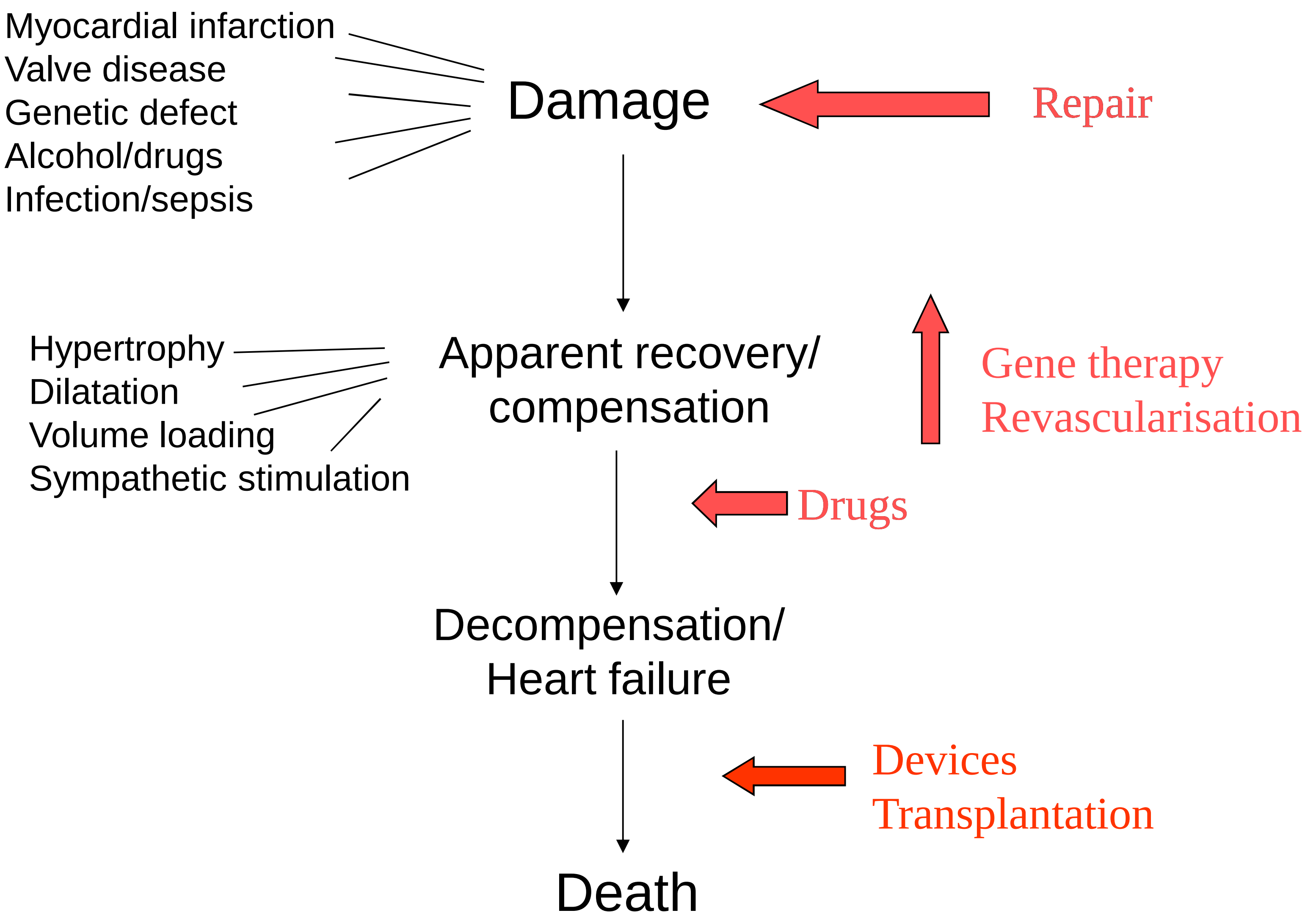
Tissue engineering challenges for cardiac repair

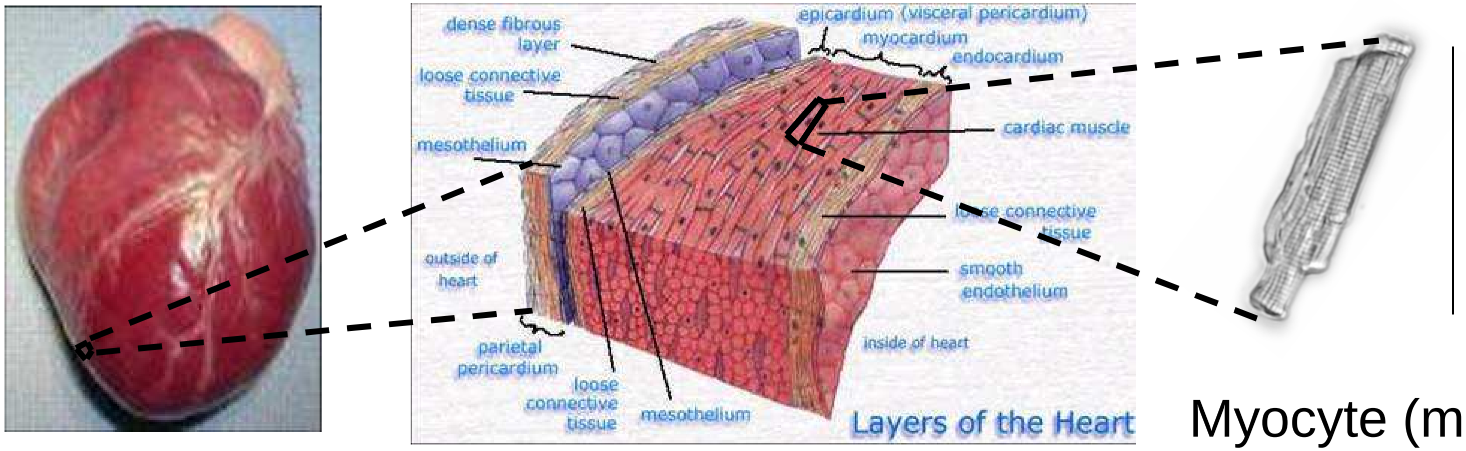
Professor Sian E. Harding

Imperial College
London

Natural history of heart failure

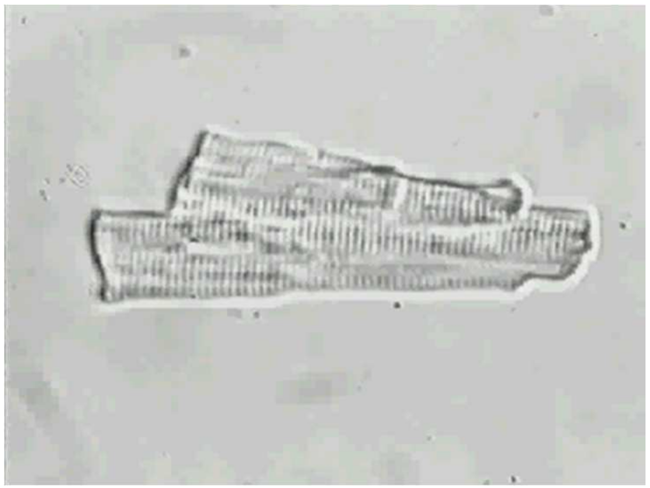


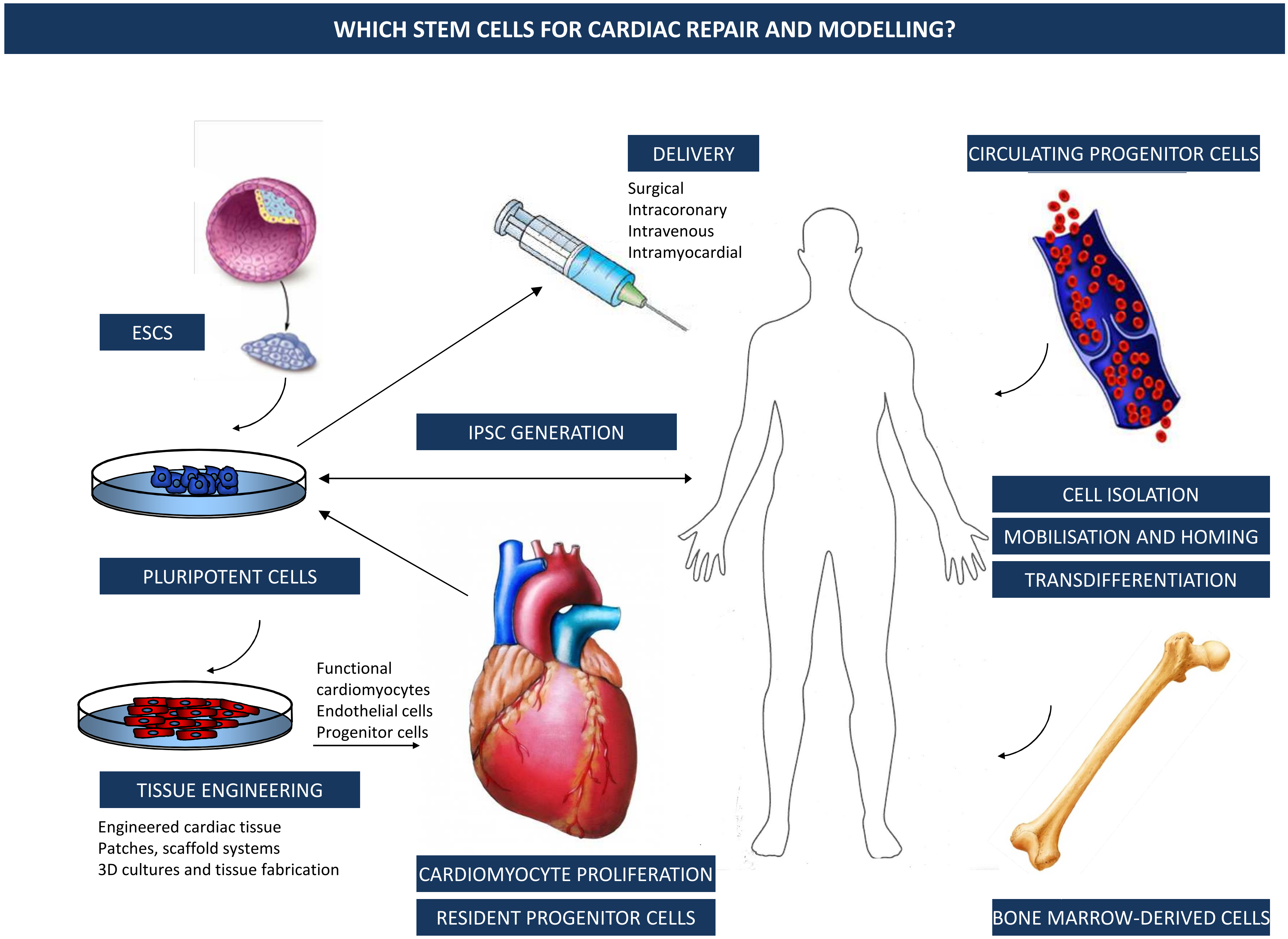
Structure of the contracting myocardium



0.1mm

Myocyte (muscle cell)

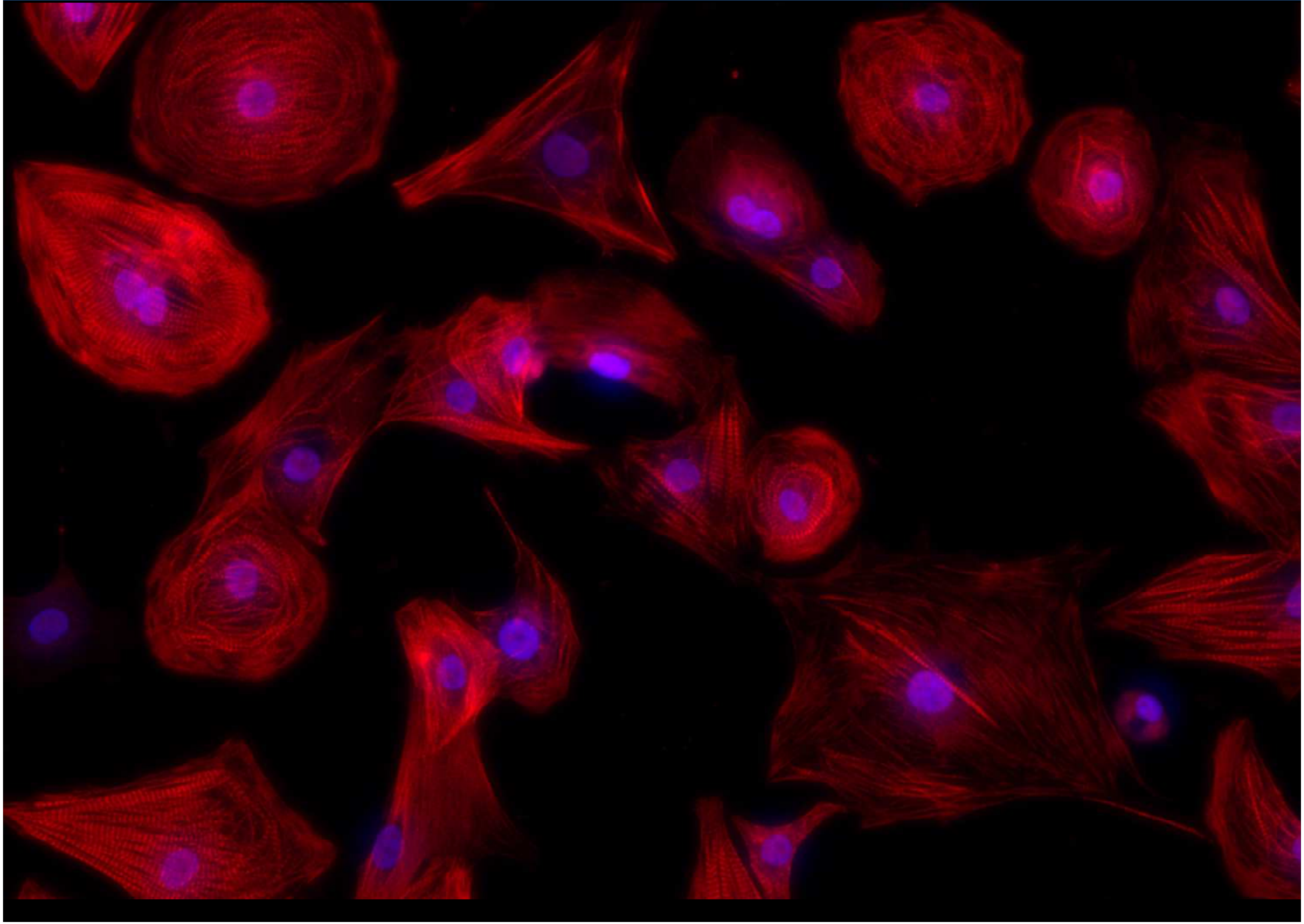


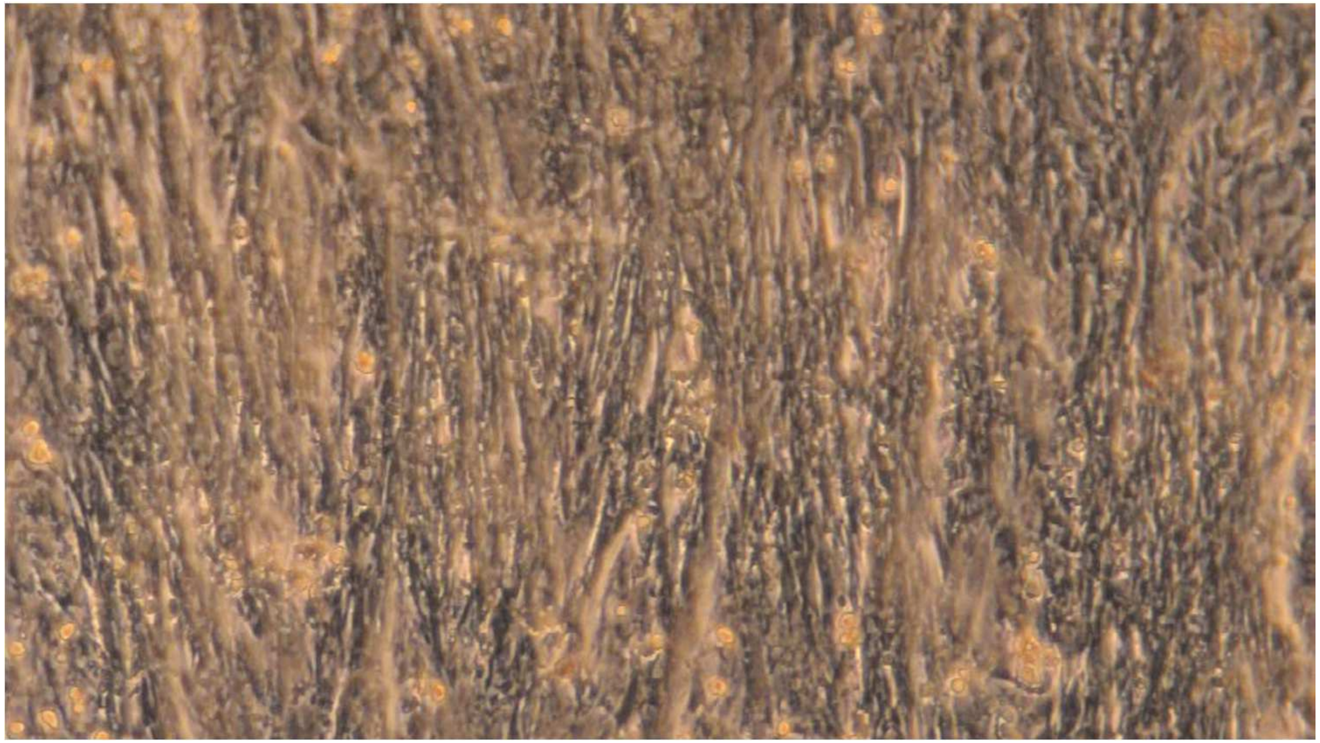

WHICH STEM CELLS FOR CARDIAC REPAIR AND MODELLING?
ESCS
DELIVERY
Surgical
Intracoronary
Intravenous
Intramyocardial
CIRCULATING PROGENITOR CELLS
IPSC GENERATION
PLURIPOTENT CELLS
CELL ISOLATION
MOBILISATION AND HOMING
TRANSDIFFERENTIATION
Functional
cardiomyocytes
Endothelial cells
Progenitor cells
TISSUE ENGINEERING
Engineered cardiac tissue
Patches, scaffold systems
3D cultures and tissue fabrication
CARDIOMYOCYTE PROLIFERATION
RESIDENT PROGENITOR CELLS
BONE MARROW-DERIVED CELLS

Which Stem Cells for Cardiac Repair?

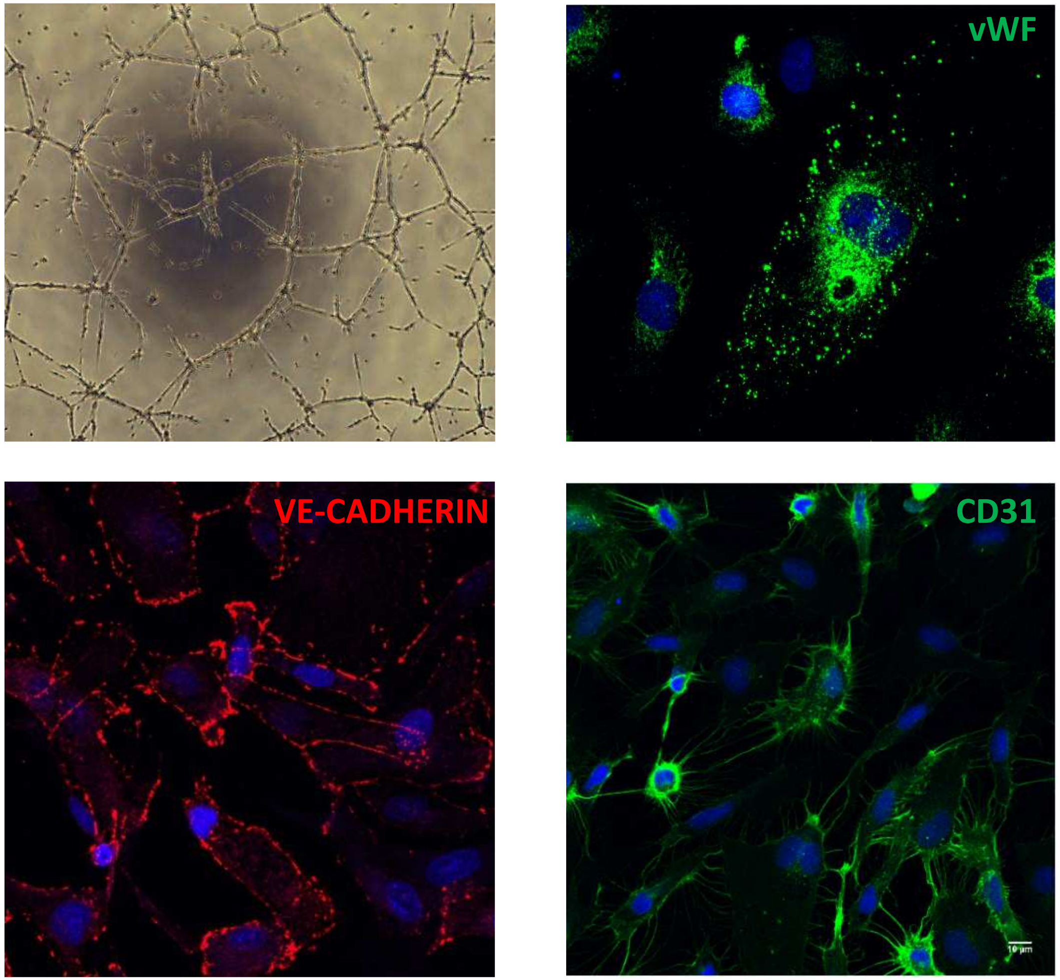
	Skeletal myoblasts	Bone marrow-derived stem cells	Mesenchymal stem cells (MSC)	Heart-derived stem cells	Embryonic stem cells	Induced pluripotent stem cells /induced cardiomyocytes
Immune matching	√	√	√	√	X	√
Forms true cardiomyocytes	X	X	?	?	√	√
Large scale production	√	X	√	√	√	√
Proliferation and motility	X	√	√	√	X	X
Ethically neutral	√	√	√	√	X	√

HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

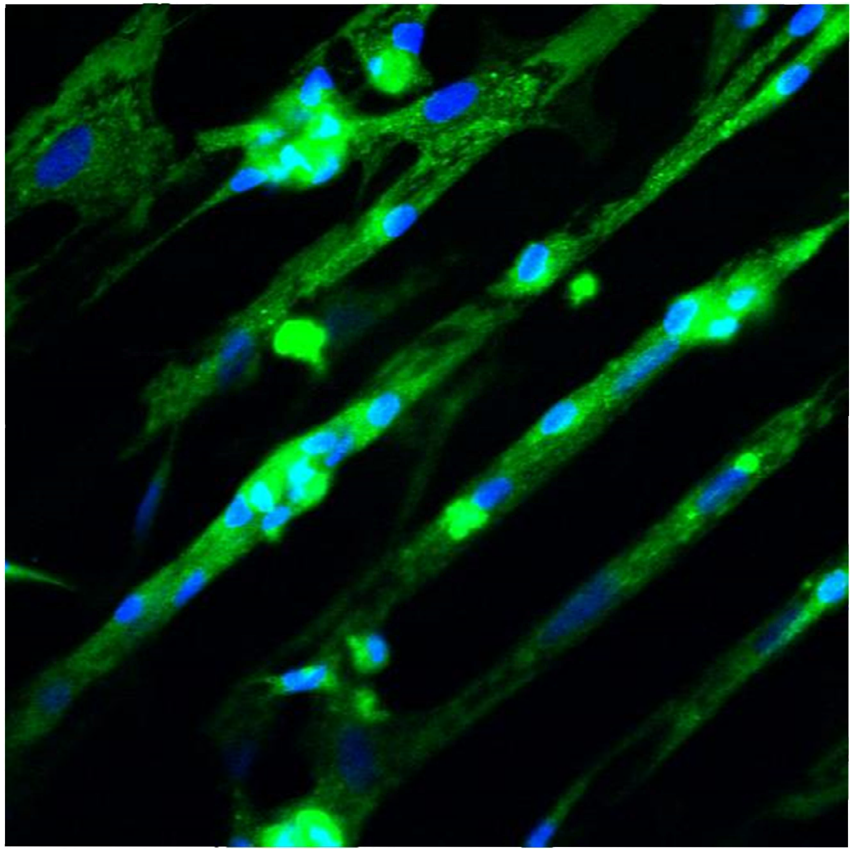




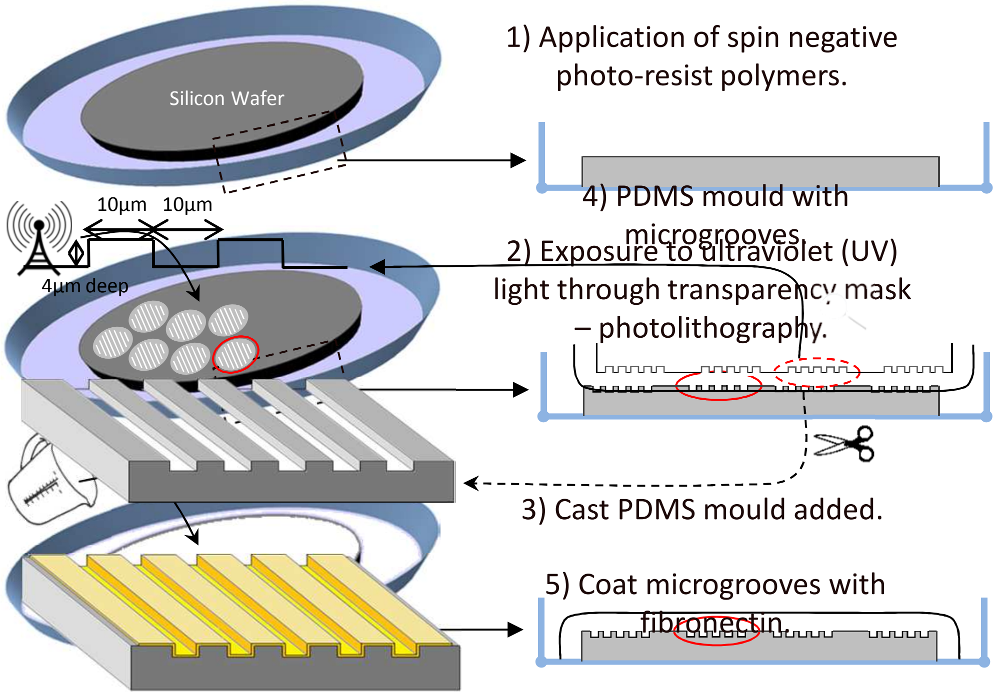
HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS



with K Lawlor, A Randi



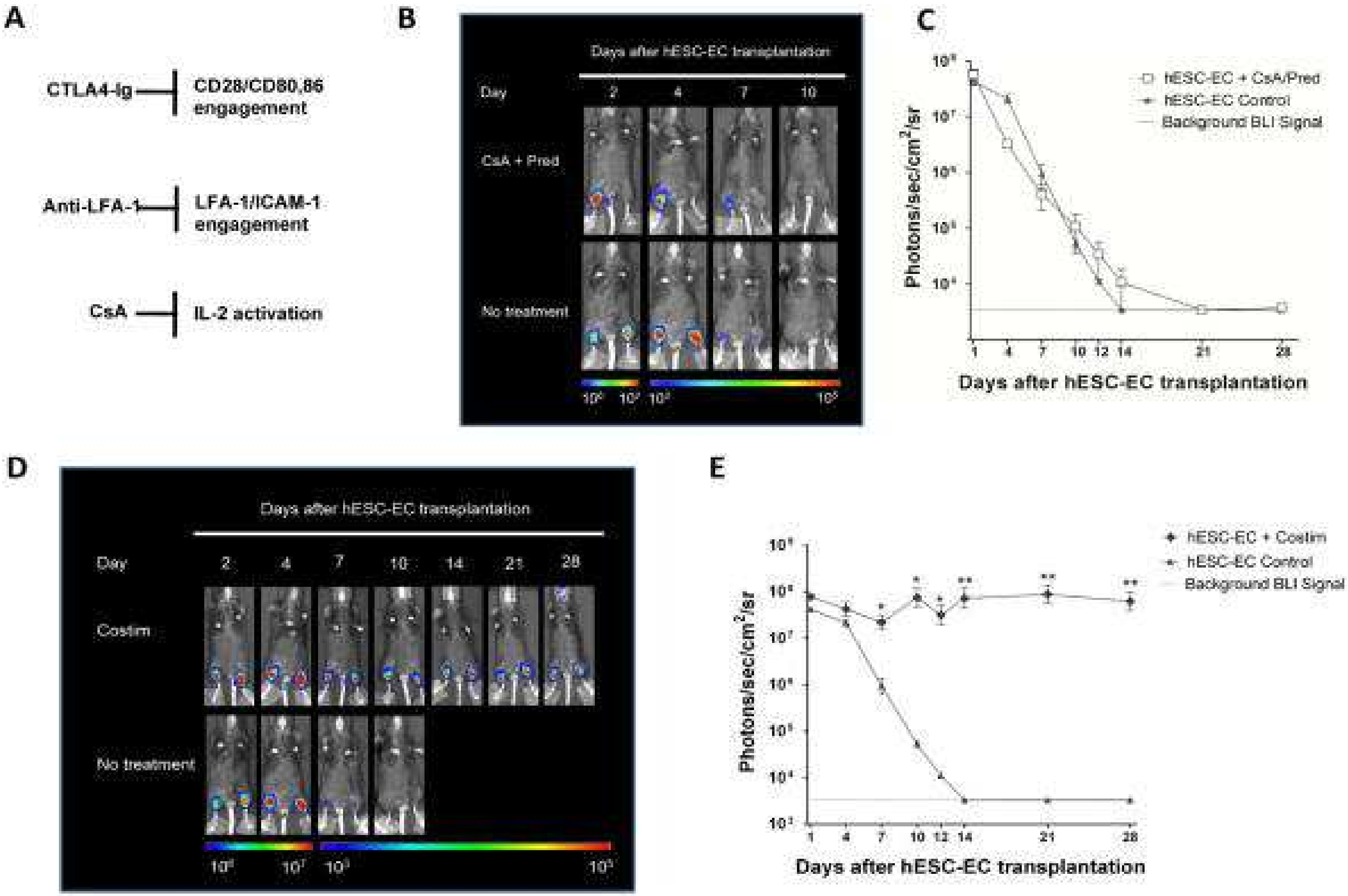
Myosin Heavy Chain
DAPI



Rao, Biomaterials 2012

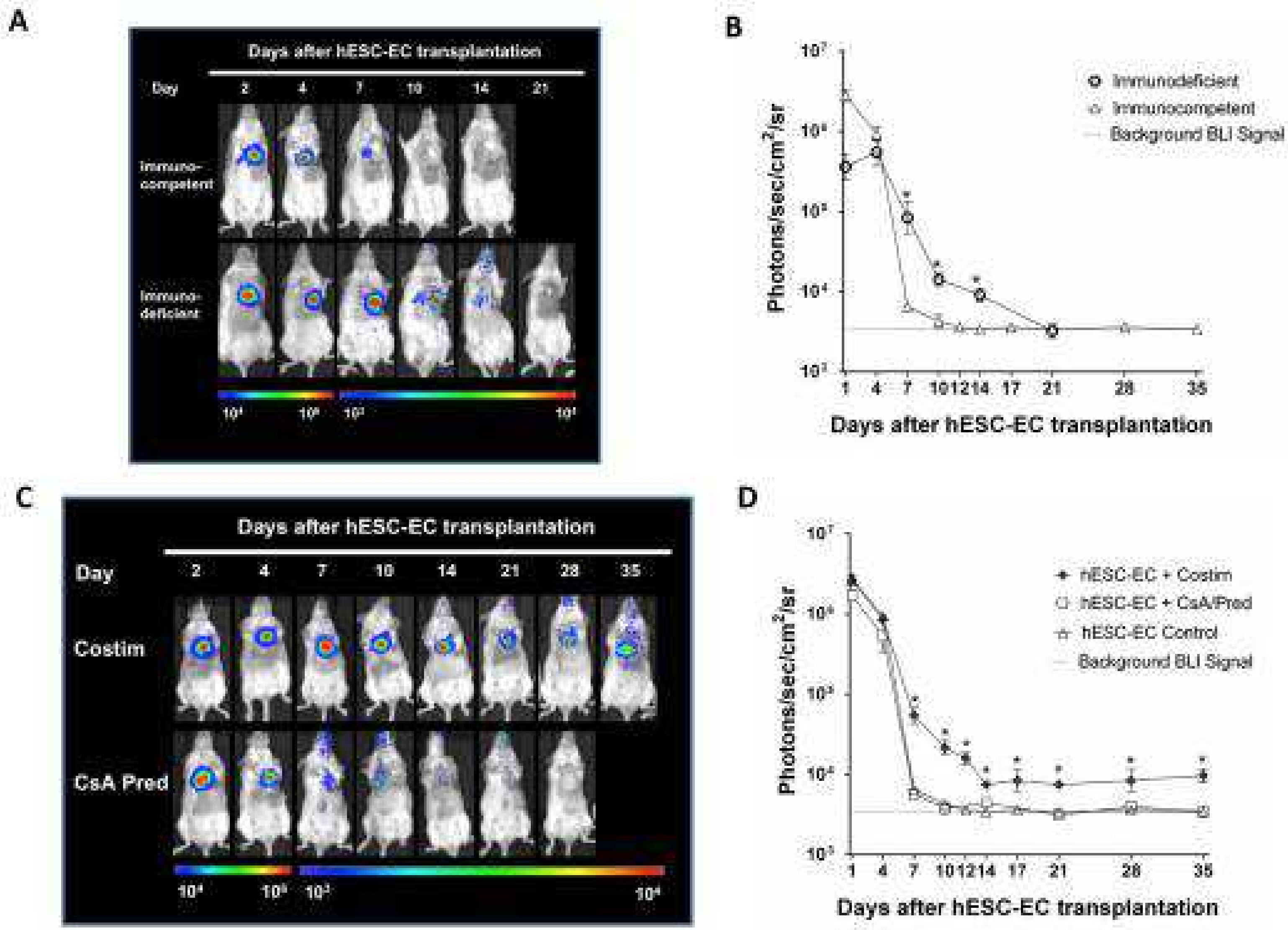
Implanting stem cells - the heart has more problems than just immune rejection!

Implantation of hESC-derived endothelial cells into hindlimb: effect of immune blockade



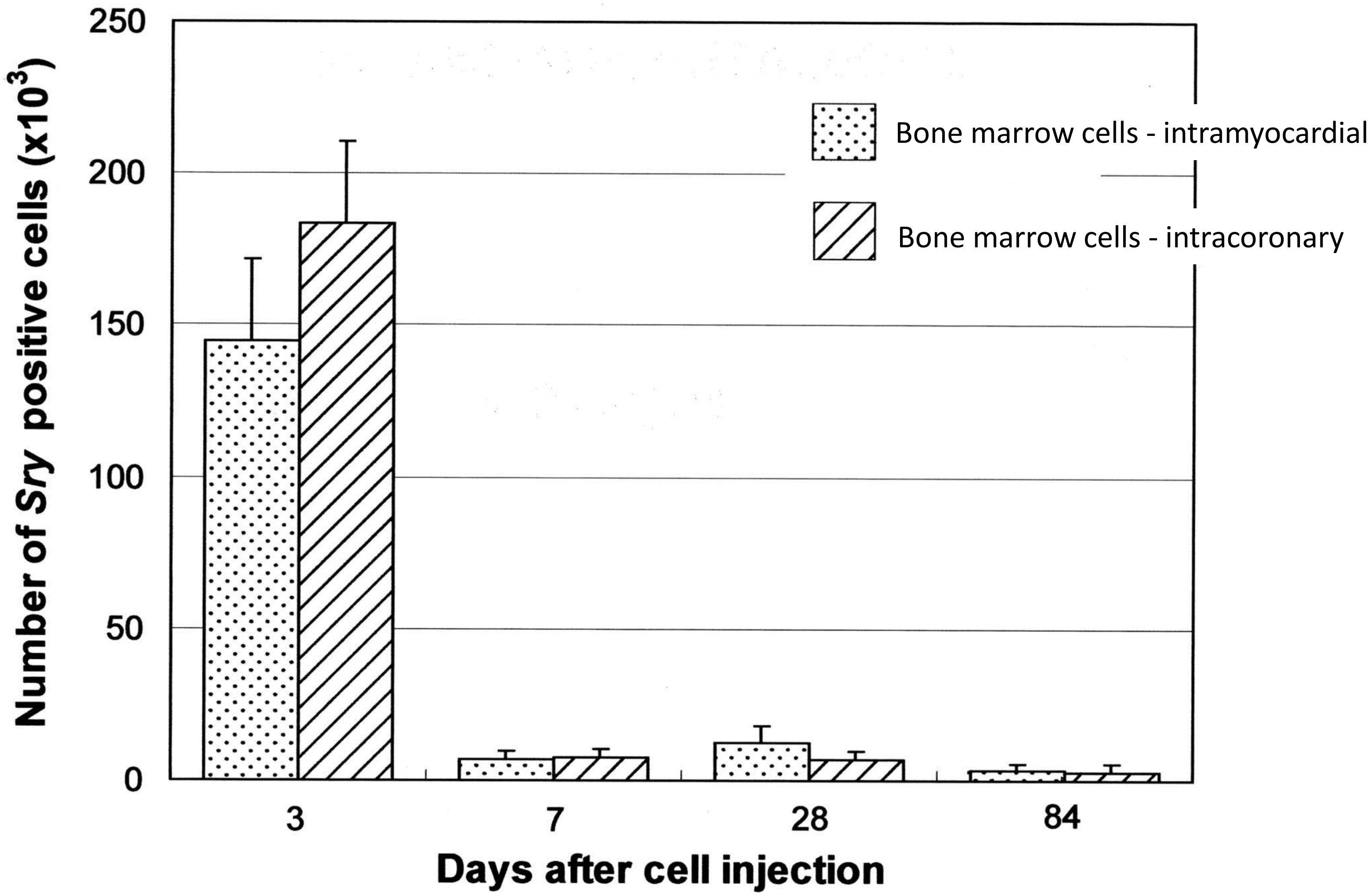
Stem Cells. 2013 Nov; 31(11): 2354–2363.

Implantation of hESC- derived endothelial cells into infarcted heart: effect of immune blockade

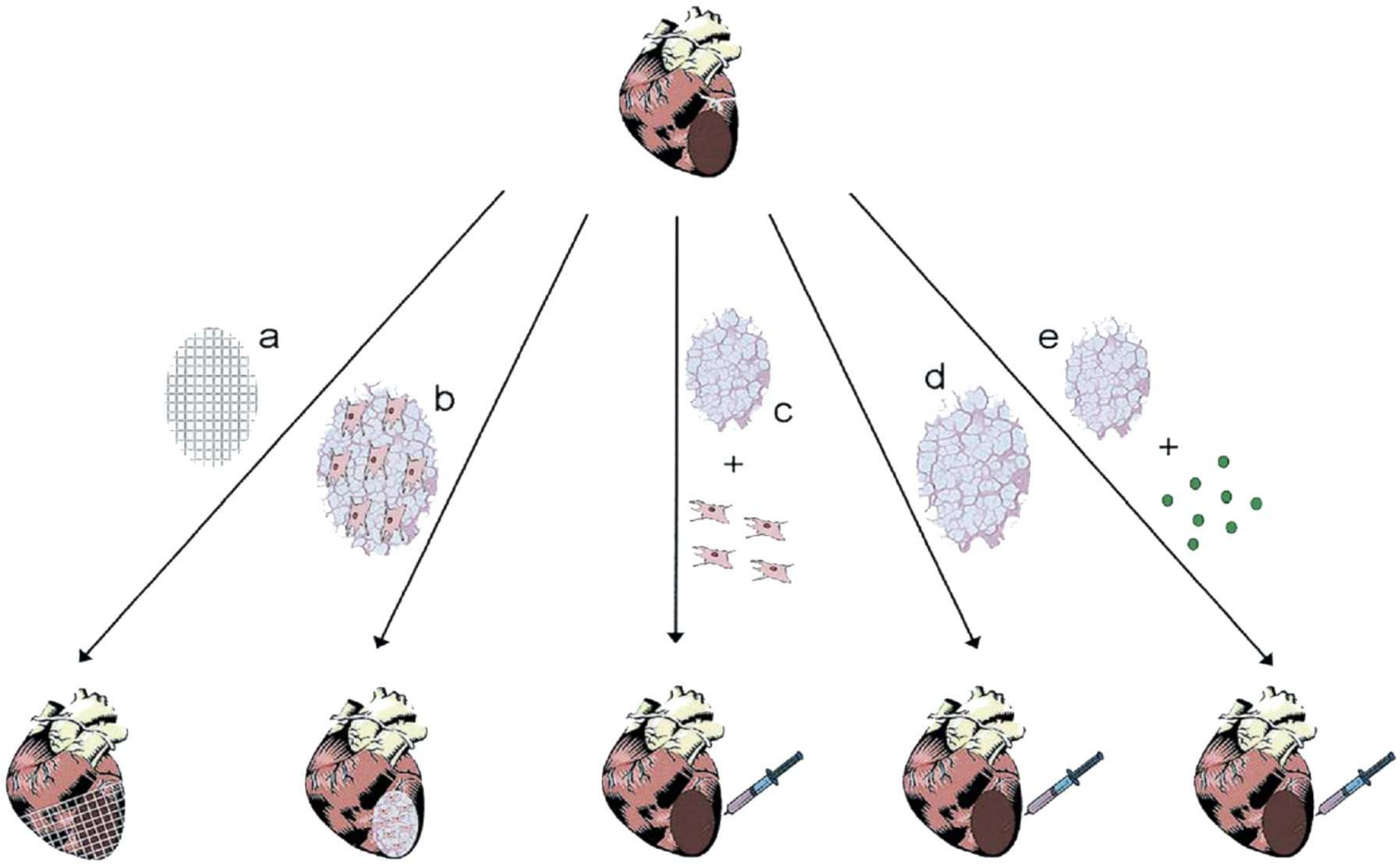


Stem Cells. 2013 Nov; 31(11): 2354–2363.

SURIVAL OF GRAFTED CELLS



Fukushima, S. et al. Circulation 2007;115:2254-2261



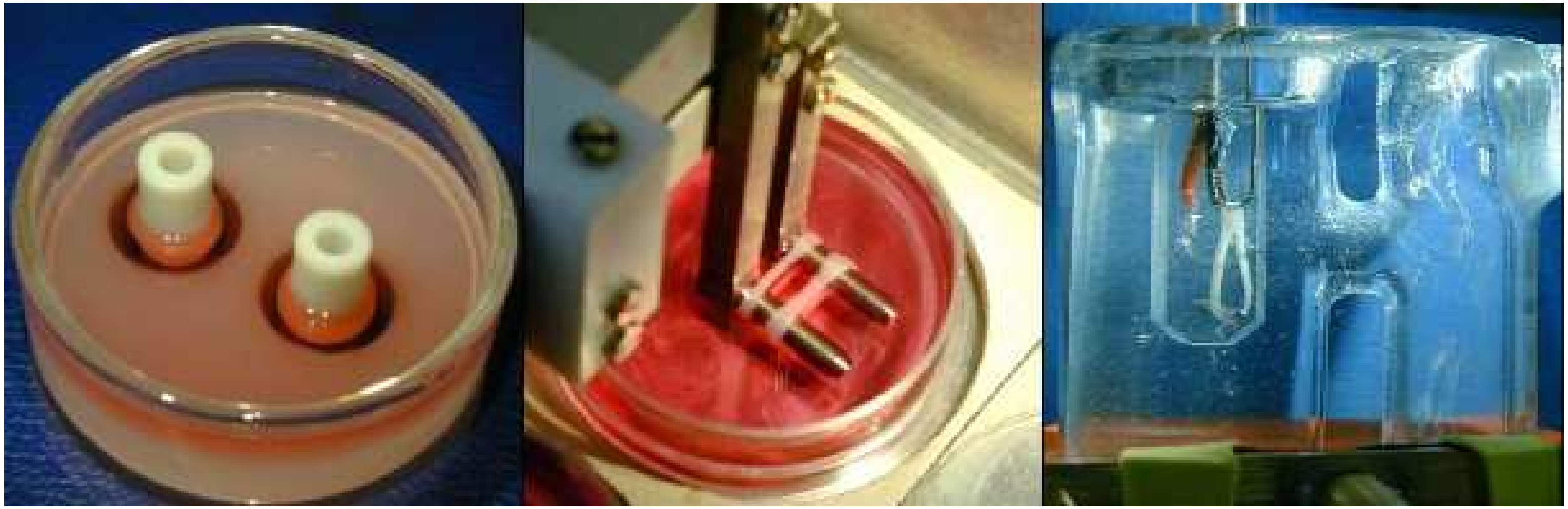
A. Use of a polymer mesh as a ventricular restraint to prevent ventricular dilatation
B. In vitro culturing of cells on a biomaterial scaffold prior to surgical attachment to the epicardium
C. Direct intramyocardial injection of cells with biomaterial scaffold
D. Direct intramyocardial injection of biomaterial alone
E. Direct intramyocardial injection of other agents such as proteins or gene therapy

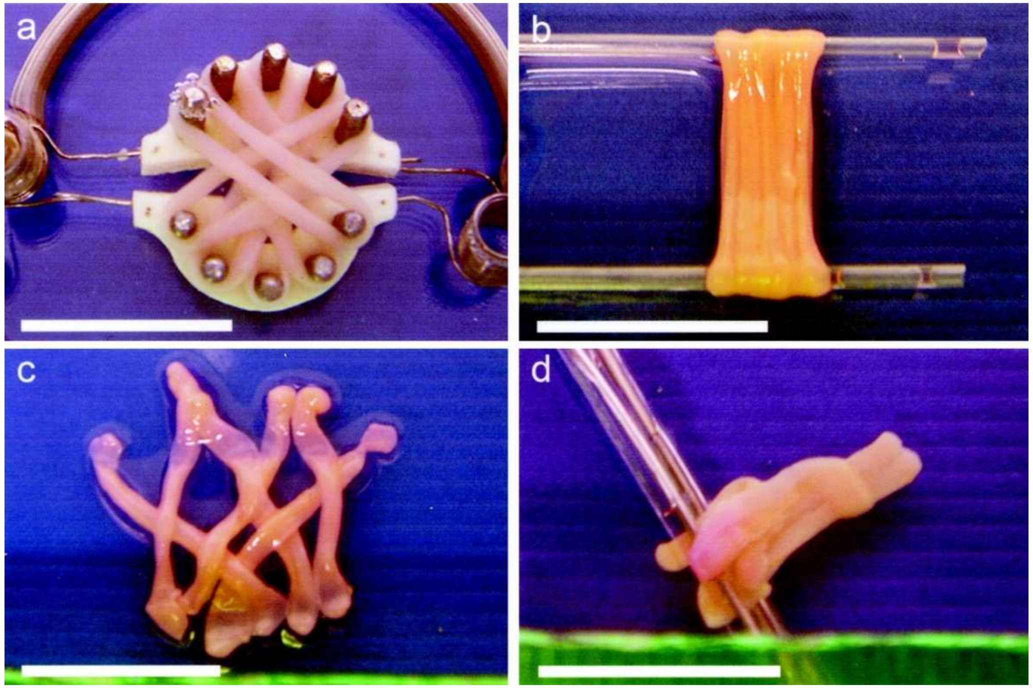
Christman *et al*. J. Am. Coll. Cardiol 2006

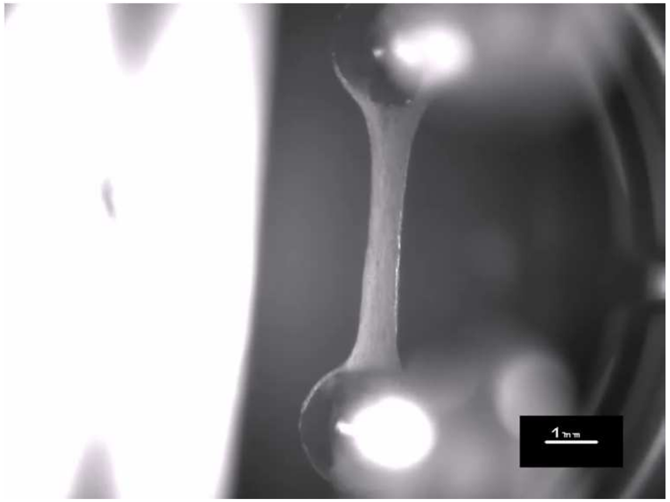
MATERIALS TO ENHANCE CELL ATTACHMENT OR SURVIVAL

MATERIAL	ADVANTAGES	DISADVANTAGES
Naturally occurring materials • Collagen • Alginate • Hyaluronic acid • Fibrin • Gelatin • Chitosan • Matrigel • decellularised tissue	Biocompatibility Porous Biodegradable Bioresorbable	Poor processibility Poor mechanical properties Possible immunogenic problems
Biodegradable synthetic polymers • Poly(lactic acid) • Poly(ethylene terephthalate) • Poly(glycerol sebacate) • Poly(lactic-co-glycolic acid) • Polypropylene fumarate • Poly(orthoesters) • Poly(anhydrides)	Good biocompatibility Off-the-shelf availability Good processibility Bioresorbable Biodegradable (wide range of rates) Added value from material tailoring • Controlled porosity • Mechanical support •Electrical conductivity •Controlled release of factors	Inflammation or nanotoxicity from degradation products Loss of mechanical properties after degradation
Non-degradable synthetic polymers	Off-the-shelf availability No foreign-body reactions Tailored mechanical properties	Effect of long term presence in the body

ENGINEERED HEART TISSUE: NEONATAL RAT CARDIOMYOCYTES IN COLLAGEN



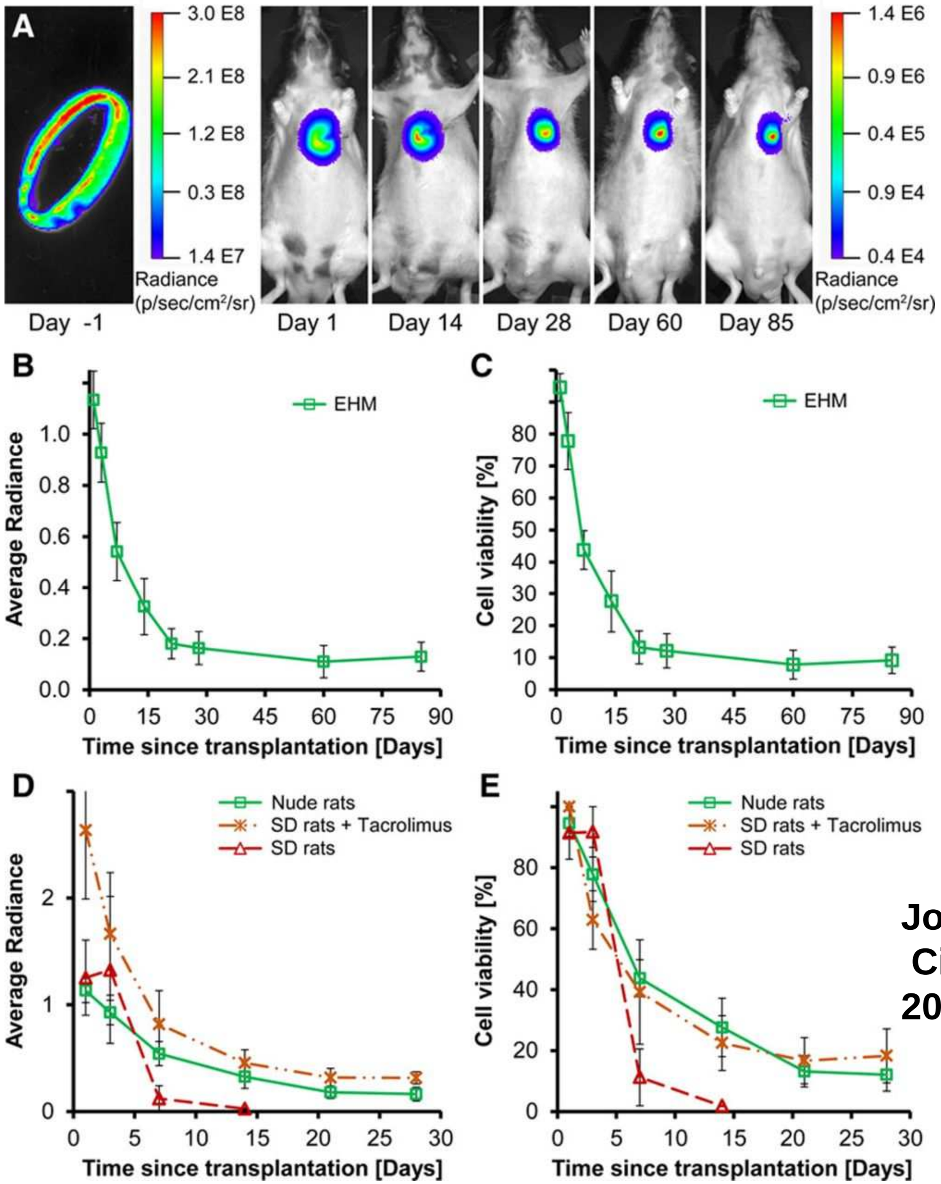




Human iPSC-CM in fibrin

Naito, H. et al. Circulation 2006;114:I-72-I-78
T Eschenhagen, WH Zimmermann

Human engineered heart muscles (EHMs) show long-term engraftment and survival.

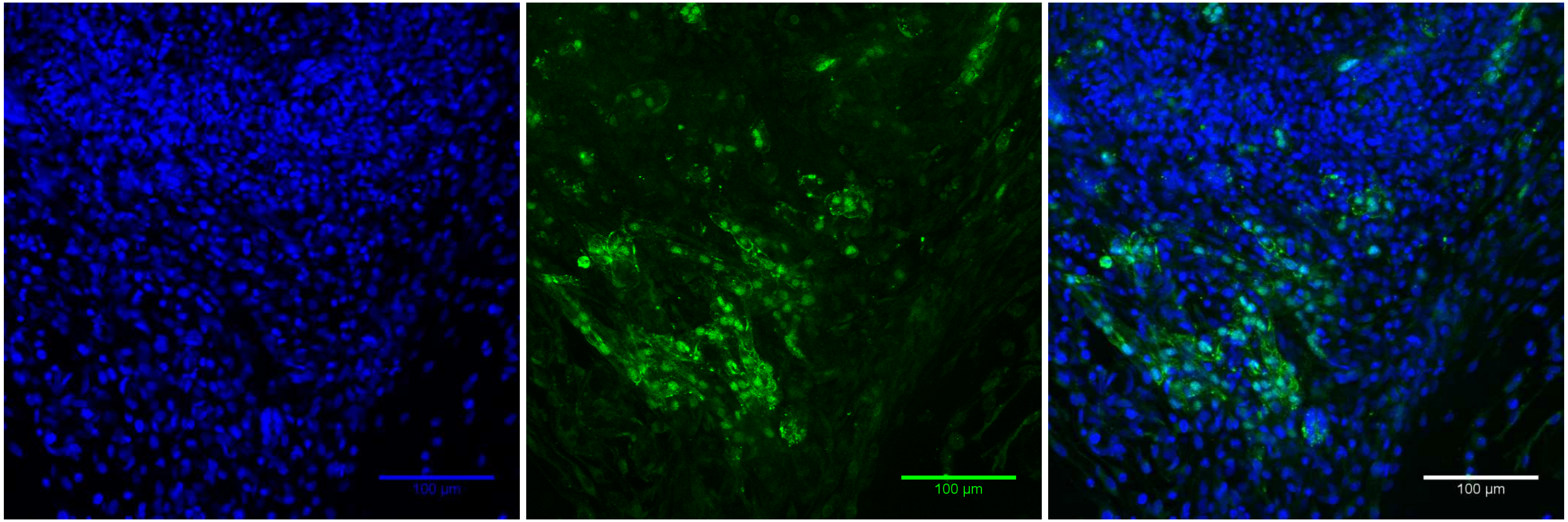


Johannes Riegler et al. Circulation Research. 2015;117:720-730

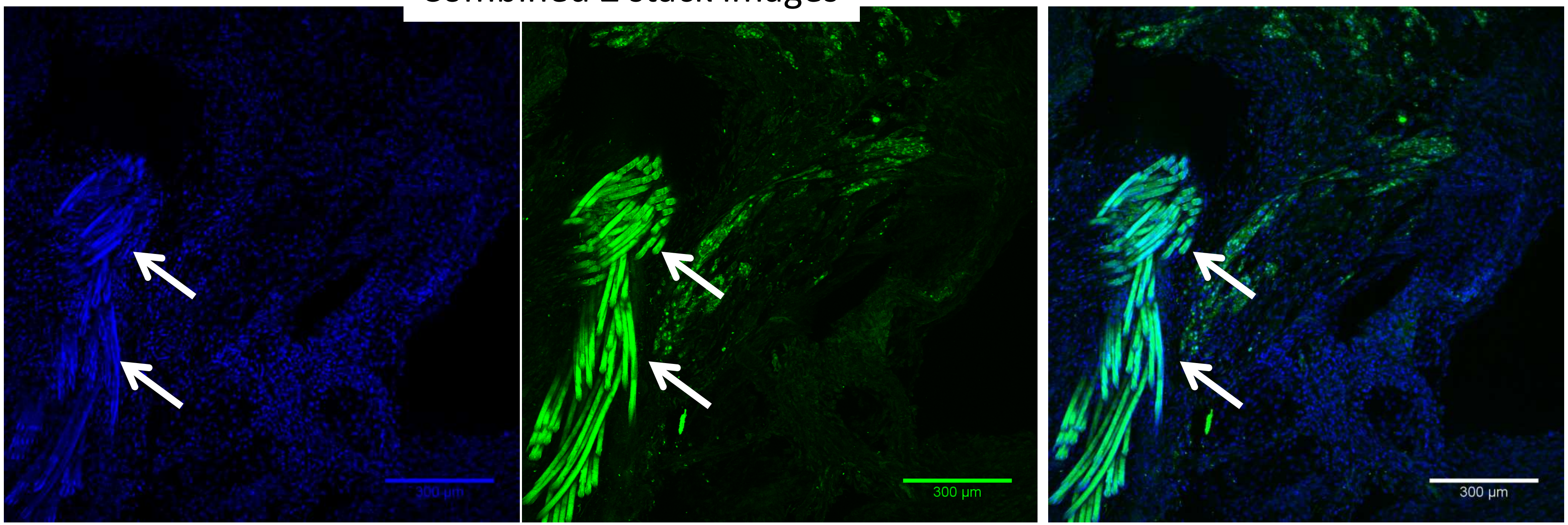
American Heart Association

Rabbit heart with human EHT

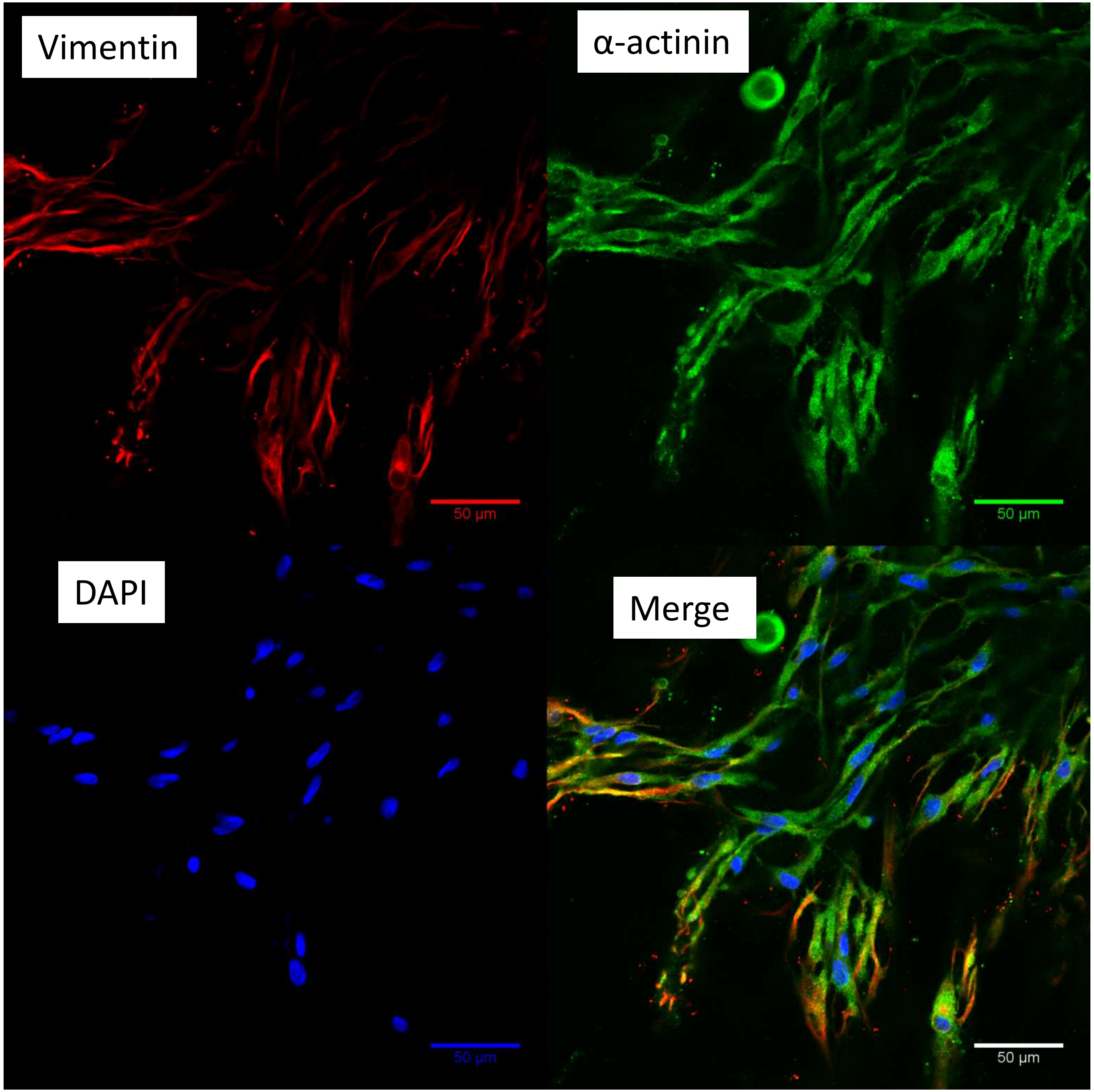
DAPI | Human Nuclei staining only | • Merge



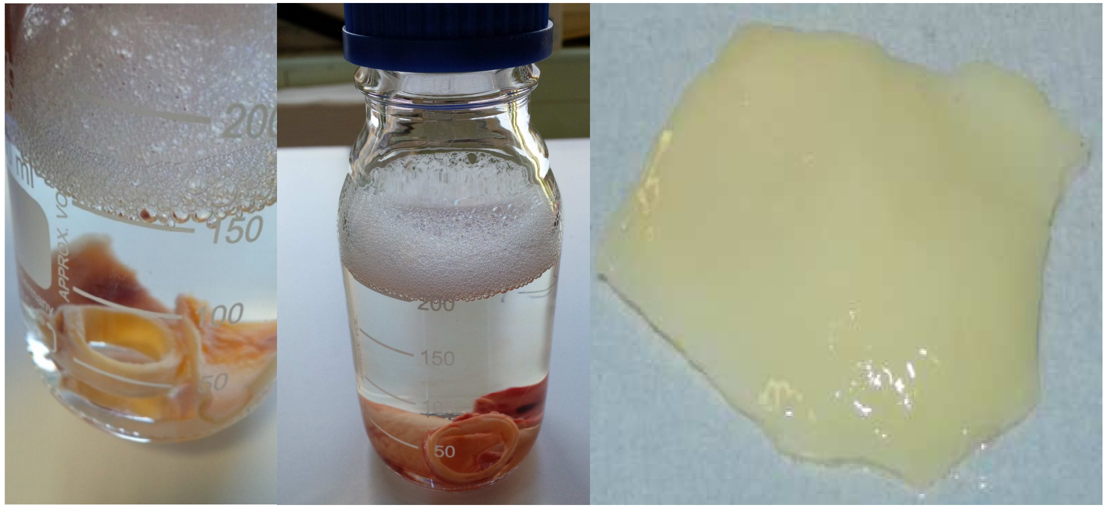
Combined Z stack images

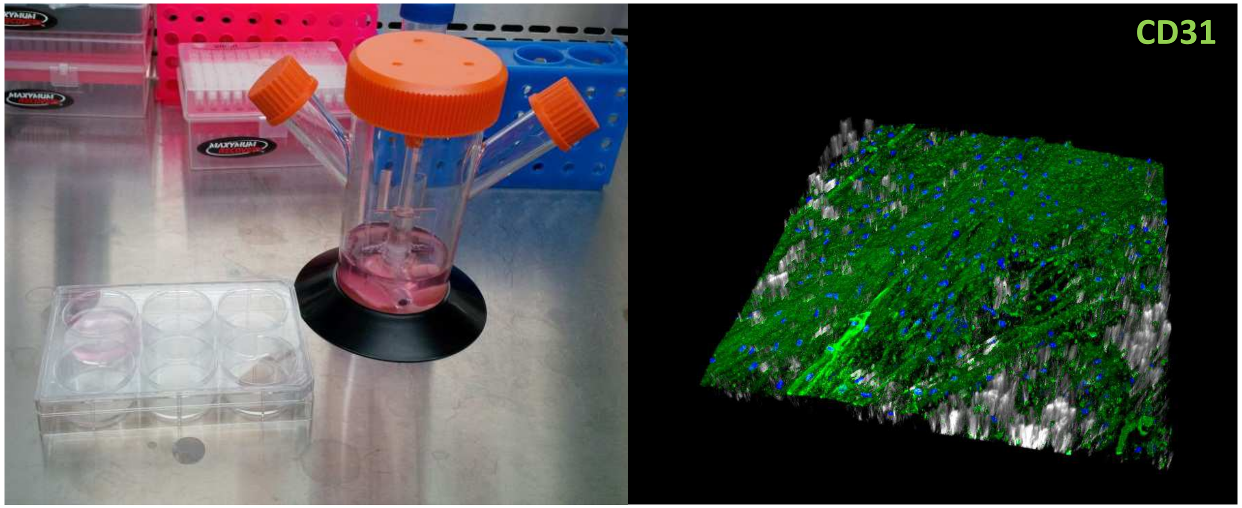


Transverse section of human EHT attached to the rabbit heart (higher magnification)

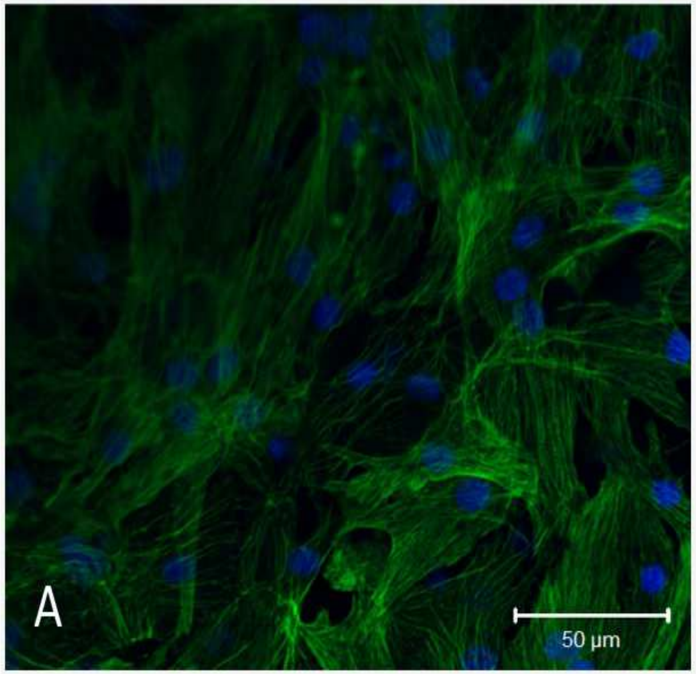


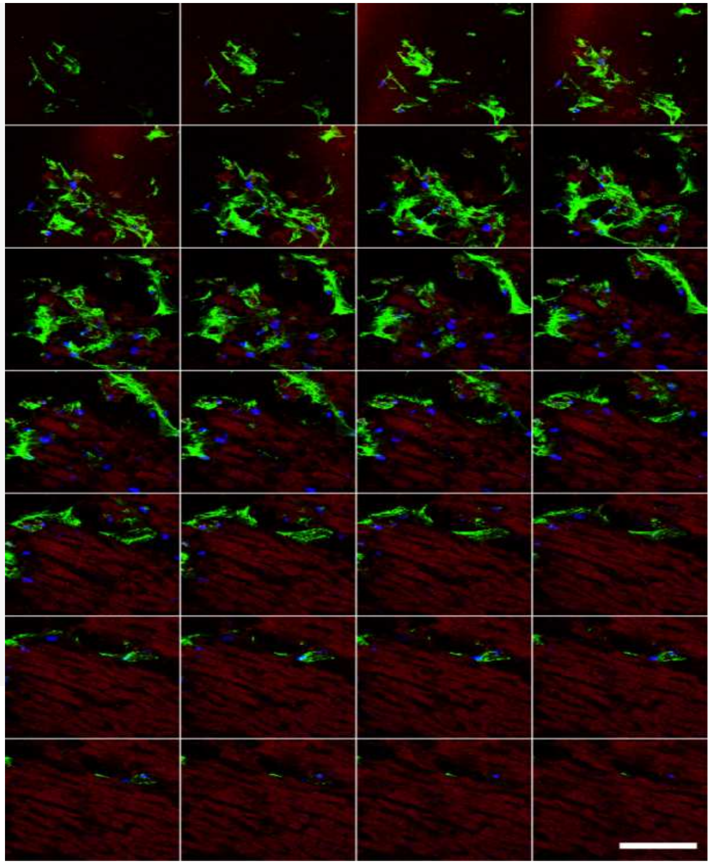
DECELLULARISED AORTA REPOPULATED WITH HIPSC-DERIVED ENDOTHELIAL CELLS

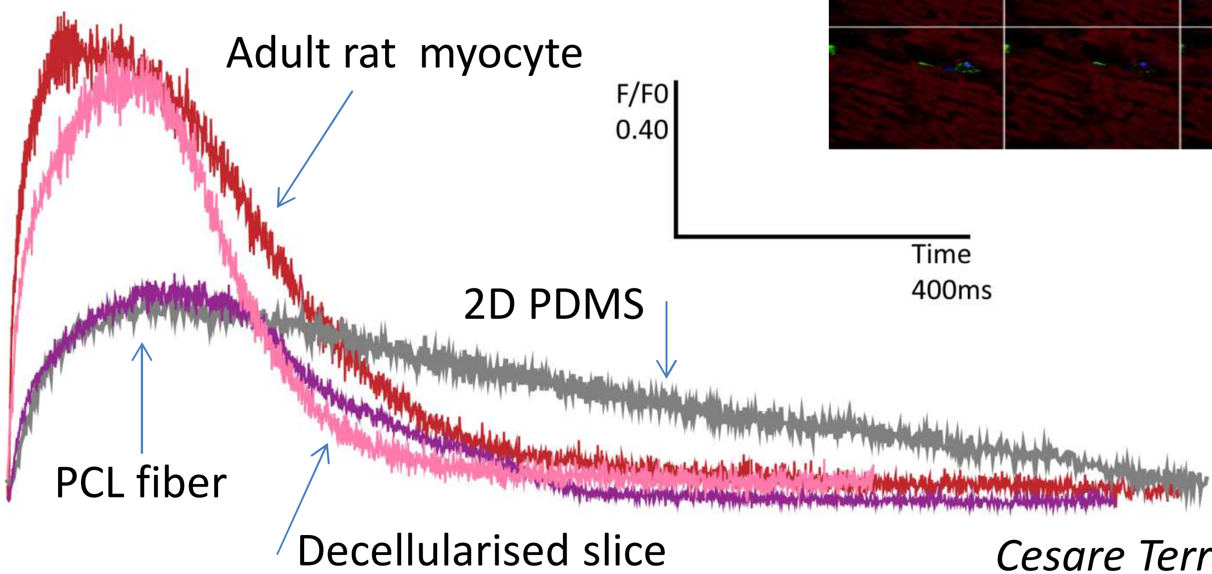


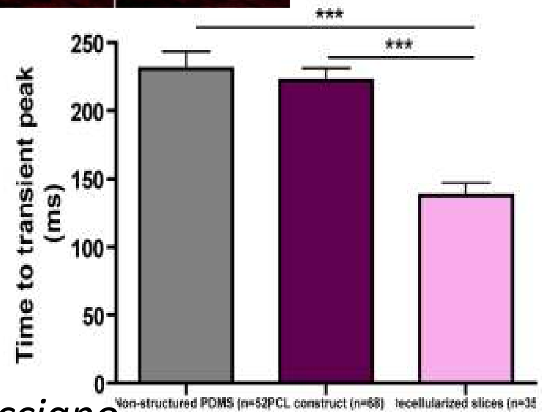


Neonatal rat ventricular myocytes seeded onto a decellularized myocardial slice.
Green – α-SMA, Blue – DAPI.



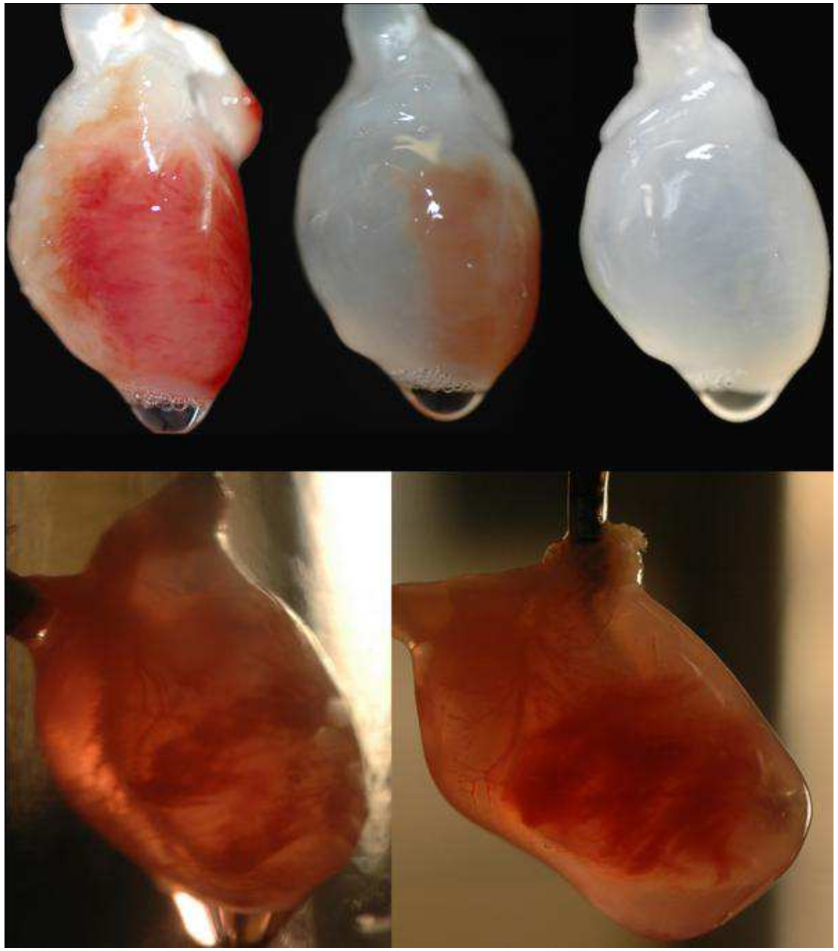






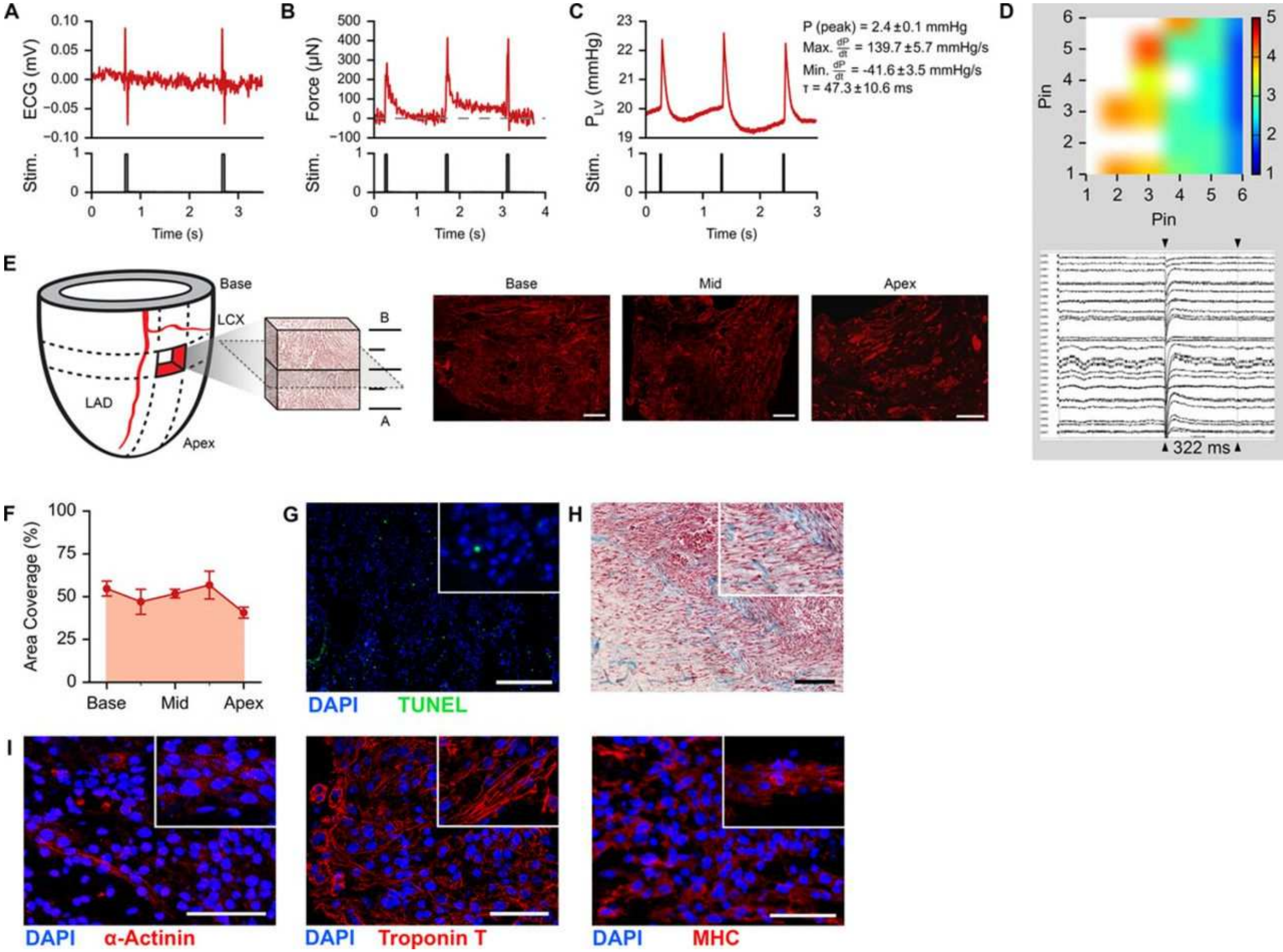
Cesare Terracciano

DECELLULARISED RAT HEART REPOPULATED WITH NEONATAL RAT CARDIOMYOCYTES



Ott. Nature Medicine 14, 213 - 221 (2008)

Repopulation of decellularized human myocardium in whole hearts with human induced pluripotent stem cell (iPSC)–derived cardiomyocytes.



Jacques P. Guyette et al. Circulation Research. 2016;118:56-72

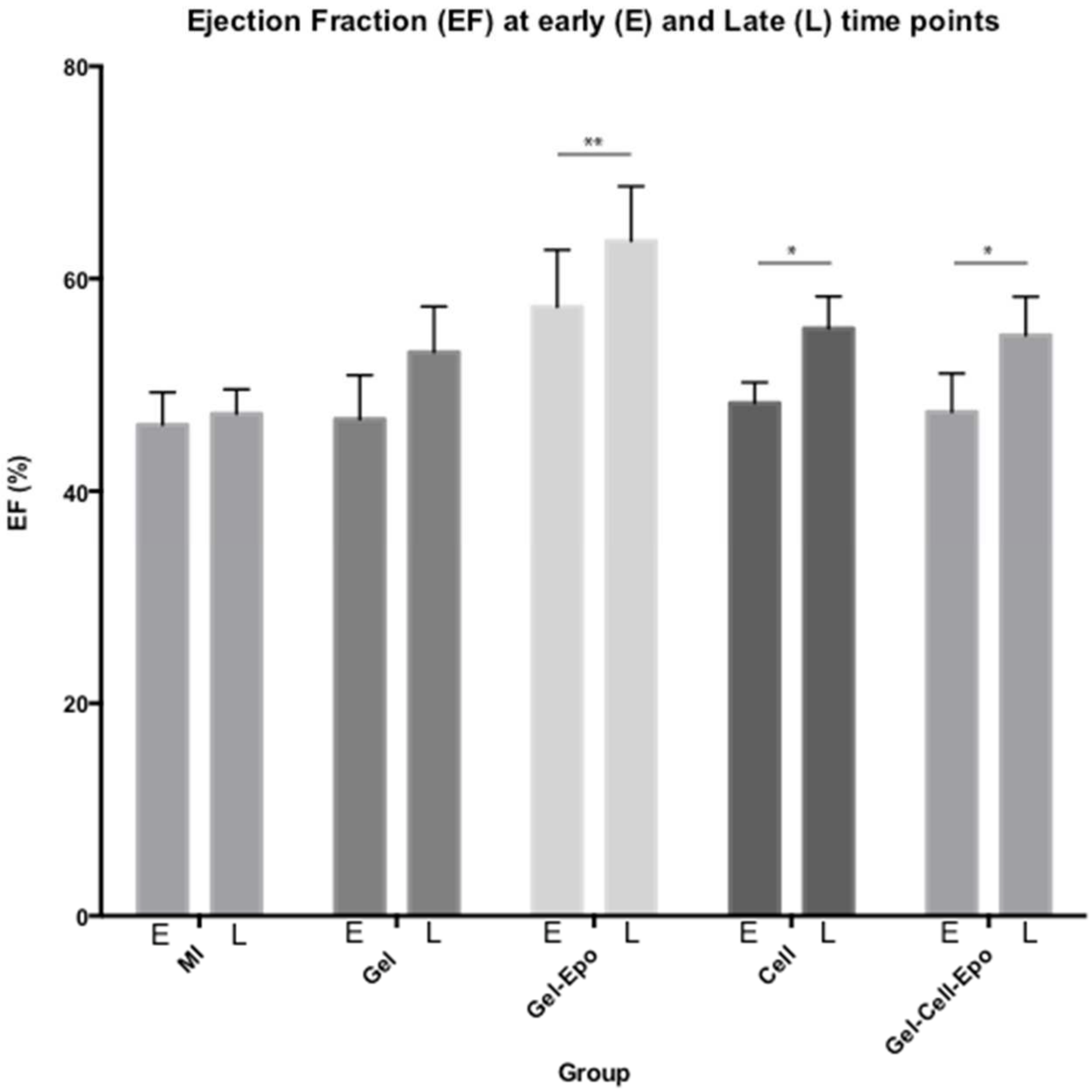
MATERIALS TO ENHANCE CELL ATTACHMENT OR SURVIVAL

MATERIAL	ADVANTAGES	DISADVANTAGES
Naturally occurring materials • Collagen • Alginate • Hyaluronic acid • Fibrin • Gelatin • Chitosan • Matrigel • Peritoneal membranes	Biocompatibility Porous Biodegradable Bioresorbable	Poor processibility Poor mechanical properties Possible immunogenic problems
Biodegradable synthetic polymers • Poly(lactic acid) • Poly(ethylene terephthalate) • Poly(glycerol sebacate) • Poly(lactic-co-glycolic acid) • Polypropylene fumarate • Poly(orthoesters) • Poly(anhydrides)	Good biocompatibility Off-the-shelf availability Good processibility Bioresorbable Biodegradable (wide range of rates) Added value from material tailoring • Controlled porosity • Mechanical support •Electrical conductivity •Controlled release of factors	Inflammation or nanotoxicity from degradation products Loss of mechanical properties after degradation
Non-degradable synthetic polymers	Off-the-shelf availability No foreign-body reactions Tailored mechanical properties	Effect of long term presence in the body

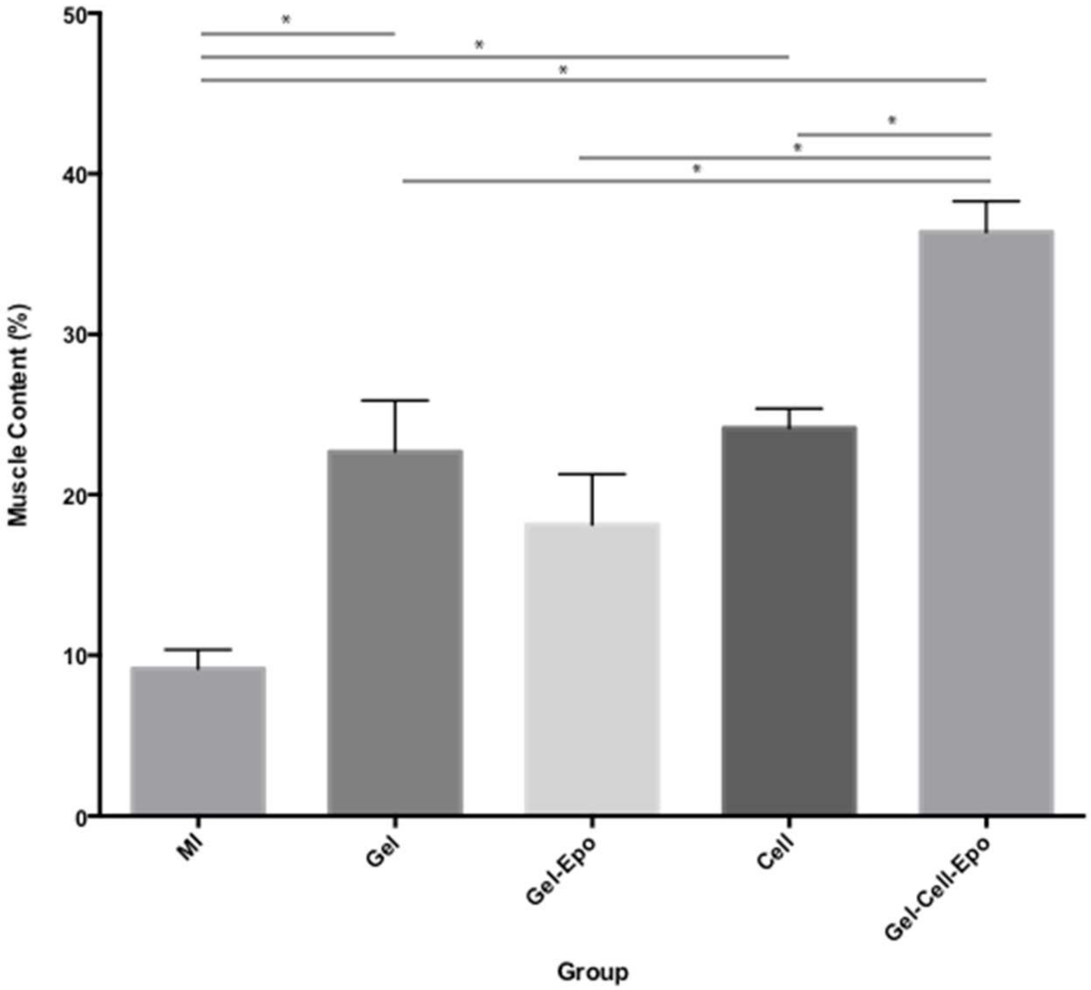
HYDROGEL WITH CARDIOPROTECTIVE ERYTHROPOETIN

Hydrogel – liquid at room temp, gel at 37C, with cardioprotective erythropoetin
With or without 1M hiPSC-derived cardiomyocytes
Athymic nude rats with myocardial infarction
Gel injected at the same time in border zone
Imaged at 1 and 8 weeks by MRI

A Chow


Ejection Fraction (EF) at early (E) and Late (L) time points
EF (%)
0
20
40
60
80
**
*
*
E
L
MI
Gel
Gel-Epo
Cell
Gel-Cell-Epo
Group

INFARCT MUSCLE CONTENT



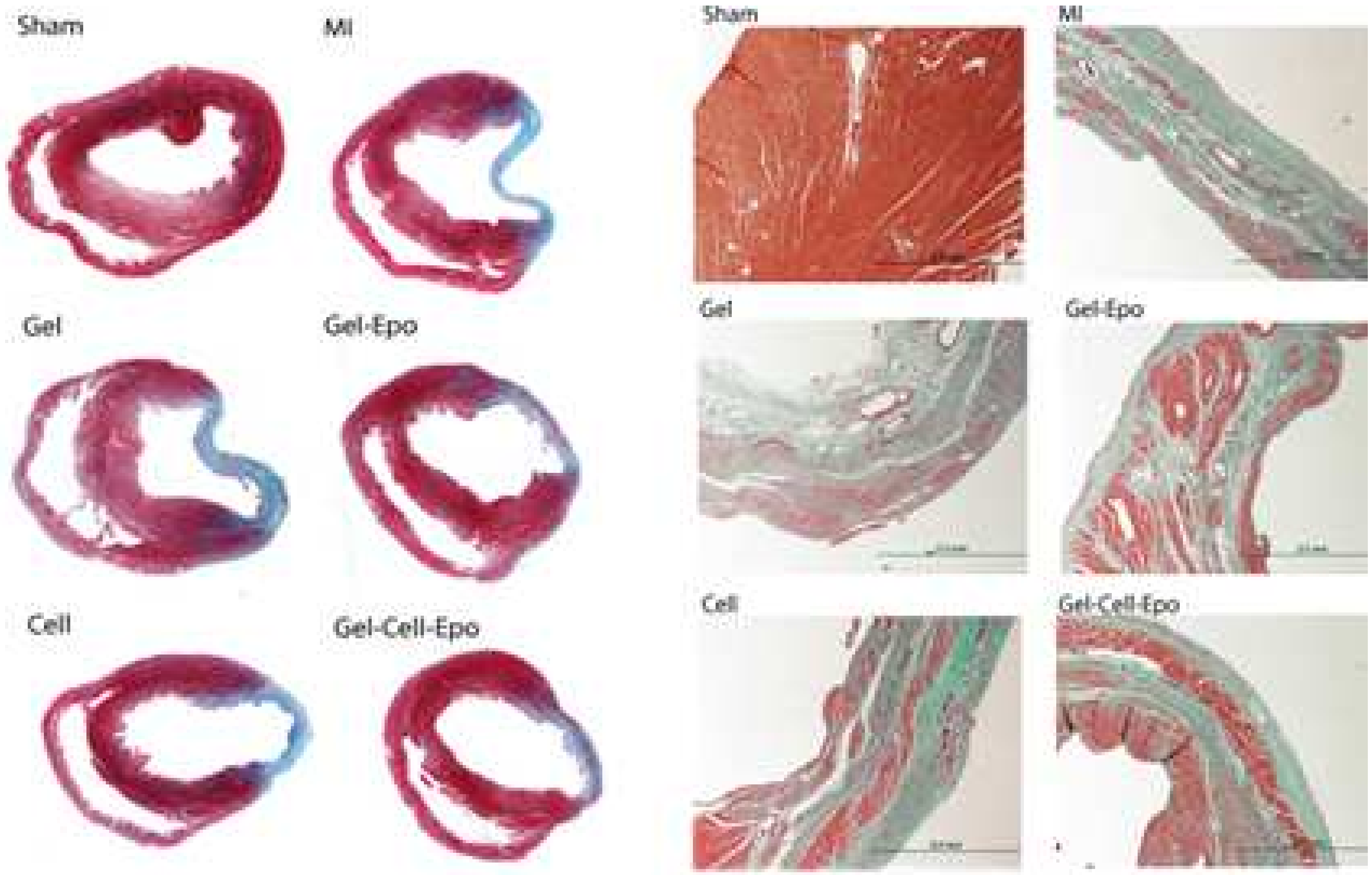
Hydrogel plus epo alone had some beneficial effects

More muscle in scar with gel+epo+iPSC-CM

Modest improvements in cardiac function, similar between conditions

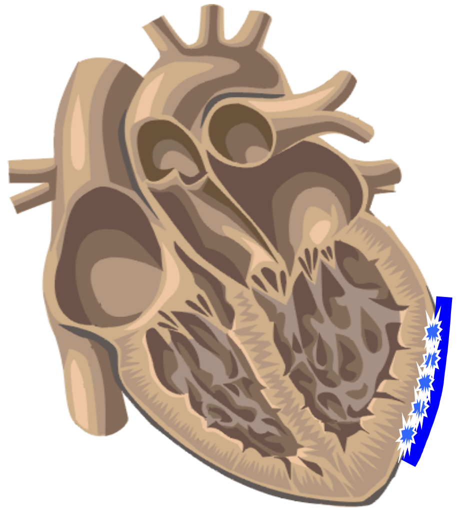
A Chow

But no human muscle!



A patch for stem cell delivery to the heart

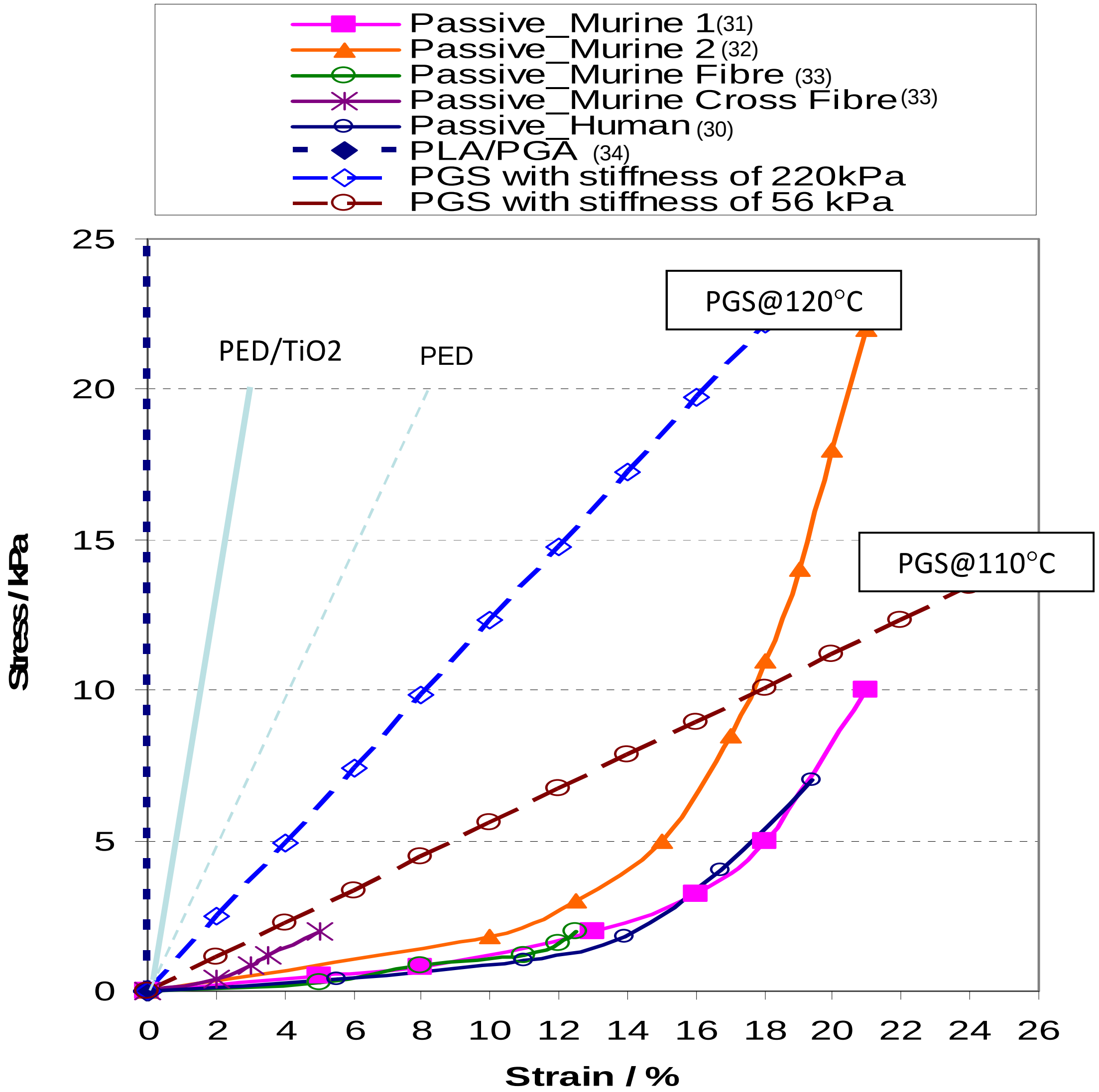
- Differentiated stem cell-derived cardiomyocytes may block microcirculation
- Not all cells will have homing ability
- Intramyocardial injection inefficient
- Advantage of patch
  - Can be prepared in advance
  - Applies cells directly to infarcted area
  - Maintains cells in position until integrated
- Added value from material?



Materials - aims

- To create materials which:-
  - Have tensile strength sufficient to prevent scar expansion
  - Are biocompatible
  - Allow hESC-CM contraction/proliferation
  - Biodegrade over appropriate timescale
  - Do not produce toxic degradation products

Elastomeric polymers - passive Stress-Strain Curves

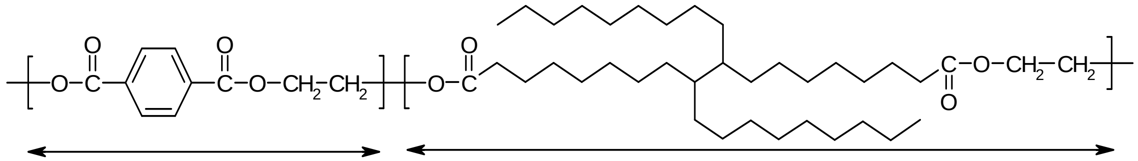


Chen et al Biomaterials 2008 and 2010

PED and PED-TiO_2

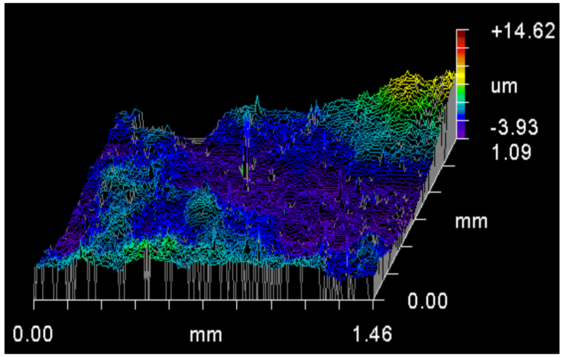
Hard [poly(ethylene terephthalate) (PET)] and soft [dilinoleic acid (DLA)] segments that have different degradation and mechanical properties.

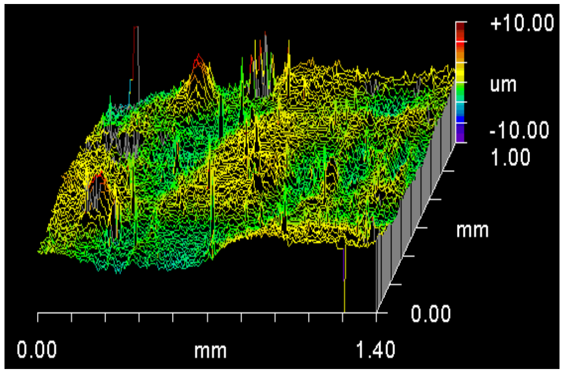
Prof M El-Fray



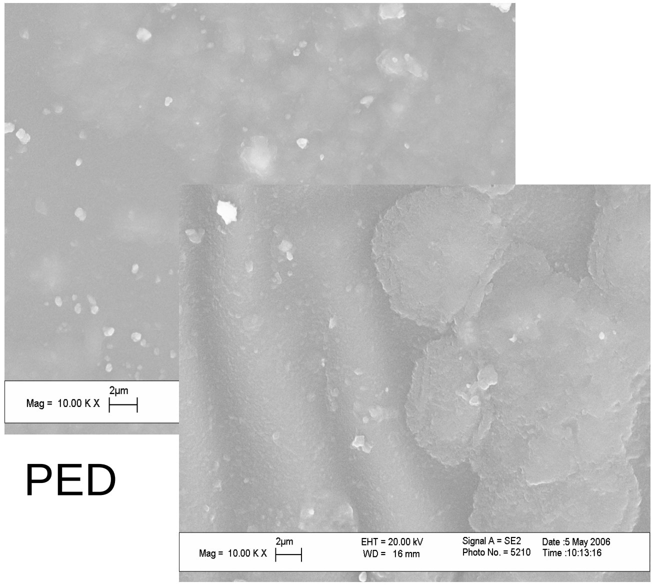
hard segment soft segment

White light interferometry





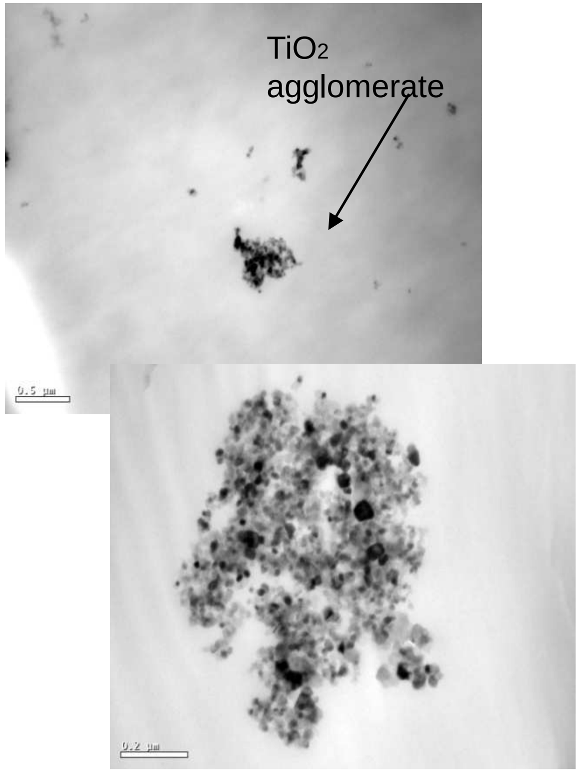
SEM



PED

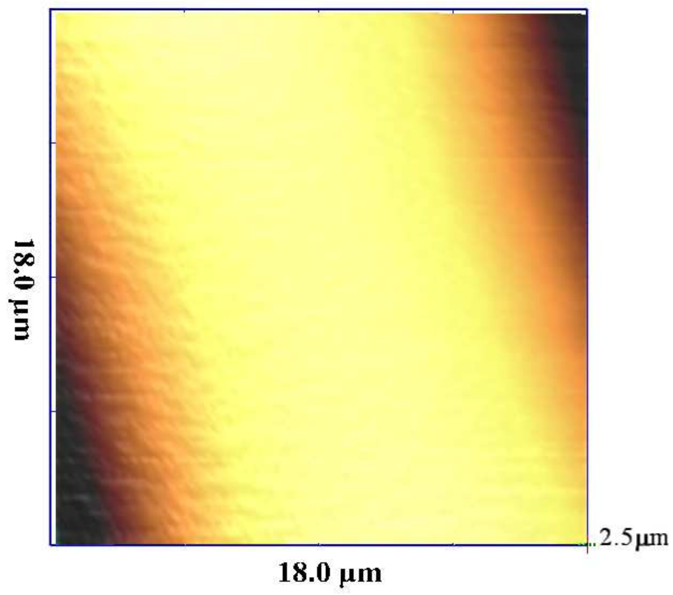
PED-0.2%TiO_2

TEM

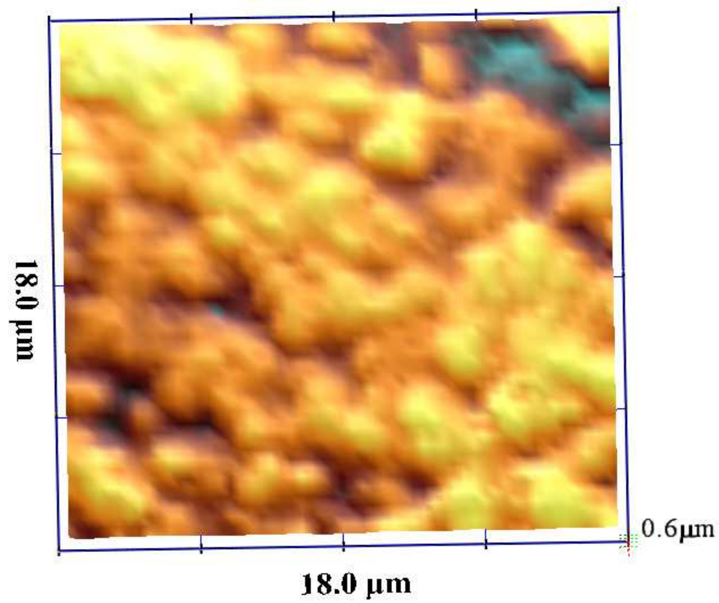


Scanning ion conductance microscope images

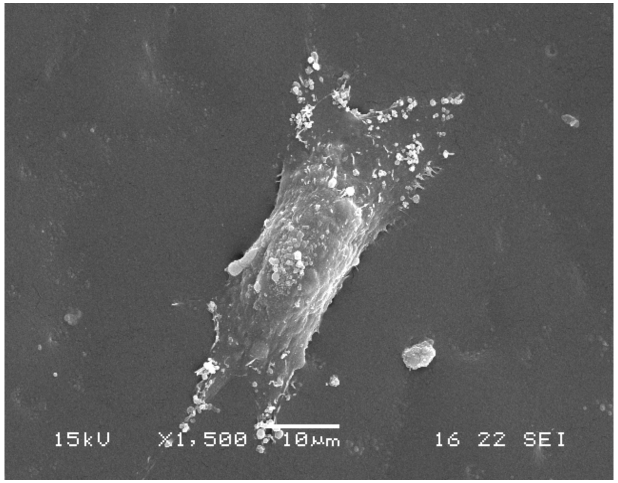
Polymer



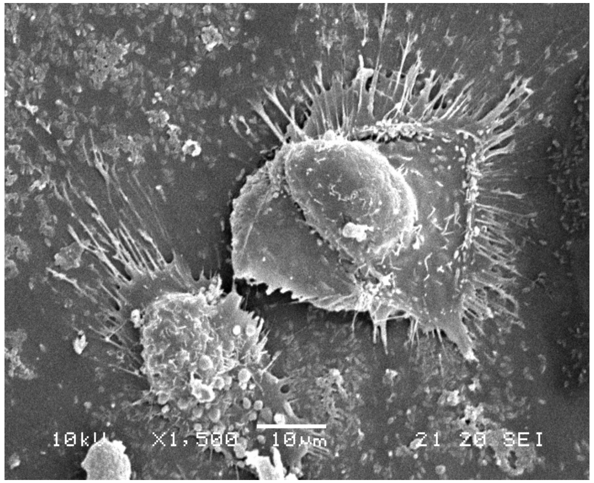
Polymer+titanium oxide



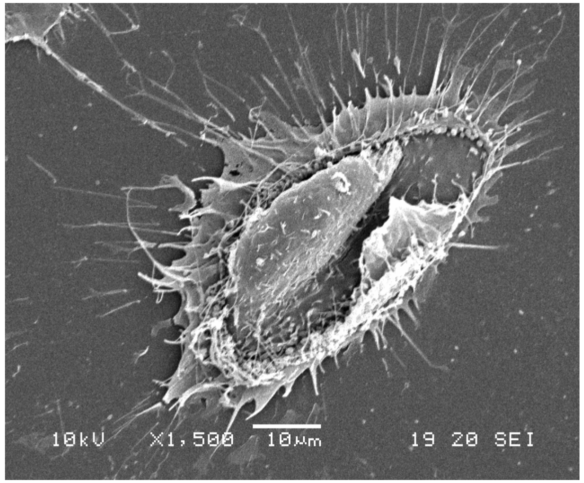
ADHESION AND SPREAD OF STEM CELL-DERIVED CARDIOMYOCYTES



PED



PED-0.2%TiO_2



Glass cover slip

PED/DLA and PED/DLA-0.2% TiO2 support beating hESC-CM for several months in culture

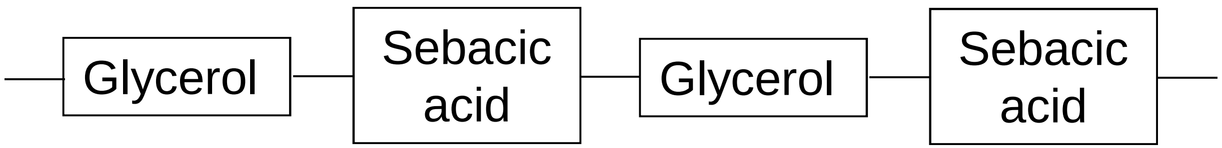
Proliferation of fibroblasts poorer than tissue culture plastic but better than present commercial material

Toxicity with TiO_2: only high levels affect adult myocytes, some evidence for slowing beating rate in hESC-CM with moderate levels

Hedeer Jawad, Nadire Ali

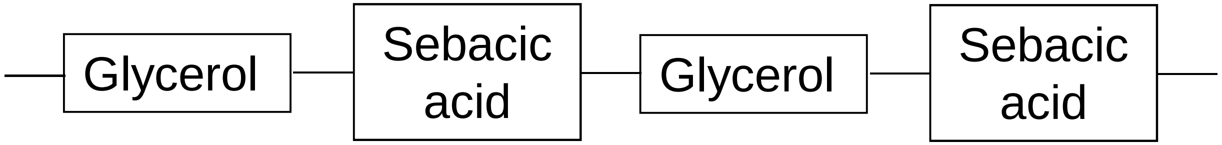
Polycondensation of PGS

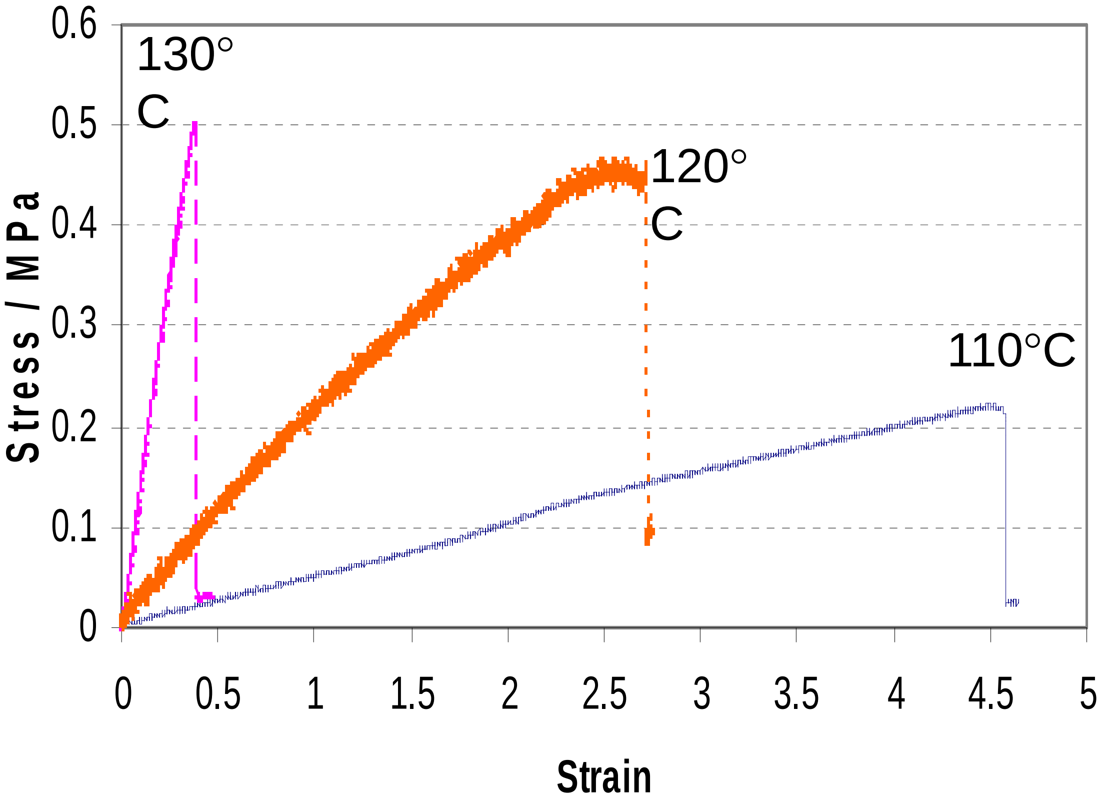
- **An equimolar mixture of glycerol and sebacic acid**

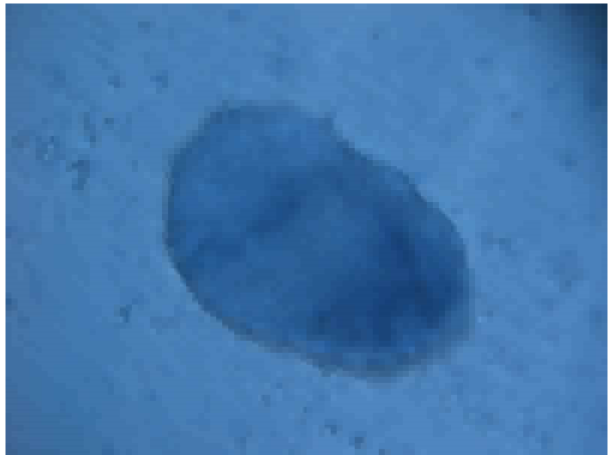


Chen QZ, Bismarck A, Hansen U, Harding SE, Ali NN, Boccaccini AR. Characterisation of a soft elastomer poly(glycerol sebacate) mechanically designed to match myocardial tissue. Biomaterials 2008

Poly (glycerol sebacate) - PGS



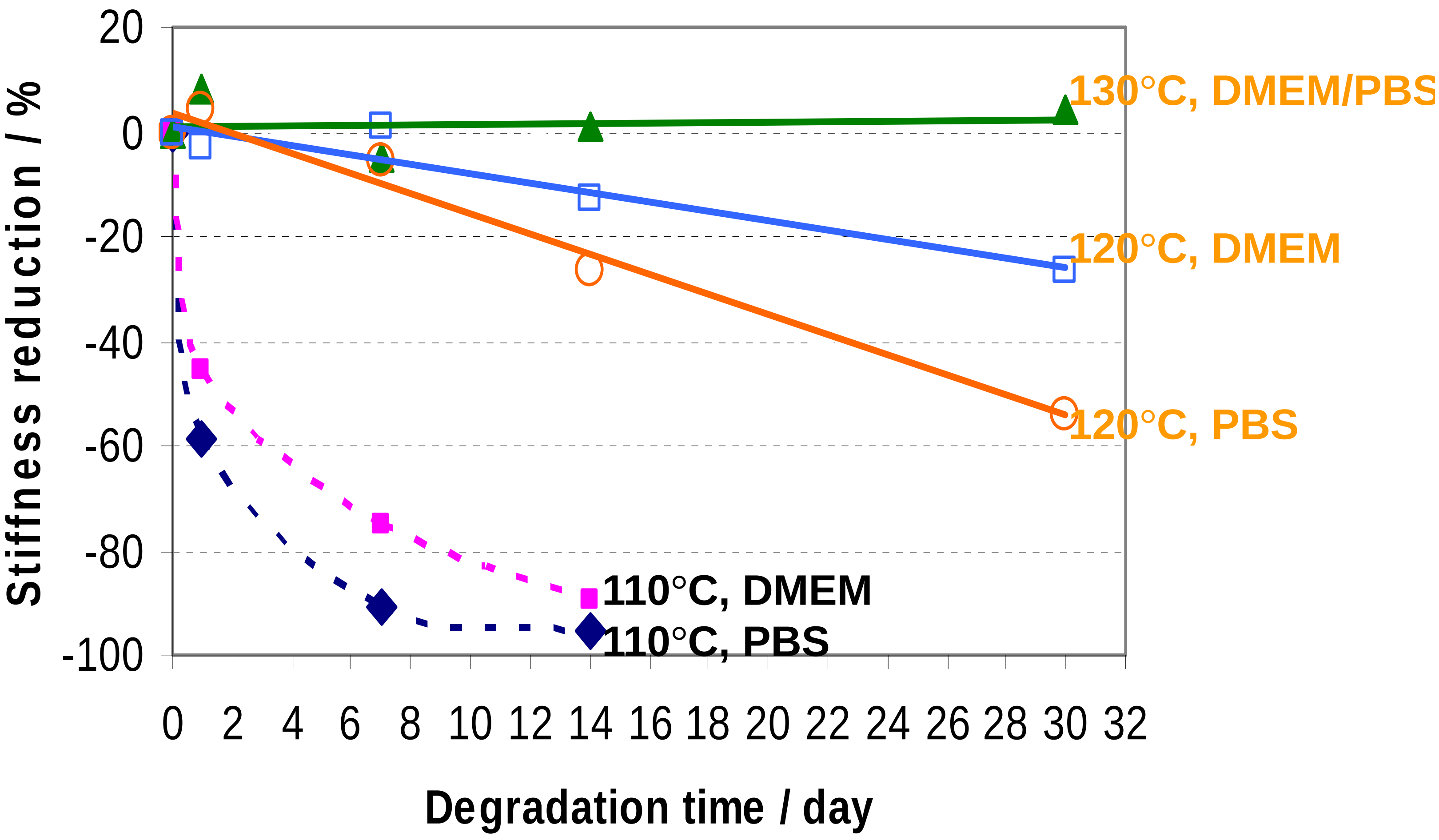




HESC-CM

Chen et al Biomaterials 2008 and 2010

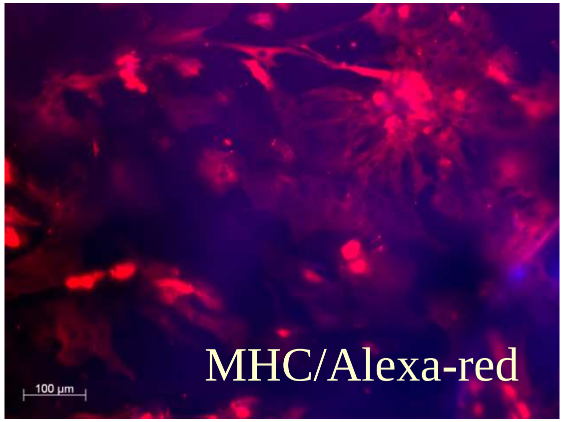
Biodegradation

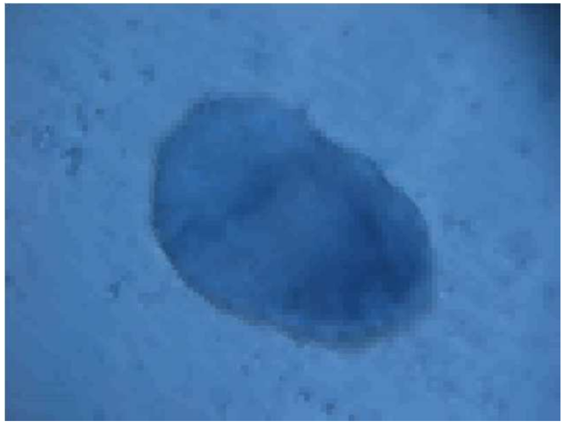


Chen et al Biomaterials 2008 and 2010

Summary 2 - PGS

- PGS has material properties that match those of human myocardium more closely than PET/DLA
- PGS can be fine tuned by synthesis temperature to produce a range of stiffness characteristics and rates of biodegradation
- PGS shows good biocompatibility and support of hESC-CM function
- An advantage for experimental studies is its relative transparency



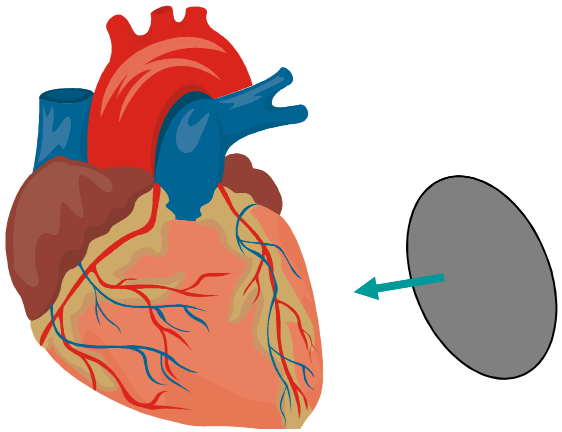


Patch testing on normal rat heart – in vivo

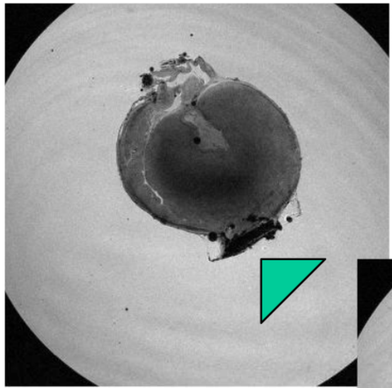
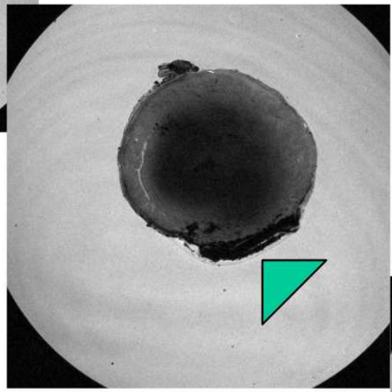
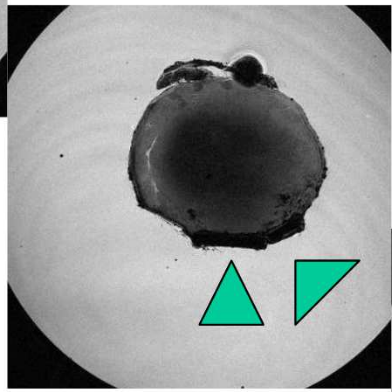
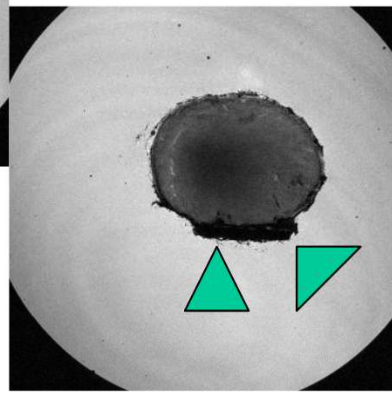
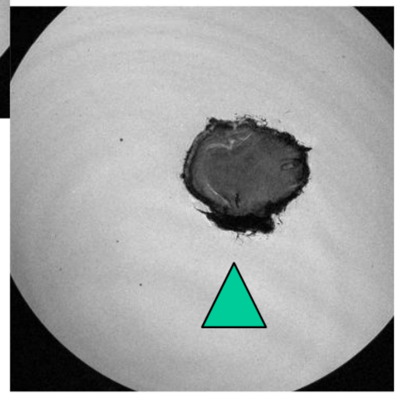
Heart Patch Transplantation

Pressure-volume study

2 weeks



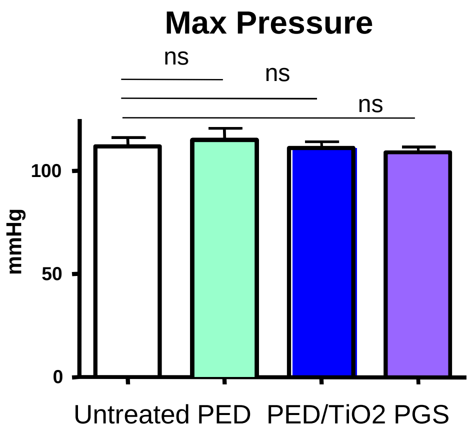
PED patch on rat heart

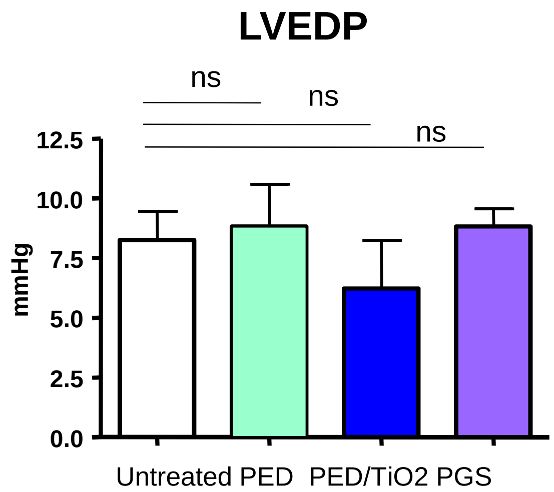






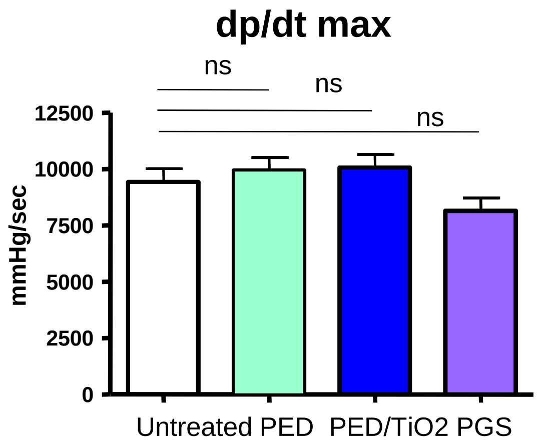
MRI – hearts examined after explant
Dan Stuckey, Oxford

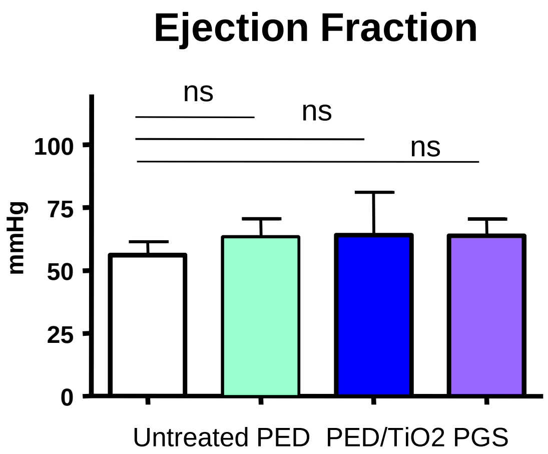
In vivo experiments on normal heart

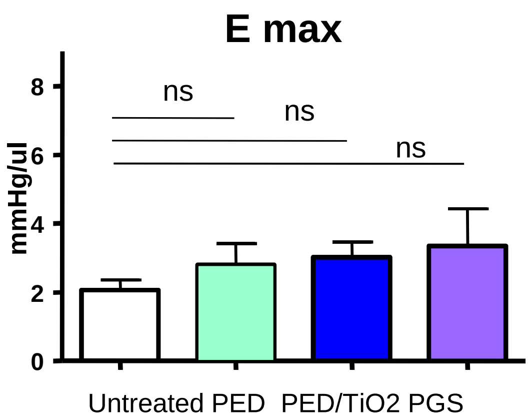
1 cm diameter patch, 0.5mm thick, sutured onto left ventricle, 2 weeks









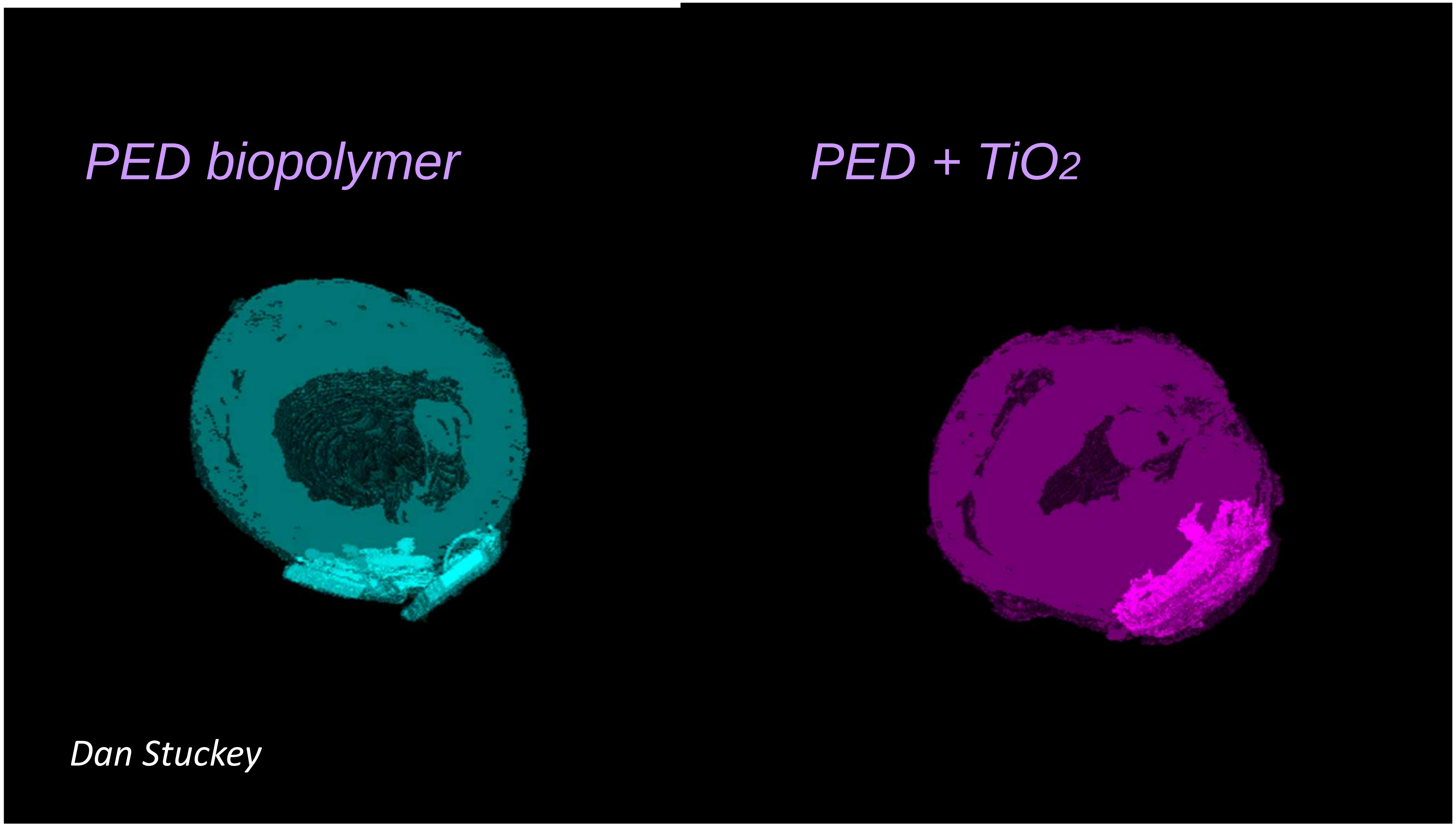


(N=6-8 per column)

Hikaru Ishii

MRI of cardiac scaffolds

In vitro detection

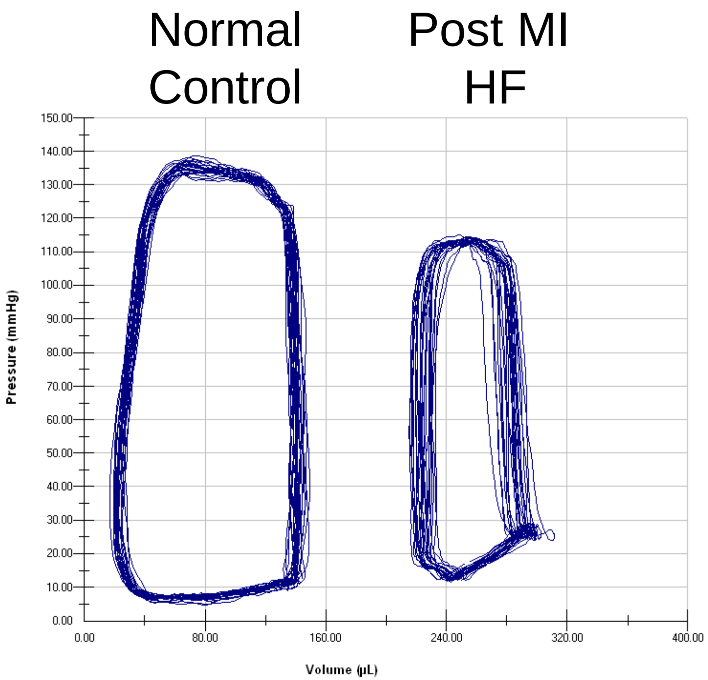


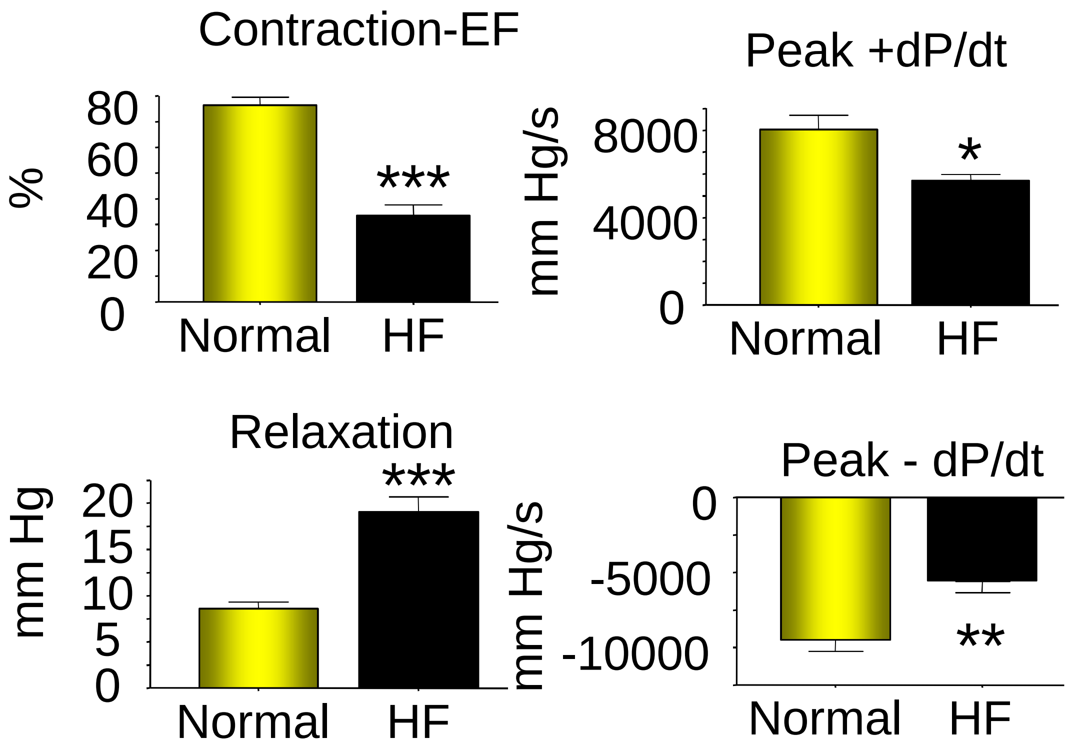
		Patch condition	Infection	Adhesion?	Comment
PED	Case 1	intact	-	+	
	Case 2	fractured	-	+	
	Case 3	fractured	-	+	
	Case 4	fractured	-	+	
	Case 5	fractured	-	+	
	Case 6	fractured	-	+	
PED/ TiO2	Case 1	Intact	-	+	
	Case 2	Intact	-	++	Infarct
	Case 3	Intact	-	+++	
	Case 4	Intact	-	+++	
	Case 5	Intact	-	+++	
	Case 6	Intact	-	+	
	Case 7	Intact	-	+	
PGS	Case 1	Partly torn	-	+	
	Case 2	Intact	-	+	
	Case 3	Intact	-	+	
	Case 4	Intact	-	+	
	Case 5	Intact	-	+	
	Case 6	Intact	-	+	

Chen , Ishii, Harding Biomaterials 2010

Rat Post MI Heart Failure Model
Steady State Pressure-Volume Analysis

n=6 per study arm. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs normal controls

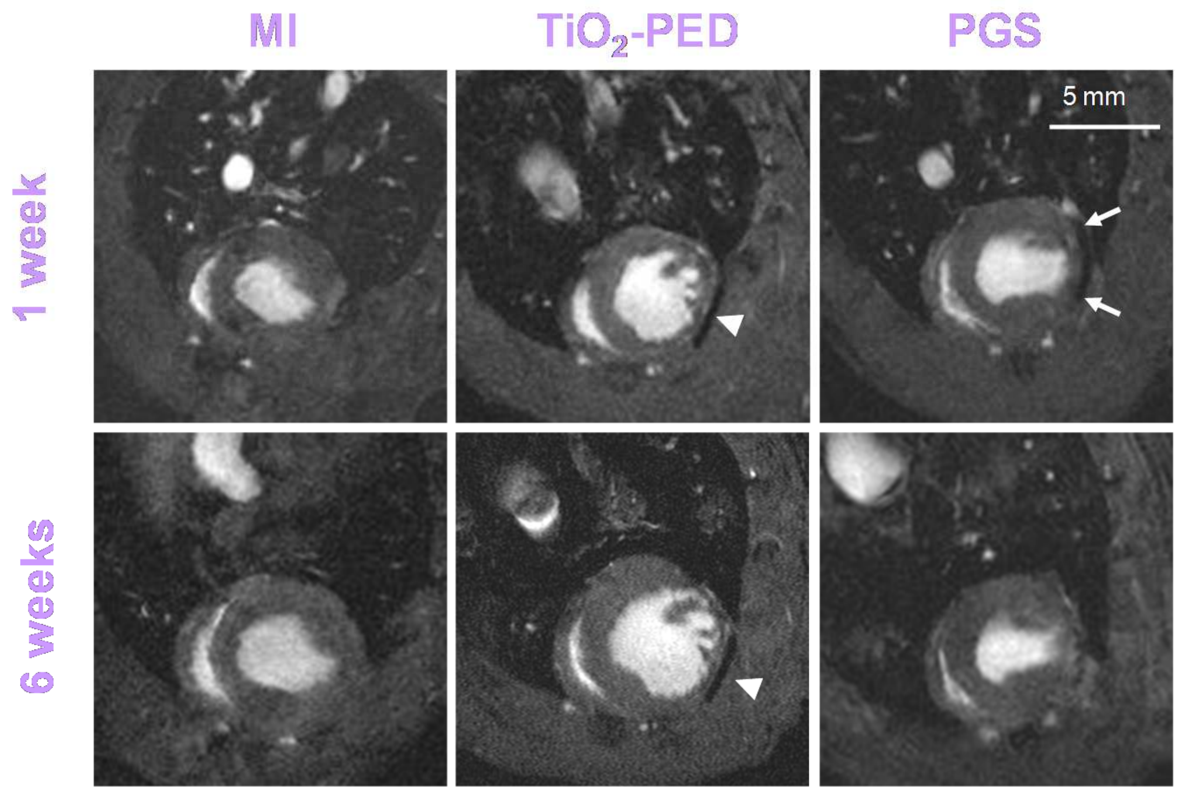


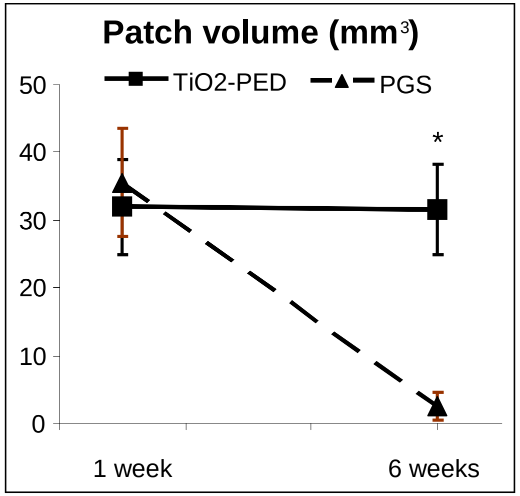


Lyon et al PNAS 2009

In vivo scaffold degradation

Hearts imaged *in vivo* at 1 and 6 weeks
PGS scaffold degraded



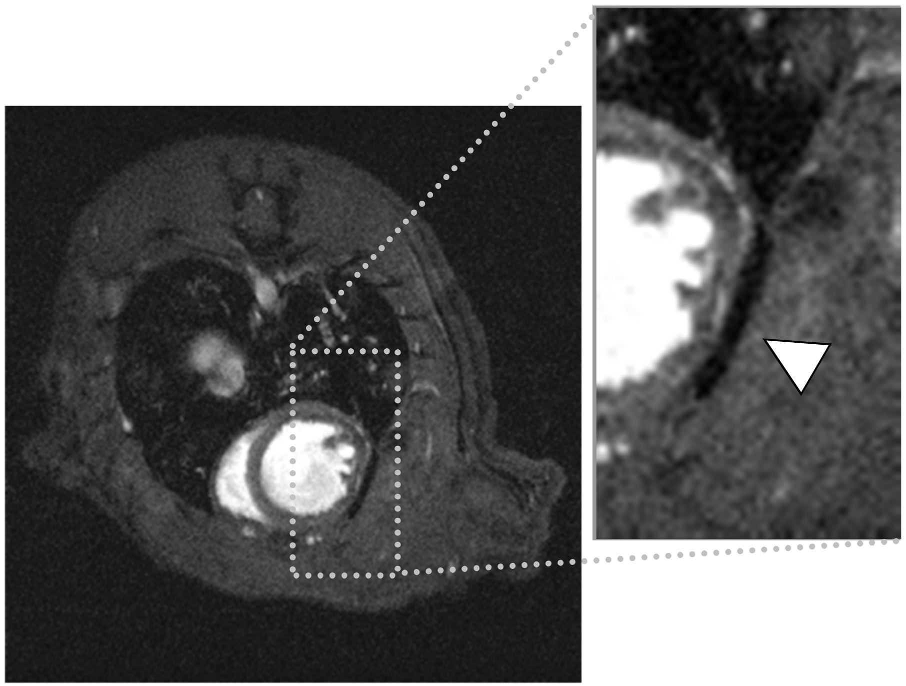


Dan Stuckey, Carolyn Carr

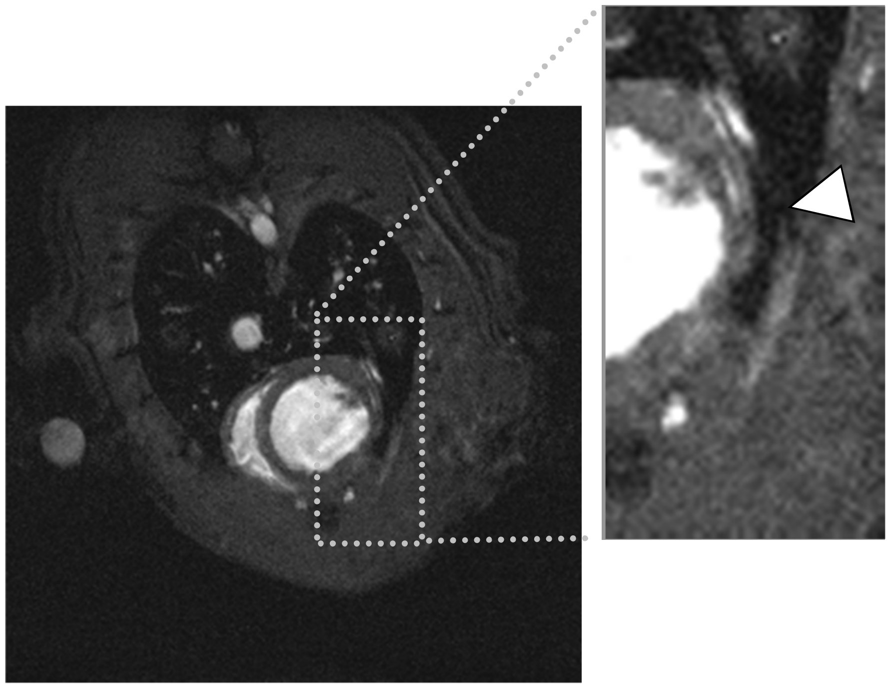
In vivo detection of scaffold movement

Scaffolds attached infarcted rat heart epicardium (n = 12)
Hearts imaged *in vivo* at 1 week at 11.7T

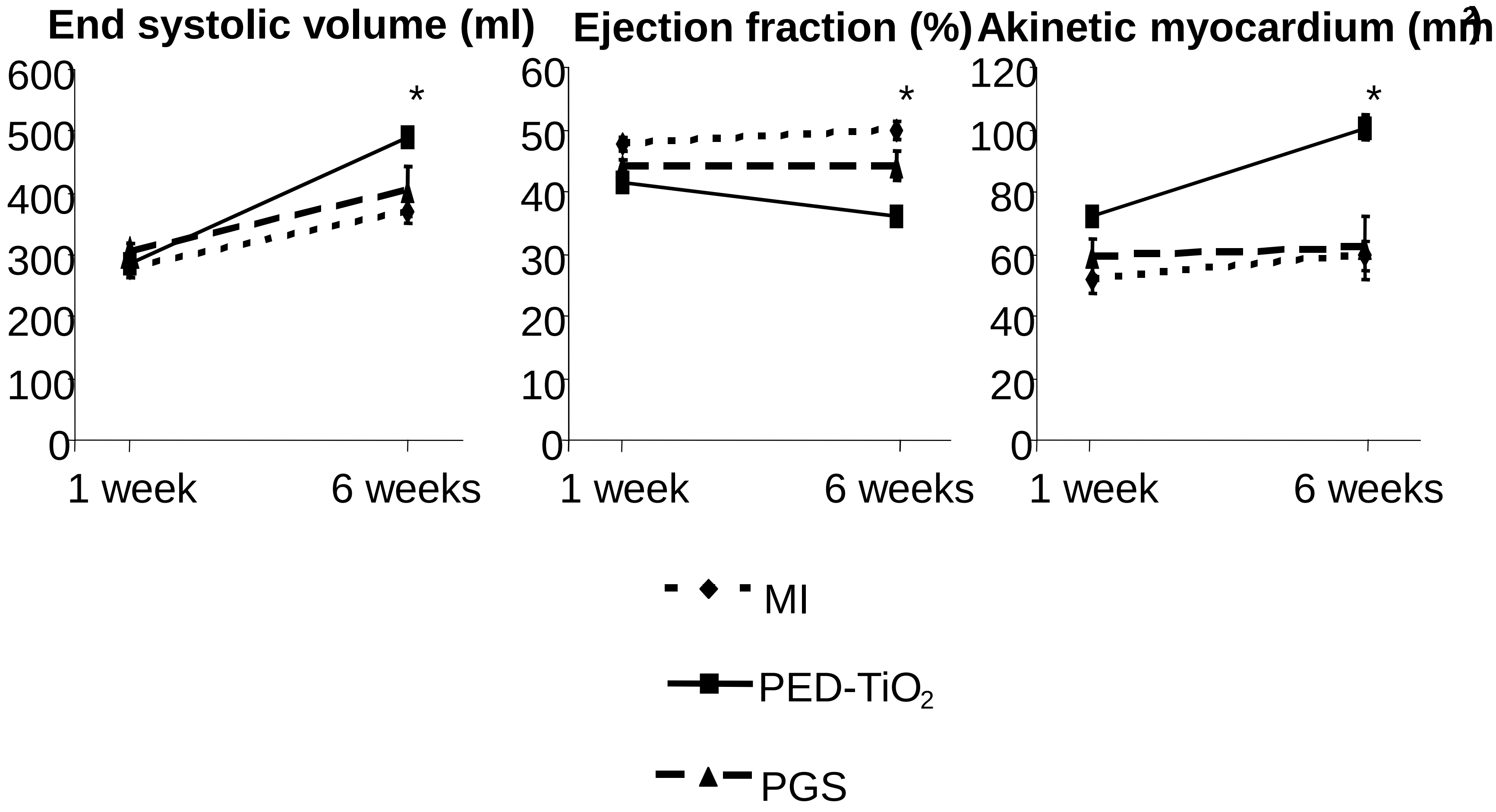
PED + TiO_2



PGS

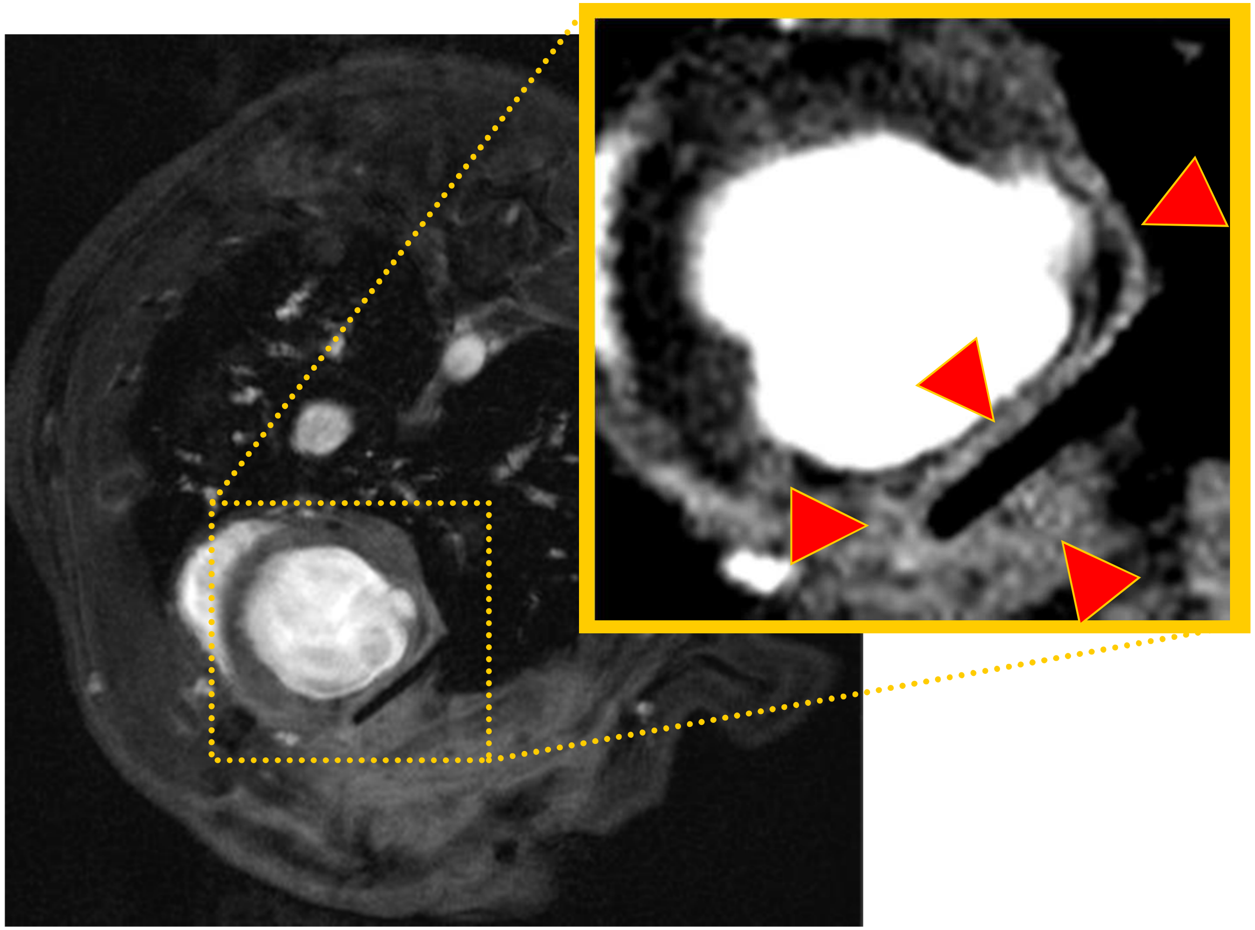


Stuckey et al Tissue Engineering 2010



Stuckey et al Tissue Engineering 2010

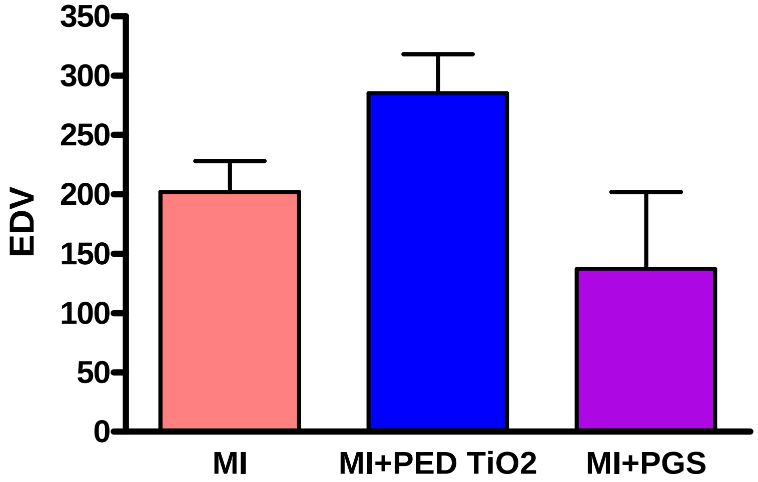
Delayed enhancement indicates necrosis adjacent to PED-TiO_2 patches



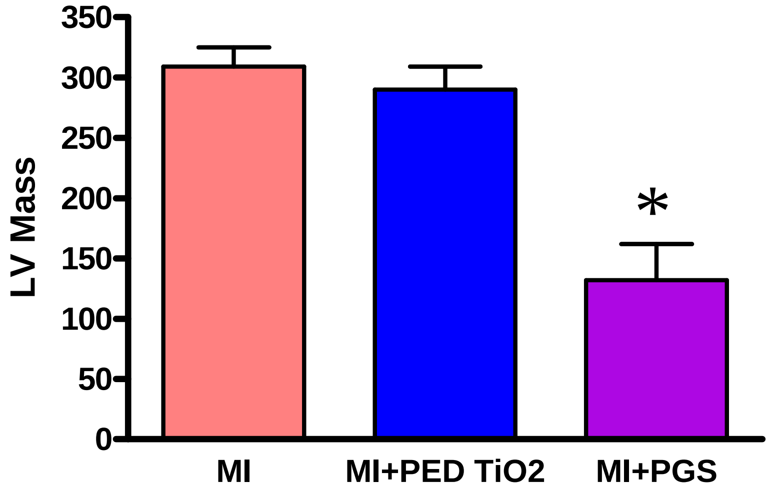
Stuckey et al Tissue Engineering 2010

Reduction of MI-induced remodelling by PGS

Change in end diastolic volume



Change in left ventricular mass



Stuckey et al Tissue Engineering 2010

Summary

- PED, PED-TiO_2 and PGS are biocompatible with hESC-CM
- Application of biomaterial patches produced only minor changes in contractile function of normal rat hearts ex vivo and in vivo.
- The PED patch was too weak to withstand in vivo forces.
- Addition of TiO_2 improved surface properties and in vivo durability, but adhesions were increased.
- In infarcted animals, PED-TiO_2 exacerbated injury and had a deleterious effect on function.
- The PED-TiO_2 did not mould to the ventricle – problems of scale with TE in small animals
- PGS can survive in vivo, and mould to ventricle, but degradation rate is increased over in vitro studies. Biodegradability may be an advantage for cell delivery.
- PGS reduced remodelling, although without improvement of contractile function.

Surface functionalization

Surface functionalization

- Polyhydroxyalkanoates (**PHAs**) are an **emerging class of biomedical polymers**

- The Roy laboratory have pioneered the use of Gram positive bacteria, especially, *Bacillus* sp. for the production of non-immunogenic PHAs

- *Bacillus subtilis OK2 and Psuedomonas mendocina* are relatively unexplored bacteria and have been successfully used for the production of a range of SCL and MCL-PHAs and in large scale

- The **SCL-PHA, P(3HB)** and **MCL-PHAs, P(3HO) and P(3HN-co-3HHP)** are being explored for use in **Cardiac Tissue engineering** and have been found to be **promising future materials** for the development of cardiac patches

Imperial College London

British Heart Foundation

UCL

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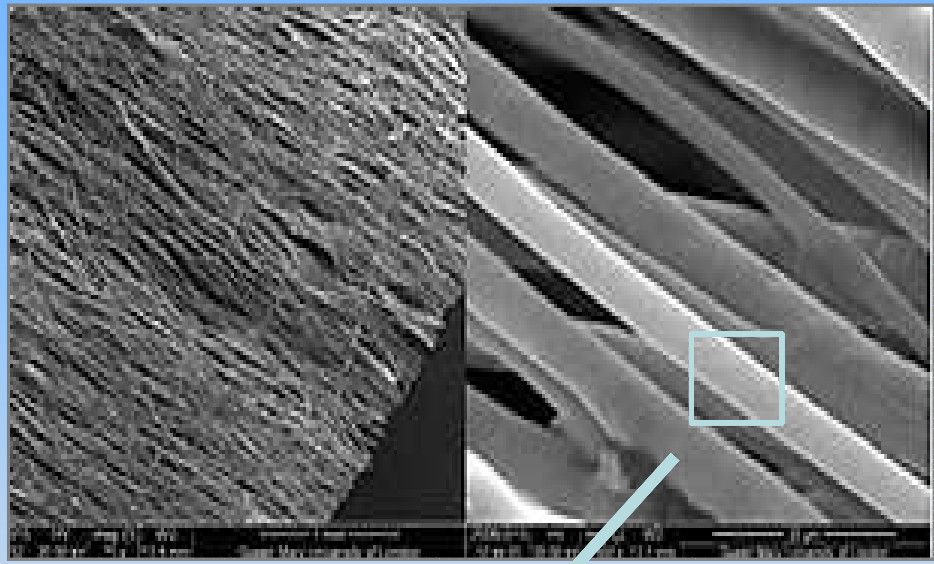
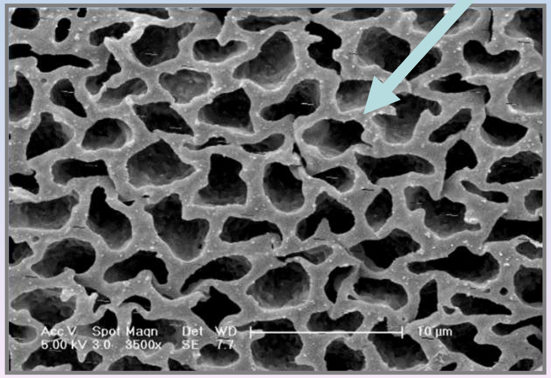
Production of P(3HB) based cell sheets

Aligned P(3HB) fibre sheets

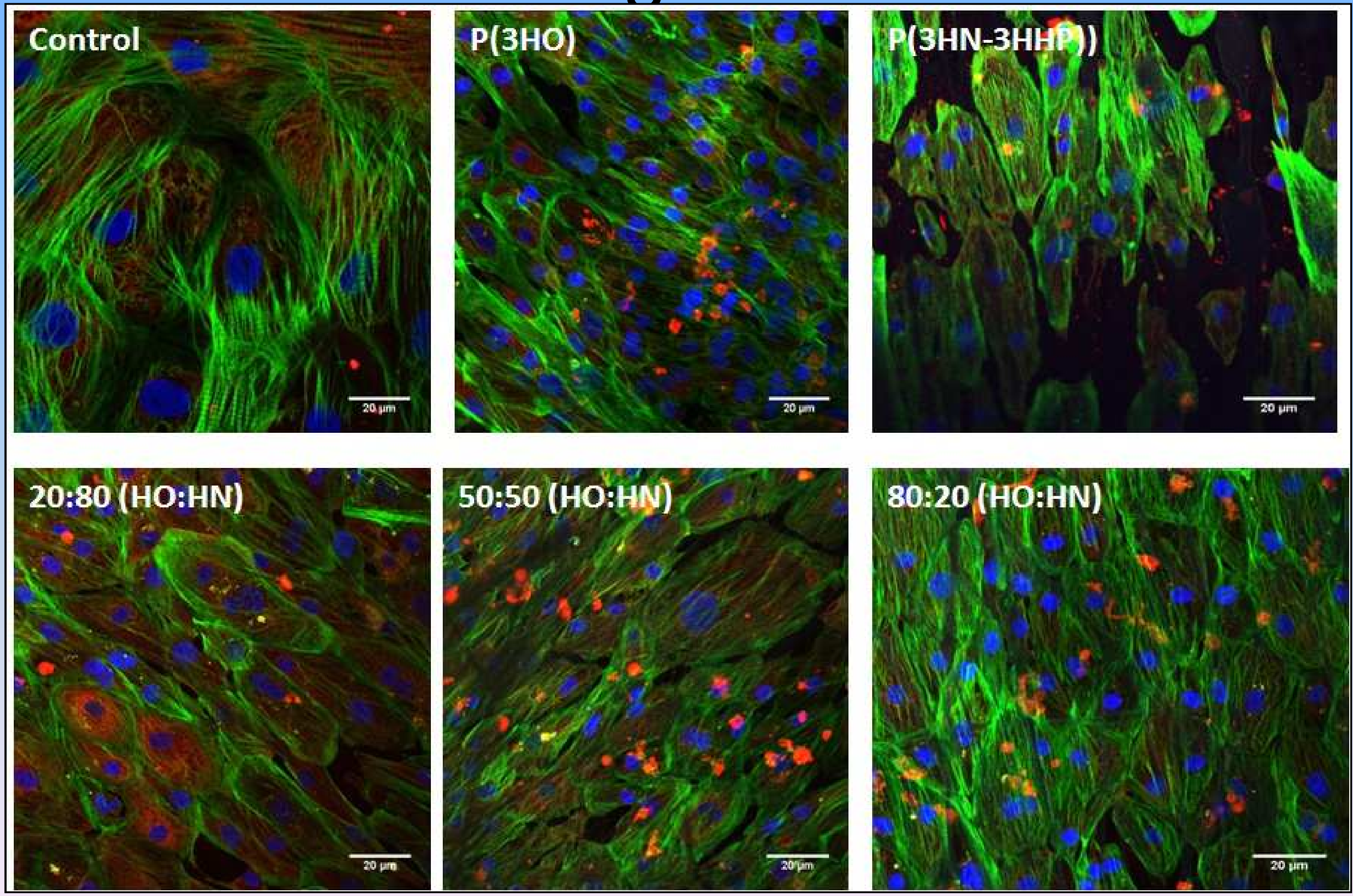




SEM image of the P(3HB) fibre sheets

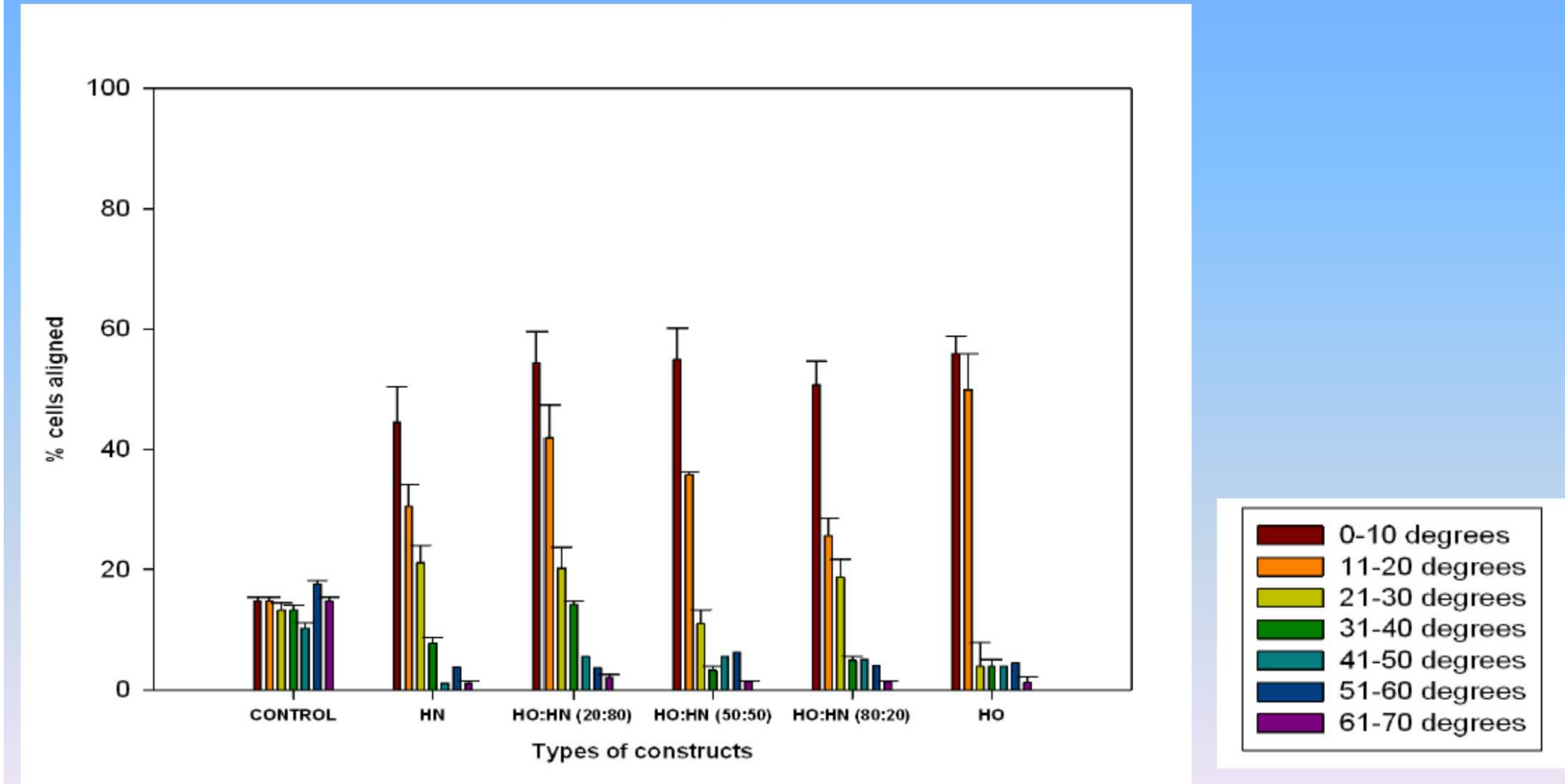
Basnett and Mahalingam *et al*., 2015, unpublished data

hiPSC-CMs on P(3HO)/P(3HN-co-3HHP) blend-Aligned Fibres



Dubey *et al*., 2015, unpublished data

hiPSC-CMs on P(3HO)/P(3HN-co-3HHP) blend-Aligned Fibres



Dubey *et al.*, 2015, unpublished data

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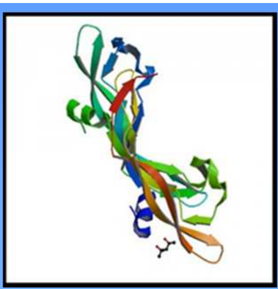

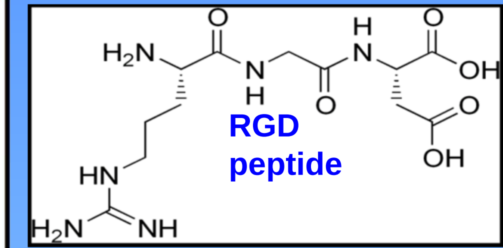

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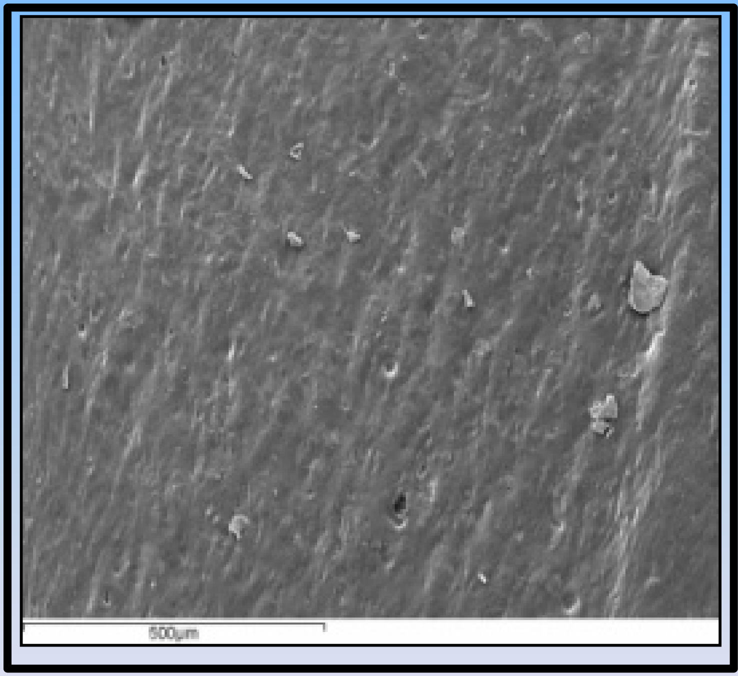
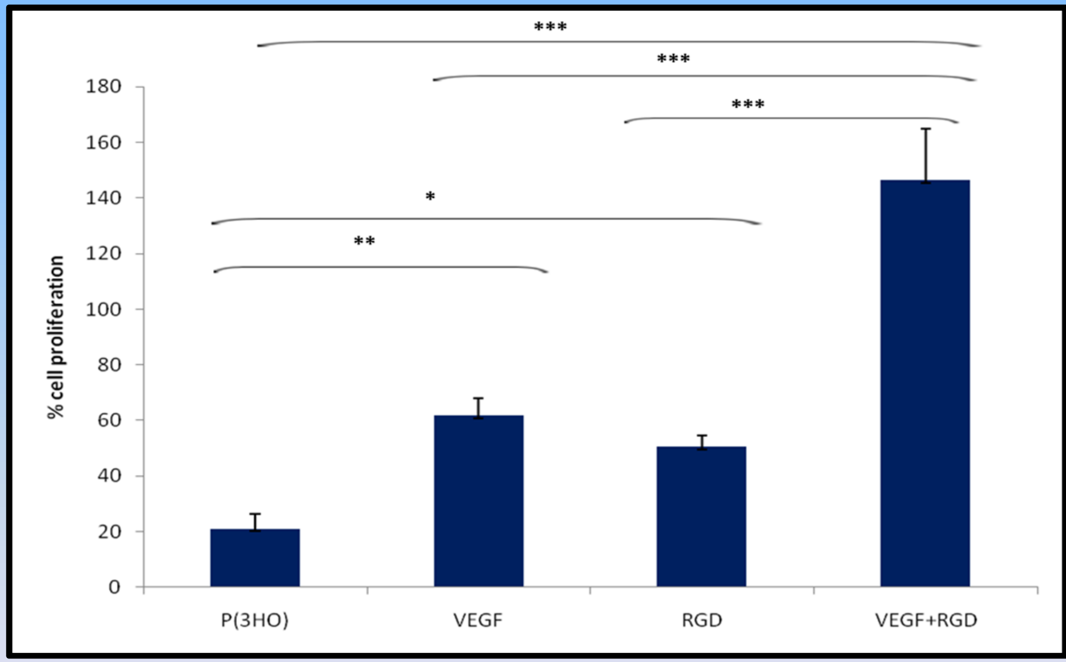
P(3HO) cardiac patches with RGD peptide and VEGF



SEM images of RGD and VEGF containing P(3HO) film

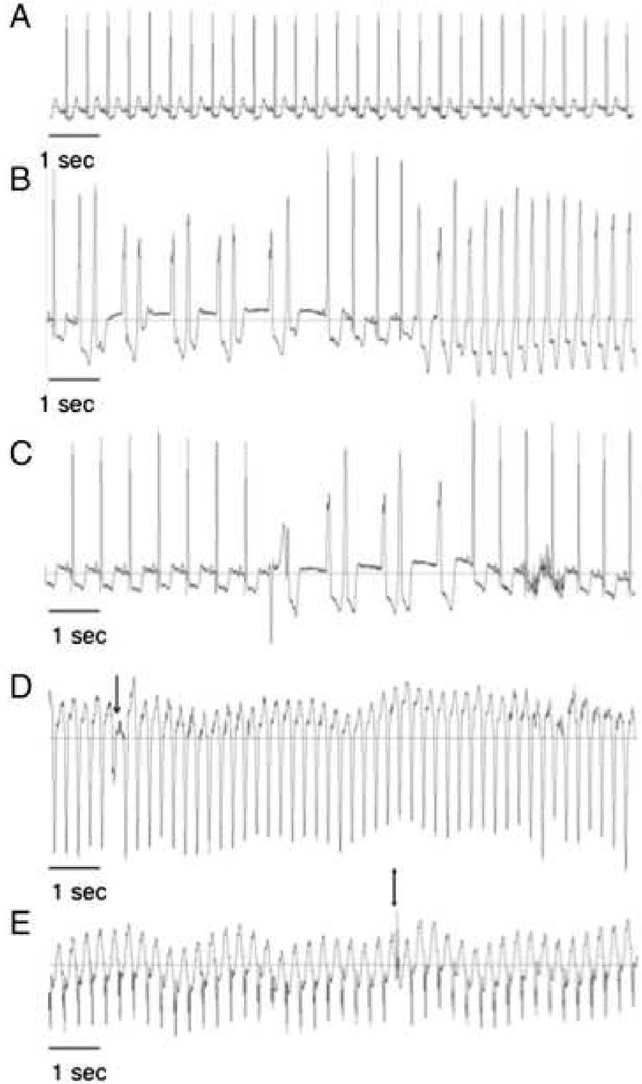


% Cell proliferation of C2C12 cell line at 24 hr

Bagdadi *et al.*, 2015, unpublished data

Conductive polymers

Arrhythmias occur early after engraftment of human cardiomyocytes in the infarcted monkey heart.



James J.H. Chong , Charles E. Murry

Cardiac regeneration using pluripotent stem cells—Progression to large animal models

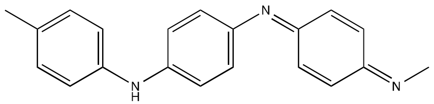
Stem Cell Research, Volume 13, Issue 3, Part B, 2014, 654 - 665

Imperial College London

Sutureless Conductive Polymer Patch

A sutureless conductive patch which can be attached to the surface of the heart by photoadhesion using a laser

Conductive Polymer: Polyaniline



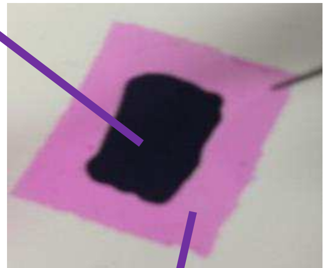
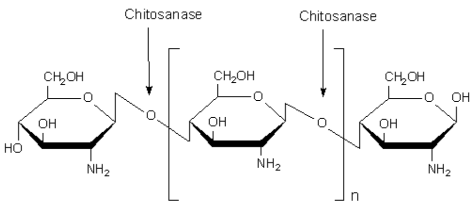
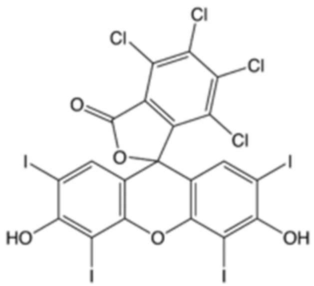


Photo-activated adhesive: Chitosan + Rose Bengal dye

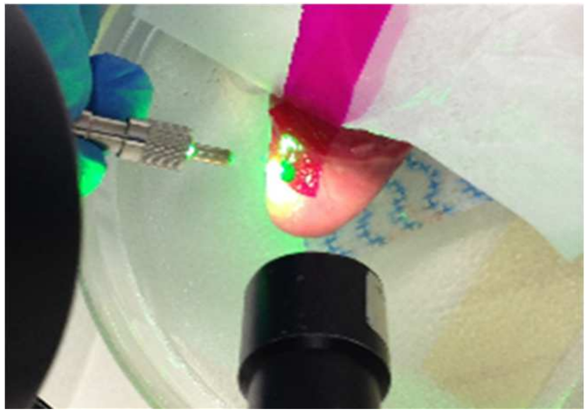




i) A flexible biomaterial

ii) A conductive substrate shown to have a stable response to extended stimulation regimes

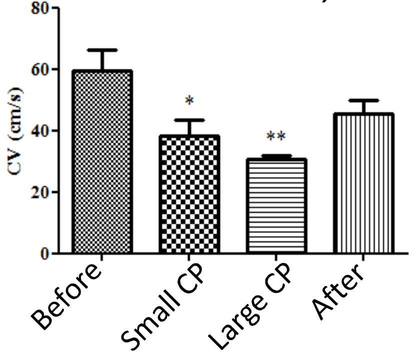
iii) An adhesive property offering potential advantages over other patches that often require the use of sutures

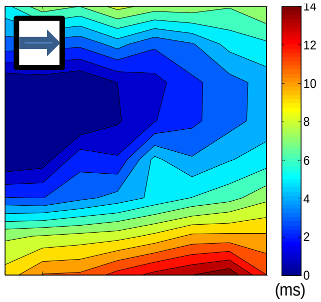


Photoadhesion using green laser (λ=532nm)

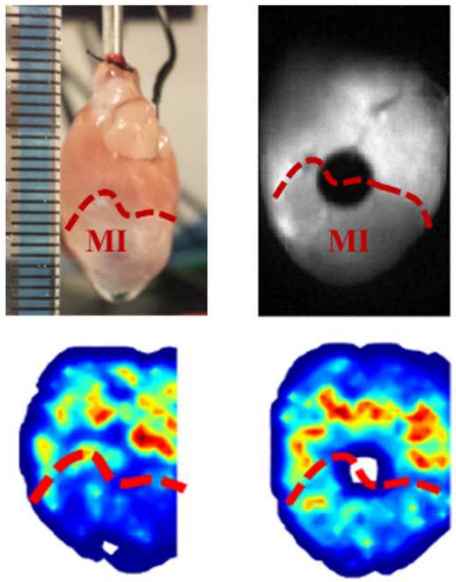
In vitro testing of conductive polymer (CP) on rat heart and myocardial slice

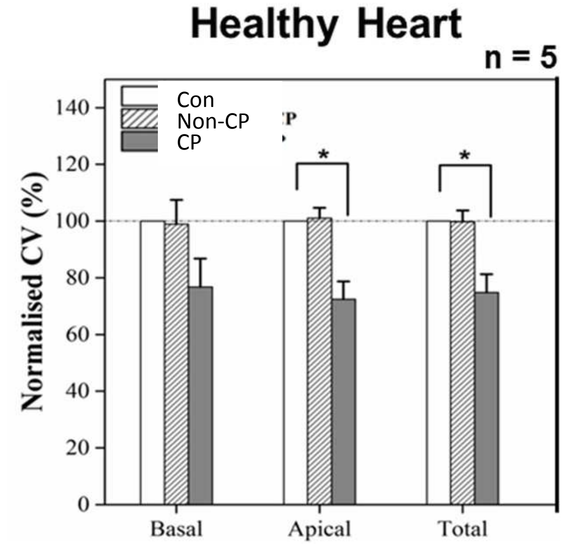
Myocardial slice from healthy heart
Conduction velocity

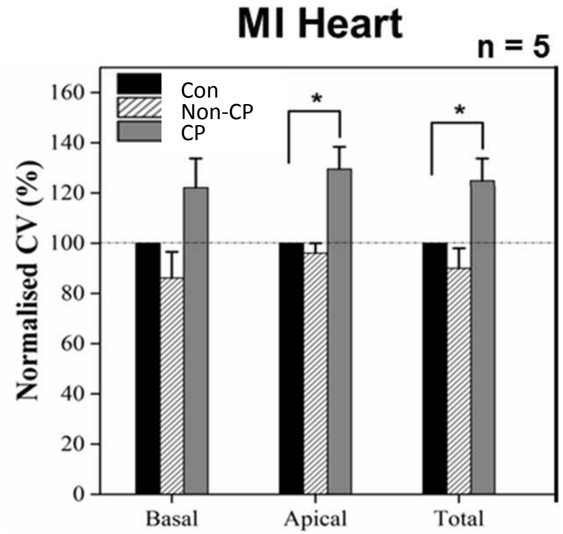




Patch applied to centre of LV bridging infarcted and non-infarcted rat myocardium





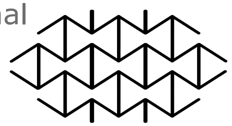


In vivo results, 2 weeks normal heart:

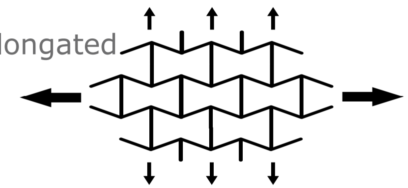
- Minor increase in EF; no arrhythmia
- Patch encapsulated – too stiff/brittle

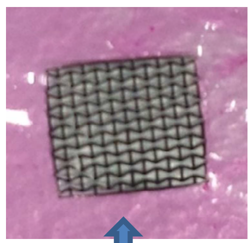
New formulation – auxetic patterning

Original



Elongated





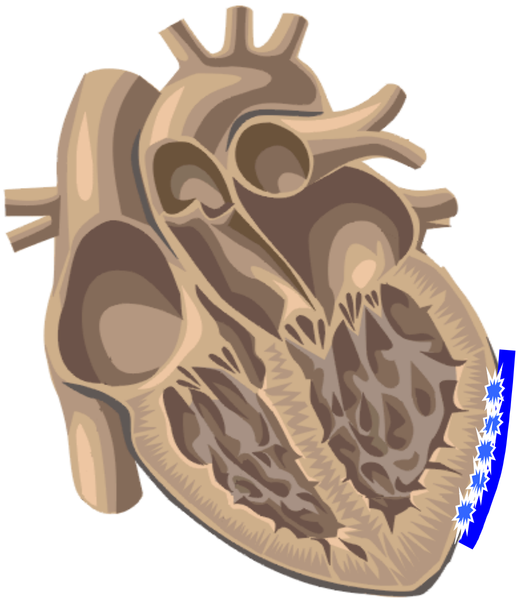
Laser adhesion border

Mawad, Stevens, Terracciano, Harding
Submitted for publication

A PATCH FOR STEM CELL DELIVERY TO THE HEART

Added value from material

- Have tensile strength sufficient to prevent scar expansion
- Are biocompatible
- Allow hPSC-CM contraction/proliferation
- Biodegrade over appropriate timescale
- Tethered protective agents – hydrogels with RGD motif
- Agents to promote vascularisation
- Polymers with electrical coupling properties
- Do not produce toxic degradation products



Advantage of patch

- Can be prepared in advance
- Applies cells directly to infarcted area
- Maintains cells in position until integrated

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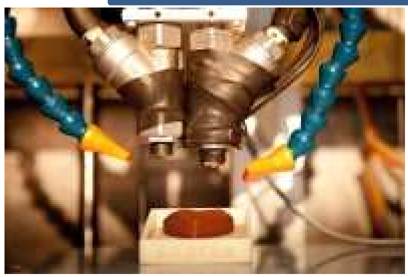
Home > News > Chocolate 3D printer arrives

NEWS

Chocolate 3D printer arrives at last

By Charlie Sorrel | 07 July 11

"Now we have an opportunity to combine chocolate with digital technology, including the design, digital manufacturing and social networking. " Dr Hao



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