Development and Testing of a Cardiac Construct for the Treatment of HF

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Background – Heart Failure

- 500,000 new cases annually
- Largest expenditure for Medicare
- Best current HF treatments focus on preventing deterioration in left ventricular (LV) function
Normal vs. Infarcted Heart. The left ventricle has a thick muscular wall, shown (A). After a myocardial infarction, heart muscle cells in the left ventricle are deprived of oxygen and die (B), eventually causing the ventricular wall to become thinner and the LV to dilate (C).

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Clinical Trials - Disappointing

Barriers to myocardial regeneration

- Rapid cell wash out
- Fibrotic tissue
- Lack of blood flow
- Low number of cells
The Idea

• Proposing a new/different approach to cell-based therapy

• Tissue engineered, patch/scaffold delivery methods
Fibroblast Patch

- Bio-absorbable Vicryl mesh overgrown with newborn human dermal fibroblasts
- Metabolically active - secretes collagen & fibronectin
- Fibroblasts produce angiogenic growth factors
  - VEGF, HGF, bFGF, and angiopoietin-1

Lancaster et al. *Tissue Engineering* 2010
Fibroblast Patch

Chronic MI model

- Does not reverse maladaptive LV remodeling
- Does not increase contractility
- Promotes angiogenesis
- Increases myocardial blood flow

Lancaster et al. *Tissue Engineering* 2010
Hypothesis

• Fibroblast patch can be seeded and co-cultured with cardiomyocytes

• Implantation of a biologically active cardiomyocyte patch will improve left ventricular function in a CHF model
Cardiomyocyte – Fibroblast Patch
Spontaneous Contractions
Cardiomyocyte – Fibroblast Patch
Electrical Stimulation
Cardiomyocyte – Fibroblast Patch
Gap Junction Mediated Dye Transfer
MEA Electrical Mapping

Lancaster et al. Circulation Heart Failure 2012 - Submitted
MEA Electrical Mapping

Consistent beat-to-beat sequence activation occurring at 78bpm
Chronic Heart Failure Model

- Left coronary ligation
- Patch placement
- Baseline Echo
- 3wk Echo <35%
- 6wk Echo
- 10wk Echo
- 18wk Echo + Hemodynamics
Improvement in LVEF at 18wks

Data are mean±SE. α and β denote statistical significance (p<0.05) CHF vs. NCM+3DFC and Sham vs. CHF respectively. CHF = chronic heart failure, NCM = neonatal cardiomyocytes, 3DFC = 3 dimensional fibroblast construct. Sham, N=8; CHF, N= 2-20; NCM-3DFC, N=3-26.

Lancaster et al. Circulation Heart Failure 2012 - Submitted
Table 1. Three week endpoint hemodynamics for rats treated with NCM-3DFC

<table>
<thead>
<tr>
<th></th>
<th>MAP</th>
<th>SYS</th>
<th>EDP</th>
<th>CI</th>
<th>dP/dt (+)</th>
<th>dP/dt (-)</th>
<th>Tau</th>
<th>PDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>129±4</td>
<td>128±4</td>
<td>5±1</td>
<td>0.52±0.04</td>
<td>7146±285</td>
<td>6368±468</td>
<td>15±1</td>
<td>171±5</td>
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<tr>
<td>CHF</td>
<td>103±4β</td>
<td>124±5</td>
<td>27±2β</td>
<td>0.45±0.05β</td>
<td>4651±250β</td>
<td>2853±148β</td>
<td>25±1β</td>
<td>112±8β</td>
</tr>
<tr>
<td>NCM+3DFC</td>
<td>100±5</td>
<td>126±4</td>
<td>15±3α</td>
<td>0.61±0.06α</td>
<td>5806±192α</td>
<td>3517±230α</td>
<td>21±1α</td>
<td>146±5α</td>
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</tbody>
</table>

Table 2. Eighteen week endpoint hemodynamics for rats treated with NCM-3DFC

<table>
<thead>
<tr>
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<th>MAP</th>
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<th>EDP</th>
<th>CI</th>
<th>dP/dt (+)</th>
<th>dP/dt (-)</th>
<th>Tau</th>
<th>PDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>119±2</td>
<td>119±2</td>
<td>8±1</td>
<td>0.35±0.03</td>
<td>7355±400</td>
<td>6103±287</td>
<td>16±1</td>
<td>178±6</td>
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<tr>
<td>CHF</td>
<td>84±2β</td>
<td>105±7β</td>
<td>28±3β</td>
<td>0.32±0.03</td>
<td>4048±217β</td>
<td>2172±130β</td>
<td>29±1β</td>
<td>98±9β</td>
</tr>
<tr>
<td>NCM+3DFC</td>
<td>101±3α</td>
<td>111±6</td>
<td>17±7</td>
<td>0.33±0.06</td>
<td>4713±118α</td>
<td>2915±219α</td>
<td>25±2</td>
<td>137±5α</td>
</tr>
</tbody>
</table>

Data are mean ± SE. α, p<0.05 CHF vs. NCM+3DFC and β, p<0.05 Sham vs. CHF. At three wks Sham, N = 7-20; CHF, N = 6-12; NCM-3DFC, N = 7-13. At eighteen wks Sham, N= 8; CHF, N=3-6, NCM+3DFC, N=2-3.
Tissue Characterization at 3 & 18 wks post implantation

Trichromed LV cross sections 3 and 18 weeks after implantation

Lancaster et al. *Circulation Heart Failure* 2012 - Submitted
Inducible Pluripotent Stem Cells (iPSCs)

- IPS cells are uniquely useful stem cells
  - Derived from adult tissue via non-invasive methods
  - Can be expanded indefinitely
  - Can be differentiated into any cell type in the body
  - Fully pluripotent
- IPS cells offer distinct advantages to ES cells
  - Can be created via streamlined & non-invasive methods
  - Eliminates political/social issues regarding tissue source
  - Enables diversity of genotype and phenotype

Draw 1 small sample from 1 person → Reprogram sample tissue into IPS cells → IPS cells multiply and expand in culture indefinitely → Differentiate IPS cells into any cell type in the body (unlimited numbers)
iPSCs - 4 days in Culture

iPSC derived cardiomyocyte patch demonstrates spontaneous and synchronized contractions after 4 days in culture.

Lancaster et al. Unpublished data
Functional Improvements?

• Currently implanting iCELL patches in Rats with CHF
  – Varying seeding densities
  – Functional Benefits? Echo & Hemodynamics
  – Cell Survival (RFP)
Conclusion

- Demonstrated cardiac patches can be made
- Beats spontaneously and synchronously
- Respond to electrical stimulation
- Displays function gap junctions
- Electrically stable
- Improves cardiac function in rats with CHF
- BUT... at this point a proof of concept, must use clinically relevant cell type(s)
- Reproducible tissue culture techniques with iPSC derived cardiomyocytes
Financial Support

- VA Merit Review
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- Sarver Heart Center
  - Shaftner Memorial Award 2011
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Questions?

Spontaneous Contractions
Pacing
Functional Gap Junctions
MEA – Electrical Mapping
Ejection Fraction
Hemodynamics
Tissue Characterization
iPSCs
iPSCs – Force Generation

Lancaster et al. Unpublished data
Future Work

• Live cell tracking
• Ion channel expression
• Gene expression - Connexin-43, Akt, Pim-1, ILK, SDF-1, and CXCR4
• Larger animal model
Mechanism of Action

- Cellular replacement (cardiomyocytes)
  - Survival
  - Maturation
  - Integration
- Cytokine activation
- Stimulation of intrinsic cardiomyocyte population
Ideal Cardiac Construct

• Contractile
• Electrophysiologically stable
• Mechanically robust yet flexible
• Vascularized or at least quickly vascularized after implantation
• Non-immunogenic
  – Cells
  – Degradation (cytotoxic)

Leor et al. 2005
Challenges of Myocardial Tissue Engineering

- Polymer scaffolds exhibit host inflammatory response
- Scaffold degradation products release potential toxic substances
- Scaffolds compliance is different than myocardial tissue
- Cardiomyocytes remain isolated; tissue does not beat as syncytium

Cardiomyocyte seeded dermal fibroblast patch does NOT face these challenges.
Cytokine, chemokine, and growth factor stimulation in kinetic versus static strain (10% 1Hz) of the 3DFC in vitro involved in angiogenesis.

3DFC + NCM
3DFC + additional fibroblasts
3DFC + conditioned media
3DFC + NCM on non-infarcted RV
Salk iPS-derived Cardiomyocytes on Fibroblast Patch *in vitro*
iPSCs: Salk Institute

One day after seeding, spontaneous contractions were seen

Dr. Juan Carlos Izpisua Belmont, Salk Institute, La Jolla, CA
CDI iPS-derived Cardiomyocytes
Fibroblast Patch

**Acute MI model**
- Improves myocardial blood flow
- Improves LV function
- Reverses maladaptive remodeling

**Chronic MI model**
- Increases myocardial blood flow
- Promotes angiogenesis
- Does not reverse maladaptive LV remodeling
- Does not increase contractility

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Cell-to-Cell Communication & Mechanical-Electrical Coupling

Understanding the electrical activity of a scaffold *in vitro* may predict the utility of cell-based therapy in HF.
Cell-to-Cell Communication & Mechanical-Electrical Coupling

Spontaneous Contractions

Transverse conduction voltage of 776±6 μV
Shortest latency occurring on E7

Consistent beat-to-beat sequence activation
Cell-to-Cell Communication & Mechanical-Electrical Coupling

Normothermic (37+2°C)  
Hypothermic (27+5°C)

*in vitro* mechanical-electrical response to environment
The *Ideal* Myocardial Engineered Tissue

1. Structural support
2. Increase myocardial blood flow
3. Force development
4. Excitability

*Proof of concept ➔ Second generation patch with clinical utility*
Cardiomyocyte – Fibroblast Patch
3 Week Functional Data

Ejection Fraction

Cardiac Index

Data are mean + SE. SO, n=21; UN, n=12; 3DFC, n=9; NCM-3DFC, N=9. CHF = chronic heart failure, NCM = neonatal cardiomyocytes, 3DFC = 3 dimensional fibroblast construct. * p<0.05 vs. SO, @ p<0.05 vs UN, # p<0.05 vs 3DFC
Cardiomyocyte – Fibroblast Patch
3 Week Functional Data

Data are mean ± SE. ; SO = Sham Operated; UN = Untreated;
3DFC = 3-Dimensional Fibroblast Construct; NCM-3DFC = Neonatal Cardiomyocyte 3DFC.
SO, n=19; UN, n=12; 3DFC, n=9; NCM-3DFC, n=9.
* P<0.05 vs SO; @ P<0.05 vs UN; # P<0.05 vs 3DFC.
Clinical Trials of Cardiac Stem Cell Therapy

ALCADIA = AutoLogous human CArdiac-Derived stem cells to treat Ischemic cArdiomyopathy.
CADUCEUS = CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction.
SCIPIO = cardiac Stem Cell Infusion in Patients with Ischemic cardiomyopathy.

Conclusions

• Successful tissue engineered scaffold requires:
  – Anisotropic structure and function
  – Force development
  – Excitability

• NCM patch is “proof of concept”
Conclusions

• Second generation patch with clinical utility

• Patch may provide biological substitutes:
  – *in vitro* and *in vivo* application
  – Disease modeling
  – Drug development
  – Therapeutic tissue reconstruction