The First International Iranian
Tissue Engineering and
Regenerative Medicine Congress
1st ITERM
July 18-20, 2018, Tehran, Iran

# The Future of Regenerative Medicine in Cardiac surgery

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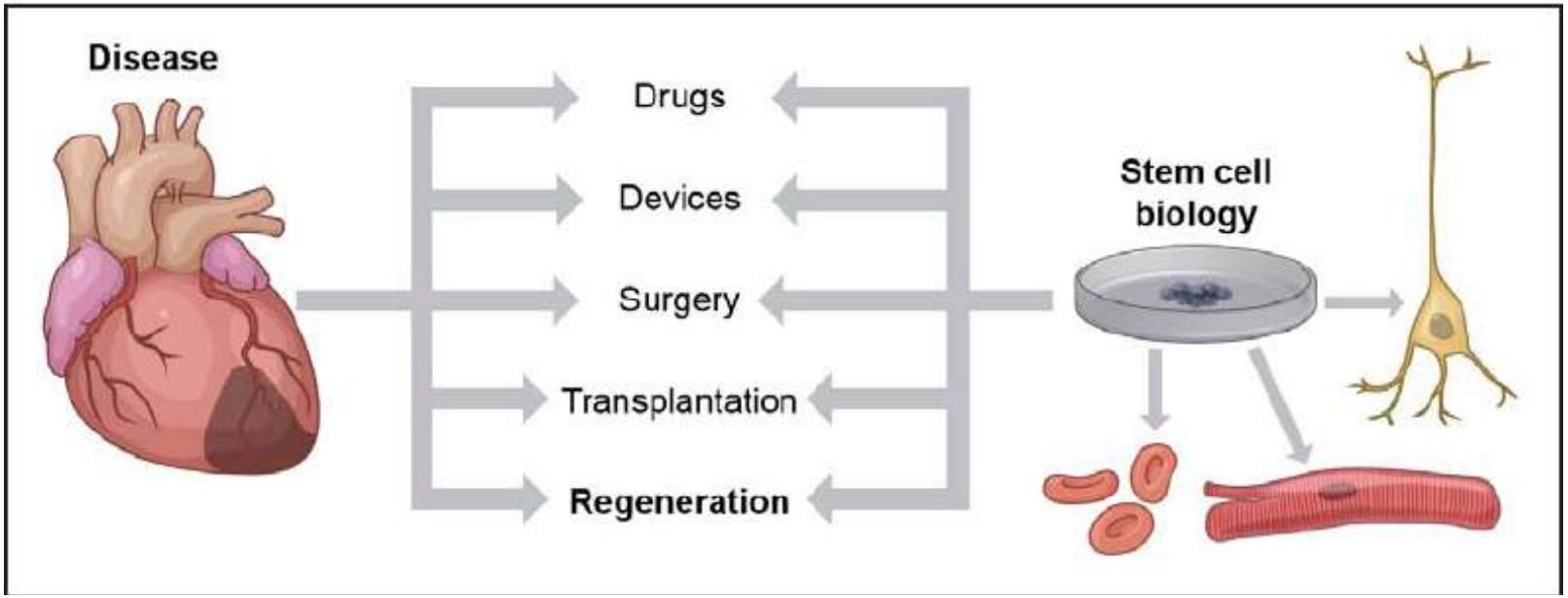
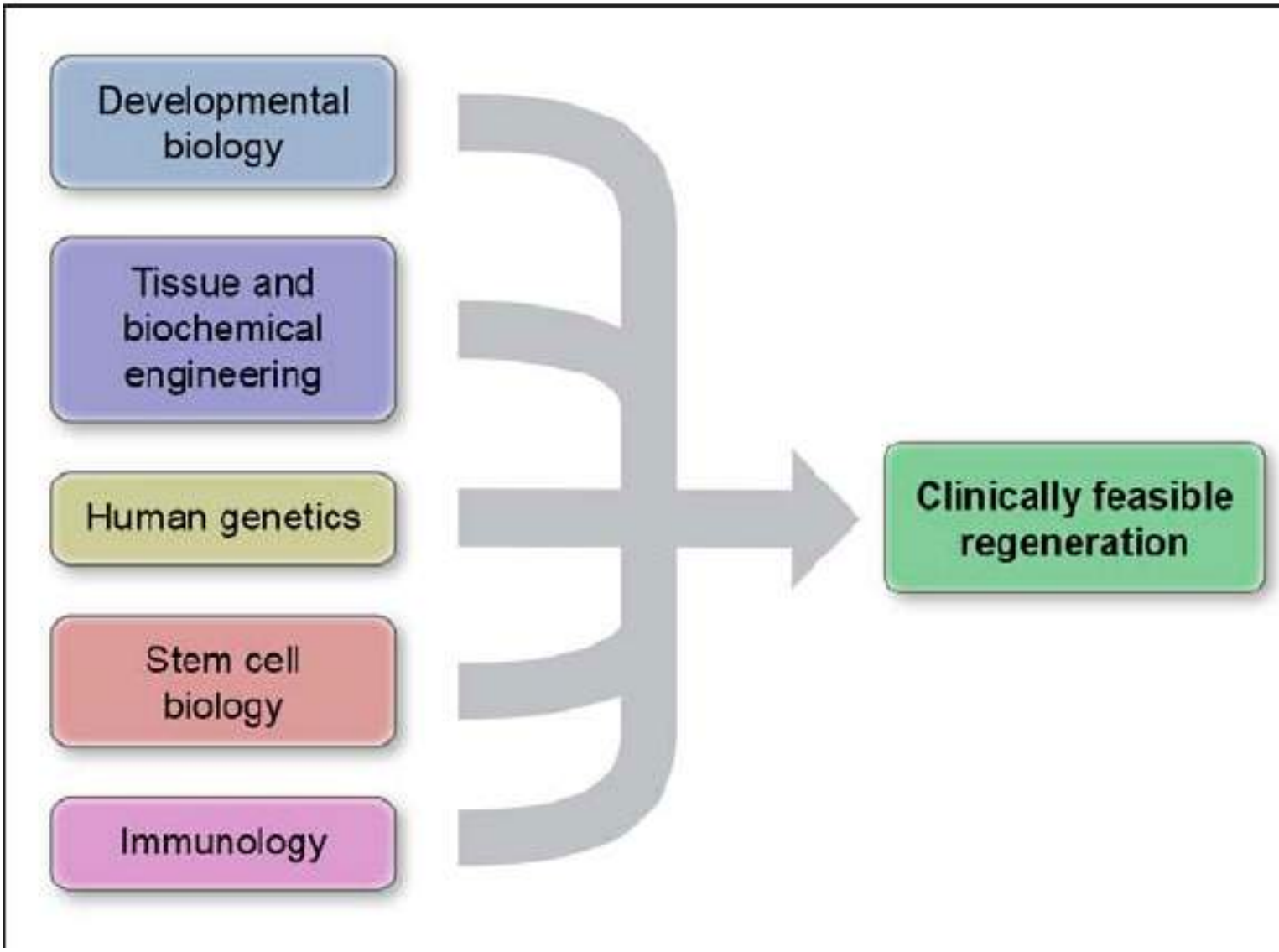
Tir 1397 July 2018

**rhc.ac.ir**

Regenerative treatments will emerge but will be most useful in areas where more traditional approaches leave voids of unmet needs; thus, progress in other fields will play a major role in determining where regenerative breakthroughs can occur




Developmental biology
Tissue and biochemical engineering
Human genetics
Stem cell biology
Immunology
Clinically feasible regeneration

In 2001, Orlic *et al.* reported that bone marrow stem cells injected into the infarcted myocardium

Unfortunately, although some randomized clinical trials disclosed a functional improvement due to bone marrow-derived stem cells, the initial enthusiasm for heart regeneration by stem cell transplantation has since been dampened by the modest clinical benefits observed to date

injected stem cells do not persist for long in the myocardium and do not work through a transdifferentiation process into new cardiomyocytes but rather through paracrine effectors

Austin Journal of Clinical Cardiology

Open Access

Austin Publishing Group

Editorial

# Cardiovascular Surgery Contributing to Regenerative Medicine

Acute ischemic injury and chronic cardiomyopathies lead to permanent loss of cardiac tissue and, consequently, heart failure. Cell transplantation is thought to be an ideal therapeutic method

Death Valley

Many clinical studies so far: TOPCARE-AMI, BOOST, REPAIR- m cells), REGENT (endothelial progenitor cells), POSEIDON (mesenchymal stem cells), MAGIC CAuSMIC (skeletal myoblasts), CADUCEUS, SCIPIO (cardiac stem/ progenitor cells) and so on

It is commonly believed that the low rates of grafted cell survival and engraftment diminish their potential and are serious technical limitations of stem cell therapy. Over 70% of injected cells have been reported to die progressively during the first 48 hours after needle injection due to the hypoxic, inflammatory, and/or fibrotic environment.

Therefore, new strategies such as combining the cells with bioengineering techniques have been developed and shown improvement of the efficiency of stem cell therapies

Starting with initial experiments performed by combining the cell injection with injectable biomaterials (collagen, fibrin, gelatin or Matrigel), brand-new techniques such as the creation of microtissues (cell sheets, patches or engineered cardiac tissue) are now being developed in order to enhance both cell survival and the homogeneous and organized distribution of the cells.

We have utilized a scaffold-free cell sheet technology using culture dishes covalently grafted with the temperature-responsive polymer poly (N-isopropylacrylamide) (PIPAAm), which enables the preparation of cell sheets without enzymatic digestion

We showed that mouse embryonic stem cell (ESC)-derived cell sheet transplantation to a rat myocardial infarction model improved cardiac function through indirect paracrine mechanisms such as tissue neovascularization

The direct mechanical support of the transplanted cell sheets would be desirable for more effective cardiac regeneration.

However, no evidence of the reinforcement of contraction by the physical integration of the cell sheet and host myocardium was reported to date.

To realize that, more increased survival of cell sheets would be essential, and supplemental strategies together with current cell sheet transplantation, such as vascularization of cell sheet, might be promising.

Obtaining sufficient volume of cardiac cells which can survive after transplantation and creating patches/organs that mimic the structure and function of the heart remain challenging.

In this regard, induced pluripotent stem cells (iPSCs) possess great potential for cardiac regeneration as they can be expanded geometrically and repeatedly *in vitro*, can give rise to multiple cardiac cell lineage cells once allowed to differentiate, and lack the ethical and immunogenic issues associated with the use of ESCs

numerous (>$10^8$) heart-composing cells might be required to fully repair a damaged human heart. This approach to repairing cardiac tissue has been tested in preclinical studies with encouraging results including our result

However, no human trials of the use of ESCs or iPSCs for cardiac repair have been attempted so far.

One future direction of regenerative medicine supported by cardiovascular surgery is the combination of stem cell therapy and conventional surgical procedures

Concomitant coronary artery bypass grafting (CABG) and stem cell administration has been studied in patients with chronic myocardial ischemia, but the results were too marginal to justify full-scale therapeutic implementation

The combination of cell therapy and various surgical procedures other than CABG, such as left ventricular assist device implantation, left ventricular reconstruction or mitral repair for ischemic mitral insufficiency, might be a promising strategy in the future and could provide hope for many patients, especially those with severe chronic heart failure who are ineligible for heart transplantation.

Another direction of future research is the further elucidation of the mechanisms of cardiac repair through cell therapy

Previous studies of somatic stem cell therapy relied on injections of heterogeneous cell populations, which limited the insights they could provide into the cellular and molecular behaviors and mechanisms of action of transplanted cells. An understanding of the roles of each cell population as well as the various complex intercellular interactions in the heterogeneous populations transplanted would be a breakthrough in the improvement of cardiac cell therapy.

Utilizing the mouse ESC differentiation system to obtain defined cardiovascular populations, we recently found a major cellular mechanism, that is, cardiomyocytes are essential for sufficient cardiac restoration after sub-acute myocardial infarction mainly through angiogenesis

There are many promising approaches to cardiac regeneration besides cell therapies discussed above.

The generation of human hearts from other animals by using interspecific chimera technology with blastocyst complementation is one such approach

. Another avenue of regenerative medicine is gene therapy, which is emerging as a potential treatment option in patients suffering from a wide spectrum of cardiovascular diseases, including coronary artery disease, peripheral vascular disease, vein graft failure, and in-stent restenosis

MicroRNAs (miRNAs), which are small, non-coding RNAs that regulate gene expression in a sequence-dependent manner, are also being investigated as a new modality of gene therapy for ischemic heart disease or vascular diseases.

In 2010, Ieda et al. reported that a combination of three developmental transcription factors (Gata4, Mef2c, and Tbx5) directly reprogrammed postnatal cardiac or dermal fibroblasts into differentiated cardiomyocyte-like cells in vitro (direct reprogramming)

This technology was recently applied to an *in-vivo* mouse MI model in which the three genes were delivered by a retroviral vector, resulting in the direct reprogramming of cardiac fibroblasts within the infarction site into cardiomyocyte-like cells and the amelioration of cardiac dysfunction. Therefore, despite concerns over the ethics and safety of gene therapy, it is a promising segment of the broad field of cardiovascular disease research.

Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010; 142: 375-386.

# Characterizing Regenerative Medicine

1. **Regenerative medicine** is a broad definition for innovative medical therapies that will enable the body to repair, replace, restore and regenerate damaged or diseased cells, tissues and organs. (Mayo Clinic)

2. **Tools and Procedures (**Biofabrication or Additive Manufacturing**) of Regenerative Medicine**

- Tissue Engineering: Tissue Repair/Replacement and Lab Grown Organs
- Technologies

✓ Stem cells

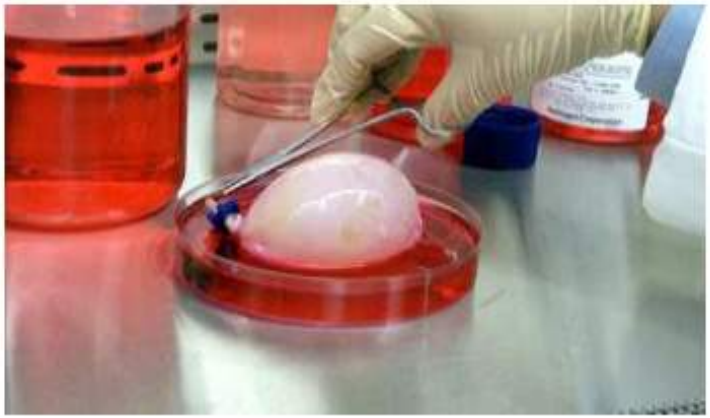
✓ Natural and Synthetic Scaffolds

✓ 3-D Printing and Chip Technologies

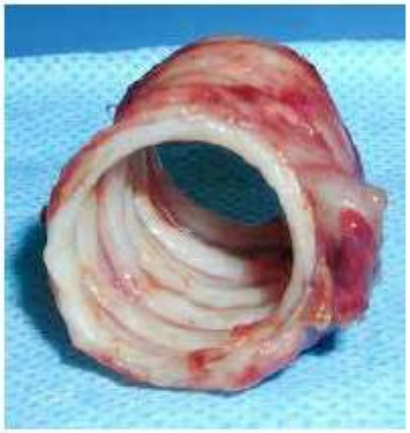
# Areas of Regenerative Medicine

## 1. *Artificial Organs: Medical Devices*

(Lab Grown Bladder)



## 2. *Tissue Engineering & Biomaterials*

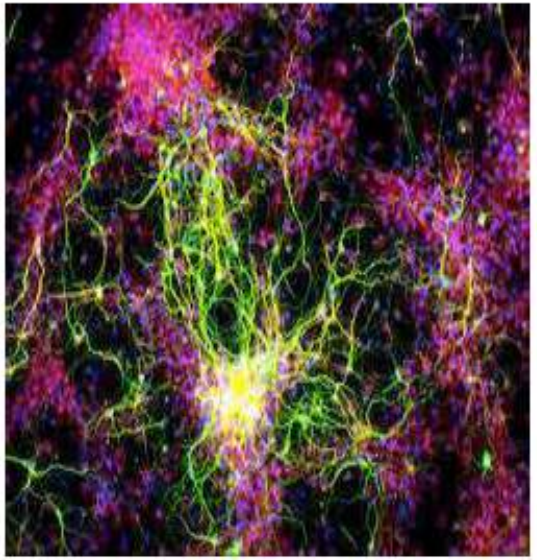


Scaffolds

# Areas of Regenerative Medicine

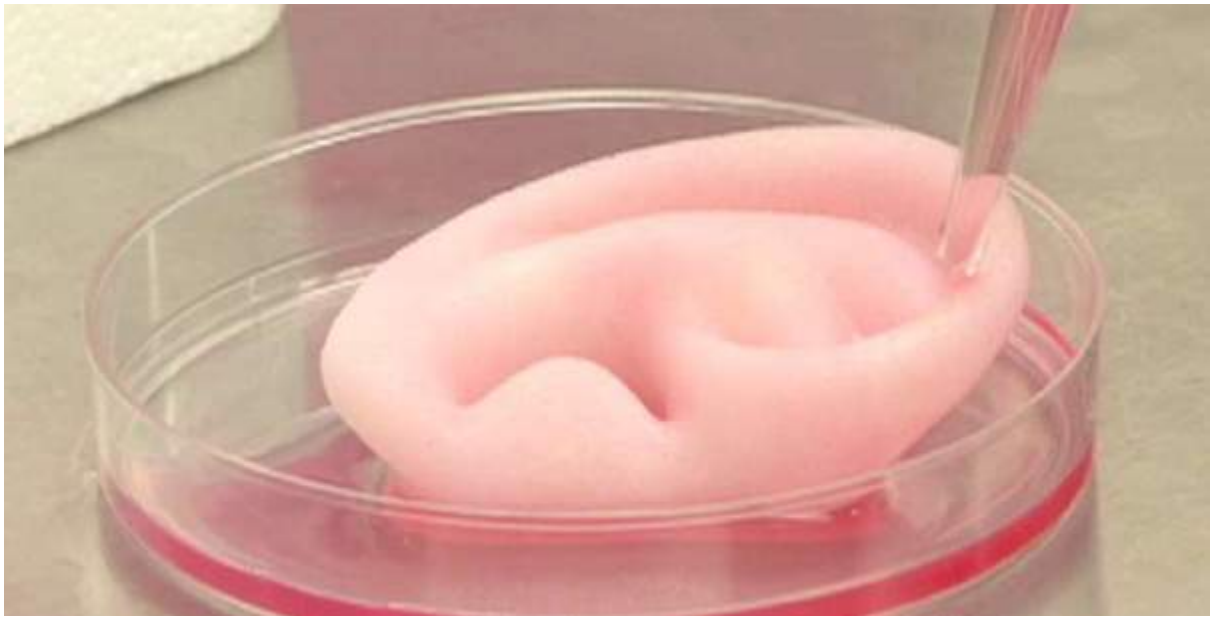
### 3. *Cellular Therapies*

- Use of Stem Cells (From Patient)
- Development of Regenerative Medicine Treatments.
- Enhance Regeneration of Tissues and Organs.



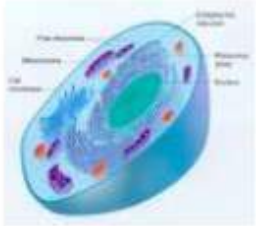
### 4. *Clinical Trials*

- Many Currently in Progress.
- NIH and Private Organizations.



# 3 Tools of Tissue Engineering

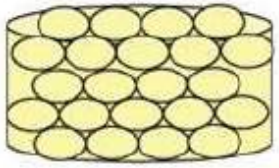
- Cells
  - Living part of tissue
  - Produces protein and provides function of cells
  - Gives tissue reparative properties
- Scaffold
  - Provides structural support and shape to construct
  - Provides place for cell attachment and growth
  - Usually biodegradable and biocompatible
- Cell Signaling
  - Signals that tell the cell what to do
  - Proteins or Mechanical Stimulation



# Components of a TE construct

**scaffold/matrix**

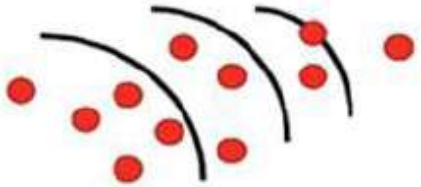
→ usually degradable, porous



**soluble factors**

→ made by cells or synthetic

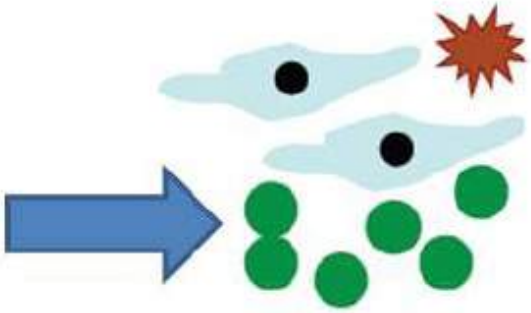
→ various release profiles



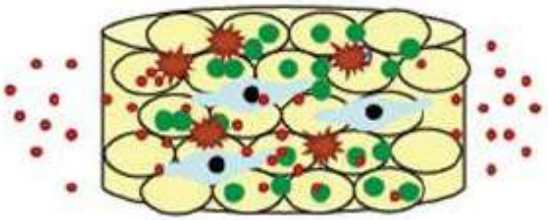
**cells**

→ precursors and/or differentiated

→ usually autologous



**integrated implantable or injectable device**



# Strategies of TE

- Isolated cells and cells substitues
  - Allows for an infusion of specific cells into the patient without the complication of surgery
- Tissue growth factor
  - Massive quantities  in targeted delivery
  - Use of gene delivery system to upregulate the local production
- Cell-matrix interaction with Scaffolds
  - To grow and eventually replace a biodegradable scaffold

## Nanotechnology and Microfluidics in Tissue Engineering

- Advances in fabrication technologies have brought a new dimension to the field of tissue engineering.
- Fabricate tissue engineering scaffolds with complex 3-D architectures and customized chemistries that mimic the in vivo tissue environment.

## What do we want in a scaffold?

- 1. Biocompatible
- 2. Biodegradable
- 3. Chemical and Mechanical Properties
- 4. Proper architecture



## Role of the Scaffold

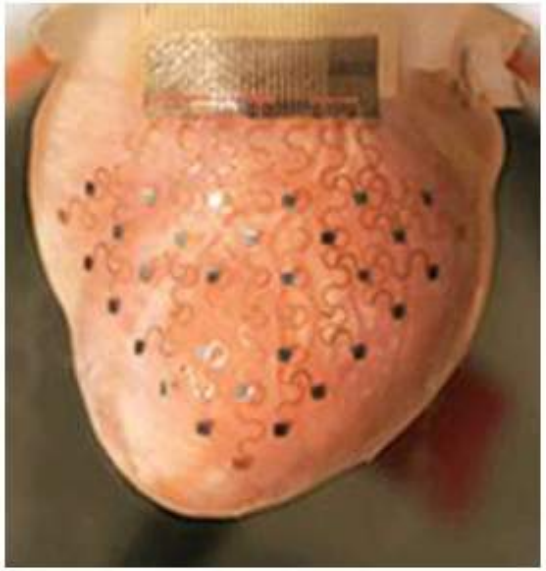
- Present a surface/structure that closely resembles the extracellular matrix (ECM)
- Surfaces that could maximize favorable biological responses (cell-matrix interaction, Protein-matrix interaction)

# Regenerative Medicine Challenges

1. Design of Biomaterials that Function in the Body
2. Getting Enough Cells and Cell Types for Engineering Tissues and Organs
3. Vascularity: Engineering Blood Vessels that Supply Nutrients, Oxygen and Signals to Bioengineered Tissues and Organs
4. Cost of Tissue and Organ Development Procedures

# Promise of Regenerative Medicine

## 3-D Printer Creates Heart Membrane



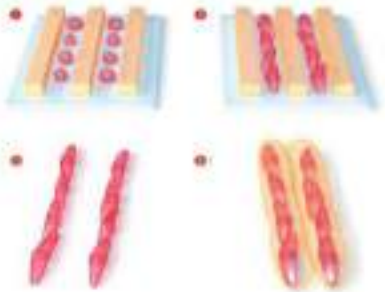
[Prof. Igor Efimov, Washington University in St. Louish: ttps://news.wustl.edu/news/Pages/26554.aspx]

*Lizhi Xu and al., Nature Communications, 2014, 3329, doi:10.1038/ncomms4329*

## 3-D Elastic Membrane Fits Heart's Epicardium

## Regenerative Medicine Pioneers

Dr. Ali Khademhosseini (Wyss Institute at Harvard)



[MIT Technology Review: www2.technologyreview.com/tr35/profil

- 2007: Creating living tissues
- Organs in the lab

## Regenerative Medicine Pioneers

Dr. Paolo Macchiarini (Karolinska Institute)

First Donor Trachea

v Has Normal Respiratory

## Regenerative Medicine Pioneers

1993: Dr. Robet Langer (Langer Lab, MIT)

Tissue engineering, controlled release systems and transdermal delivery systems

## Regenerative Medici

McGowan Institute of Regener
(University of Pittsburg)

- Removed Cells from Pig Bladder Extra Cellular Matrix (ECM)
- Re-grows Severed Digits and
- New Muscle Tissue Development of 3-D bioscaffolds for liver and heart regeneration

## Medicine Pioneers

for Regenerative Medicine (WFIRM)

Dr. Anthony Atala

- 2006-2007: First to Engineer/Transplant Lab-Grown Organ into a Human
- Transplant was Successful
- Currently Developing Organ on a Chip Program

## Nobel research on Stem cell biology

Sir John B. Gurdon

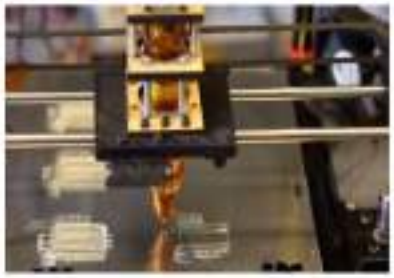
Shinya Yamanaka

*The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent"*

## Regenerative Medicine Innovators

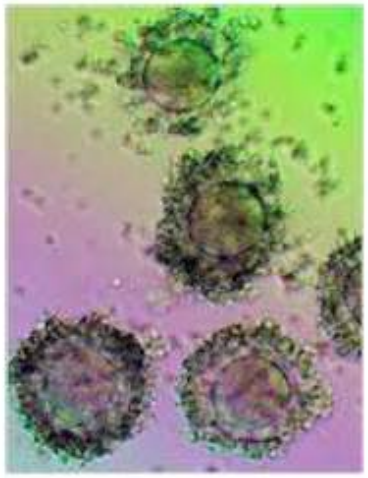
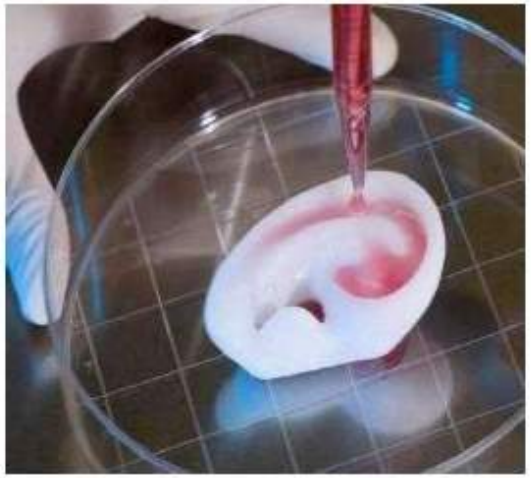
Dr. Jordan Miller (Rice University)

- 2013: Uses 3-D Print Technology
- Engineers Blood Vessels Using Sugar



# Futuristic!

Stem Cells + Organ Scaffold + 3D Printer


= Libraries of Replacement Organs?

Advanced Drug Delivery Reviews 120 (2017) 3–24

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

# Stem cells and heart disease - Brake or accelerator?☆

Gustav Steinhoff [a,*], Julia Nesteruk [a], Markus Wolfien [b], Jana Große [a], Ulrike Ruch [a], Praveen Vasudevan [a], Paula Möller [a]

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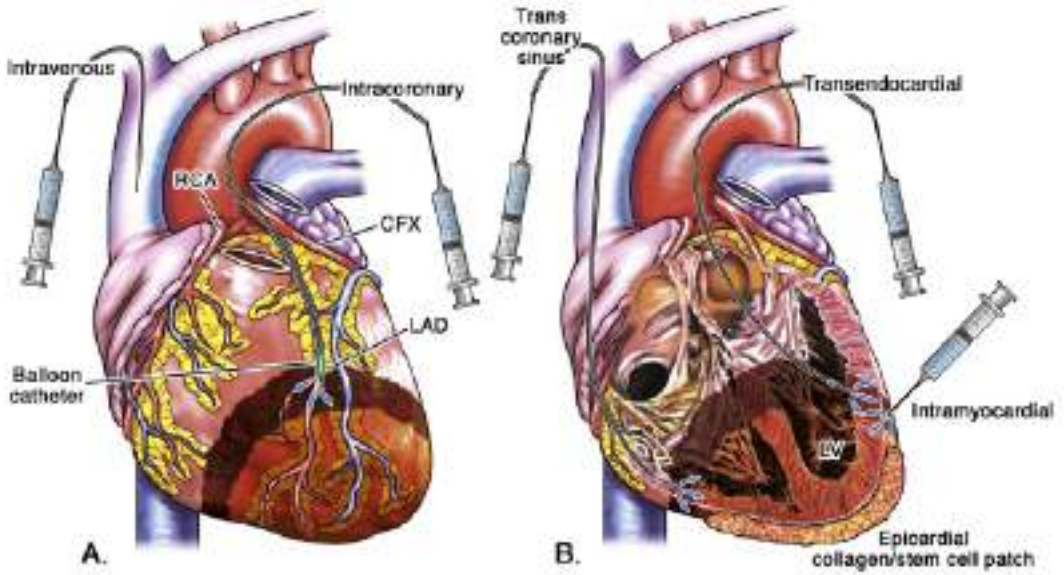
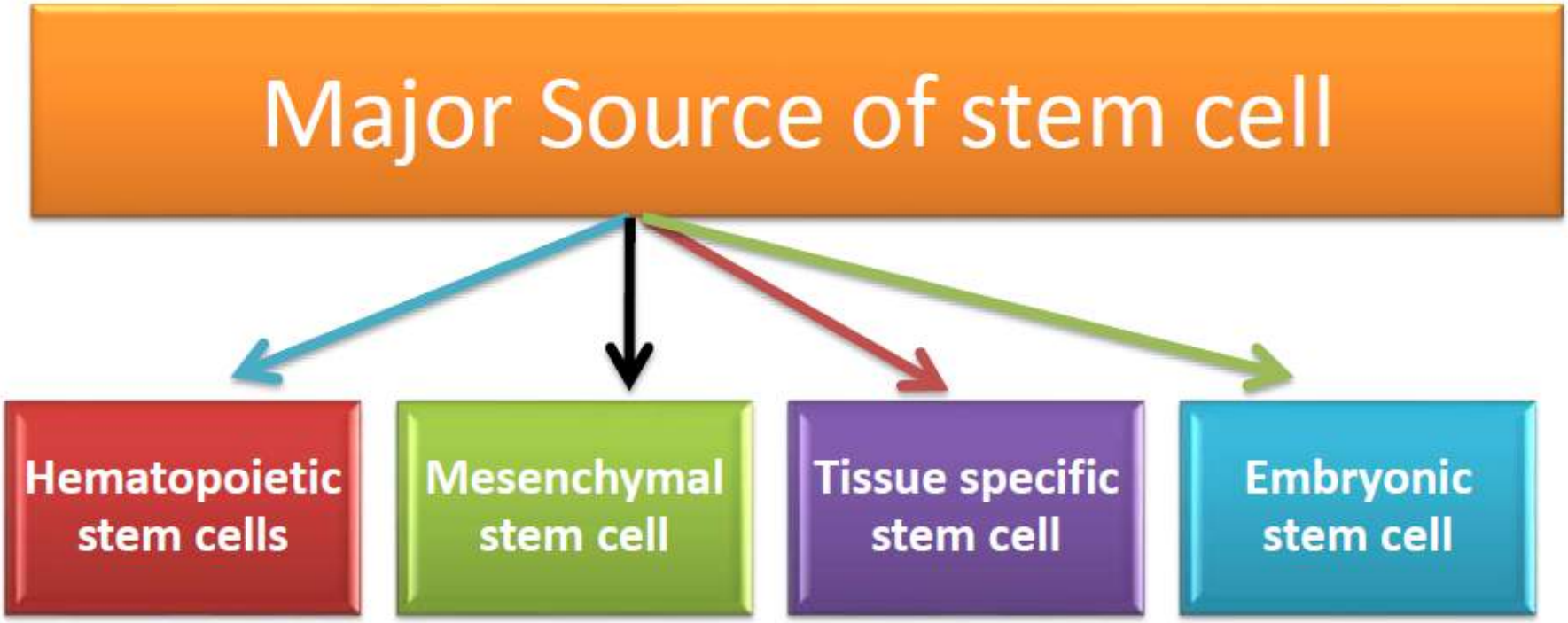



Basic Research (GSP) → Preclinical Research (GLP; GMP) → Clinical Research (GMP; GCP) → MA / Market (GMP; GVP) → ?


Major Source of stem cell
Hematopoietic stem cells
Mesenchymal stem cell
Tissue specific stem cell
Embryonic stem cell

# Goals of tissue engineering

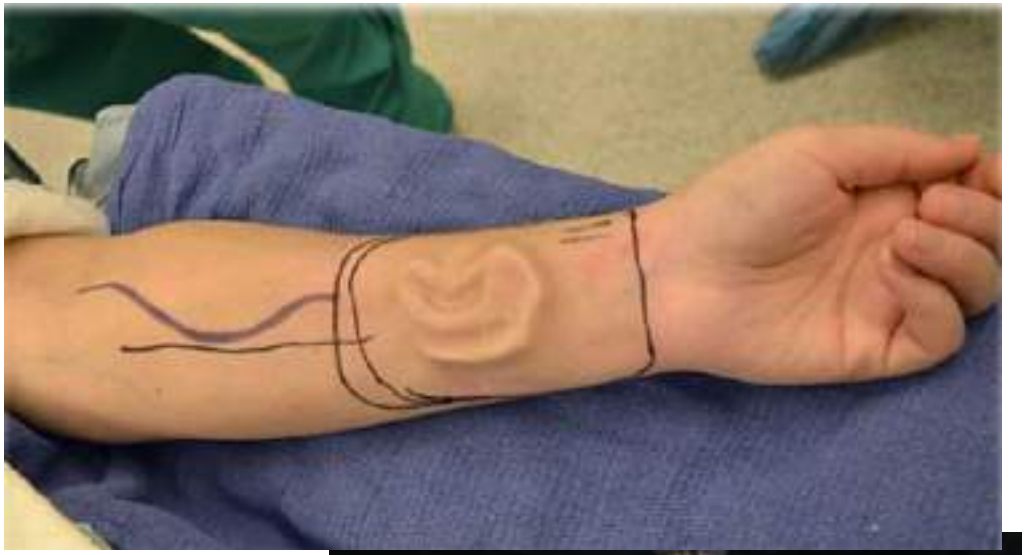
- *Growth of cell in three dimensional systems*
- *Delivery systems for protein therapeutics*
- *Cell cultivation methods for culturing recalcitrant cells*
- *Transgenic protein expression in transplantable cells*
- *Vehicles for delivering transplantable cells*
- *Avoiding immunogenicity in transplantation systems*
- *Development of markers for tracking transplanted cell*
- *Developing in vivo and ex vivo biosensors for monitoring cell behaviour during tissue production.*

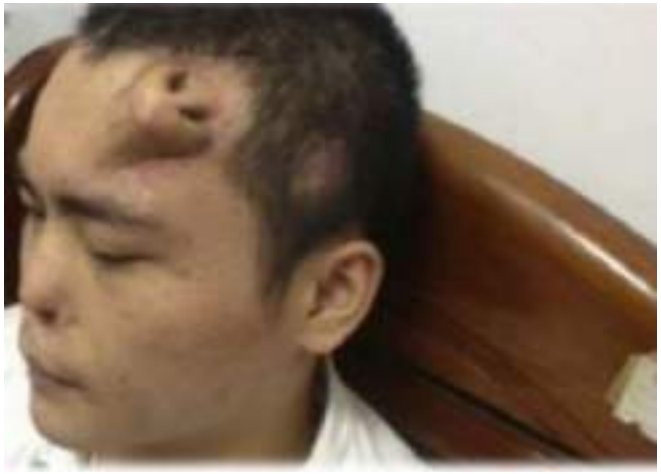
# Advantage of tissue engineering

- *Tissue engineering provides long term and much safer solution than other options.*
- *The traditional transplantation complications are minimized.*
- *The donar can be patients himself or herself.*
- *The need for donar tissue is minimal.*
- *Immuno suppression problem can be minimized.*
- *The presence of residual foreign material can be minimized.*

# Disadvantage of tissue engineering

- *Cell isolation and preparation , biomaterial of nutrients transport and transplantation is very complex process.*
- *It is difficult to achieve cell diffrentiation into desired cell type and ensuring their nutrient supply after implantation into the body.*
- *There may be obstacles to growing cells in sufficient quantities.*
- *The time necessary to develops cells in culture before they can be used and possible lack of function at the donar site are some other limitations.*

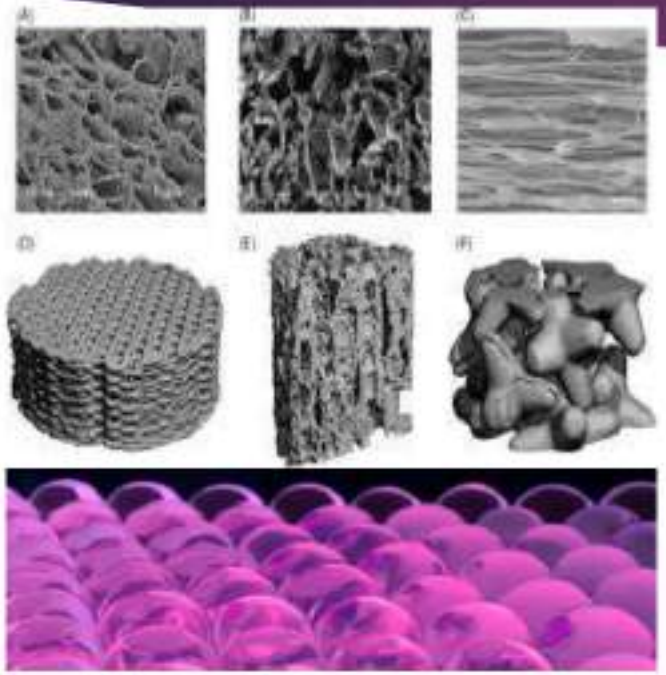




# Introduction

- Heart disease is the leading cause of death and disability all over the world accounting for approximately 40% of all human mortality
- **Treatment limitations:**
- Cardiomyocytes cannot divide to replace injured cells
- Restricted intrinsic capacity of the heart
- Lack of organs for transplantation and
- Complications associated with immune suppressive treatments
- **The main targets for tissue engineering**
- Blood vessels
- Heart muscles- myocardium and
- Heart valve

# Biomaterials

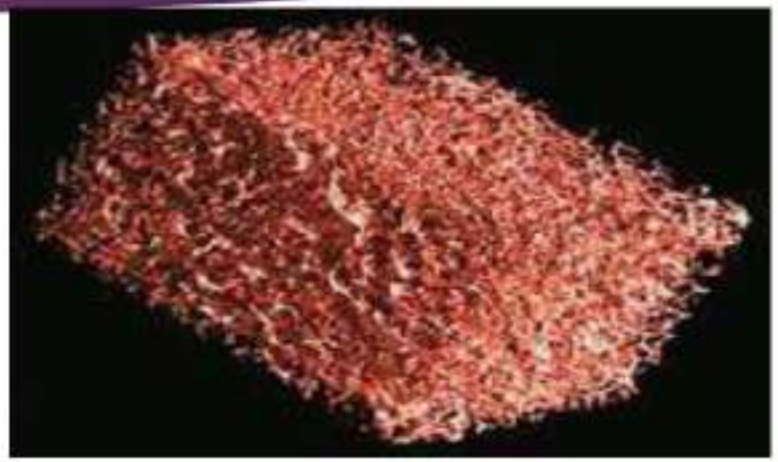


Most commonly used biomaterials for cardiovascular tissue engineering are

- **Biodegradable Polymeric scaffolds** (Polyglycolic acid PGA)
- **Hydrogels** (seeded with collagen, fibrin, alginate)
- **Decellularized tissue** (composed of natural ECM proteins: collagen, fibronectin etc.)

# Biomaterials-Scaffolds

- **Scaffold** provides structure for cells/tissue to grow and deliver biomolecules (growth factors, cytokines, etc.)
- Properties (chemical, mechanical, biological) should be adjusted to provide appropriate performance.



# TE Product Requirements

- **Biocompatible**

  Should not elicit immune or inflammatory response

- **Functional**

  Adequate mechanical and hemodynamic function, mature ECM, durability

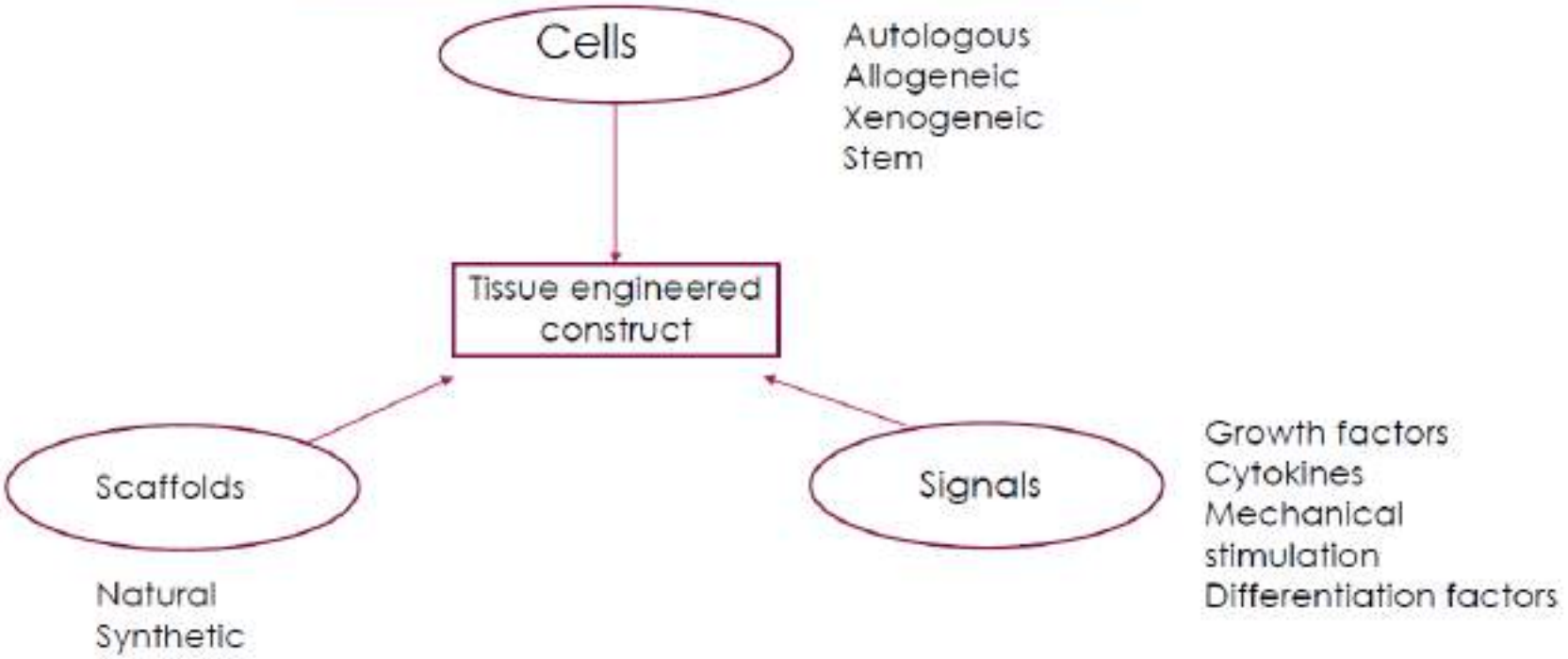
- **Living**

  Growth and remodelling capabilities of the construct should mimic the native heart valve, blood vessel or myocardium structure

# Continued

- **Blood Vessels**
  - Must be able to withstand high-pressure fluid dynamics, turbulence
  - Biocompatible, functional, living
- **Valves**
  - Must be able to operate in a **very dynamic and severe environment**
  - Open and close at 1Hz, exposed to mechanical stresses, high pressure fluid dynamics, turbulence etc.
- **Myocardium Patch**
  - High vascularity is critical
  - Mechanical and electrical anisotropy
  - High metabolic demand

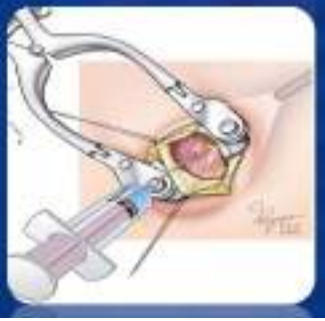
# Overview


Cells
Autologous
Allogeneic
Xenogeneic
Stem
Tissue engineered construct
Scaffolds
Natural
Synthetic
Signals
Growth factors
Cytokines
Mechanical stimulation
Differentiation factors

## 1) Direct myocardial injection:

- The most accurate type of delivery that can be achieved by direct visualization during thoracotomy.
- Can be done either as an adjunct to CABG or as sole therapy.


- ***Disadvantages:***
  - Possible arrhythmogenicity.
  - Need for concomitant open-heart surgery.
  - Can't be repeated if needed.



- The ideal modality of cell delivery should have the following characteristics:
  - Safe with minimal complications.
  - Easily performed.
  - Cost benefit ratio within range.
  - Applicable across a wide range of clinical scenarios
  - Targeting of the cells precisely in an adequate cardiac environment.

## 2) Transcatheter endomyocardial injection:

- Less invasive technique.
- Electromechanical testing to confirm the presence of ischemic or dead myocardium should be done before cell delivery.


- *Disadvantages:*
  - Complex procedure as electromechanical map of the left ventricular chamber is required.

## 3) Intracoronary cell injection:

- Most popular mode of cell delivery especially after AMI.
- It can be performed at the same time of (PCI).
- *Disadvantages:*
  Not suitable for all cell types as larger cells as MSCs may occlude the Microcirculation.

## 4) Intravenous injection:

- The simplest and least invasive delivery route and produces minimal complications.
- Can easily be repeated if necessary.
- *Disadvantage:*
  low percentage of cells delivered.

# Scaffold in Stem Cell Tx

I. Natural Scaffold
II. Synthetic Scaffold
    1. Non Biodegradable Scaffold
    2. Biodegradable Scaffold

# The major roles for supporting matrices (scaffold)

1. It serves as a **framework**, which maintains the shape of the defect. It provides physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site.
2. It serves as a 3D substratum for **cellular adhesion, migration, proliferation and production of extracellular matrix**.
3. It serves as **a barrier to restrict cellular migration** in a selective manner.
4. It potentially serves as a **delivery vehicle for growth factors.**
5. **Temporary Nutrition**

# Natural Scaffold : Extracellular Matrix

**Natural Scaffold for Bone tissue engineering :**
**the extracellular matrix (ECM) of bone, the unique microenvironmental niche for bone morphogenesis**

- Decellularized Bone
- Decellularized Bronchial Tree from corpse
- Decellularized Cardiac Valves from corpse
- Etc.

# Scaffold for Myocardial Regeneration – in the form of sheet

1. It serves as a **framework**, which maintains the shape of the defect. It provides physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site.
2. It serves as a 3D substratum for **cellular adhesion, migration, proliferation and production of extracellular matrix**.
3. It serves as **a barrier to restrict cellular migration** in a selective manner.
4. It potentially serves as a **delivery vehicle for growth factors.**
5. **Temporary Nutrition**

# Current Interventional and Surgical Management of Congenital Heart Disease

## Specific Focus on Valvular Disease and Cardiac Arrhythmias

Kimberly A. Holst, Sameh M. Said, Timothy J. Nelson, Bryan C. Cannon, Joseph A. Dearani

*Circulation Research* March 17, 2017

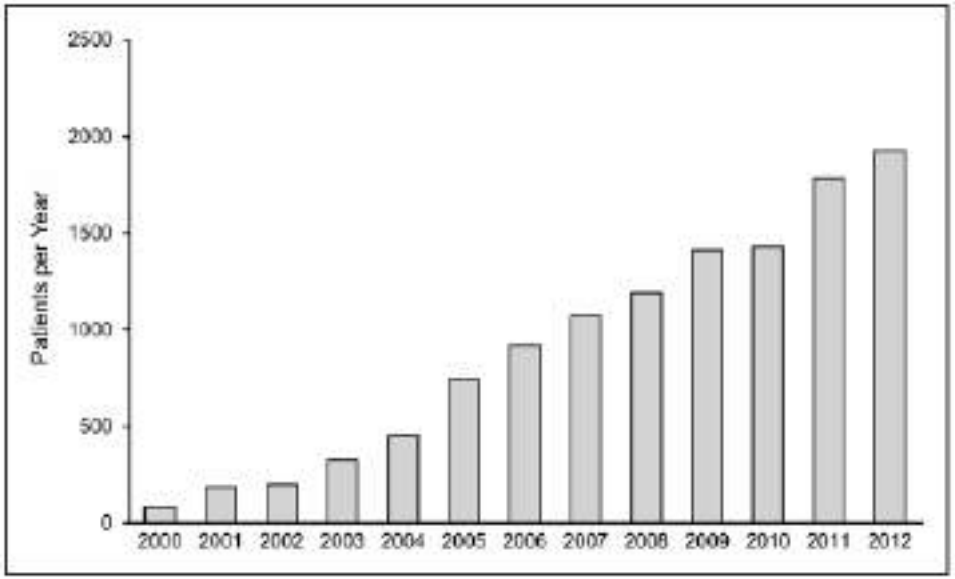



**Figure 1. Adult congenital volume through time.** Adult patients with congenital heart disease continue to increase through time. Adult patient volumes as reported to The Society of Thoracic Surgeons Congenital Heart Surgery Database from 2000 to 2012 from 116 congenital heart surgery centers. Reproduced from Fuller et al[9] with permission of the publisher. Copyright ©2015, The Society of Thoracic Surgeons.

The pediatric heart is more resistant to fibrosis in response to injury and is defined by increased density of progenitor cells or cardiomyocytes that retain proliferative potential. Notably, pressure overload in pediatric RV demonstrate a significant increase in cardiac stem cells.[87] The endogenous regenerative potential of the youthful heart may not be sufficient to overcome the challenges of severe CHD, such as HLHS

The surgical repair of CHD is continuously challenged by a growing heart with increased physiological demands. Therefore, customized regenerative products that can meet the emerging gaps of yesterday's treatments will afford new possibilities to preserve and extent the viability of each patient's suboptimal cardiac tissues. As

PERSEUS trial;
(Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease)

autologous cardiosphere derived cells and intracoronary delivery of $3.0\times10_{5}$ cells/kgmonths after either stage 2 or stage 3 palliation

≈6% increase of ejection fraction of the
single ventricle by 3 independent measures

# TICAP trial

(Transcoronary Infusion of Cardiac Progenitor Cells in Patients With Single Ventricle Physiology)

Patients with HLHS undergoing stage 2 surgical palliation and having autologous umbilical cord blood collected and processed may be eligible for direct injections into the myocardium during the surgical procedure

*Review Article*

# The Rapidly Evolving Concept of Whole Heart Engineering

**Laura Iop,[1,2] Eleonora Dal Sasso,[1,2] Roberta Menabò,[3] Fabio Di Lisa,[3,4] and Gino Gerosa[1,2]**

Stem Cells International
Volume 2017, Article ID 8920940, 18 pages
https://doi.org/10.1155/2017/8920940

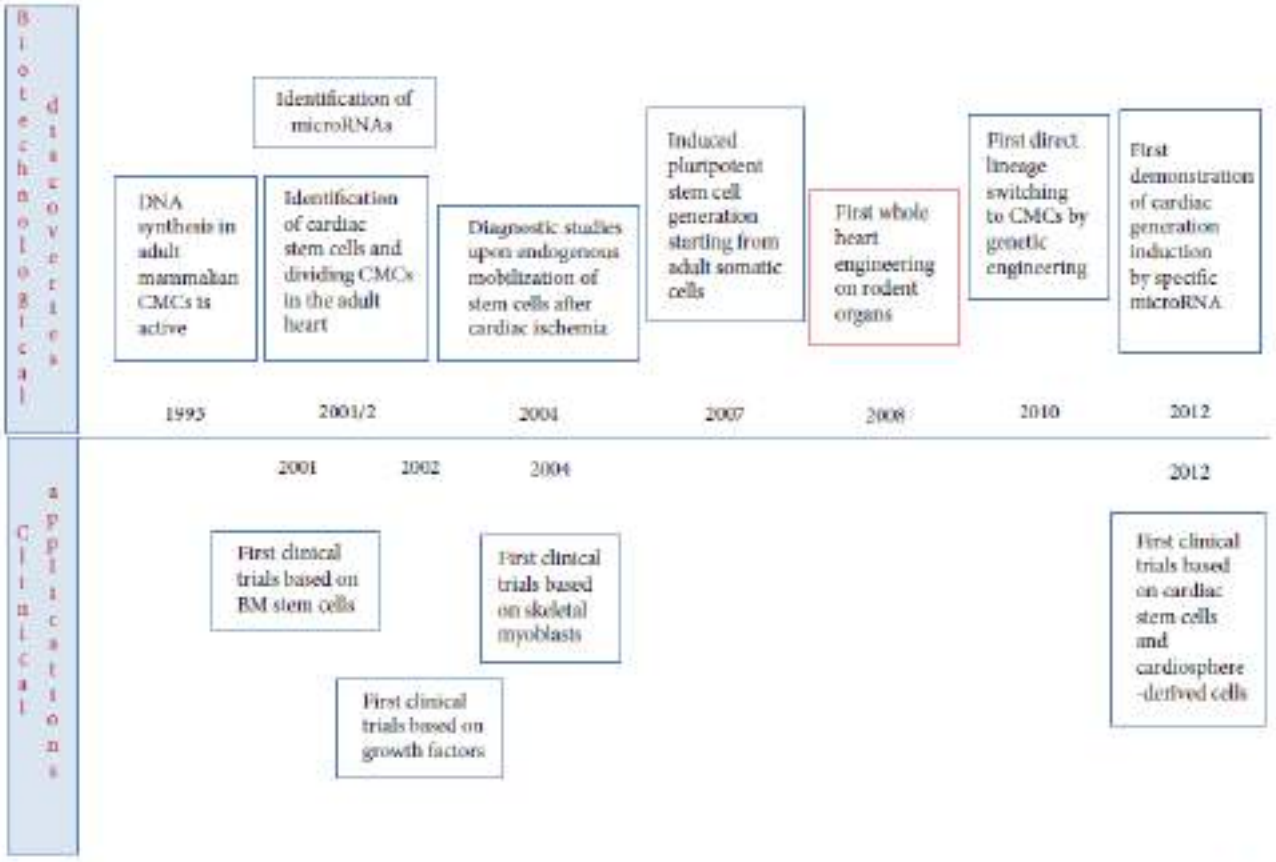



Figure 1: The most striking technological advancements and clinical applications anticipating the birth and evolution of the whole


Poor retention and survival
of transplanted cells in the heart which can decrease to 39% at 1 h following injection as seen in human studies

The
increased cell death is swayed by various inflammatory response mediators, mechanical injury, hypoxia and ischemia-reperfusion stressors, and influenced as well by the donor cell source and quality[69]

TISSUE ENGINEERING: Part B
Volume 22, Number 6, 2016

DOI: 10.1089/ten.teb.2016.0136

termis.
Tissue Engineering
& Regenerative Medicine
International Society

***REVIEW ARTICLE***

# Regenerative Medicine Strategies for Hypoplastic Left Heart Syndrome

Josue Chery, MD,[1] Joshua Wong, MD,[2] Shan Huang,[1] Shuyun Wang, MD, PhD,[1] and Ming-Sing Si, MD[1]

Nevertheless, the high morbidity and mortality associated with HLHS remains unsatisfactory with an emerging epidemic of RV failure in these patients with no available alternative treatment options. This challenging clinical scenario presents as an ideal situation where regenerative medicine approaches can be applied. Promising preclinical and early clinical evidence suggest that stem cell therapy may be able to preserve the function of the pressure-overloaded RV. Initial case reports and subsequent clinical trials highlight the potential benefits of regenerative medicine in patients with HLHS, while reminding us of the challenges that remain to be conquered

Review

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Regenerative Medicine

# Regulation of the microenvironment for cardiac tissue engineering

*Regen. Med.* (2017) 12(2), 187–201

Engineering cardiac tissue that mimics native myocardium tissue depends on modulating several microenvironmental cues, including biochemical, electrical, spatial and biomechanical factors, along with intercellular interactions. The implementation of these different microenviromental cues can influence CM differentiation, maturation, organization and electrophysiology, in order to improve the engineered cardiac tissue's functionality and ultimately myocardial function.

## Executive summary

### Background

- Cardiovascular tissue engineering employs the use of stem cells, microenviromental cues and intercellular interactions to generate functional tissue constructs.
- Cardiomyocyte (CM) sources include resident cardiac stem cells, adult stem cells and pluripotent stem cells.

### Microenvironmental cues

- Biochemical molecules and growth factors have been employed to promote stem cell differentiation into CMs. These molecules are present in biochemical signaling pathways that regulate embryonic development of the myocardium.
- The extracellular matrix, which is a scaffolding structure of extracellular proteins in which cells reside, modulate cell function in part through spatial factors. Spatial factors rely on the use of spatial patterning to mimic the cell-to-cell and cell-to-extracellular matrix interactions of CMs.
- Biomechanical stimulation of CMs is accomplished by altering substrate stiffness and stretching CMs.
- Intercellular interactions between CMs and non-CMs occur through electromechanical signaling and paracrine signaling.

### Applications for cardiac tissue engineering

- Microenvironmental stimulation of CMs affect CM proliferation, maturation, organization, survival and electrophysiological characteristics.
- Microenvironmental stimulation of CMs and incorporation of intercellular interactions from non-CMs are used to generate cardiac constructs that can be implanted into the diseased myocardium.

### Challenges

- CM cell survival *in vivo*, maturation and organization.

*Review Article*

# The Light and Shadow of Senescence and Inflammation in Cardiovascular Pathology and Regenerative Medicine

**Laura Iop,[1] Eleonora Dal Sasso,[1] Leonardo Schirone,[2] Maurizio Forte,[3] Mariangela Peruzzi,[2] Elena Cavarretta,[2] Silvia Palmerio,[2,3] Gino Gerosa,[1] Sebastiano Sciarretta,[2,3] and Giacomo Frati[2,3]**

[1]*Cardiovascular Regenerative Medicine Group, Department of Cardiac, Thoracic and Vascular Surgery, University of Padua and Venetian Institute of Molecular Medicine, Padua, Italy*
[2]*Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy*
[3]*IRCCS Neuromed, Pozzilli, Italy*

Mediators of Inflammation
Volume 2017, Article ID 7953486, 13 pages

*Original papers*

*Med Ultrason 2015, Vol. 17, no. 2, 160-166*
DOI: 10.11152/mu.2013.2066.172.effbm

# Effectiveness of bone marrow mononuclear cells delivered through a graft vessel for patients with previous myocardial infarction and chronic heart failure: an echocardiographic study of left ventricular remodeling.

**Fujian Duan*[1], Zhi Qi*[1], Sheng Liu[2], Xiuzhang Lv[1], Hao Wang[1], Yiming Gao[1], Jianpeng Wang[1]**

[1]Department of Echocardiography, [2]Department of Cardiovascular Surgery, Fuwai Hospital & Cardiovascular Institute, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences & Tsinghua University, Peking Union Medical College, Beijing, China.

**Abstract**

**Aims:** The graft of stem cells to treat ischemic cardiomyopathy is popular in many clinical trials. The aim of this study was to evaluate the effectiveness of isolated coronary artery bypass graft combined with bone marrow mononuclear cells (BMMNC) delivered through graft vessels to improve left ventricular remodeling of patients with previous myocardial infarction and chronic heart failure using echocardiography. **Material and methods:** Patients with previous myocardial infarction and chronic heart failure were randomly allocated to one of the two groups: CABG only (18 patients), or CABG with BMMNC transplantation (24 patients). Echocardiographic parameters were measured on B-mode imaging, 3D imaging and color flow imaging. **Results** Post-operative LVEDD (end-diastolic dimension of left ventricle), LVESD (end-systolic dimension of left ventricle), LVEDV (end-diastolic volume of left ventricle), LVESV (end-systolic volume of left ventricle), LVEDVI (LVEDV indexed to body surface area), LVESVI (LVESV indexed to body surface area), LV-mass (mass of left ventricle) and LV-massI (LV-mass indexed to body surface area) were significantly improved compared with those obtained prior to operation in CABG+BMMNC group (al $p<0.05$). The same parameters were not significantly different pre- and postoperative in the CABG group (al $p>0.05$). Postoperative mitral regurgitation score was not significantly different from those prior to operation in both groups (al $p>0.05$). In Chi-square tests, LVEDD, LVESD, LVEDV, LVESV, LVEDVI, LVESVI, LV-mass, LV-massI were determinants of the left ventricular remodeling. **Conclusion:** The improvement of left ventricular remodeling in

Table I. Baseline Characteristics

| Clinical date | CABG+BMMNC group (n=24) | CABG group (n=18) | P value |
|---|---|---|---|
| Age,y | 57.88±8.52 | 56.56±9.09 | 0.881 |
| Man,% | 95.8 | 94.4 | 0.679 |
| BSA,m2 | 1.81±0.12 | 1.78±0.13 | 0.723 |
| NYHA function class | 3 (2,3) | 2 (2,3) | 0.239 |
| No.of grafts | 4 (4,5) | 4 (4,5) | 0.331 |
| CPB time,min | 90(61,103) | 89(78,116) | 0.198 |
| Clamping time,min | 60(50,67) | 55(48,70) | 0.868 |
| Ventilation time,h | 16(13,20) | 17(14,20) | 0.332 |
| ICU stay,days | 3(3,5) | 3(3,4) | 0.221 |
| 6-min walking test | 452(408,495) | 433(382,497) | 0.206 |
| BNP,ng/L | 1302(714,1676) | 890(680,1646) | 0.431 |
| Hypertension,% | 16.7 | 11.1 | 0.481 |

Table III. Echocardiographic parameters in CABG group

| | pre-surgen (mean±SD) | 1 year later (mean±SD) | P value |
|---|---|---|---|
| LVEDD (mm) | 56.66±6.76 | 54.61±6.64 | 0.364 |
| LVESD (mm) | 45.67±6.27 | 41.66±8.05 | 0.105 |
| LVEDV (ml) | 167.61±42.10 | 156.78±36.30 | 0.414 |
| LVESV (ml) | 106.94±27.68 | 95.56±28.92 | 0.236 |
| LVEDVI (ml/m$^2$) | 93.76±23.65 | 87.17±15.36 | 0.285 |
| LVESVI (ml/m$^2$) | 59.84±13.59 | 53.02±13.48 | 0.140 |
| LV-mass (g) | 242.11±60.56 | 233.50±60.51 | 0.672 |
| LV-massI (g/m$^2$) | 135.41±28.56 | 129.95±27.43 | 0.562 |
| MR score | 1.28±0.75 | 1.17±1.29 | 0.755 |

Table II. Echocardiographic parameters in CABG+BMMNC group

| | pre-surgen (mean±SD) | 1 year later (mean±SD) | P value |
|---|---|---|---|
| LVEDD (mm) | 60.96±5.26 | 52.29±5.94 | 0.000 |
| LVESD (mm) | 46.70±5.77 | 37.86±6.47 | 0.000 |
| LVEDV (ml) | 196.17±41.26 | 145.38±40.81 | 0.000 |
| LVESV (ml) | 126.04±28.22 | 82.04±34.02 | 0.000 |
| LVEDVI (ml/m2) | 108.14±20.94 | 80.72±22.59 | 0.000 |
| LVESVI (ml/m2) | 69.47±14.52 | 45.62±19.13 | 0.000 |
| LV-mass (g) | 267.25±67.97 | 222.88±60.44 | 0.021 |
| LV-massI (g/m2) | 147.37±35.33 | 123.53±32.45 | 0.019 |
| MR score | 1.42±0.65 | 1.04±0.71 | 0.061 |

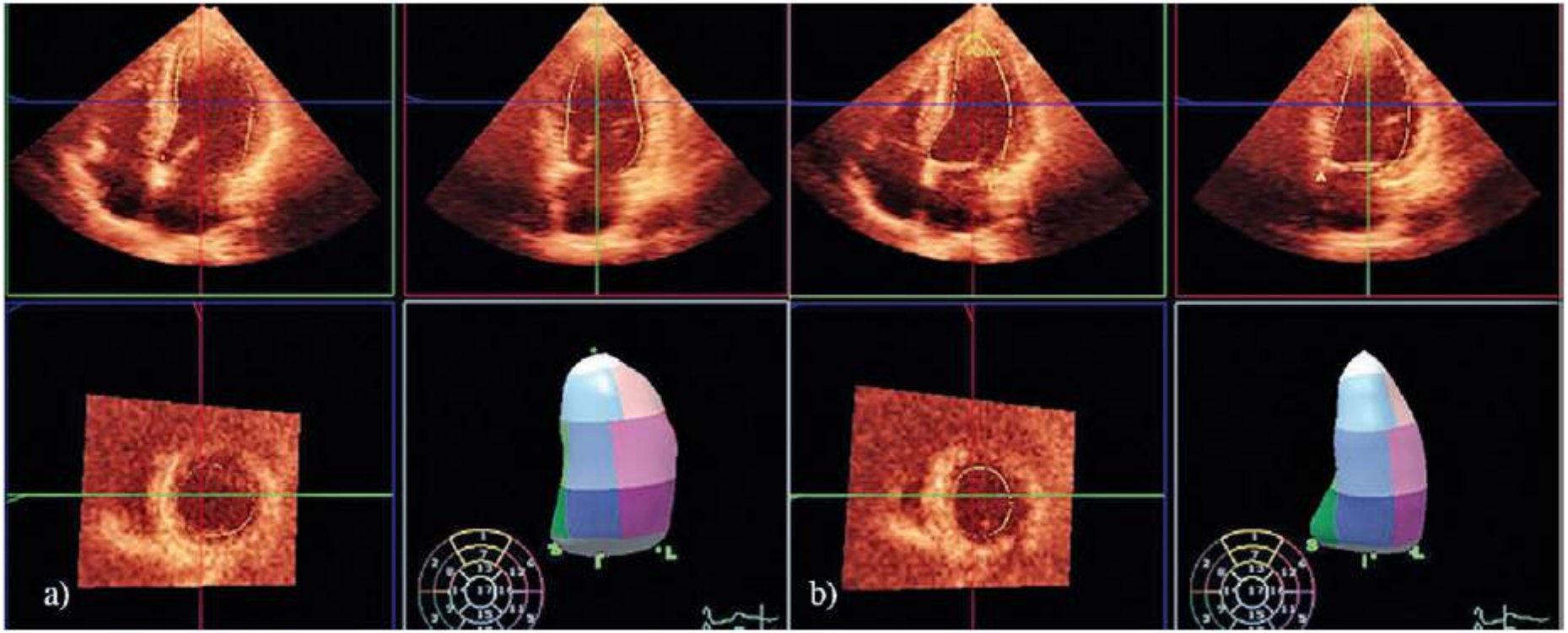



**Fig 1.** a) Apical 3D full-volume data set. Triplane is displayed. Para-sternal short-axis views are shown for completeness and to indicate the image plane position in the 3D images. End-diastolic volume of left ventricle is calculated and shown in the 3D imagings; b) Apical 3D full-volume data set. Triplane is displayed. Para-sternal short-axis views are shown for completeness and to indicate the image plane position in the 3D images. LVESV is calculated and shown in the 3D imagings.

*Med Ultrason 2015, Vol. 17, no. 2, 160-166*
DOI: 10.11152/mu.2013.2066.172.effbm

## Conclusions

The effectiveness of isolated CABG combined with BMMNC delivered through graft vessels in improvement of the LV remodeling of patients with previous myocardial infarction and chronic heart failure was verified in this study. The improvement of LV remodeling in CABG+BMMNC group was better than in the CABG group and this improvement can by verified by using echocardiography.

**Conflict of interest**: none

European Heart Journal (2009) **30**, 662–670
doi:10.1093/eurheartj/ehn532

**CLINICAL RESEARCH**
*Coronary heart disease*

# Improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study

**Aims** To investigate whether intracoronary transfer of bone marrow progenitor cells (BMPCs) early after reperfusion of an acute myocardial infarction improves regional myocardial function in a randomized double-blind, placebo-controlled strain rate imaging study.

**Methods and results** Regional myocardial deformation was measured using velocity-derived strain rate imaging in 67 STEMI patients randomized 1:1 to intracoronary infusion of BMPC ($n = 33$) or placebo ($n = 34$). Myocardial segments were grouped into infarct ($n = 232$), border ($n = 250$), and remote ($n = 526$) based on MRI-delayed enhancement and the perfusion territory of the infarct-related vessel.

Four months after revascularization and progenitor cell/placebo transfer, regional myocardial deformation (rate) improved significantly more in the infarct segments of BMPC patients (treatment effect on end-systolic strain: $-3.7 \pm 1.0\%$, $P = 0.0003$; peak-systolic strain rate: $-0.20 \pm 0.07\ s^{-1}$, $P = 0.0035$). These findings were confirmed by a significantly greater improvement of longitudinal mitral valve ring displacement in the infarct walls of BMPC patients (treatment effect: 0.93 mm, $P = 0.034$).

**Conclusion** Intracoronary infusion of BMPC early after reperfusion of a STEMI improves recuperation of regional myocardial function at 4 months' follow-up. Quantitative assessment of regional systolic function might be more sensitive than global LV ejection fraction for the evaluation of BMPC therapy after STEMI.

**Keywords** Stem cell therapy • Regional myocardial function • Strain rate imaging • Acute myocardial infarction

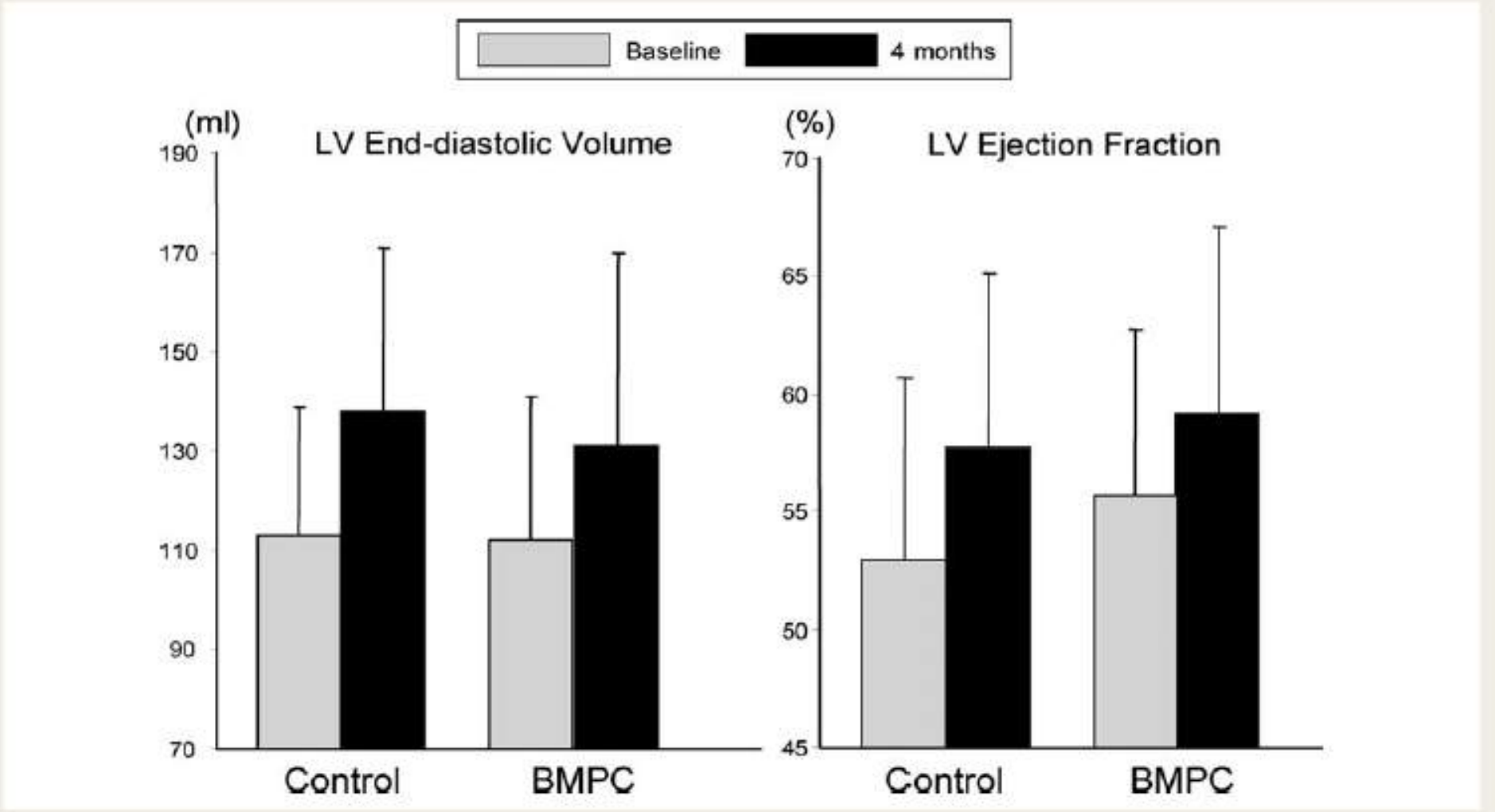



**Figure 3** LV end-diastolic volume (mL) and LV ejection fraction (%) by ultrasound modified Simpson's method at baseline and at 4 months' follow-up in patients treated with bone-marrow progenitor cells (BMPCs) and in controls. Data are mean ± SD.

Clinical Track

# Autologous Mesenchymal Stem Cells Produce Concordant Improvements in Regional Function, Tissue Perfusion, and Fibrotic Burden When Administered to Patients Undergoing Coronary Artery Bypass Grafting

## The Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) Trial

Vasileios Karantalis, Darcy L. DiFede, Gary Gerstenblith, Si Pham, James Symes,

*Clinical Trial Registration: Circ Res*. 2014;114:1302-1310

- Preclinical studies provide evidence that bone marrow stem cells contribute to cardiac function and reverse remodeling after ischemic damage acting both locally and remotely (possibly through paracrine mechanisms.

- In studies to date, investigators have either infused or injected bone marrow–derived cells in areas that were undergoing revascularization

- ***Here, we test the hypothesis that intramyocardial injections of autologous MSCs delivered to segments of myocardium not receiving surgical revascularization improve regional cardiac structure and function*.**

- ***Methods and Results:*** **patients were injected with autologous MSCs into akinetic/hypokinetic myocardial territories not receiving bypass graft for clinical reasons.**

- **MRI was used to measure scar, perfusion, wall thickness, and contractility at baseline, at 3, 6, and 18 months and to compare structural and functional recovery in regions that received MSC injections alone, revascularization alone, or neither.**

- **After 18 months, subjects receiving MSCs exhibited increased LV ejection fraction (+9.4±1.7%, *P*=0.0002) and decreased scar mass (–47.5±8.1%; *P*<0.0001) compared with baseline.**

- **MSC-injected segments had concordant reduction in scar size, perfusion, and contractile improvement (concordant score: 2.93±0.07), whereas revascularized (0.5±0.21) and nontreated segments (–0.07±0.34) demonstrated nonconcordant changes (*P*<0.0001 versus injected segments).**

European Heart Journal (2014) **35**, 1263–1274
doi:10.1093/eurheartj/ehu007

**CLINICAL RESEARCH**
*Stem cells*

# Autologous CD133$^+$ bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: the Cardio133 trial†

[5]Berlin-Brandenburg Center for Regenerative Therapies, Berlin 13353, Germany

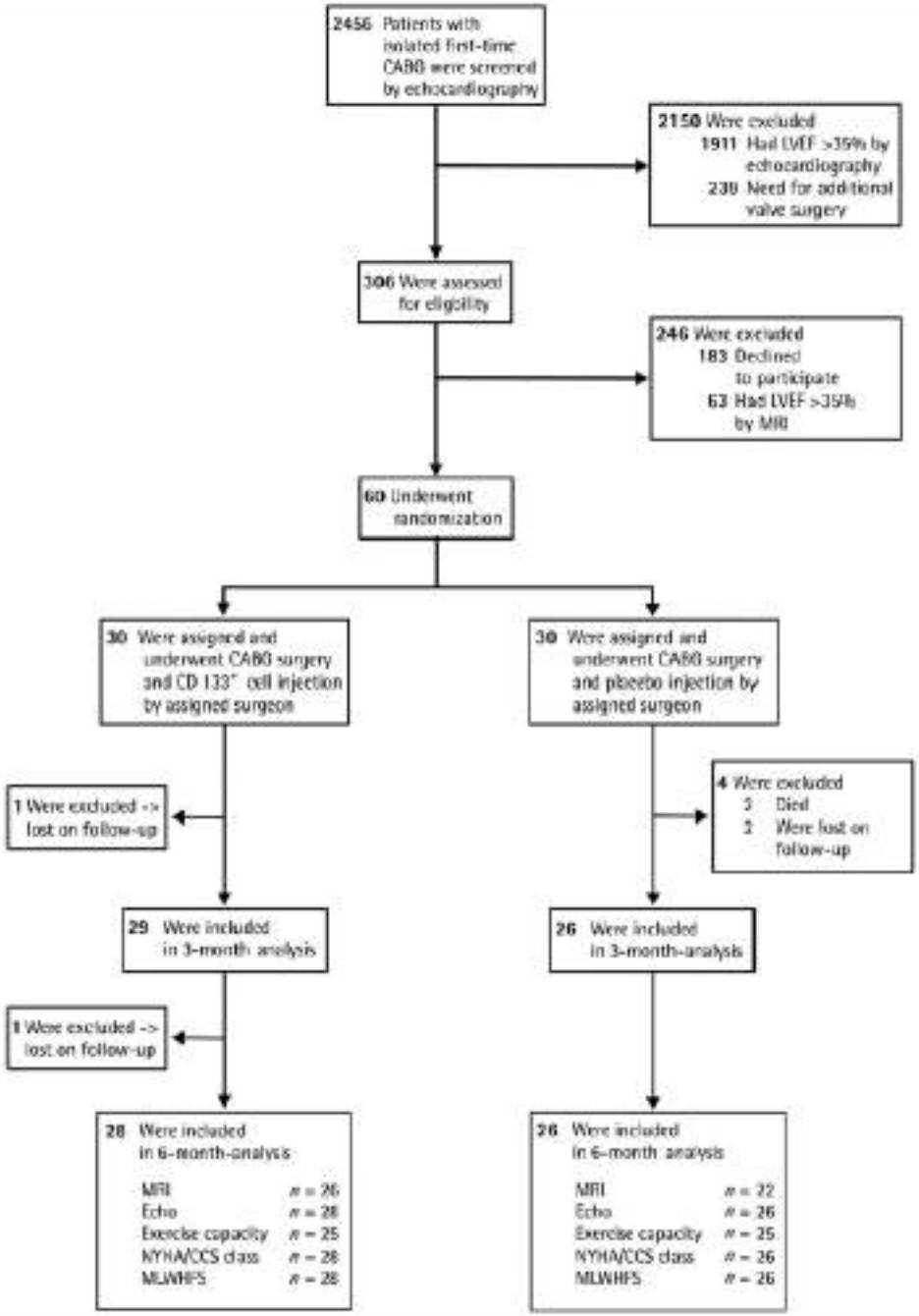
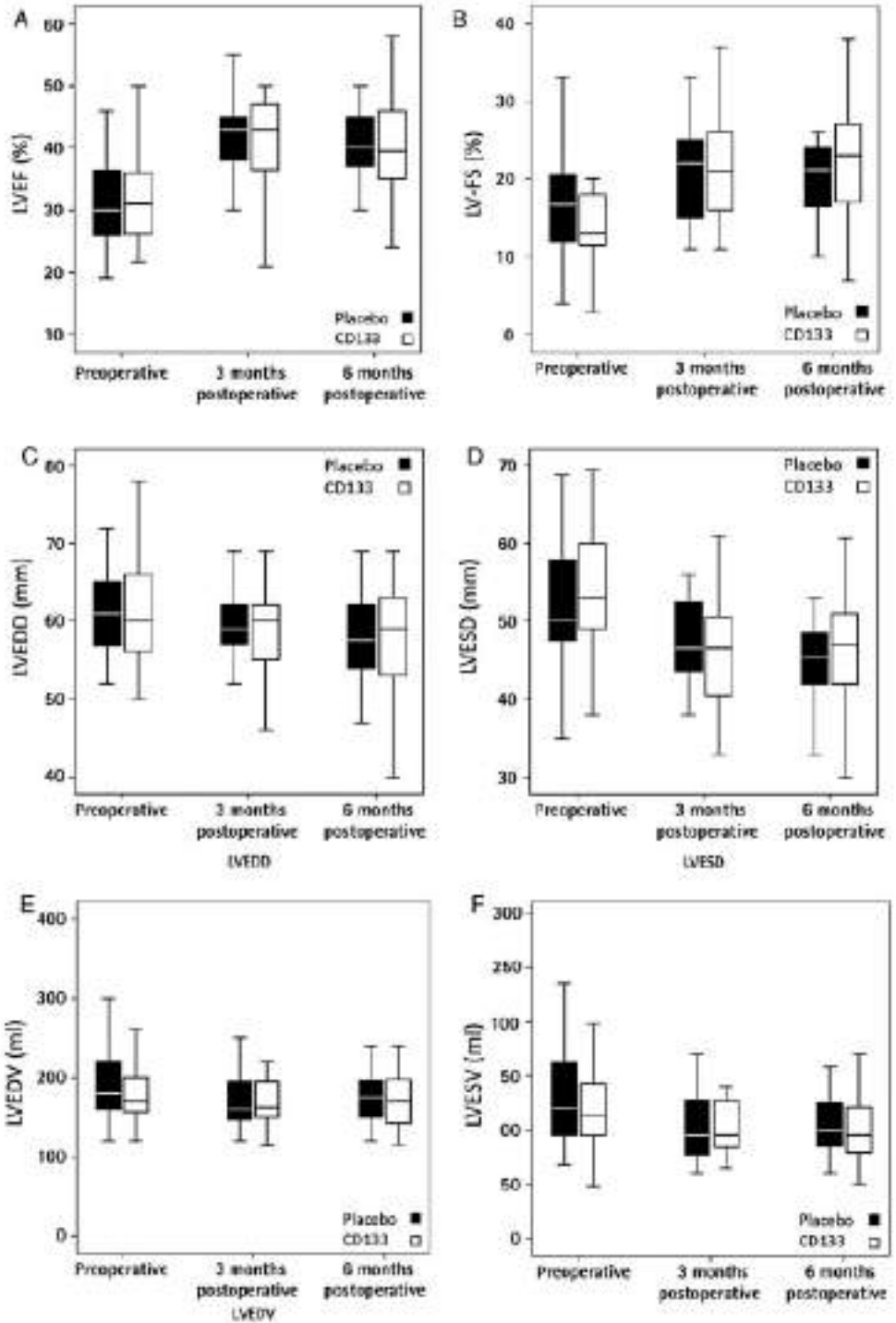
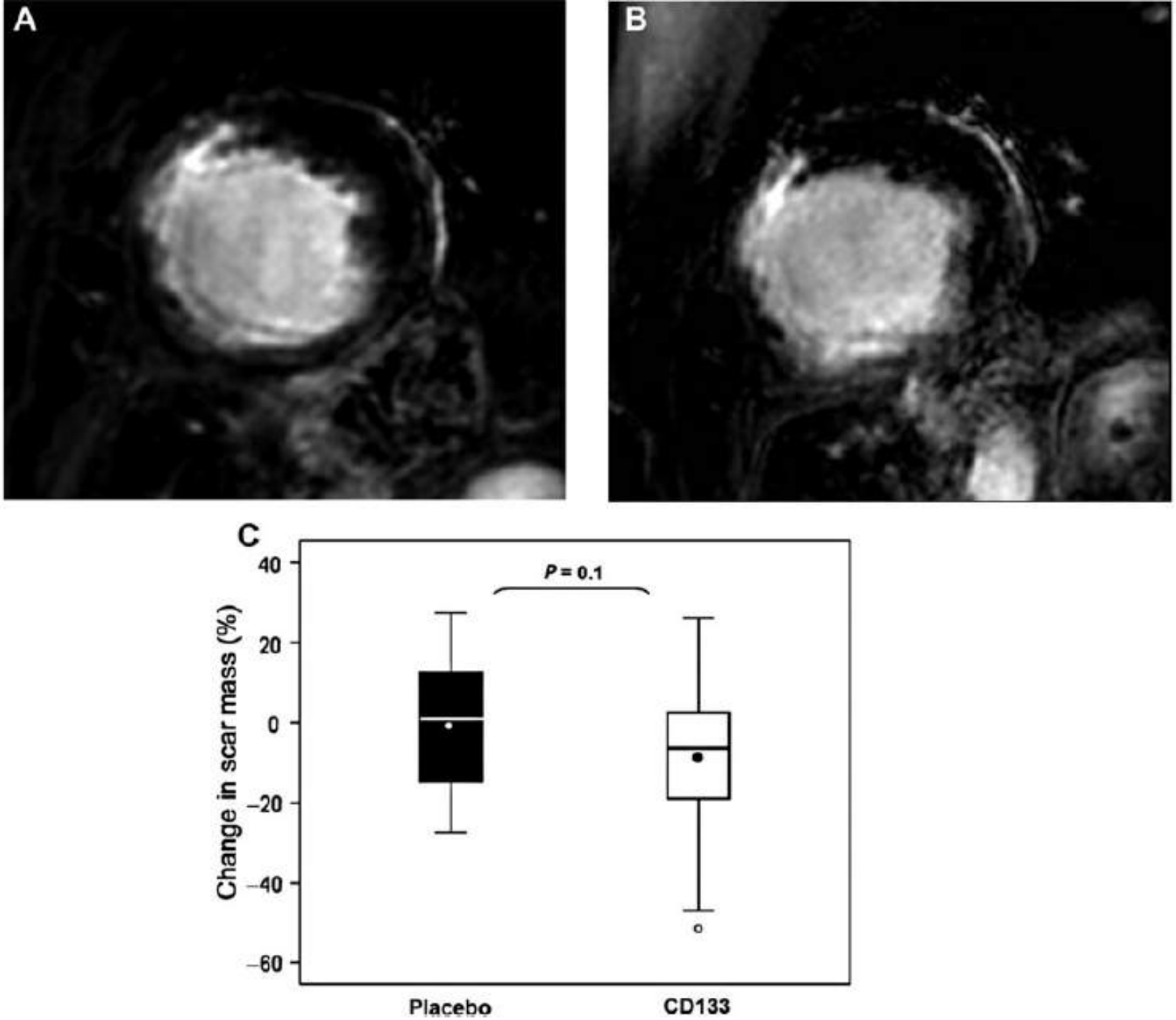



**Figure 1** Study design: Consort flow chart of the CARDIO133 trial.


A
LVEF (%)
Placebo
CD133
Preoperative
3 months postoperative
6 months postoperative
B
LV-FS (%)
C
LVEDD (mm)
LVEDD
D
LVESD (mm)
LVESD
E
LVEDV (ml)
LVEDV
F
LVESV (ml)


A
B
C
40
20
0
−20
−40
−60
Change in scar mass (%)
P = 0.1
Placebo
CD133

European Heart Journal (2014) **35**, 1263–1274
doi:10.1093/eurheartj/ehu007



**Conclusion** Although there may be some improvements in scar size and regional perfusion, intra-myocardial injection of $CD133^+$ BMC has no effect on global LV function and clinical symptoms. Improvements in regional myocardial function are only detectable in patients with posterior infarction, probably because the interventricular septum after anterior infarction is not accessible by trans-epicardial injection.

BMJ

*BMJ* 2014;348:g2688 doi: 10.1136/bmj.g2688 (Published 29 April 2014)



RESEARCH

# Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis

**Conclusions** Avoiding discrepancies is difficult but is important because discrepancy count is related to effect size. The mechanism is unknown but should be explored in the design of future trials because in the five trials without discrepancies the effect of bone marrow stem cell therapy on ejection fraction is zero.

Kardiologia Polska 2013; 71, 5: 485–492; DOI: 10.5603/KP.2013.0095 ISSN 0022–9032

ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

# The combined use of transmyocardial laser revascularisation and intramyocardial injection of bone-marrow derived stem cells in patients with end-stage coronary artery disease: one year follow-up

**Methods:** Five male patients (age 49–78 years) with end-stage diffuse CAD, severe angina (CCS III/IV) despite intensive medical therapy and disqualified from prior coronary artery bypass grafting (CABG) or percutaneous coronary intervention were included. After heart exposure, at sites where CABG was impossible, TMLR was performed with the Holmium: YAG laser combined with injection of 1 mL of bone marrow concentrate into the border zone of a laser channel using a Phoenix handpiece.

**Results:** No deaths in the follow-up period were observed. All patients were in I CCS Class. One year after the procedure, left ventricular (LV) segments treated by BMLR tended to demonstrate stronger myocardial thickening compared to baseline ($53.0 \pm 7.5\%$ vs. $45.0 \pm 9.5\%$; $p = 0.06$). Using late gadolinium-enhanced imaging, new myocardial infarction was found after one year only in one LV segment treated by BMLR. The BMLR treated regions in the remaining subjects, as well as regions subtended by left internal thoracic artery in two subjects, did not show new myocardial infarction areas. In contrast, all subjects who underwent only BMLR procedure revealed new and/or more extensive myocardial infarct in regions not treated by BMLR.

**Conclusions:** Intramyocardial delivery of bone marrow stem-cells together with laser therapy is a safe procedure, with improvement in quality of life during follow-up. One year after the procedure, myocardial regions where BMLR was performed tended to demonstrate stronger myocardial thickening observed in cardiac magnetic resonance imaging.

Journal of the American College of Cardiology
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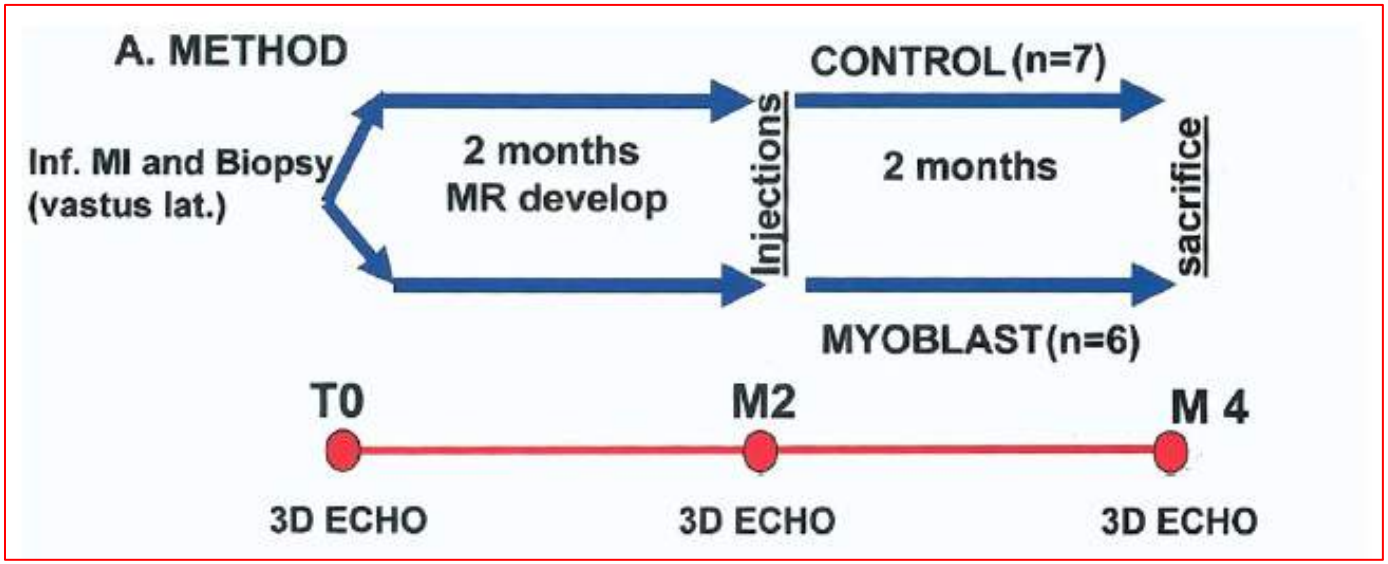
Published by Elsevier Inc.

Vol. 47, No. 10, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2005.12.063

# Autologous Myoblast Transplantation for Chronic Ischemic Mitral Regurgitation

Emmanuel Messas, MD, MSc,*†‡§ Alain Bel, MD,*†‡§ Miguel Cortes Morichetti, MD,†‡§ Claire Carrion, PhD,‖ Marc D. Handschumacher, BS,¶ Séverine Peyrard, BS,# Jean Thomas Vilquin, PhD,‖ Michel Desnos, MD,*†‡§ Patrice Bruneval, MD,*§** Alain Carpentier, MD, PhD, FACC,*†‡§ Philippe Menasché, MD, PhD,*†‡§ Robert A. Levine, MD,¶ Albert A. Hagège, MD, PhD†‡§

*Paris, France; and Boston, Massachusetts*



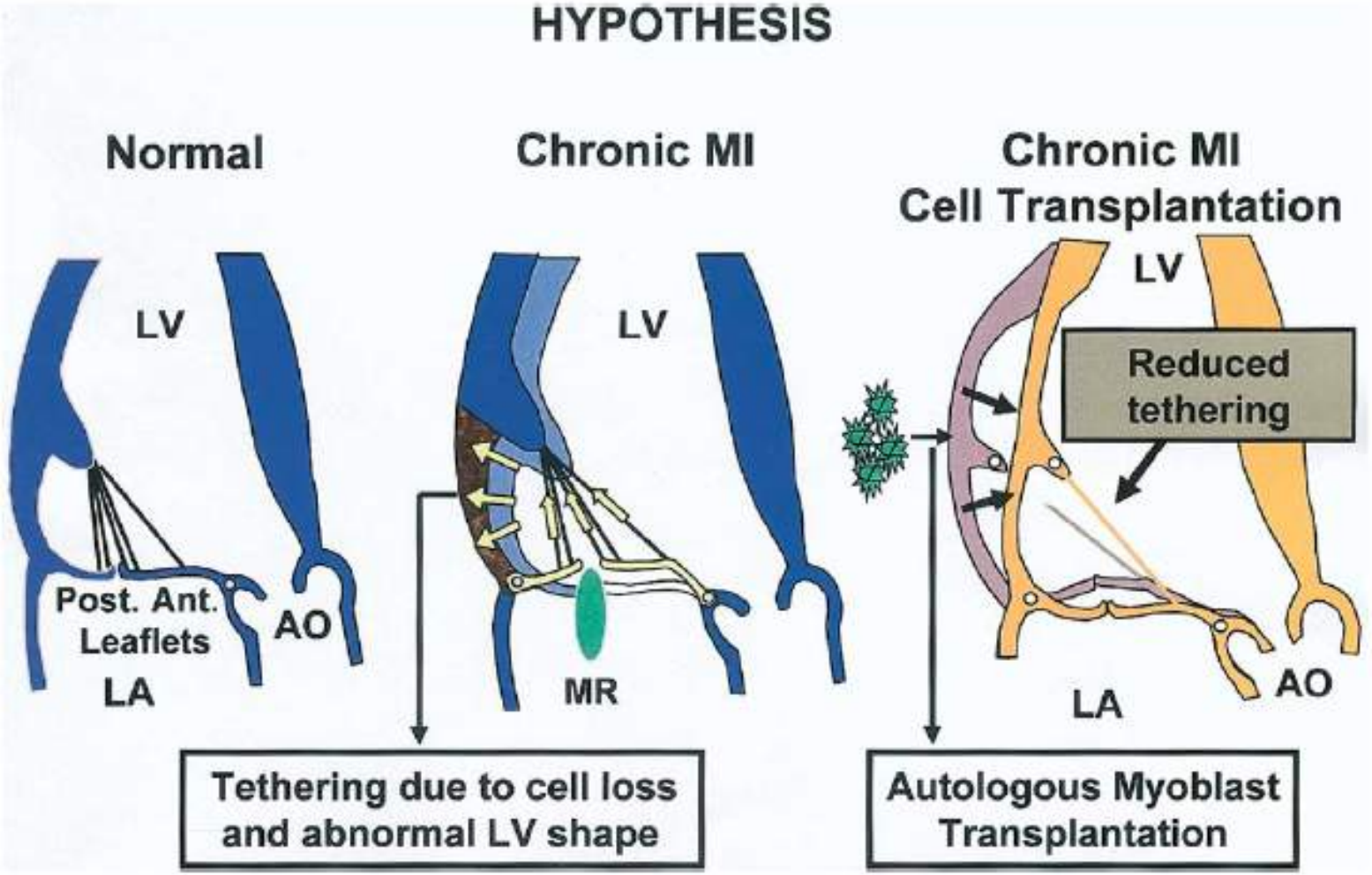

HYPOTHESIS
Normal
Chronic MI
Chronic MI
Cell Transplantation
LV
LV
LV
Post. Ant.
Leaflets
LA
AO
MR
Reduced
tethering
LA
AO
Tethering due to cell loss
and abnormal LV shape
Autologous Myoblast
Transplantation

**Table 1.** Echocardiographic Measurements

| | Baseline | | Chronic MI | | Sacrifice | |
|---|---|---|---|---|---|---|
| | Control | Cell Transplantation | Control | Cell Transplantation | Control | Cell Transplantation |
| HR | 99.5 ± 1.6 | 99.1 ± 2.7 | 103.1 ± 3.4 | 100.6 ± 3.6 | 109.7 ± 2.9 | 100.8 ± 4.9 |
| EDV(ml) | 32.01 ± 0.5 | 32.2 ± 1.6 | 63.4 ± 3.1* | 62.6 ± 5.1* | 111.0 ± 3.8† | 102.3 ± 5.1† |
| ESV(ml) | 10.5 ± 0.33 | 11.8 ± 0.5 | 39.1 ± 1.7* | 39.7 ± 3.1* | 75.4 ± 4.1† | 63 ± 2.9† |
| EF | 0.67 ± 0.01 | 0.63 ± 0.02 | .38 ± 0.02* | .36 ± 0.02* | .33 ± 0.01 | .38 ± 0.01 |
| WMS indexed | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.25 ± 0.08* | 1.19 ± 0.05* | 1.39 ± 0.11† | .94 ± 0.13† |
| Tethering dist (cm) | 2.48 ± 0.03 | 2.5 ± 0.05 | 3.07 ± 0.05* | 2.9 ± 0.06* | 3.5 ± 1.1† | 2.51 ± 0.04† |
| MAA ($cm^2$) | 5.8 ± 0.04 | 5.8 ± 0.19 | 6.8 ± 0.19* | 6.3 ± 0.12* | 7.2 ± 0.12† | 6.6 ± 0.18† |
| MRSV (ml) | 1.14 ± 0.2 | 1.33 ± 0.41 | 7.2 ± 0.59* | 5.8 ± 0.59* | 13.1 ± 0.77† | 4 ± 0.51† |
| RF% | 5.4 ± 0.9 | 6.3 ± 1.8 | 30 ± 0.5* | 27.4 ± 4.8* | 36 ± 1 | 10.5 ± 1.6† |

All two-way ANOVAs but one (heart rate) were significant $p < 0.05$. Significant changes $p < 0.025$ (Bonferroni corrected) are indicated for the two-way comparisons: *Baseline


**CONCLUSIONS** Autologous skeletal myoblast transplantation attenuates mild-to-moderate chronic ischemic MR, which otherwise is progressive, by decreasing tethering distance and improving EF and wall motion score, thereby enhancing valve coaptation. These data shed additional light on the mechanism by which skeletal myoblast transplantation may be cardioprotective. (J Am

doi:10.1510/icvts.2010.245936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Tra ...ation

retrospective studies showed no effect on long-term mortality in patients affected by severe MR and considerable left ventricular dysfunction undergoing mitral valve repair [5]. These results reflect the etiology of ischemic MR which is secondary to ventricular dysfunction and indicate that myocardial factors form fundamental determinants regarding the outcomes of patients with cardiomyopathy undergoing mitral valve surgery. Mitral valve repair without addressing myocardial remodelling processes most likely results only in a temporary reduction of the MR grade [4]. Therefore, patients with ischemic MR offering no option to address the underlying pathology by revascularisation pro-

European Heart Journal (2011) **32**, 2830–2840
doi:10.1093/eurheartj/ehr059

**BASIC SCIENCE**

# Injectable living marrow stromal cell-based autologous tissue engineered heart valves: *first experiences with a one-step intervention in primates*

**Aims** A living heart valve with regeneration capacity based on autologous cells and minimally invasive implantation technology would represent a substantial improvement upon contemporary heart valve prostheses. This study investigates the feasibility of injectable, marrow stromal cell-based, autologous, living tissue engineered heart valves (TEHV) generated and implanted in a one-step intervention in non-human primates.

# Methods

## Scaffold fabrication

## Isolation of primate bone marrow-derived mononuclear cells

## Phenotyping of bone marrow-derived mononuclear cells

## Bone marrow-derived mononuclear cell seeding and characterization

## Tissue engineered heart valves implantation and *in vivo* functionality

## Minimally invasive delivery: the transapical implantation of tissue engineered heart valves

The transapical implantations were successful in all animals. Of all six animals five valves were deployed in the orthotopic valvular

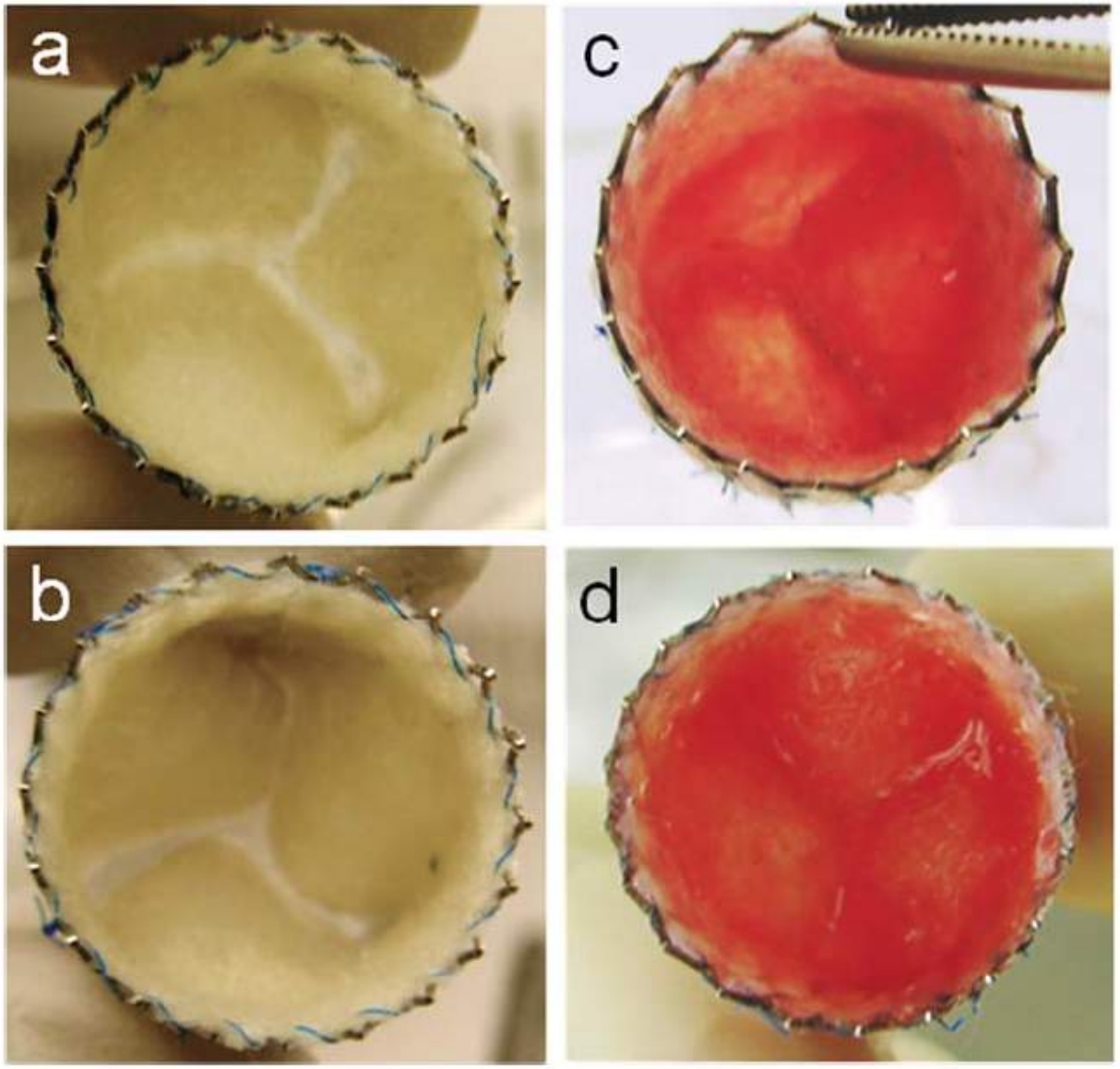
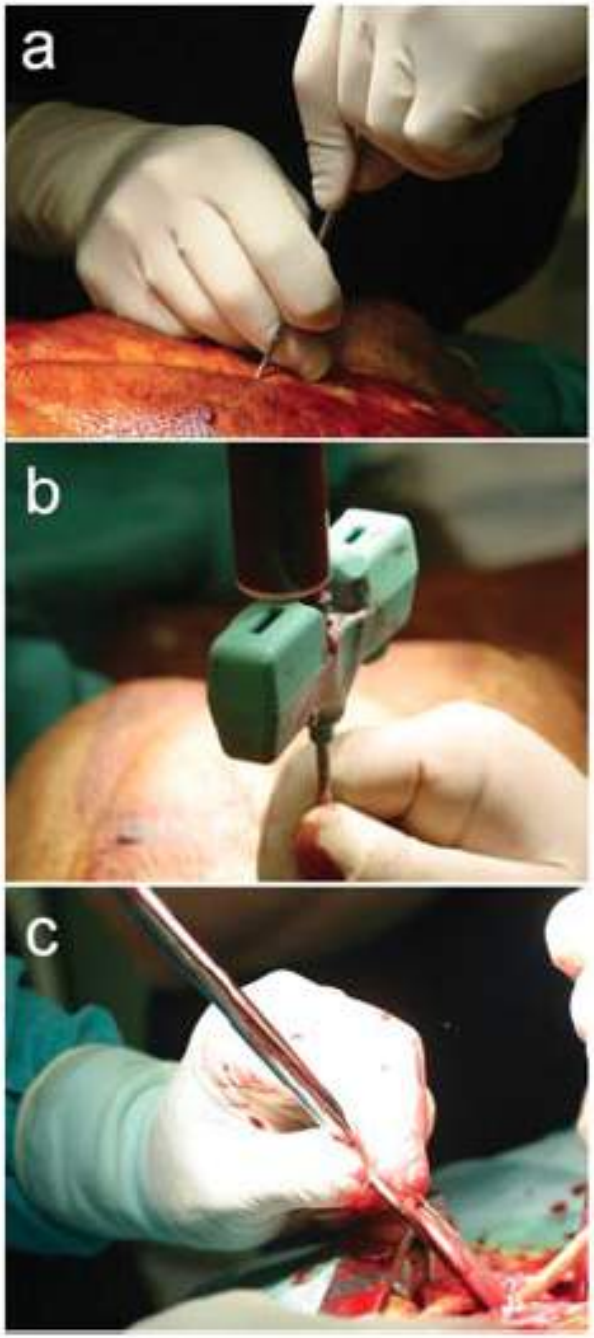



**Figure 2** Bone marrow-derived tissue engineered heart valves. After isolation of bone marrow-derived mononuclear cells, stented polyglycolic acid scaffold matrices (*A* and *B*) were seeded with cells using fibrin as a cell carrier (*C* and *D*).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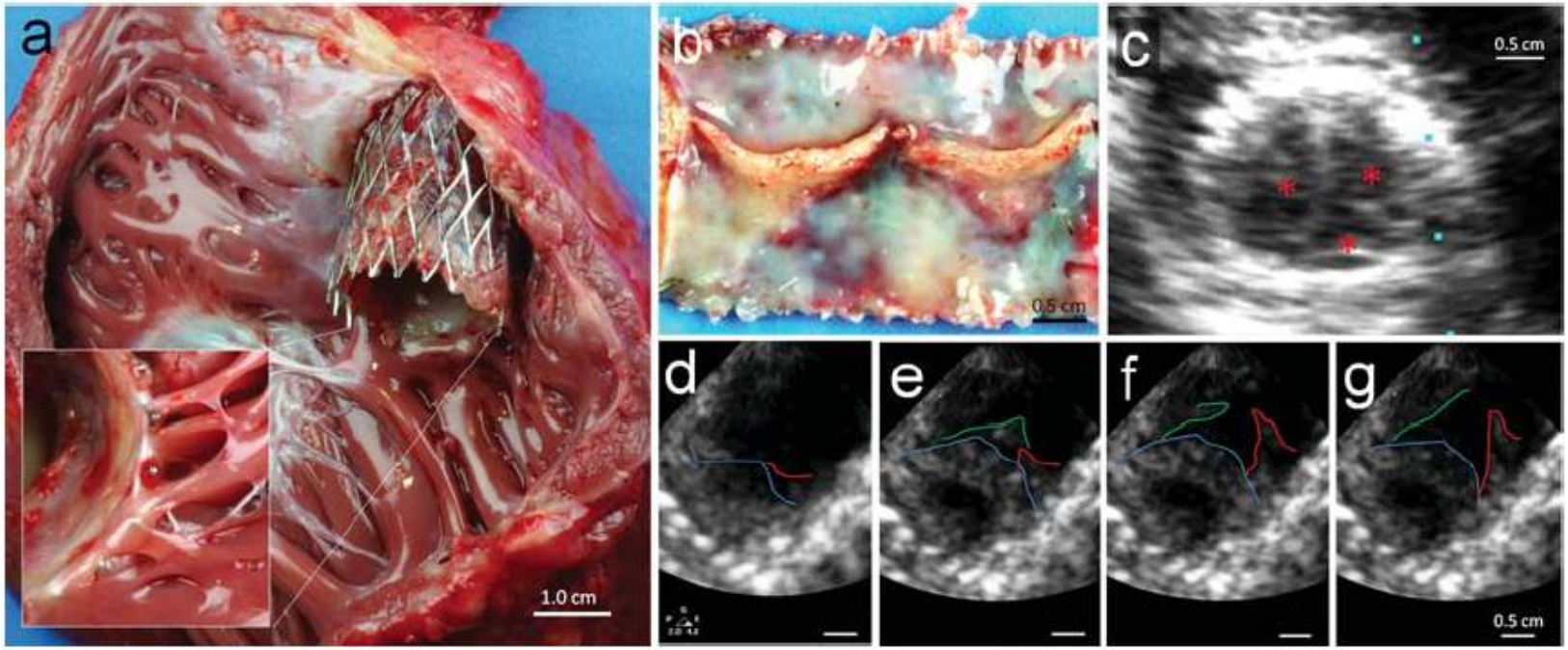


**Figure 4** Explant analysis of tissue engineered heart valves. After 4 weeks *in vivo* the stented constructs were well integrated into the adjacent tissue (*A*). Orthotopical tissue engineered heart valves (*B*) presented with a cusp-like leaflet structure, with shorter leaflets than native controls. In a final transoesophageal echocardiography-assessment the leaflet co-aptation (*C*; asterisk indicates leaflets) as well as opening movements of all three leaflets could be visualized (*D*–*G*).

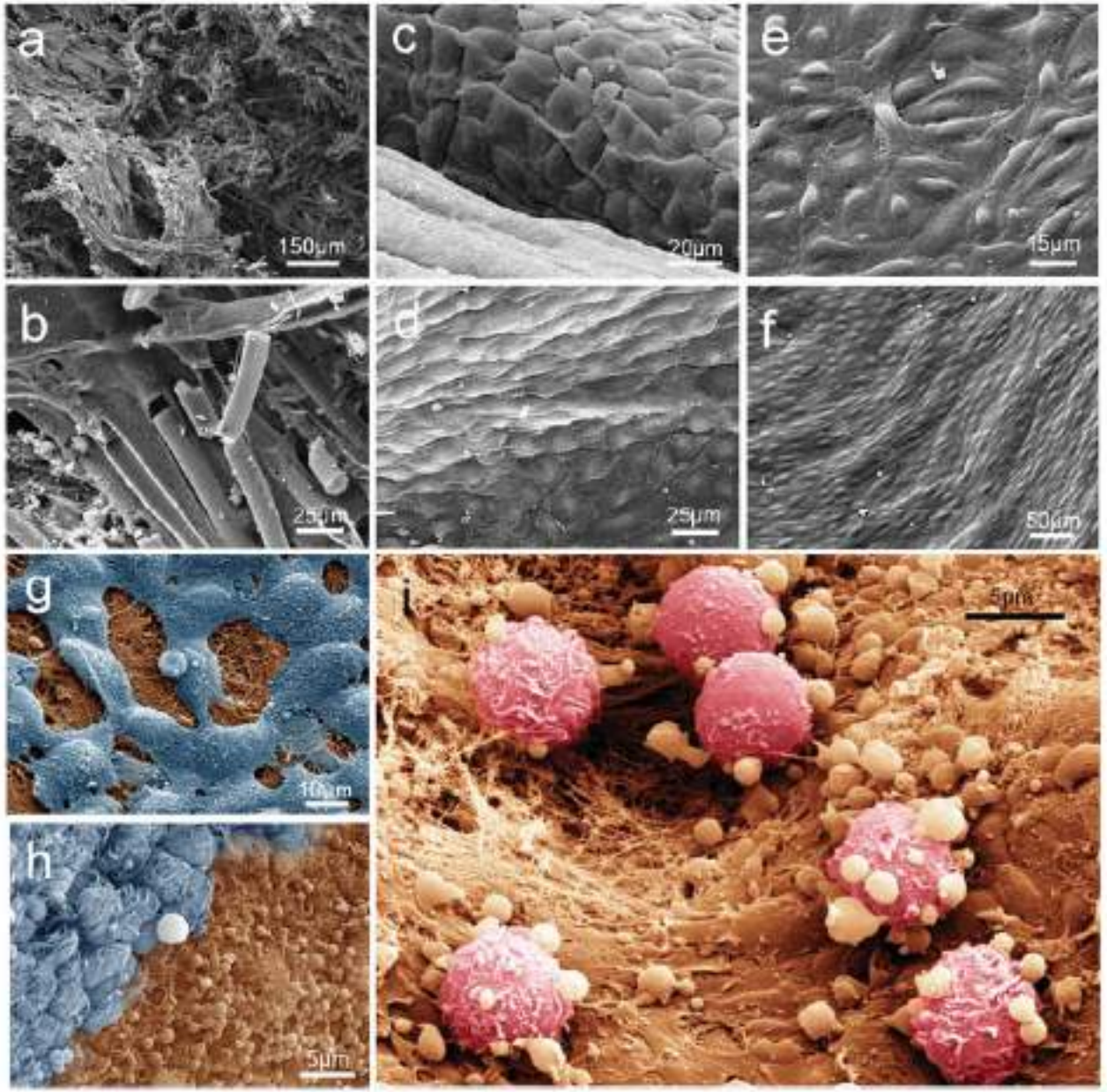


**Figure 5** Scanning electron microscopy of the polyglycolic acid–poly-4-hydroxybutyrate scaffold (*A* and *B*), primate (*C*), and human (*D*) control leaflets. In most areas the surface of the 4 week explants showed confluent (*E* and *F*) or initial (*G*) endothelial coverage. In some areas the surface remodelling was still evident involving thrombocyte attachment (*H*) and leucocyte attraction (*I*).

# Conclusions

These first results of combining minimally invasive valve replacement procedures with heart valve tissue engineering in a single intervention in a preclinical primate model are promising and demonstrate the feasibility of using BMCs for the fabrication of TEHV. Moreover, utilizing the body's natural abilities to regenerate TEHV *in vivo*, may greatly simplify, and improve the clinical feasibility of the autologous cell-based TEHV approach. Such autologous and living heart valves with repair and regeneration capacities may represent the next generation of transcatheter and transapical heart valves overcoming the time limitations of the currently used bioprosthetic valves suggesting their future clinical application also beyond elderly patients.

International Journal of Nanomedicine

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**Original Article**

# Expression of *COLLAGEN* 1 and *ELASTIN* Genes in Mitral Valvular Interstitial Cells within Microfiber Reinforced Hydrogel

**Maryam Eslami, M.D, Ph.D.[1, 2, 3*], Gholamreza Javadi, Ph.D.[1*], Nasser Agdami, Ph.D.[4], Mohammad Ali Shokrgozar, Ph.D.[5]**

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4. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
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J Tissue Eng Regen Med. 2016 Jan 22. doi: 10.1002/term.2127. [Epub ahead of print]

**Engineering natural heart valves: possibilities and challenges.**

Namiri M1,2, Ashtiani MK1, Mashinchian O1, Hasani-Sadrabadi MM1,3, Mahmoudi M4,5,6, Aghdami N1, Baharvand H1,2.

During the past three decades, tissue engineering-based approaches have shown tremendous potential to overcome these limitations by the development of a **biodegradable scaffold**, which provides biomechanical and biochemical properties of the native tissue. Among various scaffolds employed for tissue engineering, the decellularized heart valve (DHV) has attracted much attention, due to its native structure as well as comparable haemodynamic characteristics. Although the human DHV has shown optimal properties for valve replacement, the limitation of valve donors in terms of time and size is their main clinical issue. In this regard, xenogenic DHV can be a promising candidate for heart valve replacement. Xenogenic DHVs have similar composition to human valves, which will overcome the need for human DHVs. **The main concern regarding xenogeneic DHV replacement is the immunological reaction and calcification following implantation, weak mechanical properties and insufficient recellularization capacity**.

Hindawi Publishing Corporation
Stem Cells International
Volume 2016, Article ID 7168797, 11 pages
http://dx.doi.org/10.1155/2016/7168797

*Review Article*

# Improving Cell Engraftment in Cardiac Stem Cell Therapy

Myocardial infarction (MI) affects millions of people worldwide. MI causes massive cardiac cell death and heart function decrease. However, heart tissue cannot effectively regenerate by itself. While stem cell therapy has been considered an effective approach for regeneration, the efficacy of cardiac stem cell therapy remains low due to inferior cell engraftment in the infarcted region. This is mainly a result of low cell retention in the tissue and poor cell survival under ischemic, immune rejection and inflammatory conditions. Various approaches have been explored to improve cell engraftment: increase of cell retention using biomaterials as cell carriers; augmentation of cell survival under ischemic conditions by preconditioning cells, genetic modification of cells, and controlled release of growth factors and oxygen; and enhancement of cell survival by protecting cells from excessive inflammation and immune surveillance. In this paper, we review current progress, advantages, disadvantages, and potential solutions of these approaches.

*Int. J. Mol. Sci.* **2015**, *16*, 597-627; doi:10.3390/ijms16010597

OPEN ACCESS
International Journal of
**Molecular Sciences**
**ISSN 1422-0067**
www.mdpi.com/journal/ijms

*Review*

# Accelerating *in Situ* Endothelialisation of Cardiovascular Bypass Grafts

**Ee Teng Goh [1,2], Eleanor Wong [1,2], Yasmin Farhatnia [1], Aaron Tan [1,2,3] and Alexander M. Seifalian [1,4,*]**

**REVIEW**

# Luminal Surface Engineering, 'Micro and Nanopatterning': Potential for Self Endothelialising Vascular Grafts?

**D.S.T. Chong [a,b], B. Lindsey [a], M.J. Dalby [c], N. Gadegaard [d], A.M. Seifalian [b], G. Hamilton [a,b,*]**

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[b] Centre for Nanotechnology and Regenerative Medicine, Division of Surgery and Interventional Science, University College London, London, UK
[c] Centre for Cell Engineering, University of Glasgow, Glasgow, UK
[d] Division of Biomedical Engineering, School of Engineering, University of Glasgow, Glasgow, UK

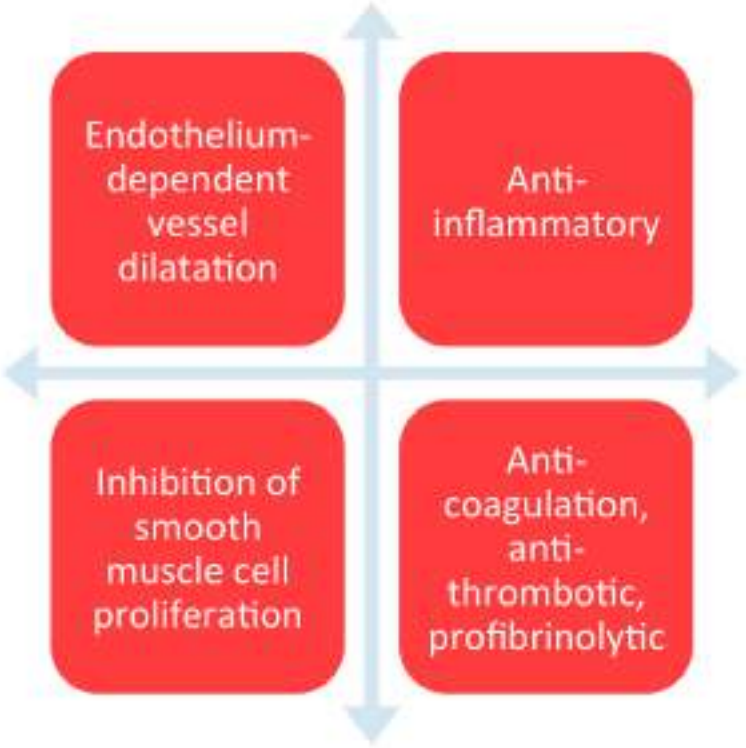


**Figure 1.** Shows the different functions of endothelium.

**Abstract:** The patency of synthetic cardiovascular grafts in the long run is synonymous with their ability to inhibit the processes of intimal hyperplasia, thrombosis and calcification. In the human body, the endothelium of blood vessels exhibits characteristics that inhibit such processes. As such it is not surprising that research in tissue engineering is directed towards replicating the functionality of the natural endothelium in cardiovascular grafts. This can be done either by seeding the endothelium within the lumen of the grafts prior to implantation or by designing the graft such that *in situ* endothelialisation takes place after implantation. Due to certain difficulties identified with *in vitro* endothelialisation, *in situ* endothelialisation, which will be the focus of this article, has garnered interest in the last years. To promote *in situ* endothelialisation, the following aspects can be taken into account: (1) Endothelial progenital cell mobilization, adhesion and proliferation; (2) Regulating differentiation of progenitor cells to mature endothelium; (3) Preventing thrombogenesis and inflammation during endothelialisation. This article aims to review and compile recent developments to

*Ann Thorac Surg*. 2012 May ; 93(5): 1524–1533. doi:10.1016/j.athoracsur.2012.01.063.

# Stem Cells in Thoracic Aortic Aneurysms and Dissections: Potential Contributors to Aortic Repair

**Ying H. Shen, MD, PhD**[1,2], **Xiaoqing Hu, MD**[1,2], **Sili Zou, MD**[1,2], **Darrell Wu, MD**[1,2,3], **Joseph S. Coselli, MD**[1,3], and **Scott A. LeMaire, MD**[1,2,3]

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## Comment

Multipotent SCs are known to play an important role in arterial remodeling after injury. The presence of circulating endothelial progenitor cells has been previously reported in a murine model of abdominal aortic aneurysms [15], in patients with abdominal aortic aneurysms [16], and, recently, in patients with ascending aortic aneurysms [17]. In this study, we have shown that SCs are abundant in two other forms of aortic disease: descending TAA and chronic TAD. Specifically, we found that there were significantly more STRO-1+ cells, c-kit+ cells, and CD34+ cells and in the media and adventitia of aortic tissue from TAA and TAD patients than in control aortic tissue. Furthermore, subsets of STRO-1+ cells, c-kit+ cells, and CD34+ cells appeared to differentiate into SMCs and fibroblasts, and a large number of STRO-1+ cells exhibited differentiation into macrophages. The presence of multipotent SCs at sites of aneurysm and dissection formation that can further differentiate into SMCs suggests the existence of an active repair process involving SCs.

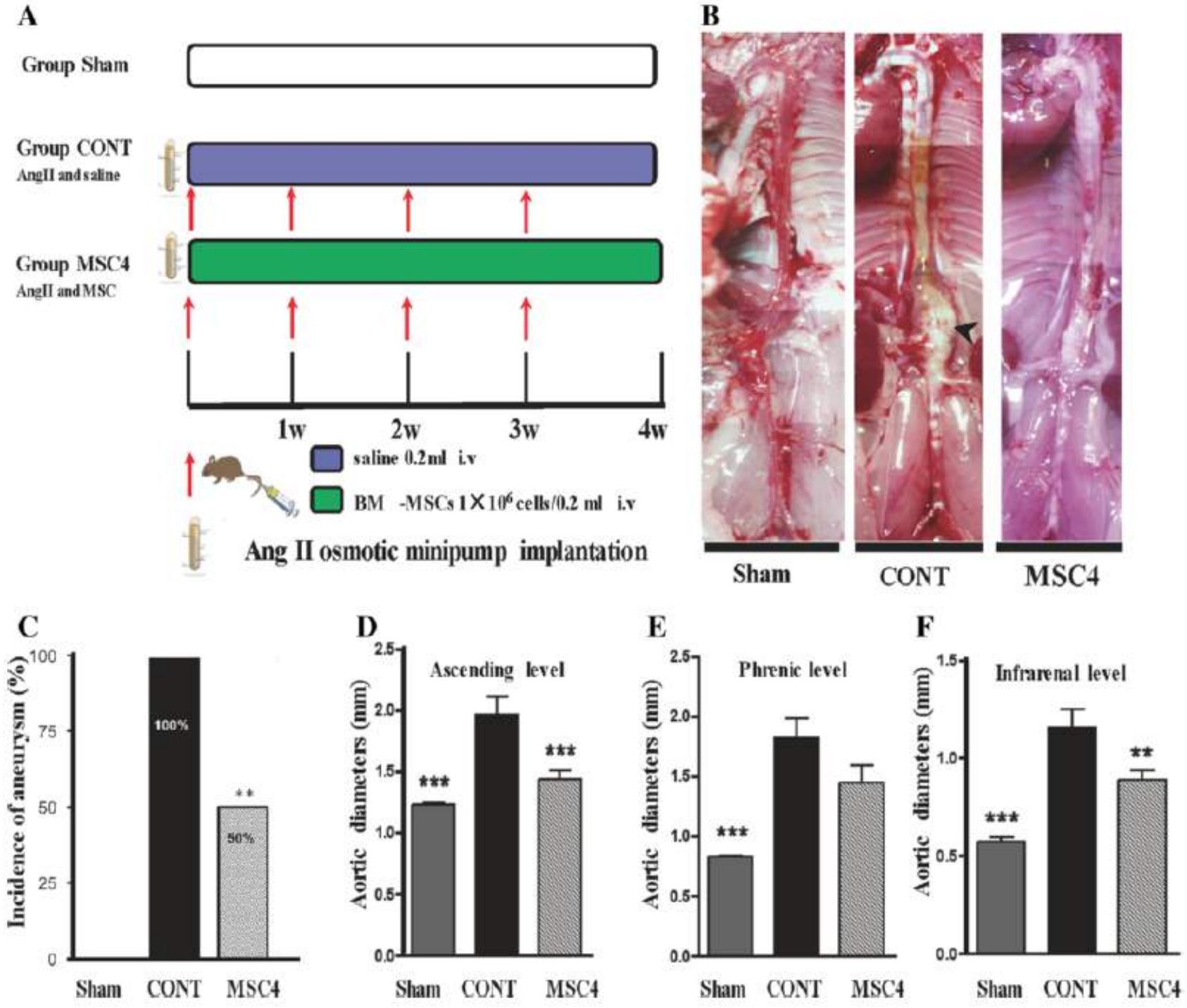
Fu et al. Journal of Translational Medicine 2013, 11:175
http://www.translational-medicine.com/content/11/1/175

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

# Intravenous administration of mesenchymal stem cells prevents angiotensin II-induced aortic aneurysm formation in apolipoprotein E-deficient mouse

Xian-ming Fu†, Aika Yamawaki-Ogata†, Hideki Oshima, Yuichi Ueda, Akihiko Usui and Yuji Narita*


A
Group Sham
Group CONT
AngII and saline
Group MSC4
AngII and MSC
1w
2w
3w
4w
saline 0.2ml i.v
BM -MSCs 1×10^6 cells/0.2 ml i.v
Ang II osmotic minipump implantation
B
Sham
CONT
MSC4
C
Incidence of aneurysm (%)
100
75
50
25
0
100%
**
50%
Sham
CONT
MSC4
D
Ascending level
Aortic diameters (mm)
2.5
2.0
1.5
1.0
0.5
0.0
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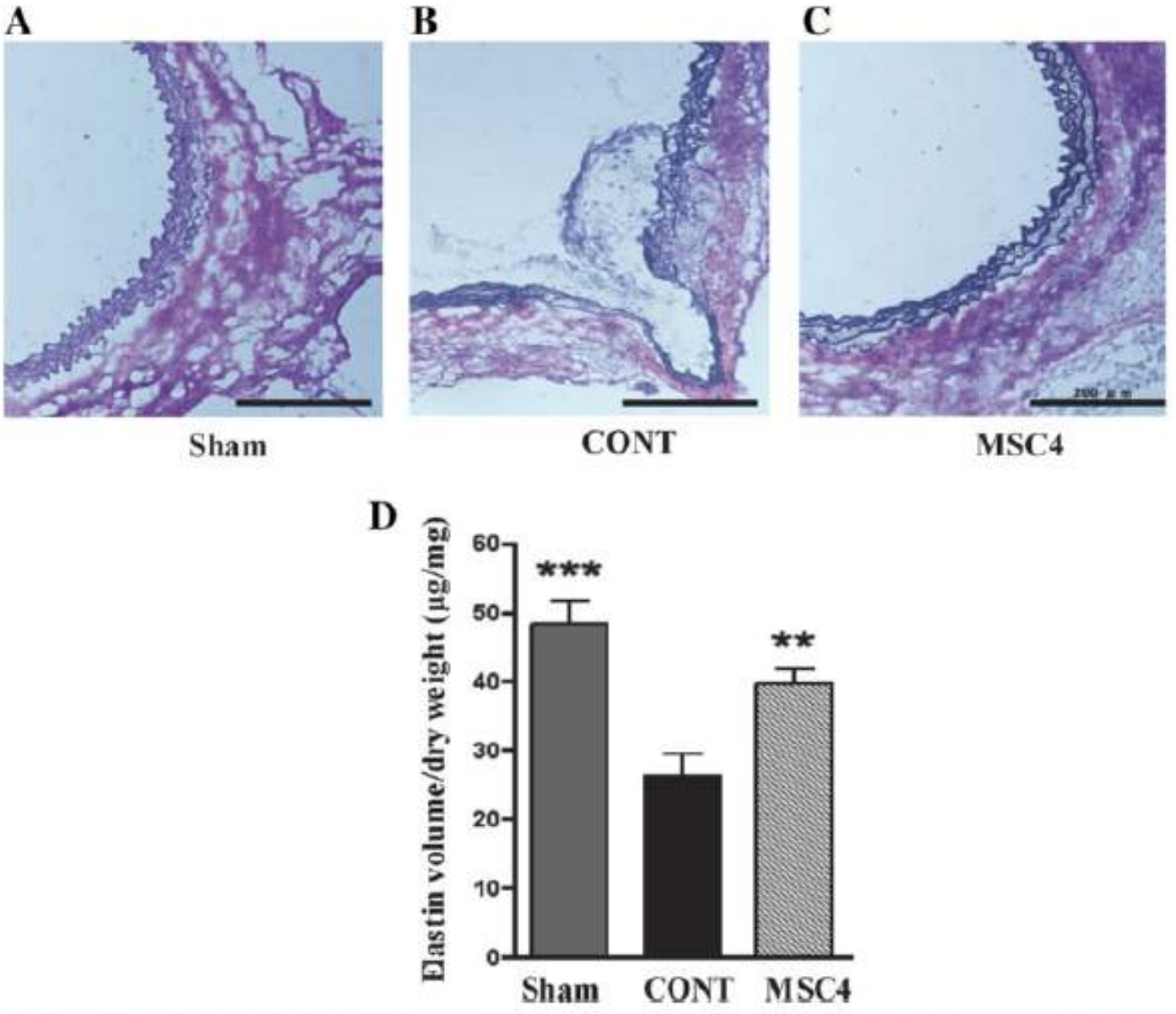


**Figure 2 Multiple intravenous administrations of BM-MSCs attenuated aortic elastin degradation in apoE$^{-/-}$ mice. A)** EVG staining shows normal wavy elastic lamina structure in group Sham, and **B)** disruption of elastic lamina and aneurysm formation in group CONT. **C)** Administration of BM-MSCs maintained wavy structure of the elastic lamellae. Scale bar = 200 µm. **D)** Measurement of elastin volume of aortic tissues showed a significant decrease in group CONT compared with group Sham, but preservation in group MSC4. Data are presented as means ± SEM ($n$ =10-12) $^{**}P<0.01$, $^{***}P<0.001$ vs. group CONT, assessed by one-way ANOVA.

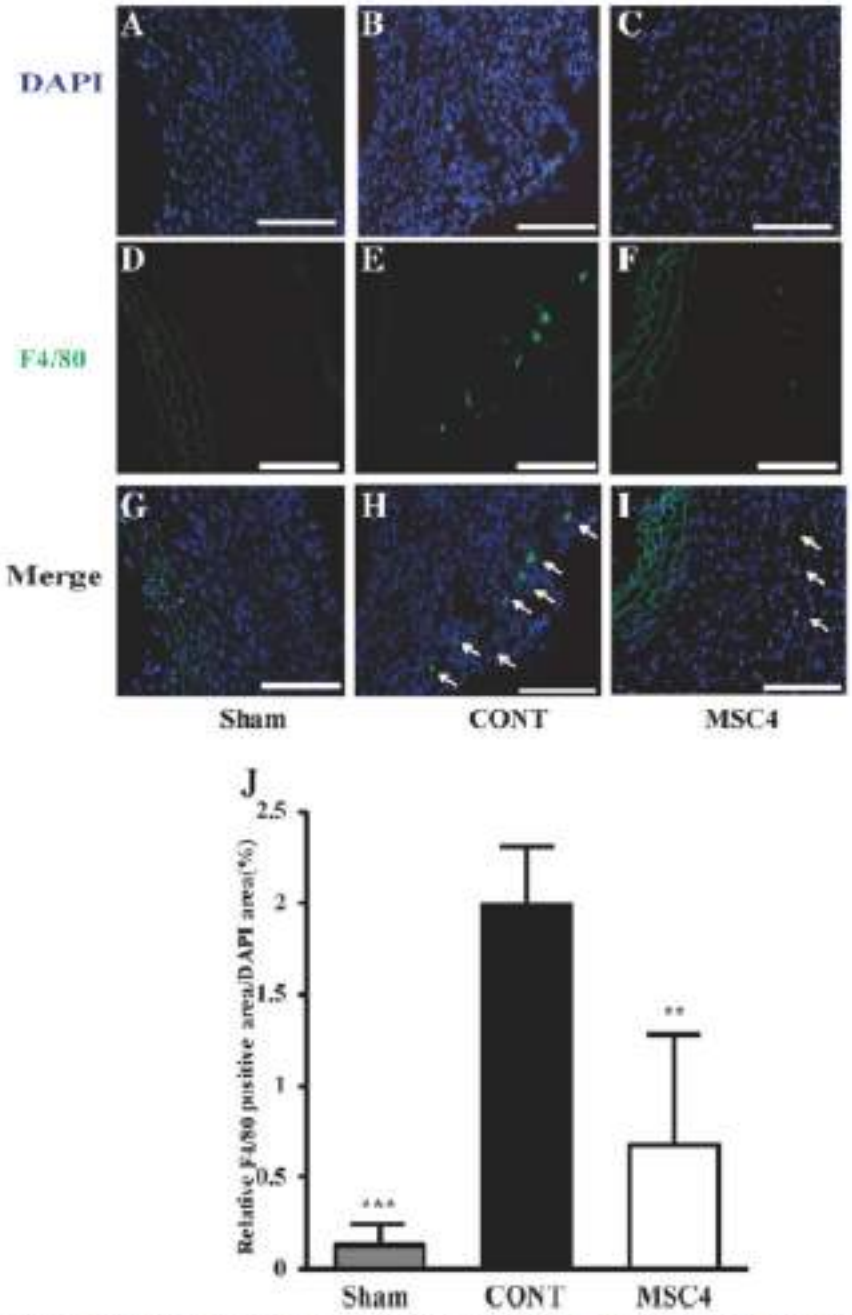


**Figure 3 Multiple intravenous administrations of BM-MSCs suppressed macrophage infiltration in aortic tissues. A-I)** Representative F4/80 immunofluorescence stained sections of suprarenal aortas from Sham, CONT, and MSC4 groups. Arrowheads indicated F4/80 macrophages. Scale bars=100 μm. **J)** Quantitation of F4/80- positive macrophages area as a ratio of the DAPI staining area. Data are presented as means ± SEM ($n$ =10-12) **P<0.01, ***P<0.001 vs. group CONT, assessed by one-way ANOVA.

JOURNAL OF TRANSLATIONAL MEDICINE

## Conclusions

Multiple intravenous administrations of BM-MSCs were effective to suppress inflammatory reactions in Ang II-infused apoE$^{-/-}$ mice, and inhibit the development of AAs. It may therefore serve as a new therapeutic strategy for patients with AA.

Biomaterials. 2015 Dec;73:175-84. doi: 10.1016/j.biomaterials.2015.09.016. Epub 2015 Sep 11.

# 6-month aortic valve implantation of an off-the-shelf tissue-engineered valve in sheep..

Departments of Biomedical Engineering, University of Minnesota, United States.

The high pressure gradients and dynamic flow across the aortic valve leaflets require engineering a tissue that has the strength and compliance to withstand high mechanical demand without compromising normal hemodynamics. A long-term preclinical evaluation of an off-the-shelf tissue-engineered aortic valve in the sheep model is presented here. The valves were made from a tube of decellularized cell-produced matrix mounted on a frame. The engineered matrix is primarily composed of collagen, with strength and organization comparable to native valve leaflets. In vitro testing showed excellent hemodynamic performance with low regurgitation, low systolic pressure gradient, and large orifice area. The implanted valves showed large-scale leaflet motion and maintained effective orifice area throughout the duration of the 6-month implant, with no calcification. After 24 weeks implantation (over 17 million cycles), the valves showed no change in tensile mechanical properties. In addition, histology and DNA quantitation showed repopulation of the engineered matrix with interstitial-like cells and endothelialization.

J Artif Organs. 2007;10(1):29-35. Epub 2007 Mar 23.

# Minimally immunogenic decellularized porcine valve provides in situ recellularization as a stentless bioprosthetic valve.

Iwai S1, Torikai K, Coppin CM, Sawa Y

To overcome these obstacles, we have developed a minimally immunogenic tissue-engineered valve that consists of an unfixed, decellularized porcine valve scaffold capable of being spontaneously revitalized in vivo after implantation. Porcine aortic root tissue was decellularized using detergents such as sodium lauryl sulfate and Triton X-100. The porcine valve was treated very gently and plenty of time was allowed for constituents to diffuse in and out of the matrix. In a preliminary study, a piece of decellularized porcine valve tissue was implanted into the rat subdermal space for 14 and 60 days and the structural integrity and calcification were evaluated. As an in vivo valve replacement model, the decellularized porcine valve was implanted in the pulmonary valve position in dogs and functional and histological evaluation was performed after 1, 2, and 6 months. Histological examination showed that the newly developed detergent treatment effectively removed cellular debris from the porcine aortic tissue. Decellularized porcine valve tissue implanted subdermally in rats showed minimal inflammatory cell infiltration and calcification. In the valve replacement model, spontaneous reendothelialization and repopulation of the medial cells were observed within 2 months, and good valve function without regurgitation was observed by echocardiography up to 6 months.

Biointerphases
A Journal of Biomaterials and Biological Interfaces

# Novel heart valve prosthesis with self-endothelialization potential made of modified polyhedral oligomeric silsesquioxane-nanocomposite material

Hossein Ghanbari, Dina Radenkovic, Sayed Mahdi Marashi, Shirin Parsno, Nima Roohpour, Gaetano Burriesci, and Alexander M. Seifalian

(Received 1 October 2015; accepted 11 December 2015; published 13 January 2016)

In the cardiovascular system, the endothelial layer provides a natural antithrombogenic surface on the inner portion of the heart and associated vessels. For a synthetic material therefore, the ability to attract and retain endothelial or endothelial progenitor cells (EPCs), ultimately creating a single endothelial layer on its surface, is of prime importance. The authors have developed a nanocomposite polymer, based on a combination of polyhedral oligomeric silsesquioxane nanoparticles and polycarbonate urea urethane (POSS-PCU), which is biocompatible and has been used in human for the world's first synthetic trachea, tear duct, and bypass graft. In this study, the authors modified the surface of this casted nanocomposite by grafting fibronectin derived bioactive peptides [glycine-arginine-glycine-aspartic acid-glycine (GRGDG) and lauric acid conjugated GRGDG (GRGDG-LA)] to enhance the endothelialization for using heart valves leaflets from circulating EPCs. Human peripheral blood mononuclear cells were separated using Ficoll–Paque centrifugation, with harvested EPCs purified using CD34 microbead labeling and magnetic-activated cell sorting. Cells were seeded onto 96 well plates coated with POSS-PCU, GRGDG/GRGDG-LA modified POSS-PCU and PCU polymers, for a period of 21 days. Cells

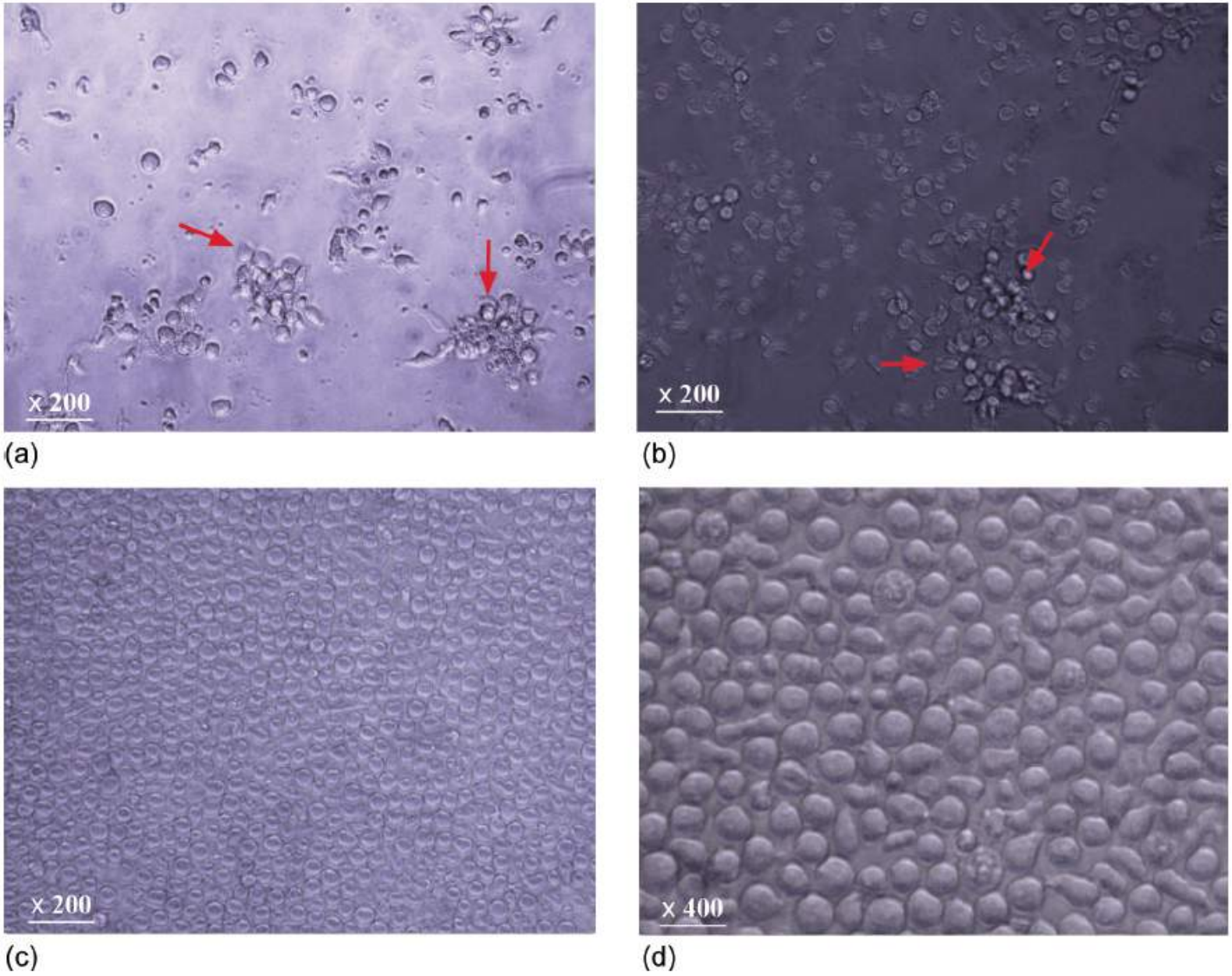


FIG. 6. Live microscopy images of the isolated cells cultured on POSS-PCU nanocomposite samples. Colonies of EPC (marked with arrows) undergoing proliferation resulted in an increased cell population over time of culture as shown on day 7 (a) and day 14 (b). The cells underwent morphological changes during the culture and spindle-shaped morphology of early EPCs on day 7 (a) was dominated by cobble stone-shaped confluent layer at day 21 (c), characteristic morphology of the endothelial cells. (d) Higher magnification of confluent layer of EC on day 21.

AVS Biointerphases
A Journal of Biomaterials and Biological Interfaces

**Novel heart valve prosthesis with self-endothelialization potential made of modified polyhedral oligomeric silsesquioxane-nanocomposite material**

Hossein Ghanbari, Dina Radenkovic, Sayed Mahdi Marashi, Shirin Parsno, Nima Roohpour, Gaetano Burriesci, and Alexander M. Seifalian

## V. CONCLUSIONS

In an *in vitro* setting, EPCs were extracted from adult peripheral blood and umbilical cord blood and cultured on the POSS-PCU nanocomposite in comparison with PCU, GRGDG, and GRGDG-LA modified polymers. EPCs' proliferation and differentiation was noticed over the time of culture. The POSS-PCU nanocomposite revealed an enhanced cell affinity and capability to provide a cell friendly environment for EPC proliferation and differentiation. According to the results, this nanocomposite material can be used for the development of synthetic leaflet heart valves, but modification with suitable peptides could result in superior in-situ endothelialization capability.

Crit Rev Biotechnol. 2015 Jul 15:1-11. [Epub ahead of print]

# Hearts beating through decellularized scaffolds: whole-organ engineering for cardiac regeneration and transplantation.

Zia S1, Mozafari M, Natasha G, Tan A, Cui Z, Seifalian AM

**Abstract**

Whole-organ decellularization and tissue engineering approaches have made significant inroads during recent years. If proven to be successful and clinically viable, it is highly likely that this field would be poised to revolutionize organ transplantation surgery. In particular, whole-heart decellularization has captured the attention and imagination of the scientific community. This technique allows for the generation of a complex three-dimensional (3D) extracellular matrix scaffold, with the preservation of the intrinsic 3D basket-weave macroarchitecture of the heart itself. The decellularized scaffold can then be recellularized by seeding it with cells and incubating it in perfusion bioreactors in order to create functional organ constructs for transplantation. Indeed, research into this strategy of whole-heart tissue engineering has consequently emerged from the pages of science fiction into a proof-of-concept laboratory undertaking. This review presents current trends and advances, and critically appraises the concepts involved in various approaches to whole-heart decellularization and tissue engineering.

European Heart Journal Advance Access published May 19, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv189

EHJ BRIEF COMMUNICATION
*Heart failure/cardiomyopathy*

# Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report

tive applications, and based on the epicardial delivery of a cell-loaded patch. Several studies have documented the superiority of this patch-based approach over intramyocardial injections with regard to cell retention, survival,[22] and, ultimately, preservation of heart function.[21] Our choice of encaging the *Isl-1*$^{+}$ cardiac

**Figure 1** Intraoperative view of the progenitor cell-loaded fibrin patch that has been slid into the pocket between an autologous pericardial flap and the epicardial surface of the infarct area.

H. Oh, M.D., Ph.D.

# STEM CELL THERAPIES IN PATIENTS WITH SINGLE VENTRICLE PHYSIOLOGY

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[a]Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan; [b]Okayama University Hospital, Okayama, Japan

Cardiac stem cell therapies for congenital heart diseases

*Shuta Ishigami, Shunji Sano, Hidemasa Oh*

Future Cardiol. 2012 Mar;8(2):161-9. doi: 10.2217/fca.12.13.

**Potential for stem cell use in congenital heart disease.**

Pincott ES1, Burch M.

# CASE REPORTS: CONGENITAL

## Regenerative therapy for hypoplastic left heart syndrome: First report of intraoperative intramyocardial injection of autologous umbilical-cord blood–derived cells

Harold M. Burkhart, MD,[a] Muhammad Yasir Qureshi, MBBS,[b] Susana Cantero Peral, MD,[c,d] Patrick W. O'Leary, MD,[b] Timothy M. Olson, MD,[b] Frank Cetta, MD,[b] and Timothy J. Nelson, MD, PhD,[b,c,d,e,f] the Wanek Program Clinical Pipeline Group, Rochester, Minn


The case study involves an HLHS newborn whose umbilical cord blood was collected at birth. The umbilical cord blood was then processed to achieve mononuclear cells and preserved at below zero temperatures until the time of delivery. At four days old, the infant underwent the Stage I Norwood procedure, allowing the right ventricle to pump blood to the lungs and the body. Then, at four months old, the patient underwent Stage II surgery, also known as the Glenn procedure, and the stem cells were injected into the baby's right ventricle. During the one and three month follow ups, the child's right ventricle function had improved.

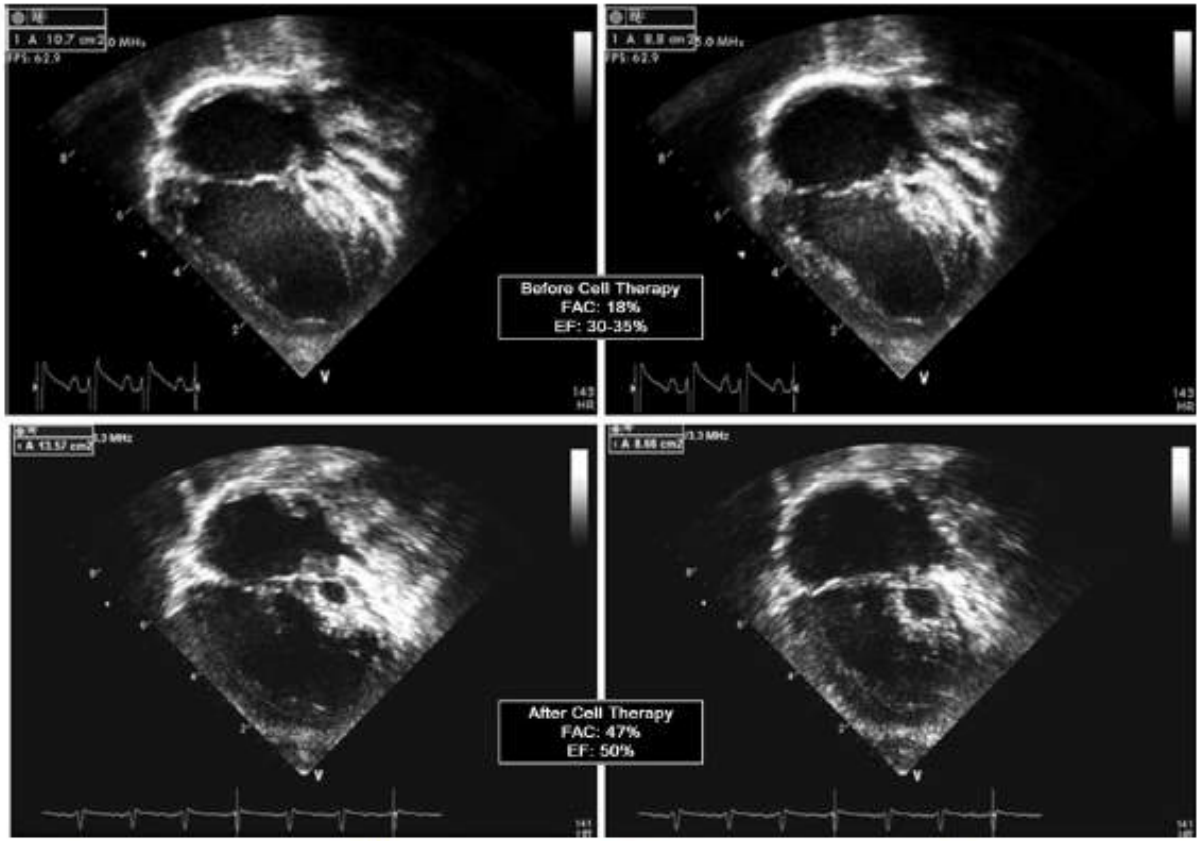


**FIGURE 1.** Apical views of echocardiogram performed before (*top panels*) and 3 months after (*bottom panels*) intramyocardial injection of umbilical-cord blood–derived stem cells. Images show improvement in right ventricular function, with an increase in the ejection fraction from 30%-35% to 50%. *FAC*, Fractional area change; *EF*, ejection fraction.


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Thank You