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

Professor Sian E. Harding

Director, Imperial BHF Centre for Cardiac Regeneration
Human stem cell-derived cardiomyocytes (SC-CM) in vivo cardiac muscle regeneration and paracrine-mediated repair
Natural history of heart failure

Damage

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

Apparent recovery/compensation

Heart thickening
Dilation
Volume loading
Sympathetic stimulation

Decompensation/
Heart failure

Repair

Gene therapy
Revascularisation

Drugs

Death

Repair

Drugs

Devices
Transplantation
Structure of the contracting myocardium

Myocyte (muscle cell)
Regeneration of cardiomyocytes? Carbon dating the heart

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?

- 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**

**Induced pluripotent stem cells**

**Pluripotent cells**

**Transdifferentiation**

**Cell isolation**

**Mobilisation and homing**

**Delivery**
- Surgical
- Intracoronary
- Intravenous
- Intramyocardial

**Circulating 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;
- Left anterior descending coronary artery;
- Circumflex artery.

Intracoronary
Intramyocardial
Transendocardial
Intravenous
Results of bone marrow stem cell implantation for heart disease

- Started around 10 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
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

Patient

Embryo Blastocyst

Stem cell Isolation

Embryonic stem Cell (hESC)

Induced pluripotent stem Cell (iPSC)

Epigenetic reprogramming

in vitro cardiac Differentiation

Cardiomyocyte
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
Patient-specific genotype

in vitro disease modelling

cardiovascular cells

drug in clinical trials

Genetic and pharmaceutical screen

Patient-specific repair

induced pluripotent stem cells

Patient-specific genotype

adult human cells
Fibrin-based mini engineered heart tissue (FBME)

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

3D constructs for implantation and modelling

- Generation under standardized conditions

Thomas Eschenhagen, Hamburg
Engineered heart tissue

Human stem cell-derived cardiomyocytes in fibrin
• 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

• 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

Rabbit in-vivo grafting protocol
Thanks to Hannah Jones, Phil Rawson, Alasdair Gallie, Lindsay Benson

- 56 days
iPSC-CM in-vitro differentiation / maturation

- 28 days
EHT creation

Day 0
EHT grafting

7-28 days
Explantation

Functional changes

Arrhythmia

Telemetry

Optical mapping

Histology/ICC

Controls
MI
Heart Failure
cyclosporin
methylprednisolone
starting day -5

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

Work-in-progress

- 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
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?*
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
Conductive Patch speeds contraction over damaged heart

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

Mawad et al, ScienceReports 2016

Significant increase in CV in MI hearts
Improving biocompatibility using auxetic patterning

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

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

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.
induced pluripotent stem cells
Patient-specific genotype

adult human cells

Patient-specific repair

drugs in clinical trials

genetic and pharmaceutical screen

Reduces animal use

cardiovascular cells

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

Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation
Elena Matsa, Divya Rajamohan, Emily Dick, Lorraine Young, Ian Mellor, Andrew Staniforth, and Chris Denning

Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome
Manouk Yannas, Brian Houston, Xiaolin Ju, Ana M. Pascual, Jonathan A. Bernstein, Joachim Hallmann, and Ricardo E. Dilmeth

Patient-specific induced pluripotent stem cell-derived models of LEOPARD syndrome
Xenia Caragiannis-Vergara, Ana Sevilla, Sunita J. D'Souza, Yen-Sie Ang, Christoph Schmied, Dong Feng Lee, Lei Yang, Alice D. Adler, Remy Bayzoo, Yong-Chen Gu, Ninette Cohen, Lisa J. Edelmann, Betty Chang, Avnishk Waghaya, Jie Su, Sheryl Pardo, Klaus D. Lichtenbelt, Marco Tartaglia, Bruce D. Goldberg, and Het B. Lamichhane

Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients
Limor Zwi-Dantsis, Irit Huber, Manhal Habib, Aaron Winterstern, Amira Gepstein, Gil Arbel, and Lior Gepstein

Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy
Ning Sun, Masayuki Yazawa, Jianwei Liu, Leng Han, Veronica Sanchez-Freire, Oscar J. Abilez, Enrique G. Navarrete, Shijun Hu, Li Wang, Andrew Lee, Aleksandra Pavlovic, Shin Lin, Rui Chen, Roger J. Hajjar, Michael P. Snyder, Ricardo E. Dilmeth, Manish J. Butte, Euan A. Ashley, Michael T. Longaker, Robert C. Robbins, and Joseph C. Wu
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.
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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

% Arrhythmogenic Events - Donor I

% Arrhythmogenic Events - Donor II

% Arrhythmogenic Events - Donor III
Calcium sensitivity in EHTs from E99K patients and a non-carrier

Original Lines

Crispr Corrected Lines

Original Lines - EC50

Crispr Corrected Lines - EC50

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.
Imperial College, NHLI

- Richard Jabbour
- Tom Owen
- Thusharika Kodagoda
- Prag Pandey
- Gabor Foldes
- Nicola Hellen

CBS Staff
- Hannah Jones
- Phil Rawson
- Alasdair Gallie
- Lindsay Benson

Chris Denning – Univ. Nottingham
Godfrey Smith – Univ. Glasgow
Thomas Eschenhagen, UKE Hamburg
Ipsita Roy – Univ. Westminster

Rosetree’s Trust

SC4SM
Stem Cells for Safer Medicines

Mending Broken Hearts

British Heart Foundation

National Centre for the Replacement, Refinement and Reduction of Animals in Research