iPS cell-derived cardiomyocytes: overcoming barriers to therapeutic use

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(i) A retrospective look at approaches for heart repair
  : autologous bone marrow-derived SC
  : cardiac-derived (ckit+) SC

(ii) Contemporary approaches
  : ES-derived CM
  : iPS-CM
    (Direct fibroblast-to-CM conversion)

(iii) Problems with cell type and maturation

(iv) Excitation-contraction coupling and the network organization of cell signalling

(v) Systematizing iPS-CM maturation

(vi) Cellular determinism: towards predicting and controlling phenotype
• Just 5/49 trials using BMSC were free from ‘discrepancies’ (~10%)

• In those 5, mean EF effect size was zero

(i) Repairing the failing heart with bone marrow-derived stem cells (BMSC): the end of the line?
Repairing the failing heart with cardiac-derived SC: the fall-from-grace of c-kit+ lineage
Human embryonic-ster cardiomyocytes regenerate

James J. H. Chong1,2,3,4,5, Xiulan Yang2,3,5, Creighton W. I. William M. Mahoney Jr1,2,5, Benjamin Van Biber1,2,5, Natha Veronica Muschelli1,2,5, G. Michael Gough5, Keith W. Vogel4,5, Lil Fabon4,5, Hans Reinecke2,3,5, Edward A. Gill2, Veronic5 & Charles E. Murry3,4,5,6,7


Diameter = 43mm
Accentuate the negatives

1. Teratomer risk

2. Long term immunosuppression

3. 100% incidence of arrhythmias (especially early on)

4. Arrhythmogenic risk increases at slower heart rates (humans)

5. Is cardiac function improved? Development of alternans

6. Long term toxicity of pro-survival cocktail (sarcoma proteins)

The response

1. Risk overplayed; 98% CM in graft; zero teratomers in > 1000 rats

4. Unlikely to see increased risk in humans

5. Further work needed

6. As point 1
Beyond ES-derived CM: grafting EHT built from IPS-CM

IPS-CM in EHT

- ‘Immature’
- In situ maturation
  - sarcomeric extension by ~ 10% but still smaller than GP CM
  - >95% conversion of MLCA to MLCV isoform
- No arrhythmias reported
- Human DNA in spleen (2/7) and lungs (4/7); none in liver or kidney
(iii) The futility of selecting nodal-, atrial- and ventricular-like cells...
Hallmarks of cardiomyocyte immaturity

Table 1. Summary of the Differences Between Immature and Adult Cardiomyocytes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Immature Cardiomyocytes</th>
<th>Adult Cardiomyocytes</th>
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<tbody>
<tr>
<td>Morphology</td>
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<td>Sarcomere</td>
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<td>Myofibrils</td>
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<td>T-tubules</td>
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<td>Mitochondria</td>
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<td>Meta</td>
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<td>Multithreaded way</td>
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<td>Electrophysiology</td>
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<td>E-C coupling</td>
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<td>Gap junction distribution</td>
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<td>Conduction velocity</td>
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<td>Responses to β-adrenergic stimulation</td>
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</table>

Data refer to human pluripotent stem cell derivatives when possible. cTnl indicates cardiac troponin I; MHC, myosin heavy chain; and ssTnl, slow skeletal troponin I.

Long-term Culture
Substrate Stiffness
Cell Patterning
Biochemical Cues
Tissue Engineering
Mechanical Loading
Electrical Stimulation

Table 2. Major Cardiac Genes Unregulated (by ≥2-fold) in

- hPSC-CM indicates human pluripotent stem cell cardiomyocyte.
- Polarized to intercalated disks
  - 0.3–1.0 m/s
- Chronotropic response
- Inotropic reaction

Lack of inotropic reaction

IPS and epigenetic imprinting: they remember where they came from........

Epigenetic memory in induced pluripotent stem cells

K. Kim^1, A. I. Takeuchi, N. Jung^2, J. S & G. Q. Dale

Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells

Ryan Lister^1*, Mattia Pelizzola^1*, Yasuyuki S. Kida^2, R. David Hawkins^3, Joseph R. Nery^1, Gary Hon^3, Jessica Antosiewicz-Bourget^4,5, Ronan O’Malley^1, Rosa Castanon^1, Sarit Klugman^1, Michael Downes^2, Ruth Yu^2, Ron Stewart^4,5, Bing Ren^3,6, James A. Thomson^4,5,7,8, Ronald M. Evans^2 & Joseph R. Ecker^1

iPS-CM ready for the clinic??

A network approach to cellular signalling
(iv) Excitation-contraction coupling and the entrainment of surface membrane and intracellular SR ‘clocks’

Inhibition of SR clock unmasks an unusual Ca\textsuperscript{2+} entrainment in IPS-CM

Control

CPA

Edwards (unpub.)
Activation via the beta-adrenoceptor pathway

Multiple downstream effectors ("Fight or flight" response)
The cell signalling ‘blueprint’ – everything linked to everything else
Excitation-contraction coupling

Ca²⁺ release

Mitochondria

Surface ion fluxes

Protein synthesis & degradation

Metabolism

Gene expression

Apoptosis

Inter-cellular synchronization

Intra-cellular synchronization

Horizontal and vertical network organization
Perturbing the homeostatic state: network adaptation

Alignment of ES-CM in culture

Maturation-linked refinement of Ca\textsuperscript{2+} handling

![Images showing cellular changes over days 2 to 7 with corresponding ΔCa\textsuperscript{2+} traces.]

- Day 2
- Day 3
- Day 4
- Day 5
- Day 6
- Day 7
SALVO-based profiling of cellular Ca\(^{2+}\): discriminating signals from noise

**Intracellular**

Frequency
\[ F = \frac{S}{s} \]

Amplitude
\[ A = \frac{amp_n - F_0}{F_0} \]

Duration
\[ D = \frac{d_a + d_b + d_c + d_n}{n} \]

Inter-transient noise (ITN)
\[ ITN = \frac{\sum(SV_1 \ldots SV_n)}{n} \]

Area
\[ Area = \frac{Area\ under\ curve}{F_0} \]

Rate of decay
\[ Rate = \frac{(k_a + k_b + k_c + k_n)}{n} \]

Temporal heterogeneity index (THI)
\[ THI = \frac{\sigma(\text{int}_1 \ldots \text{int}_n)}{n} \]

Amplitude heterogeneity index (AHI)
\[ AHI = \frac{\sigma(\text{ampa} \ldots \text{ampn})}{n} \]

Synchronization
\[ Synchronization = \frac{\text{Coincidence}(S_a \ldots S_n)}{n} \]

**Intercellular synchronization**

Cell coincidence
\[ Coincidence = 33.3\% (8/24) \]

Maturation of Ca\(^{2+}\) handling is linked to reduced Ca\(^{2+}\) signalling variability
Massively variable homeostatic Ca\textsuperscript{2+} handling

CoV = SD/mean
(v) Systematizing iPS-CM maturation: EB vs. monolayer culture

EB maturation is not associated with morphological change.
Increased post-disaggregation clustering from older EBs

A

(i) 14mm

A-area

(ii)

B-area

C-area

B

A-area

Cells per mm²

Maturation in EB (weeks)

Tau = 2.003

$\tau^2 = 0.095$

C

B-area

Cells per 0.02mm²

Maturation in EB (weeks)

Tau = 7.411

$\tau^2 = 0.083$

D

Plating heterogeneity (normalised)

Maturation in EB (weeks)

$\tau^2 = 0.959$

$\tau^2 = 0.694$

E

Proportion of B areas devoured (%)

Maturation in EB (weeks)

$\tau^2 = 0.727$

p = 0.007
Defining conditions for maximum $\text{Ca}^{2+}$ synchronization
Unmasking Ca\(^{2+}\) signalling phenotype

Switch \([\text{Ca}^{2+}]_{\text{ext}}\) from 400\(\mu\)M to 1.8mM \([\text{Ca}^{2+}]_{\text{ext}}\)
(vi) Cellular determinism
Towards predicting cellular response and heterogeneity

Homeostasis

State 1

State 2

State 3

Cause

Effect

Cell #
Predictably influencing cell-to-cell coupling in computational arrays

Variability of cell-to-cell coupling

Rotors in iPS-CM (Edwards, unpub)

SUMMARY

Progress made
• Maturity of cells
• Maturity of field
• More realistic endpoints

Barriers that still exist
• Transparency / dataset availability / understanding of ‘nuisance variables’
• Reliance on crude end-points of phenotype and function
• High levels of phenotypic variability
• Unpredictability
• Unknown impact of reprogramming / source

What’s needed?
• New ‘network’ approach to cellular variability and determinism
David Edwards  
Aled Jones  
Sarah Marsh  
Kimberley Lewis  
Catherine Hather  
Nicole Silvester  
Steven Barberini-Jammaers  
Phil Ashton  
Archana Jayanthi  
Alice Mitchell  
Jessica Wells  

**Cardiff**  
Catrin Williams  
David Lloyd  
Adrian Porch  
Dimitris Parthimos  

**Nottingham**  
Chris Denning  
Gary Duncan  
Divya Mirrington  

**Swansea (Engineering)**  
Perumal Nithiarasu  
Etienne Boileau  
Sanjay Pant  
Ankush Aggarwal
Does CM enrichment improve phenotype?

SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells

Nicole C Dubois1, April M Craft1, Parveen Sharma2, David A Elliott3, Edouard G Stanley3, Andrew G Elefant3, Anthony Gramolini1 & Gordon Keller1

To identify cell-surface markers specific to human cardiomyocytes, we screened cardiovascular cell populations derived from human embryonic stem cells (hESCs) against a panel of 370 known CD antibodies. This screen identified the signal-regulatory protein alpha (SIRPA) as a marker expressed specifically on cardiomyocytes derived from hESCs and human induced pluripotent stem cells (hiPSCs), and PECAM, THY1, PDGFRB and ITGA1 as markers of the nonmyocyte population. Cell sorting with an antibody against SIRPA allowed for the enrichment of cardiac precursors and cardiomyocytes from hESC/hiPSC differentiation cultures, yielding populations of up to 98% cardiac troponin T-positive cells. When plated in culture, SIRPA-positive cells were contracting and could be maintained over extended periods of time. These findings provide a simple method for isolating populations of cardiomyocytes from human pluripotent stem cell cultures, and thereby establish a readily adaptable technology for generating large numbers of enriched cardiomyocytes for therapeutic applications.

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5 sec  
ΔCa^{2+}  
(20 f.u.) 
Non-enriched SIRPA^{+}/VCAM^{+} SIRPA^{+}/VCAM^{-} 
Non-enriched SIRPA^{+}/VCAM^{+} ...
****
Non-enriched SIRPA^{+}/ VCAM^{+}  
0.0  
0.5  
1.0  
1.5  
2.0  
Synchronization  
(normalized)

Non-FACS (Day 7)

Non-FACS (Day 7)