Bioengineered grafts for the infarcted heart

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HEART DISEASE

Clinical Approach

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
## HF prevalence

<table>
<thead>
<tr>
<th>Age Group</th>
<th>%</th>
<th>CI 95%</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>6.4</td>
<td>4.7</td>
<td>8.0</td>
</tr>
<tr>
<td>45-54 yrs</td>
<td>1.7</td>
<td>0.1</td>
<td>3.3</td>
</tr>
<tr>
<td>55-64 yrs</td>
<td>4.1</td>
<td>2.1</td>
<td>6.1</td>
</tr>
<tr>
<td>65-74 yrs</td>
<td>6.1</td>
<td>3.9</td>
<td>8.4</td>
</tr>
<tr>
<td>74 yrs</td>
<td>18.7</td>
<td>13.9</td>
<td>23.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7.0</td>
<td>5.6</td>
<td>8.3</td>
</tr>
</tbody>
</table>
GLOBAL PREVALENCE OF HEART FAILURE, 1990 - 2018
(in millions)


- **2000**: Based on U.S. National Health And Nutrition Examination Survey (1998-1991), National Center for Health Statistics estimate of U.S. prevalence of heart failure (1.2 million), then using the higher range multiplied by a factor of 4 to estimate global prevalence of heart failure.


1 in 5 individuals > 40 years will develop HF

HF unmet needs?
1. Excessive mortality despite neurohormonal blockade

HF progresses and mortality remains high despite optimal neurohormonal blockade

2. Sudden death accounts for 50% of HF mortality

3. Too many HF admissions and readmissions
4 … and poor quality of life

Better

-100 -80 -60 -40 -20 0 20
% change in quality of life

worse

HT diabetes arthritis COPD angina HF

Heart Transplant in Spain

Figura 1. Número de trasplantes por año.
Survival in advanced HF - LVADs

Potential Device Complications

- Inflow cannula (poor position, obstruction)
- Pump/rotor dysfunction (thrombus)
- Battery dysfunction
- Outflow graft (kink, leak)
- Drive line infection / fracture
- Controller malfunction
Costs of VAD

Human heart regenerative capacity

Post-HT Chimerism
Bayes-Genis et al.
Cardiovasc Res 2002;404-10

Post-BMT Chimerism
Bayes-Genis et al.
Eur J Heart Fail 2004;6:399

Fetal Microchimerism
Bayes-Genis et al.
J Heart Lung Transplant 2005;24:2179

Bayes-Genis et al. Nature CPCM 2007;1:40
Cell loss and myocyte proliferation are part of the heart normal homeostasis

- The human heart has limited ability for autoregeneration
- Cell therapy and tissue engineering may emerge as efficient and safe therapies in addition to current treatment.
I.C. delivery of BMC or CPC
  – Randomized clinical trials after AMI
    • BOOST Lancet 2004;364:141-48
    • Janssens et al. Lancet 2006;367:113-21
    • ASTAMI NEJM 2006;355:1199-209
    • REPAIR-AMI NEJM 2006;355:1210-21
  – Randomized clinical trials in chronic IHD
    • TOPCARE-CHD NEJM 2006;355:1222-32

Intramyocardial myoblast delivery
  • MAGIC On hold

Stem cell mobilization with cytokines (G-CSF)
  • REVIVAL-2 JAMA 2006;295:1003-10
Pathways to regenerate cardiac muscle and blood cells

Key
- Self-renewal
- Gap junctions

Pluripotent ESC/iPSC

Wnt3a/β-catenin pathway

Multipotent Isl-1⁺ progenitor

Committed Isl-1⁺ vascular progenitor

Committed Isl-1⁺ ventricular progenitor

Endothelial progenitor

Vascular smooth muscle intermediate

Cardiomyocyte intermediate

Endothelial cells

Vascular smooth muscle cells

Mature cardiomyocytes

Functional muscle strips

Blood vessel
General biomaterial approaches to treatment of MI:
- LV restraints
- Cardiac patches
- Injectable approaches
Cardiac tissue engineering

Monolayer cell constructs

Imtramyocardial injection of cells in hydrogel

Ex vivo tissue in hydrogel

Extracellular matrix of natural tissues

Artificial cardiac tissue

Gálvez-Montón et al. Rev Esp Cardiol. 2013
Scaffolds

Natural scaffolds
- Fibrin
- Alginate
- Chitosan
- Collagen

Synthetic scaffolds
- PEG
- PLA
- PCL
- PLGA
- Polyglycolic acid

Extracellular matrix scaffolds
- Urine Bladder
- Small Intestinal Submucosa
- Pericardium
- Myocardium
Experimental approaches

- Nanowired 3-D cardiac patches
- CMPCs electrical stimulation
- ATDPCs electrical stimulation
- ATDPCs intramyocardial injection
- Engineered tissue grafts
- Cardiac ATDPCs within fibrin patch
- RECATAI Consortium
- Vascular pericardial flap
- Natural scaffolds
- The Smart Patch
- iPS cells in pericardial scaffolds
- FIRST-IN-MAN

Neo-organogenesis
Experimental approaches

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Neo-organogenesis

FIRST-IN-MAN
Electro-mechanical stimulation

3 x EIS Connector + power supply

6 x FrontEnd + 2 ref. channels

Electrical Stimulation Connector

Electrical Stimulation Connector
ATDPCs electrical stimulation

**Electrostimulation** on ATDPCs:

**Electrostimulation effects**

- **Cardiac ATDPCs**
  - Control
  - Electrostimulated

- **Sub ATDPCs**
  - Control
  - Electrostimulated

**Transcription factors**: MEF2A, GATA4

**Contractile apparatus**: alpha-actinin

**Cell connections/calcium handling**: actinin C43, GATA4, SERCA2

→ E = electric field direction

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Neo-organogenisis

First-In-Man
Cardiac adipose tissue derived progenitor cells

A Bayes-Genis et al. J Mol Cell Cardiol 2010
Intramyocardial injection of ATDPCs

Bayes-Genis A et al. J Mol Cell Cardiol 2010
Intramyocardial injection of ATDPCs

Bayes-Genis A et al. J Mol Cell Cardiol 2010
Engineered heart tissue: T Eschenhagen

Engineered tissue grafts

Engineered tissue grafts

Monolayered mesenchymal stem cells

Monolayered mesenchymal stem cells

Implantation of cardiac ATDPCs through a fibrin patch

To monitor (BLI) cTnI and CD31 expression of cardiac ATDPCs delivered through a fibrin patch

Bagó JR et al. Int J Cardiol. 2013
NMP-2008-2.3-1 Advanced implants and bioactive materials for critical organs
To create a scaffold delivering ATDPCs in the mouse model of myocardial infarction.

**De novo expression of cTnI**

Bioimplant vascularization

Morphometry

Cardiac Function

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FIRST-IN-MAN
Alginate hydrogel reverses LV remodeling: J Leor

Swine model post-MI: preservation of LV area + increased scar thickness

Nanowired 3-D cardiac patches

Alginate + Gold nanowires

Better alignment

Synchronous contraction

Higher levels of muscle contraction and electrical coupling proteins

Dvir T et al. Nature Nanotech 2011
Myocardial matrix hydrogel: Karen Christman

Catheter-deliverable hydrogel derived from decellularized ventricular extracellular matrix

Figure 1: Myocardial Matrix Fabrication

(A) Porcine ventricular myocardium is sliced and then (B) decellularized using sodium dodecyl sulfate. (C) Hematoxylin and eosin staining of a histological section reveals cellular removal. The decellularized extracellular matrix is then milled into a fine powder (D) and then solubilized through enzymatic digestion (E), which allows for injection via syringe and a 27-gauge needle (F).

Singelyn JM et al. JACC 2012: 59;751-63.
Gelled myocardial matrix within healthy myocardium

Figure 5  Percutaneous Transendocardial Delivery of Myocardial Matrix Hydrogel
Increased infarct wall thickness by a bio-inert material is insufficient to prevent negative left ventricular remodeling after myocardial infarction.

EMG Patch (I)

Myocardial matrix decellularization and sterilization

EMG implantation in porcine MI model

Study groups and follow-up

- EMG-ATDPC n=7
- EMG-cell free n=7
- EMG-Sham n=2

Recellularization with ATDPCs

Baseline 48h post-intervention 1 month MRI
30 days EMG-ATDPC post-implantation:

- Vascularization increase
- Cardiac function enhancement
- Fibrotic progression limitation
- Infarct size diminution
- ATDPCs: migration and cardiovascular-like phenotype

The EMG-ATDPC engraftment led to beneficial effects regarding cardiac recovery in swine pre-clinic MI model. In turn, this should ease its future clinical translation.
the “smart patch” concept

- **Smart Patch**
  - Biological membrane: decellularized human pericardium
  - Peptide hydrogel: RAD16-I
  - NanoGold: nanometric gold particles
  - Mesenchymal stem cells: ATDSC
  - Platinum microelectrodes + implantable electronic system
Human pericardium= fibrous membrane made up of 2 layers that surrounds the heart and great vessels.

MSCs derived from porcine cardiac adipose tissue
The next generation: the “smart patch” concept

**Peptide Hydrogel**

RAD16-I = **PuraMatrix™**
Spontaneously autoassembly nanopeptide, forming a molecular network with nanometric pores. Encapsulation of $2 \cdot 10^6$ cells in 400 µL of hydrogel.

**NanoGold**

*NanoGold* = colloidal solution of gold nanoparticles applied on the electrodes to improve electrical current through the patch.
The next generation: the “smart patch” concept

Microelectrodes + implantable electronic system

“The Smart Patch”

Equipment of 35mm x 38mm x 8mm with battery with autonomy for 1 month, performing a measurement every 10 minutes
The next generation: the “smart patch” concept

♀ Landrace X Large White (30 kg)
n=2 control (AMI + patch)
n=5 control (AMI + patch) + ES
n=5 treatment (AMI + patch + cells)
n=5 treatment (AMI + patch + cells) + ES

Experimental design:

- Baseline MRI
- Post-AMI MRI
- 8-week post-MI MRI
- Sacrifice

AMI
Patch +/-
Electronic system (ES)

0
the “smart patch” concept

First impedance measurements to monitor regeneration *in vivo* to date

Work in progress……….
Vascular pericardial flap

Vascular pericardial flap

Day 6

Control

Treated

Day 30

Control

Treated

Vascular pericardial flap

Clinical Trial ongoing

1. NO ethical issues
2. NO technical complications
3. NO economic issues

iPS cells in pericardial scaffolds

- iPS

Flap coverage (FLAP) (n=6)
Scaffold coverage (EM) (n=13)
Scaffold + Flap coverage (FLAP + EM) (n=7)

+ iPS

Flap coverage (FLAP) (n=9)
Scaffold coverage (EM) (n=11)
Scaffold + Flap coverage (FLAP + EM) (n=11)

MI 30 minutes

MRI

2 days

Sacrifice 4 weeks

MRI

MRI
Differentiated iPS

Fibrin scaffold + human iPS

Pigs with ischemia/reperfusion with 1 month of follow up

Cardiac function improvement

Differentiation of hiPSCs into Cardiac-Lineage CMs, ECs, and SMCs

No arrhythmias

Less apoptosis
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Neo-organogenesis

FIRST-IN-MAN
Tissue Engineering for heart muscle

Murine heart

- Ott HC et al. Nat Med. 2008


Swine heart

- Wainwright JM et al. Tissue Eng Part C Methods 2010

Images:
- a) Murine heart images with labels: Ao, RA, LA, RV, LV
- b) Murine heart images with labels: Ao, RA, LA, RV, LV
- c) Murine heart images with labels: Ao, RA, LA, RV, LV
- d) Murine heart histological image
- e) Murine heart histological image
- f) Murine heart histological image

- Swine heart images A, B, C, D, E

1% PEG in deionized water, 77.4 mm Hg, 20 °C
1% Triton X-100 in deionized water, 77.4 mm Hg, 20 °C
1% SDS in deionized water, 77.4 mm Hg, 20 °C
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FIRST-IN-MAN
MAGNUM Trial

Phase I clinical trial: JC Chachques

Demonstration of safety + efficacy of a bone marrow cell-seeded collagen matrix patch

Benefit in LVED volume and scar thickness in treated patients

Clinical Trials

FIRST-IN-MAN

AGTP-I Trial

Phase I clinical trial: A Bayes-Genis

Demonstration of safety + efficacy of a pericardial adipose flap transposition

Candidate to CABG + Chronic scar non-revascularizable

CABG + AGTP

Surgery

CABG alone

Follow-up visits

MRI 1 Week / 1 month / 3 months / 1 year

Baseline MRI

- LVEF
- LVEDV
- LVESV
- Gadolinium infarct area
Research Paper

First-in-man Safety and Efficacy of the Adipose Graft Transposition Procedure (AGTP) in Patients With a Myocardial Scar

Antoni Bayes-Genis a,b,c,*, Paloma Gastellurrutia c, Maria-Luisa Cámara d, Albert Teis a,e, Josep Lupón a,b, Cinta Llibre a, Elisabet Zamora a, Xavier Alomar e, Xavier Ruyra d, Santiago Roura c,f, Ana Revilla g, José Alberto San Román g, Carolina Gálvez-Montón c
Perspectives

**Carbon nanotube scaffolds**

- Biochemical and mechanical scaffold improvement
- Porous structures mimicking natural collagen fibers


**Cell electrospinning**

- Small and large quantity of cells generating cell-laden fibers and scaffolds
- Generation of true architectures having 3D features
- To cross-stitch the architecture during the forming stage

Major barriers to cardiac regeneration

- Ischemia/hypoxia
  - CM graft
  - Hypoxic niche
  - Neovascularization
  - hESC-EC
  - Blood vessels

- Inflammation
  - Infiltration
  - Macrophages
  - Cytokines
  - ROS
  - Neutrophils
  - T cells

- Intracardiac fibrosis
  - Fibrotic tissues
  - Secretion
  - Fibroblasts
  - SMC
  - ECM proteins

- Poor graft survival
  - Apoptotic CM
  - Anti-apoptosis and neovascularization
  - Prosurvival cocktail
  - Blood vessels
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Thank you for your attention
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