Cell Therapy Strategies to Mend a Broken Heart

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Biological Cardiac Repair

- Clinic/Pharmacology
- Interventionist:
  Angioplasty/Stents
  Surgical Revascularization

Cell replacement - “biological cardiomyoplasty”
Angiogenesis

New & Improved Approaches
Targeting the Microvasculature for Ischemic Tissue Repair

Cappilary Vasculature in the Border Zone Post-MI

Cardiomyocyte Renewal in Humans
(Evidence from integration of $^{14}$C from Nuclear Tests During the Cold War)

Renewal Capacity: 2.0 – 0.45%/yr

Exchange Rate: Fewer Than 50% during normal life span
Transient Regenerative Potential in Neonatal Mouse Heart

Cell replacement - “biological cardiomyoplasty”

Angiogenesis

- Cell as a “vector”
- Embryonic/Adult Pluripotent & Reprogrammed Cells (iPS)
- Cardiac Tissue Engineering
Post-MI Adaptations & Goals For Cardiac Cell Repair
(Complex Scenario)

- Normal heart
- Coronary artery
- LV
- Acute compensation
- Myocardial infarction
- Infarction
- Non-infarcted myocardium
- Infarcted myocardium

**Cell therapy goals**
- Prevent cardiac deterioration
  - Promote formation of new blood vessels
  - Inhibit apoptosis
  - Inhibit ROS production
- Prevent cardiac expansion
- Limit area of necrosis and scar size
- Alter scar content to improve mechanical support

Mummery, Davis & Krieger, *Sci Transl Med*, 2010
Post-MI Adaptations & Goals For Cardiac Cell Repair
(Complex Scenario)

Mummery, Davis & Krieger, Sci Transl Med, 2010
Cell Therapy in Ischemic Cardiac Disease

1. Routes & Timing for cell injection

2. BMC, Adipose Stem Cell (ASC) and genetic modified cells for cardiac repair

3. Combined use of CABG/TMLR & BMC for cardiac repair in humans
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Cell Retention Study

**Ischemia-Reperfusion (45')**

- 1d
- 2d
- 3d
- 7d

**BMCs**

- (6 x 10^6)

**Radiolabeling with ^99m^Tc-Ceretec®**

- After injection
- 24h
  - IV
  - LV
  - LV+
  - IM

**DMEM**

**Fibrin**

**Sacrifice**

**Radiometry**

Tissue Body Biodistribution of BMCs

Cardiac Retention and Biodistribution of BMCs

Injecte radioactivity (%)

Cardiac Retention of BMCs using fibrin as vehicle

Histologic sections of hearts from animals that receive AdLacZ-fibroblast after b-gal assay (A e B) and visualized on fluorescence microscopy for DAPI nuclear stain (C e D). Left panels represents sections from animals transplanted with BMCs in medium DMEM and right panel that transplanted with BMCs in fibrin.

Nakamuta et al, PLoS One, 2009
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BMC Transplantation & Cardiac Function Post-MI

MI = myocardial infarction  I = BMC implant  A = Histologic and functional assessment

<table>
<thead>
<tr>
<th>Group</th>
<th>MI</th>
<th>Content</th>
<th>Route</th>
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<tbody>
<tr>
<td>SHAM (N=5)</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M – M (N=4)</td>
<td>+</td>
<td>culture medium</td>
<td>intramyocardial (IM)</td>
</tr>
<tr>
<td>F – IM (N=4)</td>
<td>+</td>
<td>fibrin</td>
<td>intramyocardial (IM)</td>
</tr>
<tr>
<td>BMC – IV (N=5)</td>
<td>+</td>
<td>BMC (1x10^6)</td>
<td>intravenous (IV)</td>
</tr>
<tr>
<td>BMC – IM (N=4)</td>
<td>+</td>
<td>BMC (1x10^6)</td>
<td>intramyocardial (IM)</td>
</tr>
<tr>
<td>BMC+F – IM (N=4)</td>
<td>+</td>
<td>BMC + fibrin (1x10^6)</td>
<td>intramyocardial (IM)</td>
</tr>
</tbody>
</table>

Cardiac Morphometry

Nakamuta et al, PLoS One, 2009
Afterload Hemodynamic Stress to Assess Cardiac Performance

Santos et al, Can J Physiol Pharmacol, 2010

Nakamuta et al, PLoS One, 2009
Liposuction
### hASC Phenotype Homogeneity

#### FACS analysis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Average (%)</th>
<th>Standard Deviation (%)</th>
<th>N</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>CD11</td>
<td>1.7 ± 2.2</td>
<td></td>
<td>2</td>
<td>-</td>
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<tr>
<td>CD13</td>
<td>98.6 ± 1.3</td>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>CD14</td>
<td>0.9 ± 1.0</td>
<td></td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>CD29</td>
<td>87.9 ± 17.7</td>
<td>3</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>CD31</td>
<td>5.6 ± 7.7</td>
<td></td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>CD34</td>
<td>1.7 ± 1.5</td>
<td></td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>CD44</td>
<td>53.5 ± 5.1</td>
<td></td>
<td>2</td>
<td>+</td>
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<tr>
<td>CD45</td>
<td>3.4 ± 3.1</td>
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<td>2</td>
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<tr>
<td>CD49</td>
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<td>2</td>
<td>+</td>
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<tr>
<td>CD51/61</td>
<td>10.3 ± 13.0</td>
<td></td>
<td>2</td>
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<tr>
<td>CD54</td>
<td>58.3 ± 31.2</td>
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<td>2</td>
<td>+</td>
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<tr>
<td>CD73</td>
<td>95.1 ± 4.7</td>
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<td>2</td>
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<tr>
<td>CD90</td>
<td>98.9 ± 1.4</td>
<td>3</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>CD106</td>
<td>2.8 ± 3.4</td>
<td></td>
<td>2</td>
<td>-</td>
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<tr>
<td>AC133</td>
<td>0.9 ± 1.2</td>
<td></td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>HLA-ABC</td>
<td>62.0 ± 47.5</td>
<td></td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1.6 ± 1.9</td>
<td></td>
<td>2</td>
<td>-</td>
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</tbody>
</table>
hASC Selfrenewal & Pluripotency

- hASC *in vitro* presents until 14\textsuperscript{th} passage:
  - Doubling Time  \( (94.1 \pm 18.3 \text{ hours}, n=3-4) \)
  - Cumulative pop. Doubling \( (1.1+0.2 \text{ PD}, n =4-9 ) \)
  - Cell Senescence \( (3.1\pm1.7 \text{ %, n}=3-5) \)

- Differentiation potential:

  ![Adipogenic](image1)
  ![Osteogenic](image2)

*Danoviz et al, Stem Cells & Dev. 2010*
ASC Transplantation & Cardiac Function Post-MI

Danoviz et al, PloS One, 2010
Hemodynamic Variables After Pressure Overload

Prevention of Cardiac Deterioration Post-MI: What are the Molecular Mechanisms?

- Direct action
- Indirect action

ASC

Change in secretory profile

Change phenotype

Ischemia

Stretch
ASC Exposure to Controlled Stretch & Ischemia

Specific Markers
Unique Phenotypes
Secretory profile
(Protein Array & ESI Q-TOF)
Effect of Cyclic Mechanical Stretch on hASC
(12% amplitude & 1Hz)

Silva et al, unpublished
Endothelial Markers & Cyclic Stretch on hASC
(12% amplitude & 1Hz)

Silva et al, unpublished
Cardiomyocytes Markers & Cyclic Stretch on hASC
(12% amplitude & 1Hz)

Silva et al, unpublished
Contraction Assay & Cyclic Stretch on hASC
(12% amplitude & 1Hz)

A

B

Silva et al, unpublished
hASC & Expression of Angiogenic Cytokines

Silva et al, unpublished
Angiogenic Cytokines & Cyclic Stretch on hASC
(12% amplitude & 1Hz)

Silva et al, unpublished
Myocardial Infarction Modulation by ASCs

Stem Cell ASC

Stretch + Ischemia in vitro

Secretory profile

Protein-protein interaction network

Cell Differentiation

Muscle tissue development

Post translational protein modification

Interaction network for functional overrepresentation of pathways
Step 1

Endotelial Cells

VEGF stimulation/inhibition

ASCs

Cardiomyocytes

Fibroblasts

SPARC

null

ASCs injection

Cardiac Function Assessment

Nakamuta et al, unpubl.
1. Routes & Timing for cell injection

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3. Combined use of CABG/TMLR & BMC for cardiac repair in humans
Bone Marrow Progenitor Cells
(Separation of lymphofomonocitary cells)

Mononuclear Cells isolation by density gradient (Ficoll - Histopaque)

100 ml → 5 ml

Intramyocardial injection during incomplete CABG
Safety Assessment

BMC Injection:

Total = 130±3 x 10^6 cels/pt
CD34+ = 1,30 ± 0,40%
MRI Injected Area: Anterior Wall
Pat # 6 RGS

Pre-op

1 month Post-op
Total and Regional LV Ischemic Score Assessed by MRI

Total

Regional

P=0.002

P=0.0009

Efficacy – Controlled Double Blind Randomized Trials:

1. BMSC in Chronic CAD (Incomplete CABG) (N=140, 1:1)

2. BMSC in Chronic CAD (TMLR) (N=50, 1:1)
Cell Therapy in Ischemic Cardiac Disease

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Evidence for Structural & Functional Benefit (Mechanism of Action?)

Controlled & Rational Use

Cell replacement - “biological cardiomyoplasty”

Angiogenesis

1. The Naïve Approach

2. The “Awe” Approach (surprise)

3. The Rational Approach
Special Challenges Remain

Encouraging data obtained in rodents must be rapidly tested in more suitable models (e.g. swine)

Description of detailed mechanism of action (may allow the use of small molecules)

“Efficient” source of cardiomyocytes

To obtain cell/tissue integration to perform as a functional sycinction
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Combined Strategy:
TMLR & Cell Transplantation
Combined Strategy
Cardiac MRI
Injected Area: Anterior Wall

Pre-

6 mos Post-

Combined Strategy: Functional Assessment

**Angina – Functional Class**

- Basal: P = 0.0001
- 6 meses: 

**LV Ischemic Score**

- Basal: P = 0.01
- 6 meses:

**N = 9**

Gowdak et al, Int J Cardiol, 2007