Cell implantation after myocardial infarction: a 10 years experience from the ICREC laboratory

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No competing interests exist in relation to this presentation
“You never fail until you stop trying.” — Albert Einstein

“All men dream: but not equally. Those who dream by night in the dusty recesses of their minds wake in the day to find that it was vanity: but the dreamers of the day are dangerous men, for they may act their dreams with open eyes, to make it possible. This I did.” — Thomas Edward Lawrence
The ICREC laboratory: aim

HEART DISEASE

Clinical Approach
- Clinical
- Symptoms
  - Signs
  - Symptoms

Imaging
- ECG
- ECHO
- X Ray
- CT Scan
- MRI
- Angiography
- PET
- Stress test

Chemistry and Devices
- PM
- CRT
- ICD
- Pills
- Stents

BIOLOGICAL Approach
- BIO-MARKERS
  - Diagnosis
  - Prognosis
  - Monitoring
- BIO-THERAPIES
  - Stem cells
  - Tissue engineering
  - Gene therapy
The ICREC laboratory: milestones in biotherapies

Source: ICREC, unpublished
What are we facing? What are we looking for?

before therapy

after therapy
Myocardial infarction

- Myocardial infarction is caused by coronary artery occlusion provoking irreversible myocardial ischemia, loss of cardiomyocytes and formation of a non-contractile fibrous scar. It may induce ventricular remodeling and lead to heart failure.

- The human heart has a limited regenerative capacity thus, cardiac function is only fully re-established after heart transplantation. This option, however, is extremely restricted by limit number of donors and graft rejection.

- First evidences of myocardial regeneration were seen in rodents (1060s), in amphibia (1974) and, finally, in zebrafish (2002).

- Several findings changed the old dogma describing the human heart as a terminally differentiated organ:
  - resident cardiac stem cells in the heart
  - cardiomyocyte DNA synthesis
  - cardiac chimerism phenomena
Cardiovascular research: efforts

- Cellular cardiomyoplasty

- Cardiac tissue engineering
Cell therapy: basis and objectives
Cell therapy: a myriad of cellular actors
Cell therapy: clinical results

- Cumulative clinical evidence (mostly using bone marrow cells) indicates that cell therapy modestly improves cardiac function following myocardial infarction (MI)

<table>
<thead>
<tr>
<th>Study/references</th>
<th>Condition</th>
<th>Cell type</th>
<th>Delivery method</th>
<th>Safety</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamano et al. [134]</td>
<td>CMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Increased coronary perfusion (3/5)</td>
</tr>
<tr>
<td>Strauer et al. [135]</td>
<td>AMI</td>
<td>BMMNC (2.1% CD34+)</td>
<td>IC</td>
<td>+</td>
<td>Improved LVEF and contractility, reduced infarct size at 6 months</td>
</tr>
<tr>
<td>TOPCARE-AMI [136–138]</td>
<td>AMI</td>
<td>CPC/BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Similar results for both cell types: improved LVEF and local contractility, reduced infarct size at 4–12 months</td>
</tr>
<tr>
<td>Fernández-Avilés et al. [139]</td>
<td>AMI</td>
<td>BMMNC (1% CD34+)</td>
<td>IC</td>
<td>+</td>
<td>Improved LVEF and regional contractility, reduced end systolic volume</td>
</tr>
<tr>
<td>Stamm et al. [140]</td>
<td>AMI</td>
<td>CPC (CD133+)</td>
<td>IM</td>
<td>+</td>
<td>Increased perfusion, motility and wall thickness</td>
</tr>
<tr>
<td>Tse et al. [141]</td>
<td>CMI</td>
<td>BMMNC</td>
<td>IM</td>
<td>+</td>
<td>Increased motility and wall thickness</td>
</tr>
<tr>
<td>BOOST [142]</td>
<td>CMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Improved LVEF at 6 months, no difference at 18 months, improved LV function and increased regional contractility</td>
</tr>
<tr>
<td>ASTAMI [143]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>No effect on global LVEF at 6 months</td>
</tr>
<tr>
<td>REPAIR AMI [144, 145]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Improved EF and reduced infarct size at 4 months</td>
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<tr>
<td>Janssens et al. [146]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Reduced infarct size, improved regional systolic function but no augment recovery of global LV function</td>
</tr>
<tr>
<td>FINCELL trial [147–149]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Increased global LVEF at 6 months</td>
</tr>
<tr>
<td>MYSTAR [150]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IM</td>
<td>+</td>
<td>Reduced infarct size, increased myocardial viability and global EF</td>
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<tr>
<td>Hu et al. [151]</td>
<td>CMI</td>
<td>BMMNC</td>
<td>CABG</td>
<td>+</td>
<td>Increased LVEF, LV end-systolic volume index and wall motion index score at 6 months</td>
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<tr>
<td>BONAMI [152]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Increased myocardial viability at 3 months</td>
</tr>
<tr>
<td>TIME [153–155]</td>
<td>AMI</td>
<td>BMMNC</td>
<td>IC</td>
<td>+</td>
<td>Similar results for both timing of cell delivery groups: no significant effects on regional and global LV function</td>
</tr>
</tbody>
</table>

CMI = Chronic MI; AMI = acute MI; IC = intracoronary; IM = intramyocardial; CABG = coronary artery bypass graft; EF = ejection fraction; LV = left ventricular; LVEF = left ventricular ejection fraction.

1 No adverse events, including arrhythmias, calcifications and teratoma formation, were detected.

Cell therapy: the discovery of a new stem cell source

• Novel cell sources with increased potential to repair injured tissue have been sought

• We identified and characterized a progenitor cell population from biopsies of human adult cardiac adipose tissue (cardiac ATDPCs)

• Cardiac ATDPCs show a MSC-like marker profile and immunosuppressive capacity

• Remarkably, cardiac ATDPCs have an inherent cardiac-like phenotype and were able to express de novo myocardial and endothelial markers in vitro but not to differentiate into adipocytes

• Following in vivo implantation, cardiac ATDPCs improves cardiac function and diminishes scar size after MI
Cardiac ATDPCs: baseline and induced traits

Coculture with neonatal rat cardiomyocytes
Cardiac ATDPCs: immunomodulatory potential
Cardiac ATDPCs: in vivo transplantation

Bayes et al. J Mol Cell Cardiol. 2010 Nov;49(5):771-80
UCBMSCs: vascular potential

• To date, the acquisition of properties related to vascular growth has been reported for distinct cell sources, including mesenchymal stem cells (MSCs)

• MSCs comprise a population of multipotent progenitor cells derived from distinct human tissues (bone marrow, adipose tissue, umbilical cord blood, Wharton’s Jelly..)

• In vitro, UCBMSCs acquire new endothelial cell markers, increased Ac-LDL uptake, migratory/invasive capacity and self-organization into tube-like structures in Matrigel assays (vasculogenesis)

• Of note, following in vivo subcutaneous injection with Matrigel, UCBMSCs actively participate in the formation of new microvasculature connected with the host circulatory system

• By using a fibrin patch, UCBMSCs survive 4 weeks above infarcted myocardium, reduce infarct size (3-fold) and increase vessel-occupied area (2-fold)
UCBMSCs: pre-clinical results

A.

Fold gene expression (EGM-2 vs. control)

B.

150kDa —
100kDa —
150kDa —
100kDa —
50kDa —
37kDa —

CD31
VEGFR-2
VE-cadherin
β-actin

Fibroblasts UCBMSCs

C.

Egr-3 protein levels (Fold EGM-2 vs. control)

D.

Control EGM-2
UCBMSCs: pre-clinical results
UCBMSCs: pre-clinical results

Biodistribution and cardiac cell delivery: a limitation
Cell tracking system: bioluminescence

Cardiac tissue engineering: novel approaches of cell delivery

Cell delivery system: fibrin patches

Cell delivery system: fibrin patches
UCBMSMCs: pre-clinical results

A

B

C

D

UCBMSCs: pre-clinical results

![Graph showing vessel area percentage for different groups: Sham-UCBMSCs, Control-MI, MI-Fibrin, and MI-UCBMSCs. The graph compares border and distal areas.](image)

**Fibrin patch**

- GFP
- RFP
- CD31
- Hoechst + Overlay

UCBMSCs: pre-clinical results

UCBMSCs: pre-clinical results
UCBMSCs: pre-clinical results

**A**

<table>
<thead>
<tr>
<th></th>
<th>Control-MI</th>
<th>MI-Fibrin</th>
<th>MI-UCBMS Cs</th>
</tr>
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<tbody>
<tr>
<td>30 days - Baseline</td>
<td>0</td>
<td>-5</td>
<td>-5</td>
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<tr>
<td>$\Delta$LVF (%)</td>
<td></td>
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<td>p = .03</td>
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**B**

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<tr>
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<th>Control-MI</th>
<th>MI-Fibrin</th>
<th>MI-UCBMS Cs</th>
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<tbody>
<tr>
<td>30 days - Baseline</td>
<td>0</td>
<td>-10</td>
<td>-10</td>
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<tr>
<td>$\Delta$LVFS (%)</td>
<td></td>
<td></td>
<td>p = .013</td>
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</table>

**C**

*Sham-UCBMSCs*

*Control-MI*

*MI-Fibrin*

*MI-UCBMS Cs*
Electromechanical stimulation: as a pre-conditioning approach

- *In vitro* individual or combined synchronous electromechanical stimuli mimicking the cardiac environment, could mature or induce cardiac differentiation on therapeutic cells to benefit further retention and integration into the myocardium.
Electrical conditioning: *ad-hoc* device and results

Cardiac Transcription Factors increased!
Mechanical conditioning: *ad-hoc* device and results

### Cardiac Transcription Factors and Structural genes upregulated!

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tbx5</th>
<th>MEF2A</th>
<th>GATA-4</th>
<th>α-actinin</th>
<th>Cx43</th>
<th>SERCA2</th>
<th>β-MyHC</th>
<th>cTnl</th>
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<tbody>
<tr>
<td>cardiac ATDCPs Con</td>
<td>0.003 ± 0.001</td>
<td>0.089 ± 0.018</td>
<td>0.428 ± 0.196</td>
<td>2.445 ± 1.089</td>
<td>0.705 ± 0.253</td>
<td>0.397 ± 0.237</td>
<td>0.095 ± 0.049</td>
<td>0.693 ± 0.555</td>
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<td>cardiac ATDCPs MS</td>
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<td>Ratio cardiac ATDCPs</td>
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<td>P-value Con vs MS</td>
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<td>Smooth</td>
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<tr>
<td>Ratio cardiac ATDCPs</td>
<td>1.716</td>
<td>1.537</td>
<td>1.326</td>
<td>0.891</td>
<td>1.151</td>
<td>1.390</td>
<td>1.182</td>
<td>1.547</td>
</tr>
<tr>
<td>P-value Con vs MS</td>
<td>0.217</td>
<td>0.404</td>
<td>0.312</td>
<td><strong>0.001</strong></td>
<td>0.817</td>
<td>0.198</td>
<td>0.848</td>
<td>0.662</td>
</tr>
</tbody>
</table>
ICREC: several future research lines

- Translation of Allogeneic Bioengineered Myocardial/Pericardial Grafts into Clinics

Perea-Gil et al. JACC: Basic to Translational Medicine. 2016. 10.1016/j.jacbts.2016.06.005
ICREC: future research lines

- Analysis of MSC-induced Immunomodulation using well-purified Size Exclusion Chromatography-Extracellular Vesicles or exosomes (EVs)
• Is possible to increase regeneration outcomes by implantation of engineered scaffolds enriched with EVs?
ICREC: future research lines

- Set up of EV-enriched scaffolds to be implanted in a porcine MI model
‘Take-home messages’

- Cardiovascular diseases are the first cause of death worldwide with >17 million deaths in 2012

- Cellular cardiomyoplasty and cardiac tissue engineering mostly focus current efforts to enhance heart regenerative capacity

- Both cardiac ATDPCs and UCBMSCs represent useful stem cell populations with numerous pre-clinical evidences in treating post-infarcted myocardium

- Since cardiac cells are constantly submitted to physical stimuli from the cardiac environment, electrical and mechanical stimuli to monolayer stem cell cultures pre-commits them to the cardiomyogenic lineage and against the hostile cardiac milieu

- Among others, the clinical translation of allogeneic bioengineered implantable biografts and the exploitation of the immunomodulatory/regenerative potential of MSC-EVs represent novel striking research lines in the aim of treating cardiovascular diseases
“There are no impossible dreams...” — Antoni Gaudi

“… such as that envisioning the repair of broken heart” — ICREC Res Lab.
ICREC Research program
(Insuficiència Cardíaca i REgeneració Cardíaca)

Thank you!!