CM in-vitro differentiation / maturation



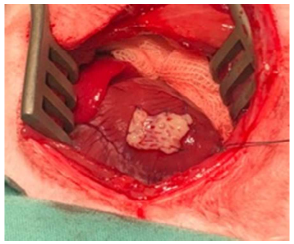
- 28 days

EHT creation



Day 0

EHT grafting



Controls
MI
Heart Failure

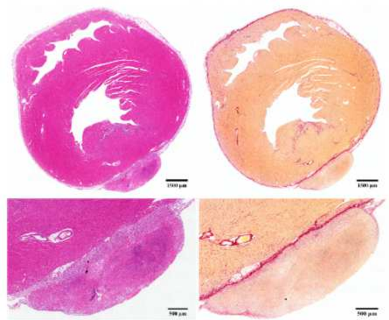
cyclosporin
methylprednisolone
starting day -5

7-28 days

Explantation

Optical mapping

Histology/ICC



https://www.dicardiology.com/content/first-reveal-linq-insertable-cardiac-monitor-implanted

Progress to date

- Development and characterization of upscaled EHT patch
- Feasibility of grafting on both control and infarcted rabbit hearts
- EHT do not appear arrhythmogenic
- EHTs are supplied by vessels that appear to be from the rabbit in origin
- Significant troponin retention at 4 weeks
- Evidence of possible synchronisation between graft and host

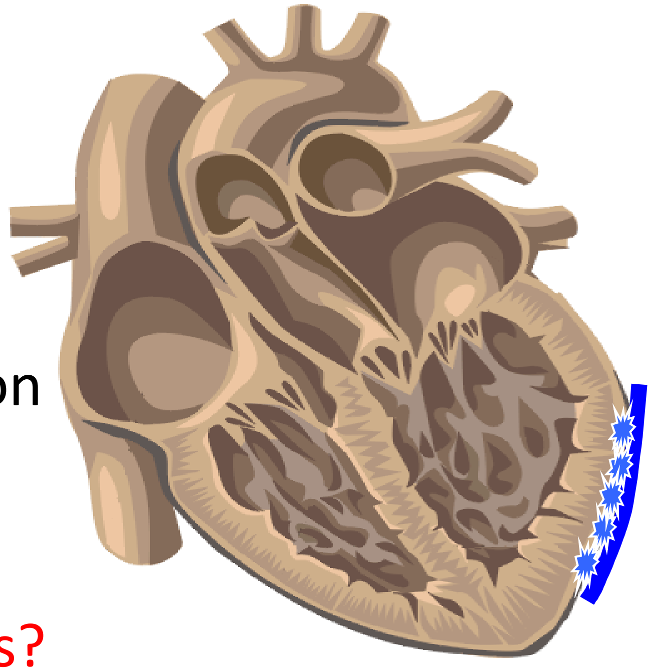
<u>Work-in-progress</u>

- Optimise immunosuppression/cell protection factors to improve retention
- Separate out in time the MI and patch placement (heart failure model)
- Addition of materials
- Move to GMP conditions
- Develop less invasive delivery methods for pig/human

CN1

A patch for stem cell delivery to the heart

- Applies cells directly to damaged area
  - *Can be prepared in advance*
- Maintains cells in the right position
- Supports the scar to prevent its expansion
- Can we use materials with conduction properties to reduce irregular heart beats?



Slide 21

CN1 This slide is a good explanation of this work for this audience

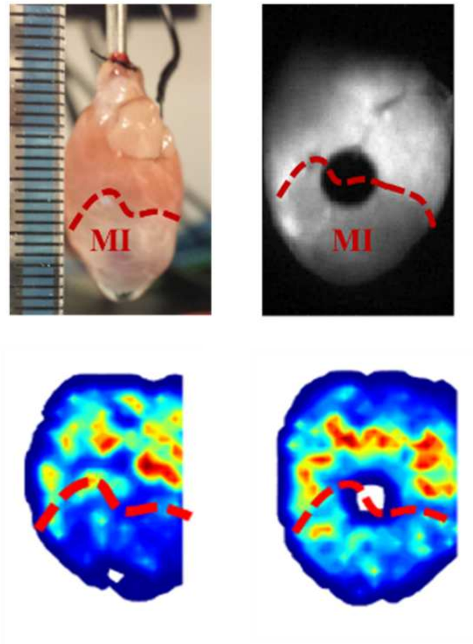
This is something they will be able to understand and see its clinical applications well.

Christie Norris, 22/02/2017

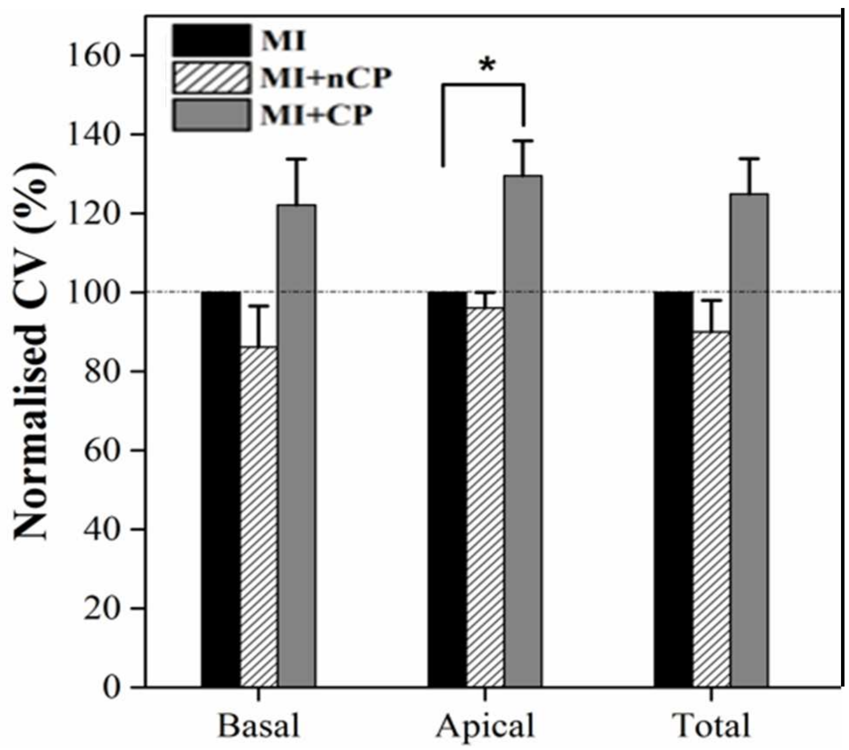
Imperial College London

Conductive Patch speeds contraction over damaged heart

Patch applied to centre of LV bridging infarcted and non-infarcted myocardium



MI Heart



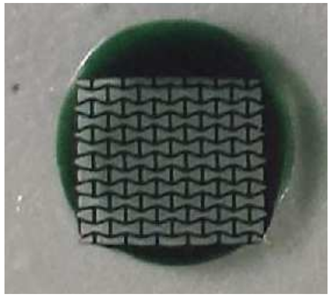
Significant <u>increase</u> in CV in MI hearts

Mawad et al, ScienceReports 2016

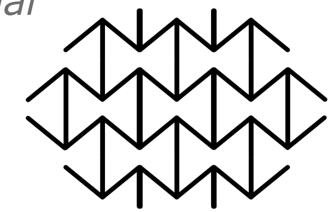
Imperial College
London

Improving biocompatability using auxetic patterning

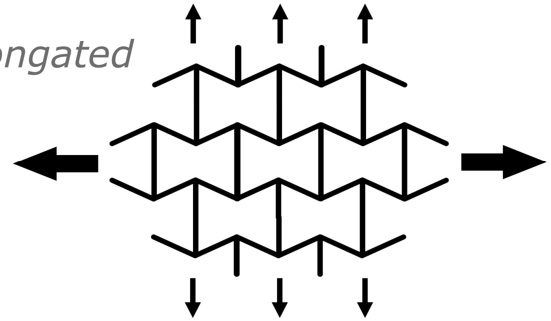
Auxetic micropatterning aims to mimic direction of cells in the normal heart

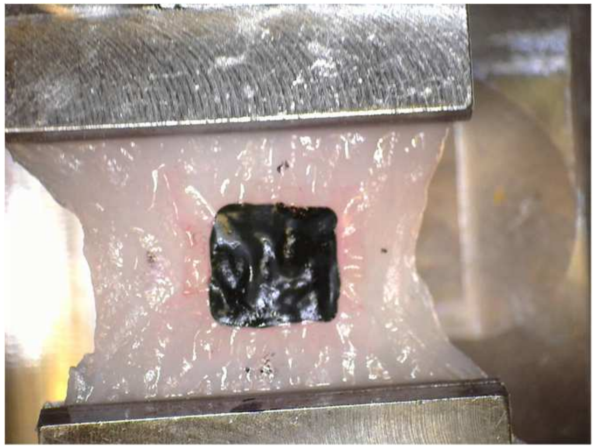


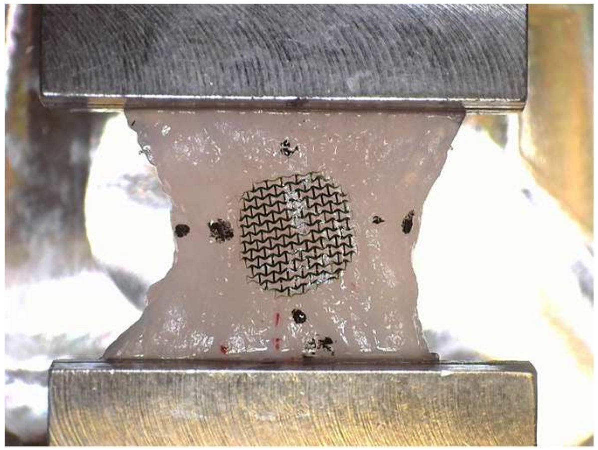
Original



Elongated







Replacement – Tesco chicken breasts

Kella Kapnisi, Catherine Mansfield

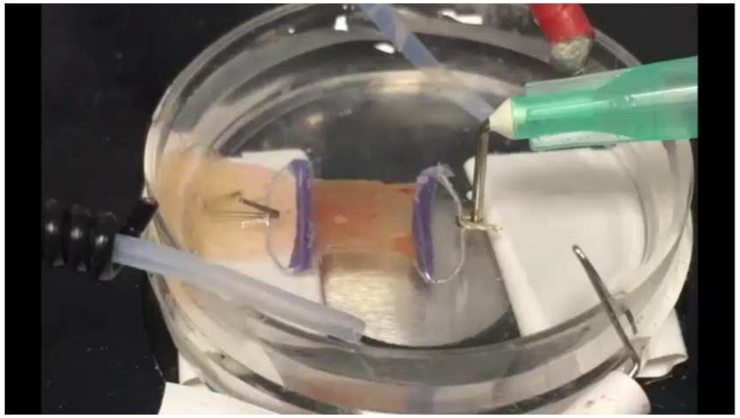
The 3Rs

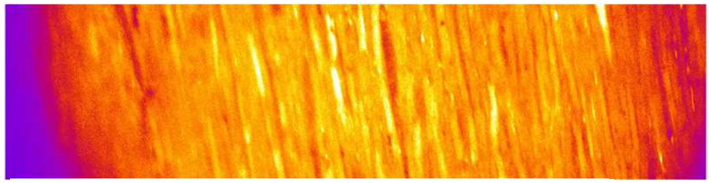
- Refinement
  - Floor pens and enrichment for rabbits
  - V-gel for intubation
  - CO2 monitoring during surgery
  - Care with diet post-op
  - Minimally invasive telemetry using linq devices

- Replacement
  - Rabbit as model suitable for regulatory submissions
  - Ex vivo ultrathin slice model for cell integration
  - Human iPSC-derived cardiomyocytes in disease modelling

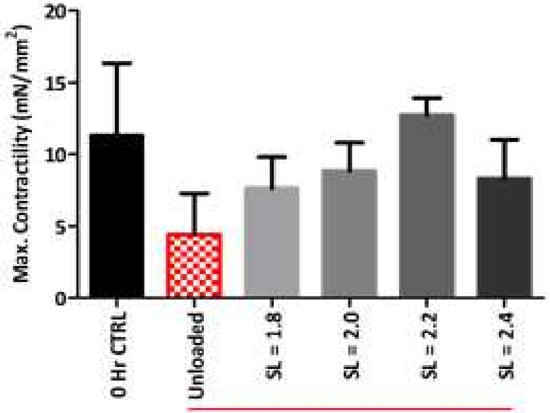
Models for iPSC-CM integration – ultrathin myocardial slices

Cesare Terracciano, Filippo Perbellini and the Cell Electrophysiology lab, NHLI, Imperial College London



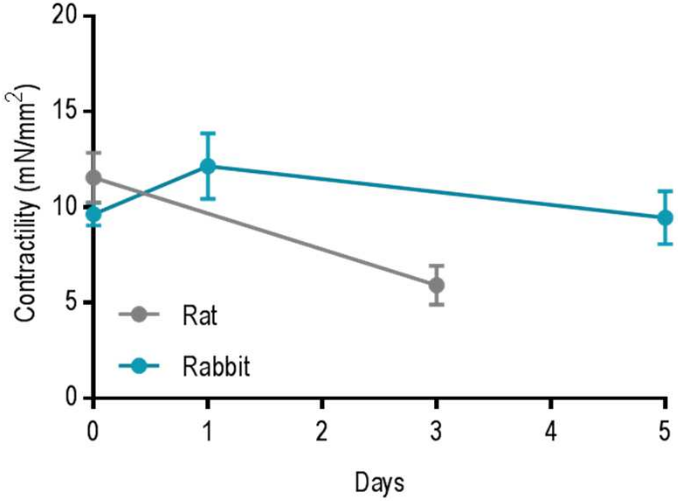


Human Heart slice



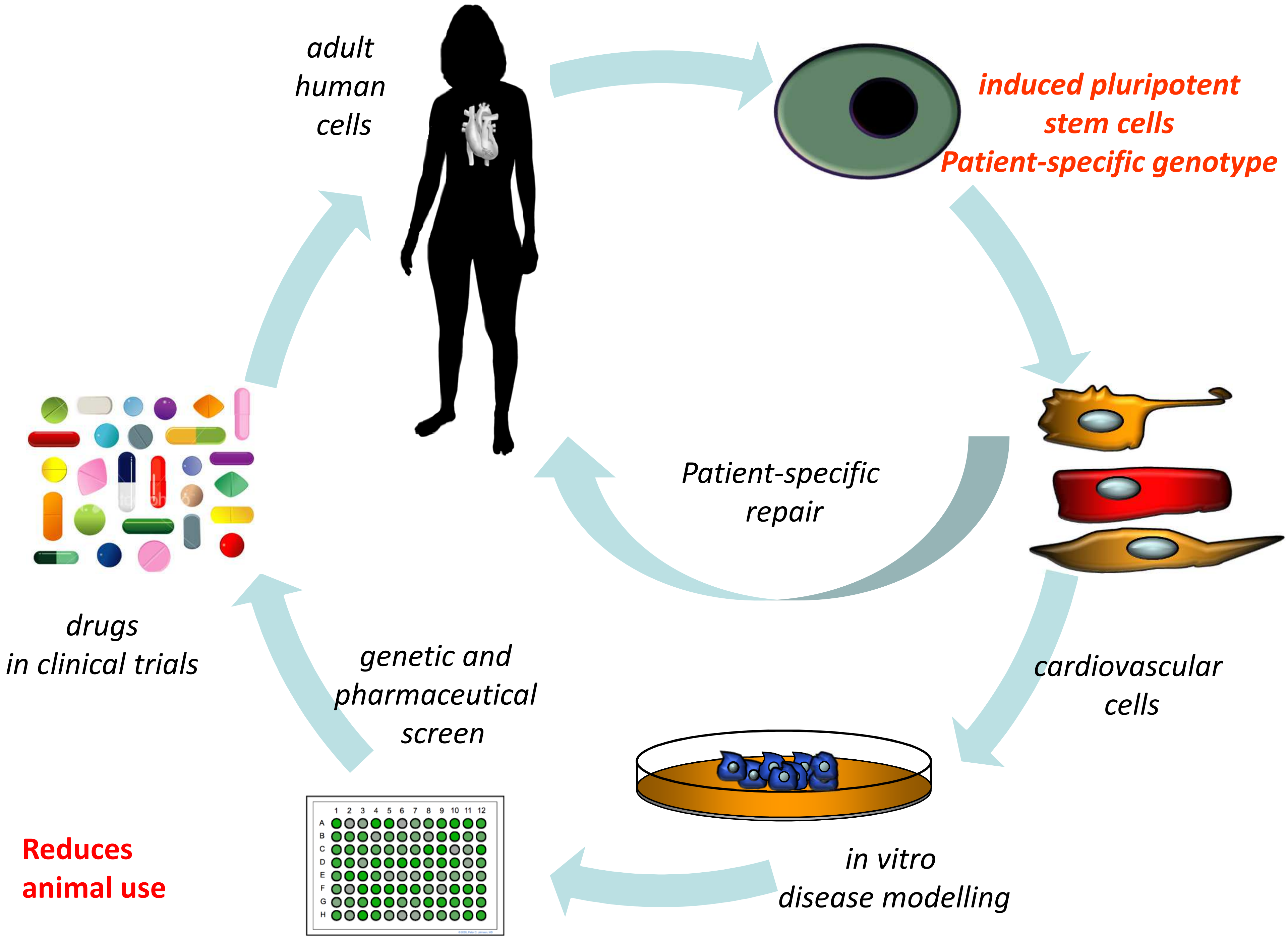
OBJECTIVES

- *Extension of the survival time of the slice through modification of electro-mechanical stimulation and culture conditions* : we have extended culture conditions of rabbit ventricular slices to 5 day culture without loss of contractile reserve.



- *Understanding of the timing and mechanisms of hESC-CM (or hiPSC-CM) engraftment:* we have found that hiPSC-CMs easily attach and beat on slices after 24hrs but not synchronously with the slice.

After 48hrs hiPSC-CMs start beating synchronously but this seems to be due to mechano-electrical activation rather than to electrical integration.


adult human cells
induced pluripotent stem cells
Patient-specific genotype
cardiovascular cells
in vitro disease modelling
genetic and pharmaceutical screen
drugs in clinical trials
Patient-specific repair
Reduces animal use

Human induced pluripotent stem cell-derived cardiomyocytes in disease modelling

Long QT syndromes
LQT1 (KCNQ1 mutations)
LQT2 (KCNH2 mutations)
LQT3 (SCN5A mutations)
LQT8 (Cav1.2 mutations, Timothy syndrome)

Arrhythmogenic right

LETTER
doi:10.1038/nature09747

Modelling the long QT syndrome with induced pluripotent stem cells

Ilanit Itzhaki[1]*, Leonid Maizels[1]*, Irit Huber[1]*, Limor Zwi-Dantsis[1], Oren Caspi[1], Aaron Winterstern[1], Oren Feldman[1], Amira Gepstein[1], Gil Arbel[1], Haim Hammerman[2], Monther Boulos[2] & Lior Gepstein[1,2]

European Heart Journal (2011) **32**, 952–962
doi:10.1093/eurheartj/ehr073
FASTTRACK BASIC SCIENCE

Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation

Elena Matsa[1], Divya Rajamohan[1], Emily Dick[1], Lorraine Young[1], Ian Mellor[2], Andrew Staniforth[3], and Chris Denning[1]*

The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 OCTOBER 7, 2010 VOL. 363 NO. 15

Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome

Alessandra Moretti, Ph.D., Milena Bellin, Ph.D., Andrea Welling, Ph.D., Christian Billy Jung, M.Sc., Jason T. Lam, Ph.D., Lorenz Bott-Flügel, M.D., Tatiana Dorn, Ph.D., Alexander Goedel, M.D.,

Cardiomyopathies

European Heart Journal
doi:10.1093/eurheartj/ehs096
BASIC SCIENCE

Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients

Limor Zwi-Dantsis[1,2], Irit Huber[1], Manhal Habib[1], Aaron Winterstern[1], Amira Gepstein[1], Gil Arbel[1], and Lior Gepstein[1,3]*

LETTER
doi:10.1038/nature09855

Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome

Masayuki Yazawa[1], Brian Hsueh[1]†, Xiaolin Jia[1]†, Anca M. Pasca[1]†, Jonathan A. Bernstein[2], Joachim Hallmayer[3] & Ricardo E. Dolmetsch[1]

nature Vol 465|10 June 2010|doi:10.1038/nature09005
LETTERS

Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome

Xonia Carvajal-Vergara[1,2], Ana Sevilla[1]*, Sunita L. D'Souza[1]*, Yen-Sin Ang[1], Christoph Schaniel[1], Dung-Fang Lee[1], Lei Yang[1], Aaron D. Kaplan[3], Eric D. Adler[3], Roye Rozov[1], YongChao Ge[4], Ninette Cohen[5], Lisa J. Edelmann[5], Betty Chang[1], Avinash Waghray[1], Jie Su[1], Sherly Pardo[5,6], Klaske D. Lichtenbelt[7], Marco Tartaglia[8], Bruce D. Gelb[5,6,9]* & Ihor R. Lemischka[1]*

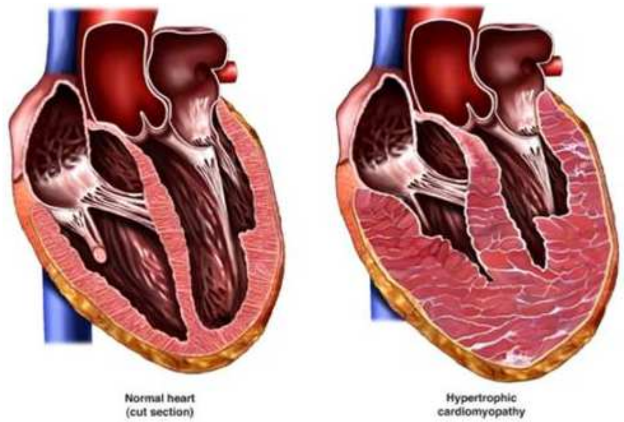
RESEARCH ARTICLE
STEM CELLS

Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy

Ning Sun,[1,2,3]* Masayuki Yazawa,[4]* Jianwei Liu,[5] Leng Han,[1,2] Veronica Sanchez-Freire,[1,2] Oscar J. Abilez,[6] Enrique G. Navarrete,[2] Shijun Hu,[1,2] Li Wang,[1,2,3] Andrew Lee,[1,2,3] Aleksandra Pavlovic,[1] Shin Lin,[1] Rui Chen,[7] Roger J. Hajjar,[8] Michael P. Snyder,[7] Ricardo E. Dolmetsch,[4] Manish J. Butte,[5] Euan A. Ashley,[1] Michael T. Longaker,[3,9] Robert C. Robbins,[10] Joseph C. Wu[1,2,3,10]†

Hypertrophic Cardiomyopathy

- Hypertrophic cardiomyopathy affects 1 in 500 of the population and is characterised by a thickening of the heart muscle.
- The E99K mutation in the ACTC gene causes apical hypertrophic cardiomyopathy.
- The ACTC E99K mutation is change in amino acid 99 from glutamine to lysine.
- There are at least 76 patients from ten families in Spain which have this mutation.



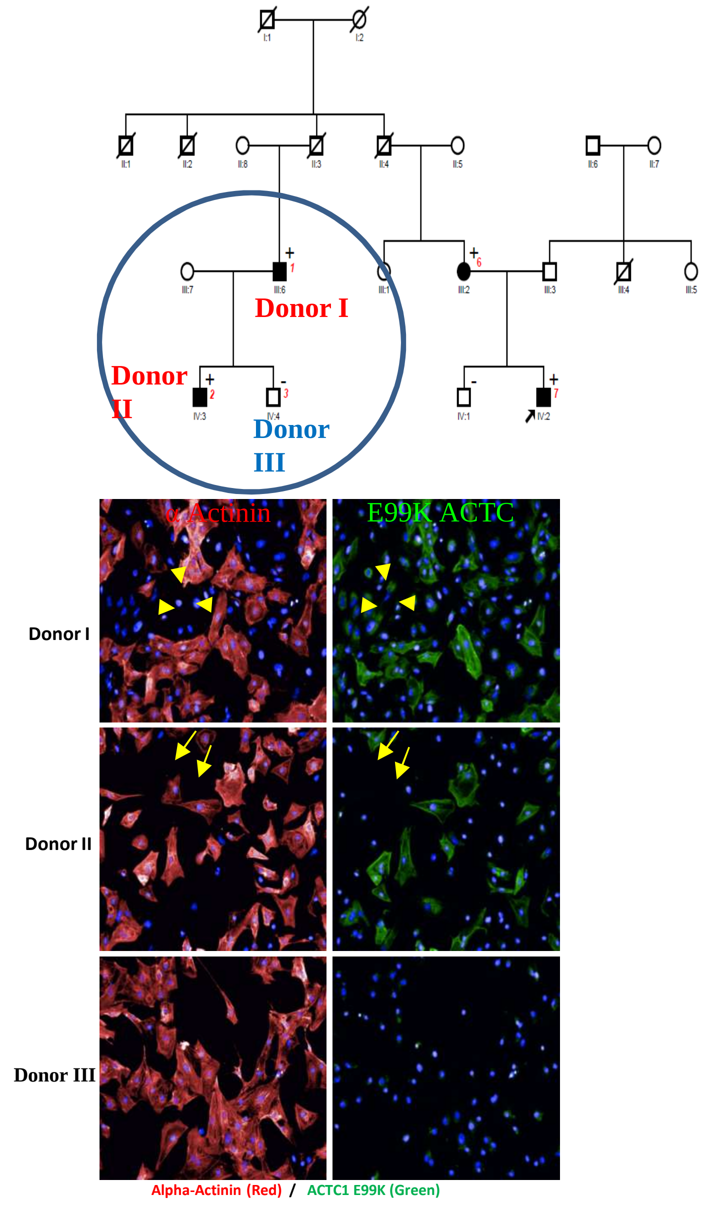
SAMPLE	FAMILY	INITIALS	AGE	GENOTYPE	PHENOTYPE
1	A	[redacted]	47	CARRIER	LVNC
2	A	[redacted]	18	CARRIER	LVNC
3	A	[redacted]	14	NON CARRIER	NORMAL
4	B	[redacted]	36	CARRIER	LVNC
5	B	[redacted]	24	CARRIER	LVNC
6	A	[redacted]	62	CARRIER	LVNC
7	A	[redacted]	34	CARRIER	LVNC
8	C	[redacted]	39	CARRIER	LVNC, ICD (NSVT, abnormal blood pressure response, family SD)
9	C	[redacted]	31	CARRIER	LVNC/ HCM, aborted SD, ICD, anoxic encephalopathy
10	C	[redacted]	70	NON CARRIER	NORMAL (mother; in this family the disease comes from the father, who died in 2006 and also suffered from LVNC and ASD
11	C	[redacted]	37	NON CARRIER	NORMAL
12	C	[redacted]	41	CARRIER	LVNC
13	B	[redacted]	37	CARRIER	LVNC
14	B	[redacted]	24	NON CARRIER	NORMAL
15	B	[redacted]	14	CARRIER	LVNC
16	B	[redacted]	38	CARRIER	LVNC + ASD (ostium secundum)
17	B	[redacted]	65	CARRIER	LVNC, atrial fibrillation
18	B	[redacted]	43	CARRIER	LVNC

Occluded for confidentiality

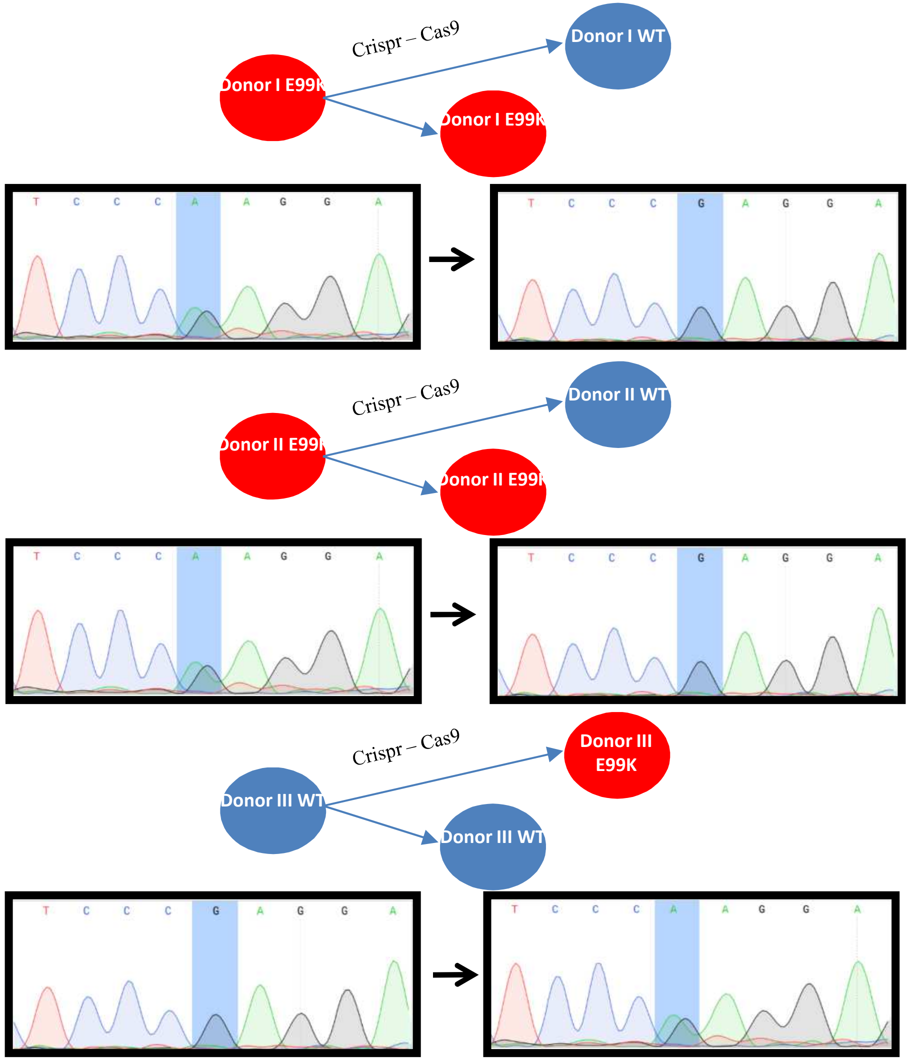
LVNC: Left ventricular non compaction SD: sudden death

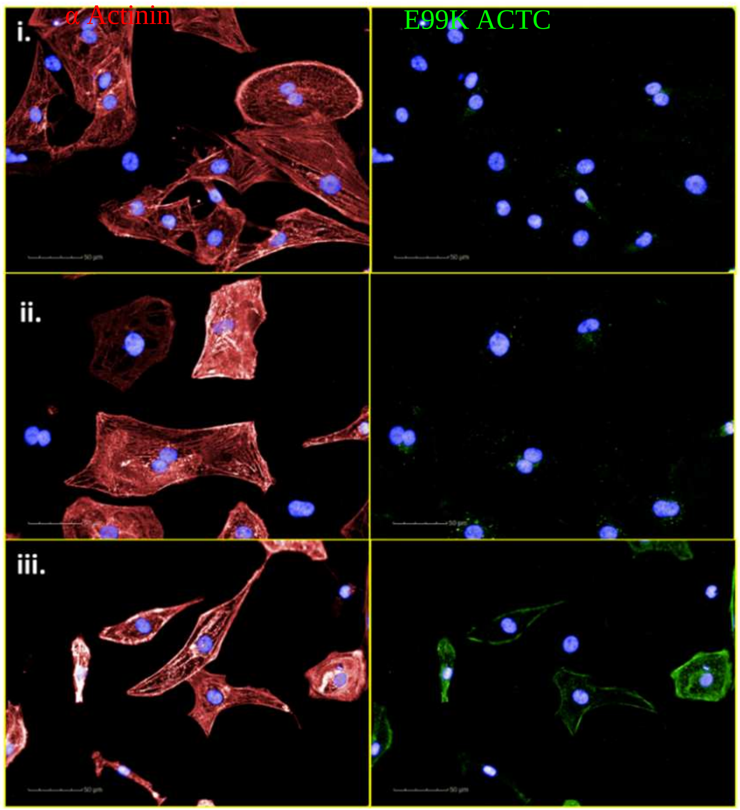
ASD: Atrial septal defect

HCM: Hypertrophic cardiomyopathy



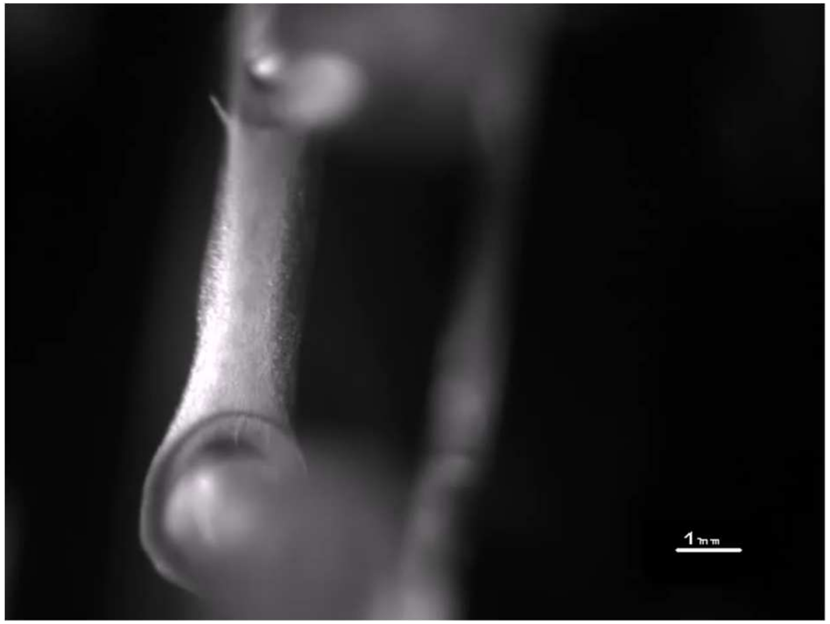
Gene edited lines to correct/insert mutation

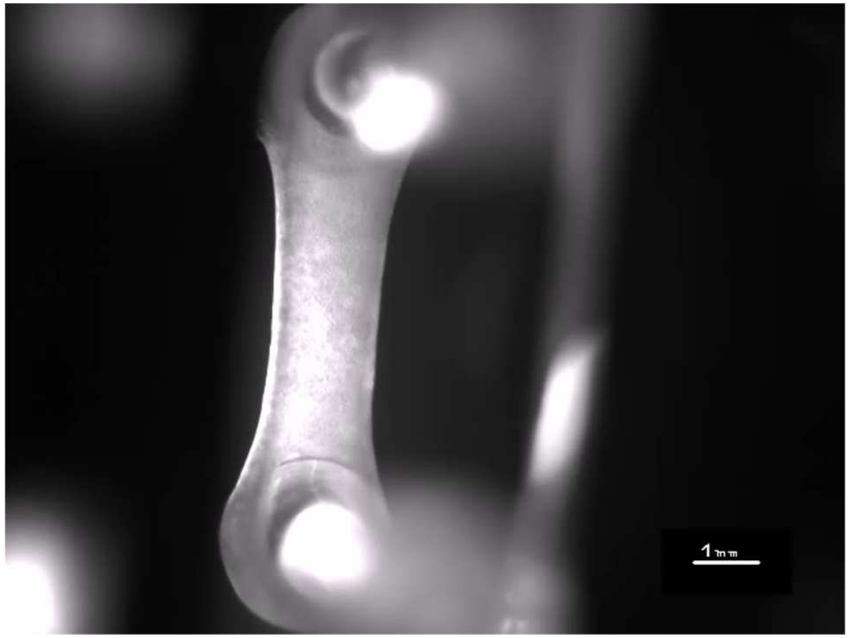


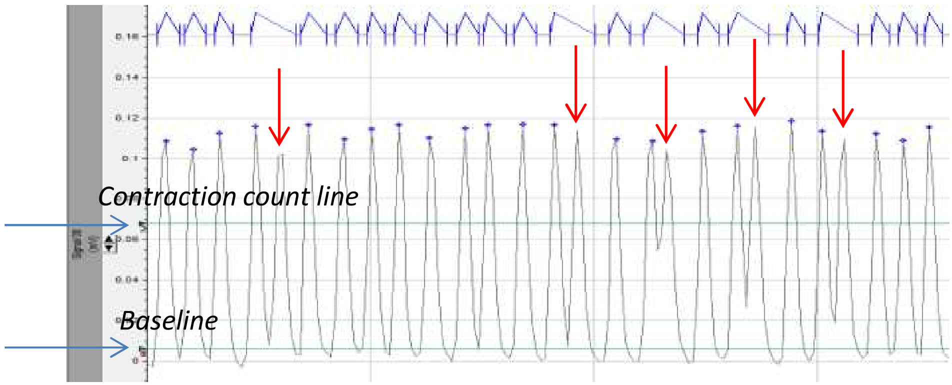


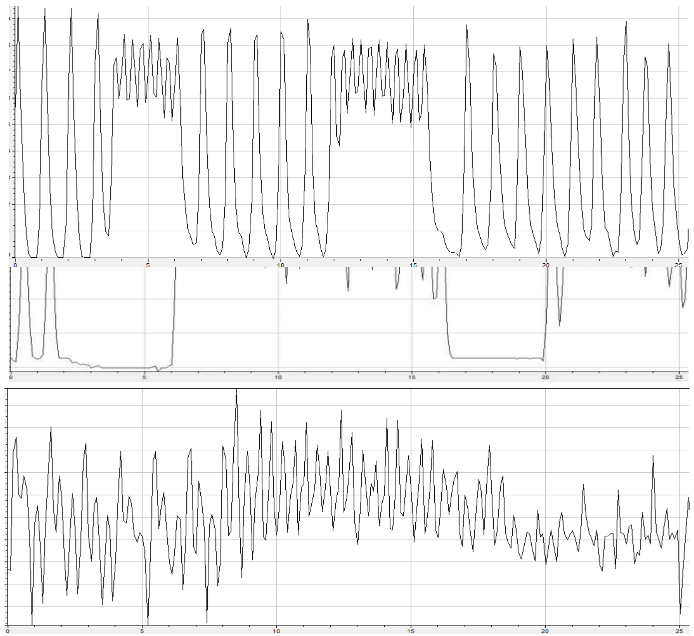
Smith, Owen et al Stem Cell Reports 2018

Arrhythmia in E99k EHTs

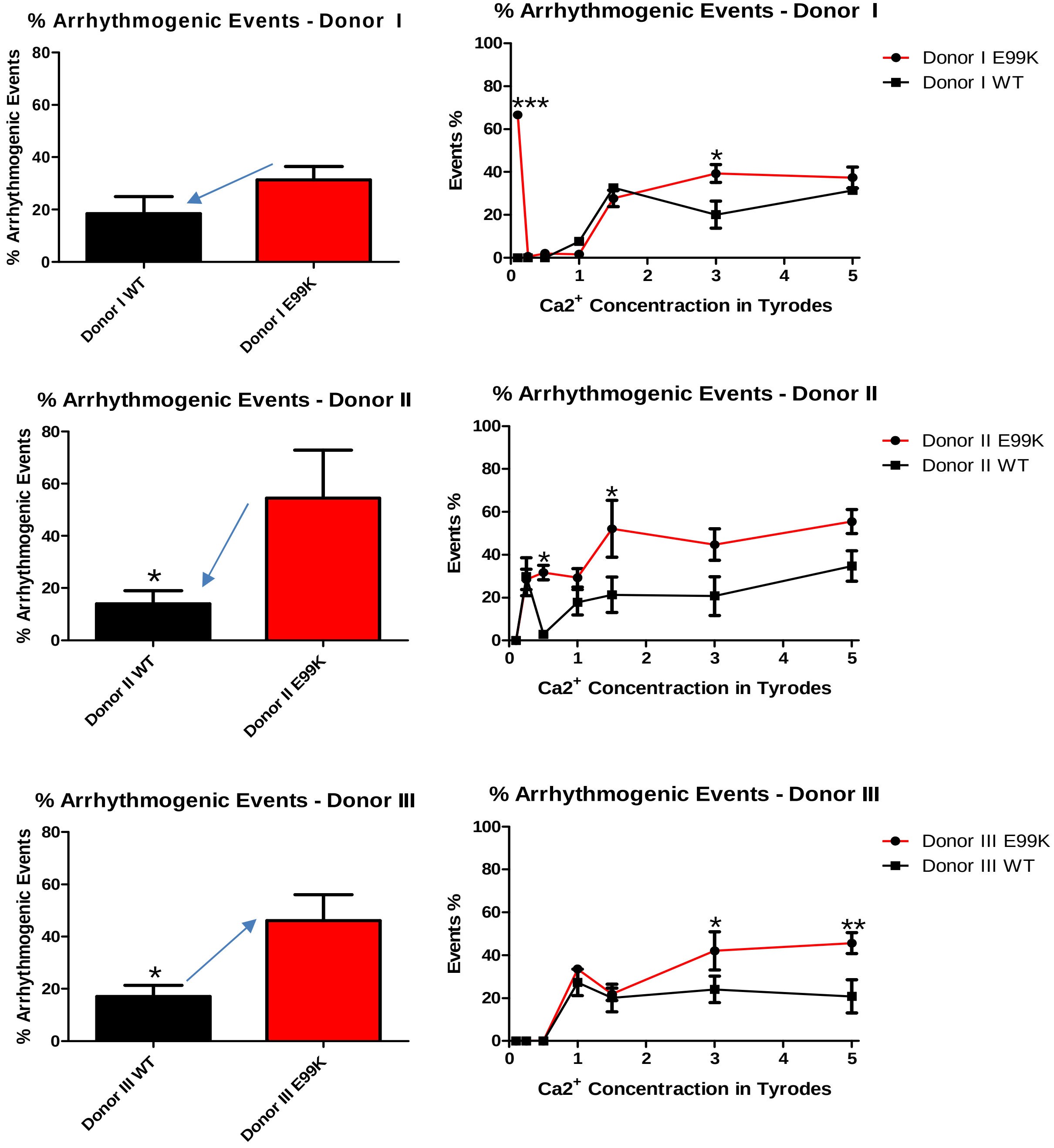




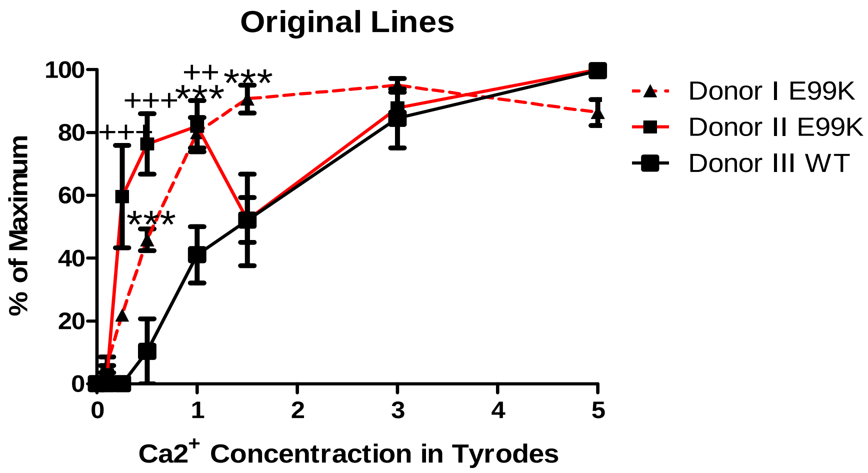


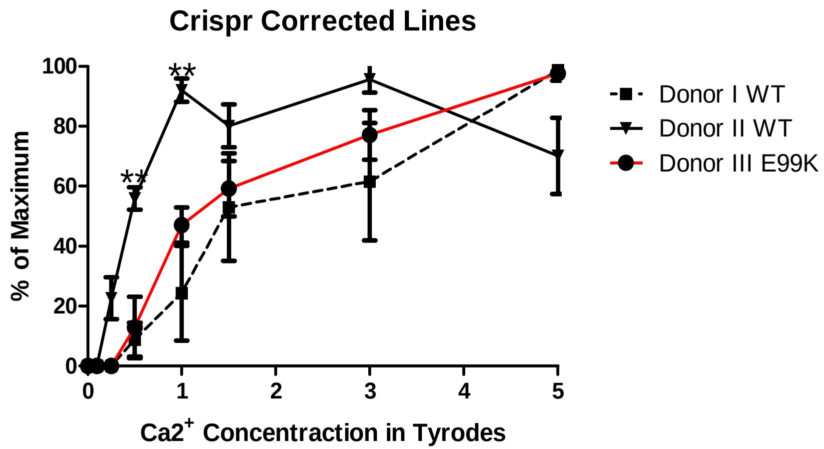


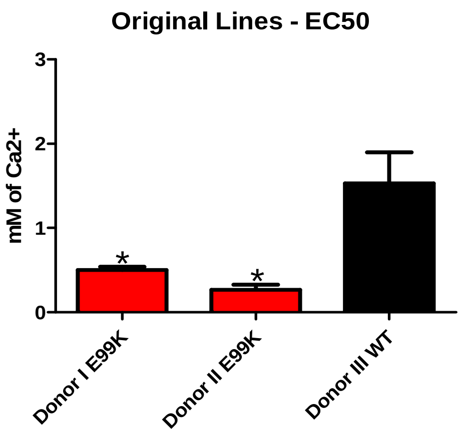
CRISPR correction of arrhythmia

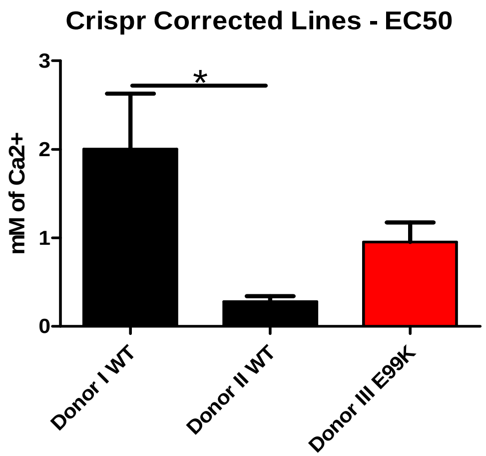


Calcium sensitivity in EHTs from E99K patients and a non-carrier









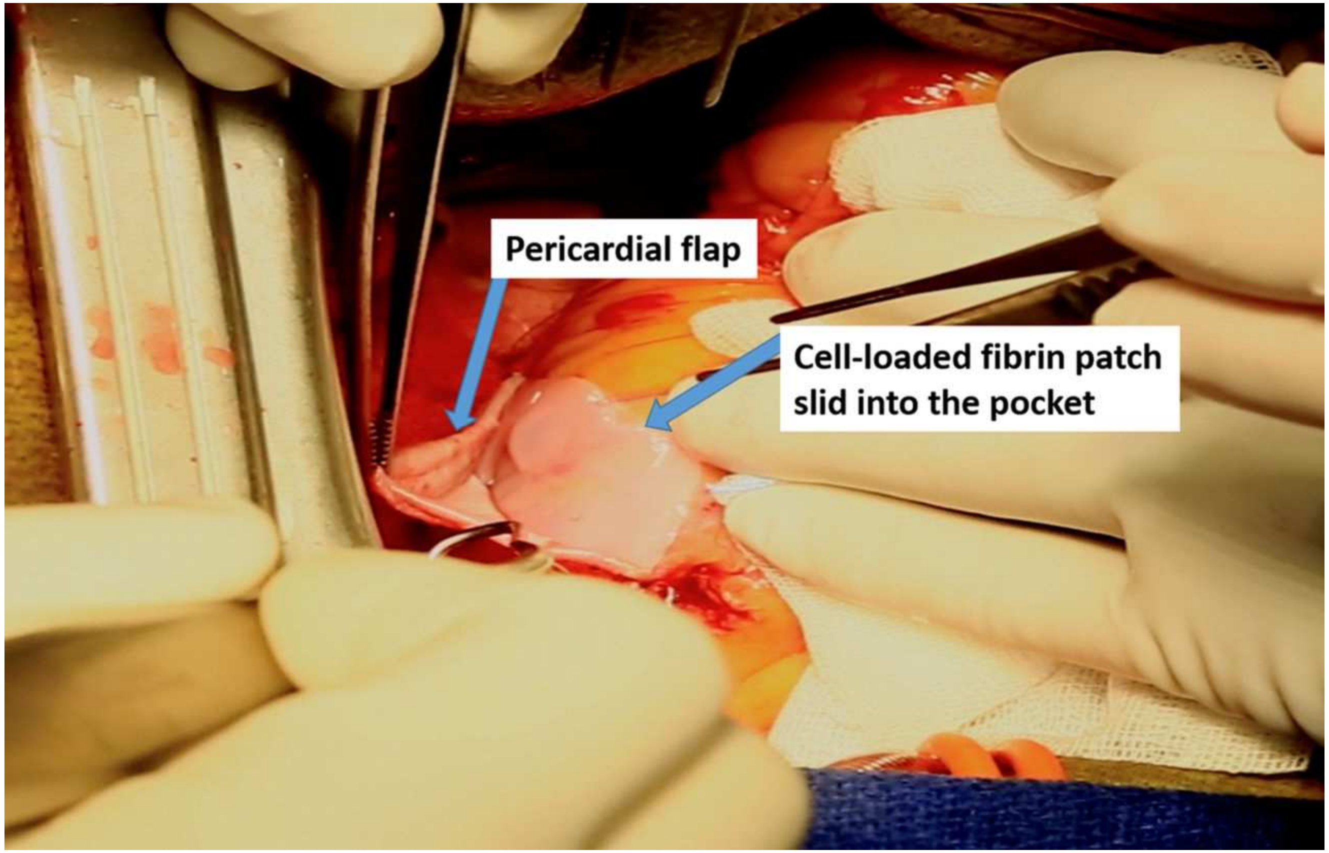
Non-carrier

Carrier

Gene editing of patient-derived iPSC lines is a powerful disease model, which can dissect effects of mutation versus background

Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report.

Intraoperative view of the progenitor cell-loaded fibrin patch that has been slid into the pocket between an autologous pericardial flap and the epicardial surface of the infarct area.



Menasche et al

European Heart Journal

Imperial College, NHLI

- **Richard Jabbour**
- **Tom Owen**
- Thusharika Kodagoda
- Prag Pandey
- Gabor Foldes
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British Heart Foundation

MENDING BROKEN HEARTS

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Stem Cells for Safer Medicines