Using pluripotent stem cells to model genetic disease of the heart

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The need for Human Cardiomyocytes

Heart failure
- ~20 million deaths annually worldwide
- Clinical benefit from skeletal or bone marrow stem cells???

Human Development / Genomics
- Insights into heart development / Nutritional modelling

Cardiotoxicity testing
- Poor in vitro tests / Preclinical: monkeys, dogs, rats, mice = 71%
  - E.g. chemotherapeutics up to 100x more tolerated by animals than humans
- Adverse drug reactions: 100,000 fatalities in US / year
- Drug withdrawals of ~$12 billion due to unexpected cardiotox

Disease modelling
- Poor understanding of numerous genetic disorders
- Availability of biopsies / Species differences
**Human vs Mouse**

- **bpm**
  - Human: ~60
  - Mouse: ~500

- **Increase bpm**
  - Human: More force
  - Mouse: Less force

- **ECG**
  - Human: ~450ms
  - Mouse: 50 - 100ms

- **K channel repolarisation**
  - Human: $I_{Kr}$, $I_{Ks}$
  - Mouse: $I_{to}$, $I_{K,slow1}$, $I_{K,slow2}$, $I_{SS}$

- **Gene expression**
  - Human: $aMHC$ (Atria), $bMHC$ (Ventricle)
  - Mouse: $aMHC$ (Ventricle & Atria)
Future Disease Modelling & Drug Discovery?

Best drugs identified & developed further

Patients with heart (or other) disease

1000s potential drugs
GWxx
PD-118057
Nicorandil
AS-siRNA
Dantrolene

Phenotype testing in lab

Patient-specific heart cells

Automation of process

Patient-specific hiPSC
or GM hESC

Best drugs identified & developed further

Future Disease Modelling & Drug Discovery?

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Modelling Genetic Disease: Long QT Syndrome

- Female 15 yrs old: 11x fainting +/- seizure
- ECG → polymorphic ventricular arrhythmia (240 bpm)
- Mutation in KCNH2 → K+ channel $I_{Kr}$ (HERG) → LQTS2

- Treatment:
  - Surgery for preventative implantable cardioverter defibrillator (ICD)
  - Prescribed life-long beta-blockers (propranolol)
    - Often fatigue, nausea, dizziness
LQTS Case Study

1) Healthy father
2) Carrier but asymptomatic mother
3) LQTS daughter

Clinic

Lab

QTc 455msec

Stress

β-blockers

QTc 571msec

Matsa (2011). Eur Heart J
How to Help?

OUTSIDE

Na⁺  Ca⁺⁺

INSIDE

K⁺  K⁺  X
Potential for Genetic Therapy?

- LQT2 [1681 G>A] = Dominant trafficking defect

**HERG**
(I\(_{\text{Kr}}\) channel)

**Pan Cadherin**
(Membrane)

*Matsa et al., 2013 Eur Heart J.*
Dominant Effect of HERG Mutation

- 4 HERG required per channel

![Diagram showing the effect of HERG mutations on channel function](image_url)

- Functional: Y (Yes), N (No)

- Need to increase WT and/or decrease M

- Transgenic overexpression
- In vivo gene targeting
- Ex vivo targeting + transplant
  - Allele-specific RNAi
Allele-Specific Knockdown in Model System

<table>
<thead>
<tr>
<th>Mut cDNA</th>
<th>5' CACCTTTGCCGCTCATCAGCACTGGCTAGCCTGCATC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3' GTGAAACCGAGTAGGTGCCTGACCGCATCGGACGTAG</td>
</tr>
</tbody>
</table>

- siRNA-A10 3' ttCCCGAGUAGUGCUGACCCG
- siRNA-A13 3' ttGAGUAGUGCGUGACCAGAUC
- siRNA-A15 3' ttGUAGUGCGUGACCAGAUCGG
- siRNA-A16 3' ttUGAGUGCGUGACCAGAUCGGA

Graphs showing the effect of different treatments on HERG expression and RQ values.
Allele-Specific Knockdown in LQT2 hiPSC-CM
Phenotypic Rescue

Matsa et al., 2013 Eur Heart J.
Duchenne muscular dystrophy

- Fatal X-linked condition
- 2.5Mb DMD gene
  - Encodes dystrophin
  - Dystrophin-Glycoprotein Complex

- Heart failure: up to 40% deaths
- Additional models beneficial
Patient mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type</th>
<th>Mutation</th>
<th>Region</th>
</tr>
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<tbody>
<tr>
<td>DMD4</td>
<td>Deletion</td>
<td>D. Exons 48-50</td>
<td>2</td>
</tr>
<tr>
<td>DMD7</td>
<td>Deletion</td>
<td>D. Exons 47-50</td>
<td>2</td>
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<tr>
<td>DMD11</td>
<td>Point Mutation</td>
<td>Exon 24: c.3217G&gt;T (p.Glu→X)</td>
<td>1</td>
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<tr>
<td>DMD15</td>
<td>Deletion</td>
<td>D. Exons 45-52</td>
<td>2</td>
</tr>
<tr>
<td>DMD16</td>
<td>Point Mutation</td>
<td>Exon 70: c.10171C&gt;T (p.Arg→X)</td>
<td>3</td>
</tr>
<tr>
<td>DMD19</td>
<td>Deletion</td>
<td>Exon 35: c.4918-4919 del TG</td>
<td>1</td>
</tr>
<tr>
<td>DMD21</td>
<td>Point Mutation</td>
<td>Exon 50: c.7437g&gt;A (p.Trp→X)</td>
<td>2</td>
</tr>
</tbody>
</table>
DMD Rescue: Microdystrophin

Nottingham - Newcastle

(Hanns Lochmuller)

Spandan Kalra
DMD Rescue: Exon Skipping
Nottingham - Leiden

Normal

| 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 |

DMD patient 4

| 41 | 42 | 43 | 44 | 45 | 46 | 47 | 51 |

DMD patient 4 - skip exon 51

| 41 | 42 | 43 | 44 | 45 | 46 | 47 | 51 |

DMD patient 7 - skip exon 51

| 41 | 42 | 43 | 44 | 45 | 46 | 51 | 52 | 53 | 51 | 52 | 53 |

Becker

exon 51 blocked
DMD Rescue: Exon Skipping

Nottingham - Leiden

DMD4 hiPSC-CM

NT +

Ex47-51 (1229bp)
Ex47-52 (996bp)

47  51

DMD7 hiPSC-CM

NT +

Ex46-51 (1079bp)
Ex46-52 (846bp)

46  51

Other chemistries
Other delivery methods

Phenotype
Key Questions

hPSC-CMs:

- ~30 hiPSC lines with electrical, structural, survival disorders
  - Long QT Syndrome
  - Duchenne muscular dystrophy
  - Adrenoceptor disorders
  - Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)
  - Ventricular Failure

- Potential in drug & gene therapy

- Beat for at least 22 months - acute & chronic tox

- Specific improvements needed, especially in hiPSC

Can systems be scaled to industrial level?
Automated Culture

With Loughborough University

Standardised hPSC culture

Scale to $3 \times 10^9$ cells

*Thomas 2009 Biotech & Bioeng*

---

**Passages**

- 0
- 4
- 8
- 12
- 16
- 20
- 24
- 28
- 32

**PDs**

- 0
- 4
- 8
- 12
- 16

---

**OCT4**

- No Iso
- +1mM Iso

**Graphs**

- +500mV
- -500mV
Future Experimental Needs

- Culture, expansion & passage hPSCs
- Cell counting
- Differentiate hPSCs
- Reduce costs by miniaturisation
- Dose wells with different reagents
- Sub-sampling of spent medium for analysis
- Cherry-pick wells
- Modular addition of equipment (e.g. Image Analysis)

→ Conclusion: Need a new robot with higher flexibility...!!!
Progress Scales

- Love for robotics
- Enthusiasm of lab members
- Thoughts of CellGEM software
- Robotic cell culture success

Relative Units

2010 2011 2012 2013

Lorraine Young, Ralph Hyde, James Smith, Qian Liu, Maria Munoz, Kasia Lis-Slimak
## Human Pluripotent Stem Cell Challenges

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumour lines (CHO, HEK…)</th>
<th>Human PSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the product?</td>
<td>Ion channel / protein</td>
<td>Cells</td>
</tr>
<tr>
<td>Harsh Split Ratio</td>
<td>Tolerate 1:100</td>
<td>Like 1:3</td>
</tr>
<tr>
<td>Clonal Plating Efficiency</td>
<td>&gt;80%</td>
<td>Survival 1-20%</td>
</tr>
<tr>
<td>Typical growth pattern</td>
<td>Cont. monolayer</td>
<td>Prefer colonies</td>
</tr>
<tr>
<td>Medium change</td>
<td>3-4 days</td>
<td>Daily</td>
</tr>
<tr>
<td>Sheer force risks</td>
<td>Low/tough cells</td>
<td>High/weak cells</td>
</tr>
<tr>
<td>Sensitivity to toxic insult</td>
<td>Low</td>
<td>Up to 1000-fold</td>
</tr>
<tr>
<td>Risk: karyotype abnormalities</td>
<td>Irrelevant</td>
<td>High</td>
</tr>
<tr>
<td>Risk: unwanted differentiation</td>
<td>None</td>
<td>High</td>
</tr>
<tr>
<td>Need for QC checks</td>
<td>Limited</td>
<td>High</td>
</tr>
<tr>
<td>Cost of dead volumes in tubes</td>
<td>Low (2p / ml)</td>
<td>High (50p / ml)</td>
</tr>
</tbody>
</table>
Cell Passaging: Roboflask

Growth Curve

Cum Pop. Doublings

Days in culture

Manual

Robot

Lorraine Young, Ralph Hyde, James Smith, Qian Liu, Maria Munoz, Kasia Lis-Slimak
Automated Hepatocyte Differentiation: 24-well

<table>
<thead>
<tr>
<th>Brightfield</th>
<th>AFP</th>
<th>ALB</th>
<th>CK18</th>
<th>HNF4a / E-Cadherin</th>
<th>CYP1A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Automated Manual Cellavista Well Selection Image Recognition Image Capture Quantification

Lorraine Young, Ralph Hyde, James Smith, Qian Liu, Maria Munoz, Kasia Lis-Slimak
Cardiac Differentiation for Automation

Forced expression of cardiac TFs: TBX5, GATA4, NKX2.5, BAF60C (Dixon et al., Mol Ther. 2011)

Growth factor induction: Density, substrate, enzymes, timing + activin A / BMP4 / FGF

Best conditions = $10^8$ / Tecan Evo 200

Cost is £50K / run Need cheaper media..

James Dixon, Vinoj George, Daphne Goh, Elena Matsa
Summary

Patient cells

Induced pluripotency

Automated culture & differentiation

Improved culture / diffn

Polymer libraries

Chemical libraries

+/- genetic modification

Disease / Safety

Nutritional Screening

Repair

Applications

Scale-up

Miniaturisation

Nutritional screening

Cystathionine

Cystine

Hepatocytes

hPSCs

Cardiomyocytes